ANALGESIC ACTIVITY, ACUTE ORAL TOXICITY AND PHYTOCHEMICAL SCREENING OF CRUDE EXTRACTS OF *Mystroxylon aethiopicum* (Thunb.) Loes. (Celastraceae.)

John Karanja Muchonjo

A thesis submitted in partial fulfilment of the requirements for the award of Master of Science Degree in Pharmacology and Toxicology

Department of Public Health, Pharmacology and Toxicology

Faculty of Veterinary Medicine

University of Nairobi

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature _____ Date: 01/11/2021

Dr. John Karanja Muchonjo

J56/12647/2018

This thesis has been submitted for examination with our approval as University supervisors.

Signature _____ Date 02/11/2021

Prof. James M. Mbaria (BVM, MSc, PhD)

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi.

Signature _____ Date 2/11/21.

Dr. Joseph M. Nguta, BVM, MSc, PhD.

Department of Public Health, Pharmacology & Toxicology,

University of Nairobi

DECLARATION OF ORIGINALITY

DECLARATION OF ORIGINALITY

UNIVERSITY OF NAIROBI

NAME OF STUDENT: DR.JOHN KARANJA MUCHONJO

REGISTRATION NUMBER: J56/12647/2018

COLLEGE: COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES

FACULTY/SCHOOL/INSTITUTE: VETERINARY MEDICINE

DEPARTMENT: PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY

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DEDICATION

This thesis is dedicated to my beloved wife Doreen and Lovely sons (Gyan and Rylan) for their love, encouragement, perseverance and prayers during the period of my studies.

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
°C	Degree centigrade
OECD	Organization of Economic Cooperation and Development
LD50	Median lethal dose
NACOSTI	National Commission for Science, Technology and Innovation
SEM	Standard error of the mean
Mg	Milligram
Kg	Kilogram
Bwt	Body weight
CGMP	Cyclic guanosine monophosphate
ATP	Adenosine triphosphate
FVM	Faculty of Veterinary Medicine
BACUC	Biosafety, Animal Care and Use Committee
IASP	International Association for the Study of Pain
UTM	Universal Traverse Mercator

ABSTRACT

Pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage. Analgesics i.e. Opioids and Non-steroidal anti-inflammatory drugs (NSAIDs) used to manage pain are less effective, inaccessible, and unaffordable and elicit deleterious side effects, hence warrants search for alternative and complementary strategies to avert pain without adverse effects and cheap.

Due to folklore history of utilization in traditional medicine, easy accessibility, affordability and presumed low toxicity profiles, Medicinal plants like *Mystroxylon aethiopicum* have better chances of offering potent analgesic. Nonetheless, there's scanty scientific data on analgesic efficacy, potency and toxicity.

This study evaluated antinociceptive activity, acute oral toxicity and qualitative phytochemical composition of crude extracts of *Mystroxylon aethiopicum*.

The plant was identified and authenticated by a taxonomist at the East Africa Herbarium. Methanolic and aqueous extracts from the plant were prepared according to standard maceration method.

Experimental female Swiss albino mice aged 4-5weeks were obtained from the animal breeding unit, PHPT Department and handled as per set guidelines (OECD 2008) i.e.23± 2^{o}C room temperature; 55-65 % Relative humidity; twelve (12)-hour daytime/night time cycle, well fed with standard mice pellets and clean drinking tap water *ad libitum*.

Determination of analgesic activity of *Mystroxylon aethiopicum* extracts was done by acetic acid-induced writhing technique. The data obtained was tabulated in Microsoft Excel 365, exported to

Graph Pad Prism statistical software version 8.4.3 for analysis; was subjected to descriptive statistics and expressed as $\bar{x} \pm SEM$. One-Way ANOVA with Tukey's *post hoc* test were used to determine significant differentials among experimental groups and for pairwise comparison and separation of means respectively. The results revealed that both crude extracts of *Mystroxylon aethiopicum* possess analgesic activity. The mice treated with aqueous extract exhibited significantly low writhing frequency compared to those that received the methanolic extract ($p<0.05$).

Acute oral toxicity was performed and analyzed as per the OECD 425 (2008) guideline monitoring for toxicity signs and fatality. There were no signs of toxicity and fatality in mice even at the cut-off dose (2000 mg/Kg bwt.) thereby conferring LD_{50} values >2000 mg/Kg bwt hence safe.

Qualitative phytochemical screening was done following standard phytochemical screening methods described by Harborne, Evans and Trease and Savithrama. The data was tabulated and revealed presence of alkaloids, saponins, tannins, glycosides, phenols, flavonoid, terpenoids, saccharides and proteins.

In conclusion, the Mystroxylon aethiopicum has analgesic activity, no toxicity signs and possesses active phytochemicals.

Recommends for herbalist to continue using *Mystroxylon aethiopicum* in analgesic, further study Sub-acute/chronic toxicological studies on the studied plant extracts be conducted to fully profile and assure their safety.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage (IASP, 1982 and Raja *et al*., 2020). It'as a major symptom of many different disease (Ströbel, 2016). It's one of the most widely diagnosed and managed symptoms of human diseases. In fact, it's the main prognostic indicator of many diseases and a key goal for healthcare. Pain epidemic status across the globe shows that close to 41.1% of adults in the developing and 37.3% of adults in developed nations suffer from chronic pain due to injuries, diseases or disorders (Tsang *et al.,* 2008). Generally, the burden of all types of pain affecting both children and adults, with or without defined cause are on the rise globally (Henschke *et al.,* 2015; Mills *et al.,* 2019). 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% to 31%. One year incidences of low back pain range from 1.5% to 38.9 % with a recurrence rate of up to 80 % (Henschke *et al.,* 2015; Mills *et al.,* 2019).

Pain arises from either the misfiring of a nerve or damage to the tissues via nociceptors (Kendroud and Hanna, 2019). Nociception is the process by which the nervous system (central and peripheral) responds to noxious stimuli including injury to tissues or elevated temperatures (Kendroud and Hanna, 2019). Such noxious conditions activate receptors responsible for relaying nociceptive information hence pain sensation (Kendroud and Hanna, 2019). 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 (Olela *et al.,* 2020).

Conventionally, pain management involves the use of analgesic and anti-inflammatory drugs, which target and modify both peripheral and central nociceptive pathways (Opioid system receptors pathway, cyclic guanosine monophosphate (cGMP) pathway, Adenosine triphosphate (ATP)-sensitive potassium (K+) channel pathway) to supress pain (Dinakar and Stillman, 2016). Even though the current analgesics and anti-inflammatories exhibit marked pain relief capabilities, they are not universally accessible (e.g. a patient may need an opioid but cannot access since it's a controlled drug thus becoming a challenge), affordable, and are associated with severe side effects, including constipation, drowsiness, dizziness, stomach upset, skin itching or rash and dry mouth (Maund, 2011; Cazacu, 2015). For example, aspirin (acetylsalicylic acid) and naproxen causes indigestion, stomach ulcers, kidney damage, hepatotoxicity, stroke, and 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 (Cazacu *et al.,* 2015). 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 (Shakya, 2016).

Medicinal plants have a rich history of folklore usage, are relatively safer, inexpensive, and readily available (Ngule *et al.,* 2013; Abdullahi, 2011; Omwenga, 2015; Mahomoodally and

Kigen, 2013). In fact, developing and developed countries has at least 80% and 40% respectively, of the global human population using plant-derived traditional medicines for their primary healthcare needs including pain management (WHO, 2019). Moreover, herbal medicines have secondary metabolites which are pharmacologically active against various diseases in addition to dietary and health promoting benefits (Moriasi *et al.,* 2020). 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 (PlantZAfrica, n.d.). Its stem bark is ethnomedically used to treat stomach-aches, chronic joint and back pains, coughs, anaemia and worm infestations in livestock (Harborne,1998; Githinji and Maina, 2018; Mhuji *et al.,* 2018). Despite these ethnomedical claims, there is no scientific prove of its pain management efficacy and safety. Therefore, the current study was performed to evaluate the analgesic activity, acute oral toxicity as well as qualitatively determine phytochemical composition of the methanolic and aqueous stem bark extracts of *M. aethiopicum* as a prospective source of safe, accessible, affordable and potent analgesic compounds for pain treatment.

1.2 Statement of the problem

Pain causes major incapacitation and conditions affecting humans and animals. It causes reduced or even loss of productivity to both the subject affected and the caregiver. As earlier stated, most of conventional agents are inaccessible, unaffordable and are associated with numerous side effects including constipation, drowsiness, dizziness, stomach upset, skin itching or rash and dry mouth and to some extent severe adverse reactions. For instance, Aspirin (acetylsalicylic acid) and naproxen causes indigestion, stomach ulcers, kidney damage, hepatotoxicity, stroke, cerebral haemorrhage, Reye's syndrome among others. On the other hand, ibuprofen and acetaminophen cause stomach and kidney problems. Moreover, opioid analgesics cause constipation, weakened immune system, nausea, drowsiness, sweating, depression, itching, euphoria and addiction. Besides, opioids cause dependence thus increasing pain brain threshold hence demand for higher potent opioids. Some conventional agents like Morphine, an opioid, is not accessible since it's a controlled drug despite its need to alleviate excruciating pain in addition to unaffordability. Therefore, owing to the high prevalence rates of pain across the world and all these bottlenecks, there is a dire need for safe, accessible, affordable and efficacious alternative for pain management. *Mystroxylon aethiopicum* is one of the sources of such ethnomedical alternatives. Despite ethnomedical claims of the plant as potent, safe and efficacious antinociceptive activity, there is absence of scientific data to validate the same in pain management as well as safety of *Mystroxylon aethiopicum*. The current studies, therefore, were focussed on evaluation of the anti-nociceptive/analgesic activity, acute oral toxicity as well as qualitatively determine the phytochemical composition of *Mystroxylon aethiopicum*'s crude methanolic and aqueous bark extracts.

1.3 Justification of the study

Based on the challenges and bottlenecks i.e. inaccessible, unaffordable and numerous side effect/adverse reaction associated with conventional medicines, there is urgent call for alternative approaches which are able to mitigate pain with fewer or no adverse reactions, accessible and inexpensive to the vast poor majority. Some medicinal plants offer alternatives or are potential sources of antinociceptive agents which are safer, accessible and affordable. 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* which possesses many phytochemicals which multi-targets several sites on pain pathways hence the current study sort to provide data for the analgesic effect and toxicity profile of *Mystroxylon aethiopicum.*

1.4 Objectives

1.4.1 General objective

To evaluate the anti-nociceptive activity, acute oral toxicity and qualitative phytochemical composition of methanolic and aqueous stem bark extract of *Mystroxylon aethiopicum*.

1.4.2 Specific objectives

1. To determine anti-nociceptive activity of methanolic and aqueous stem bark extracts of *Mystroxylon aethiopicum*.
2. To evaluate acute oral toxicity of methanolic and aqueous stem bark extracts of *Mystroxylon aethiopicum.*
3. To determine the phytochemical composition of the methanolic and aqueous stem bark extracts of *Mystroxylon aethiopicum.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Pain

Pain is an unpleasant sensation occasioned by a battery of neurochemical changes and signal transduction cascades in the peripheral and central nervous systems (Bukhari *et al.,* 2016). It's one typical manifestation of inflammation. Inflammation is a multifaceted protective response to tissue injury, pathogens or irritation aimed at defending the body and maintaining health (Bukhari *et al.,* 2016). It manifests with typical symptoms of fever, swelling, pain and redness on the site of injury (Nathan and Ding, 2010). 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 (Alemu *et al.,* 2018; Nasimolo, 2013). Pain and inflammation are induced by both endogenous and exogenous triggers (Omoigui, 2007; Oschman *et al.,* 2015). One of the potent triggers are microbial pathogens which infect the gastrointestinal tract and other body regions which have structures or toxins (Hakansson and Molin, 2011). 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 (Hakansson and Molin, 2011; Oschman *et al.,* 2015).

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 (Kilonzo and Ndakidemi, 2016).

The most common manifestation of vast majority of diseases and tissue injuries is pain, and, due to this, most diagnosed symptom of majority of diseases (Radhika *et al.,* 2017). For instance, painful disease conditions including osteoarthritis, colitis, inflammatory bowel disease, rheumatoid arthritis along with other chronic conditions like neurodegenerative disorders such as dementia and cardiovascular diseases among others are associated with inflammation (Medzhitov, 2008; Omoigui, 2007; Su *et al.,* 2019). The implication of these conditions to affected subjects is immeasurable-the huge financial burden borne by the patients or caregivers and wider society as well as physical disablement which adversely influences the quality of life and sometimes leads to death (Su *et al.,* 2019). The statistics of pain epidemics across the global arena is featured in a recent study which deduced that nearly 41.1% adults in the developing nations as well as 37.3 % of the adults in the developed countries suffers from chronic pain due to ailments, disorders ,injuries or diseases (Tsang *et al.,* 2008).

Pain manifests as either physical or psychological symptoms or both. There are varied types of pain depending on their aetiology. As such, based on clinical features, pain could be categorised as either chronic or acute; nociceptive or neuropathic; psychogenic or idiopathic. There exists two main types of physical pain i.e. neuropathic and nociceptive pain. Nociceptive pain is the leading type of physical pain in terms of occurrence. Nociceptive pain occurs when nociceptors (pain receptors for tissue injury) are stimulated by a certain trigger. Nociceptors are found all over the body more so the skin and internal organs. Upon activation by stimuli, nociceptors send signal/impulses that notifies the brain on the eminent tissue/organ injury with electrical signals

sent via the neurological system, that is, CNS (Central Nervous System); PNS (Peripheral Nervous System). Upon receipt of signals, brain perceives pain that is being felt. Nociceptive pain is further classified as either acute or chronic nociceptive pain. Additionally, nociceptive pain is also categorised further as either somatic or visceral pain. Acute pain is defined as short-lived pain that occurs suddenly due to a particular aetiology, most oftenly injury to the tissue which lasts for not more than six months. It resolves soon as the treatment of the underlying aetiological factors. Conversely, chronic pain is defined as that pain which goes beyond six months. It doesn't resolve even after the initial causative injury has been eradicated. Chronic pain may persist for years. It's worth noting that chronic pain ranges between mild to severe pain at any given time and it's usually more often. Statistics indicates that it affects an approximated 50 million adult persons in the United States, 1 in 5 South African adults (South Africa Demographic and Household Survey, 2016), 60% in Kenya (Clauw *et al.,* 2019).

On the other hand, visceral pain arises from damage of the internal organs or tissue injuries. It's usually felt in the trunk area i.e. chest, abdomen and pelvis of the body. This type of pain is often difficult to pinpoint the exact location and thus, is commonly described as pressure, aching, squeezing and cramping. Conversely, somatic pain occurs as a consequence of stimulation of the pain nociceptors in the tissues rather than on the internal organs. This encompasses the connective tissues, joints, skin, muscles and bones. Unlike in visceral pain, somatic pain is easy to pinpoint the actual location of pain. Further, somatic pain usually felt like a gnawing sensation or constant aching.

Neuropathic pain occurs due to damage of or malfunction of the neuronal system. This leads to damaged or non-functional neuronal misfiring of pain transduction signals. Infact, neuropathic

pain appears to originate from nowhere rather than in response against any noxious tissue injury. For instance, one may perceive pain whilst responding to things that usually doesn't trigger painful sensation, such as cold air or clothing against the integumentary system. Additionally, neuropathic pain is characteristically expressed by a subject as burning sensation, stabbing, tingling, shooting, freezing and/or electric shocks.

2.2 Biochemical and molecular basis of pain

Pain not only originates from inflammation but also inflammatory response (Omoigui, 2007). All painful syndromes shares a common source of origin. Thus, all pain be it nociceptive or neuropathic pain, acute or chronic, peripheral or central including windup, neuroplasticity and central sensitization are a continuance of inflammation and the inflammatory response (Omoigui, 2007). Overt pain is induced by injecting 0.6%v/v acetic acid intraperitoneally. Afterwards, this stimulant initiates a swift release of endogenic mediators of inflammation such as prostaglandins which in turn switches on the primary nociceptors (Gawade, 2012; Martinez *et al.*, 2016; Nesa *et al.*, 2018).

Tissue injury or damage, triggers the immune cells to produce several inflammatory biochemical mediators which include prostaglandin, interleukins 1- (a) and (b), Interleukins (4, 6 and 8), serotonin, histamine and nitric oxide. Consequently, nerve cells also produce biochemical mediators such as inflammatory glutamate, protein Substance P, vasoactive intestinal peptide, neurokinin A and calcitonin gene-related peptide (CGRP).

These mediators of inflammation activates local pain nociceptors and nerve endings thereby producing hyper-sensitivity on the injured site. Action of these inflammatory mediators leads to excitation of nociceptors on the nerves, muscle, skin and joints. This output in turn, stimulates the

A-delta fibers and C-fibers that transmits painful sensory information to the brain via the spinal cord. The expression of Na+ channels in neuron fibers (A-delta fibers and C-fibers) is transformed significantly, hence resulting in unusual excitability in the neurons responsible for sensory functions. Further, the neuronal messages entering the spinal cord triggers release of neuropeptides and neurotransmitters such as proteins Substance P. The release of these neuropeptides (i.e. vasoactive intestinal peptide, protein substance P), eliminates Mg^{2+} -induced inhibition thereby enabling the excitatory inflammatory amino acids such as aspartate activate N-methyl-D-Aspartate receptor (NMDAR) found in the spinal cord. The resultant effect due to activation of NMDA receptors is amplification of neuron traffic as well as painful stimulus that reaches the spinal cord from the peripheral tissues. Activated motor neurons are then transmitted to the muscles thus, superfluous muscle tensity leading to inflammation which eventually results in release of cyclooxygenase-2 (Cox-2) enzyme also known as prostaglandin –endoperoxide synthase (PTGS) enzyme. This production of PTGS enzyme leads to the release of lipid inflammatory mediators at the injured site. Comprehensive elicitation of prostaglandin – endoperoxide synthase expression on spinal cord neurons and in the central neuronal system stimulates production of elevated levels of prostaglandin E2 (PGE2) in the cerebrospinal fluid. Activated polymodal C fibers depolarizes second order projection neurons ‘wide dynamic range (WDR) neurons in the dorsal horn that projects supra-spinally. Further, supraspinal projections elicits a supra-spinally coordinated escape behaviour. For instance, local intraperitoneal injection of capsaicin or acetic acid evokes an unconditionally somatotopically-directed behaviour such as paw- licking or withdrawal; these behavioural patterns are regarded to emulate the underlying mechanism.

2.3 Pathophysiology of pain and drug targets

Peripheral nociceptive receptors respond to stimuli, that is, pH, Adenosine triphosphate and ligands thereby creating an afferent neuronal conduction to the spinal cord's dorsal root ganglia and dorsal horn. They are stimulated/activated essentially in a continuous manner (Dinakar and Stillman, 2016). Upon activation, they produce chemical mediators into the surrounding damaged area thereby invoking the release of inflammatory mediators including growth factors (e.g. CSF-1), cytokines (eg.CCL2) and prostanoids (Dinakar and Stillman, 2016). The release of glutamate and peptides within the spinal cord leads to activation of multiple nociceptive receptors, mostly, the *N*-Methyl-D-aspartate (NMDA) ionotropic receptors. This further, releases glutamate hence generating spinal hypersensitivity (Dinakar and Stillman, 2016). Upon arrival at the rexed lamina II, the first order projection neurones synapse to second-order projection neurones (WDR type) that decussates in the anterior white commissure of the spinal cord. In turn, the second-order neurons then cranially ascends in the spinothalamic tract to the ventral posterolateral (VPL) nuclei of the thalamus in the ventrolateral half of the spinal cord and relays their information to the brainstem. At the ventral posterolateral nuclei, the information is processed. It's worth to note that it's in thalamus on the spinothalamic pathway that is associated with pain perception; thalamus also plays a critical role in pain modulation and suppression. From there, the information is transmitted to cerebral cortex through fibers found in posterior limb of the internal capsule to terminate in the ipsilateral post central gyrus (primary somatosensory cortex). The somato-sensory cortex decodes the information to locate the actual site of pain being perceived. Additionally, proprioception is brought into consciousness in the somato-sensory cortex. Just like the way ascending pathway institutes conscious realization of pain towards the brain, the

descending pathway carries out modulation of pain sensation. The brain may makes a requisition for the release of specified chemicals with analgesic activity. This, in turn, inhibits painful sensation. Consequently, the hypothalamus prompts for the release of not only chemical mediators but also hormones such as GABA, norepinephrine, glycine and opioid peptides responsible for suppression of pain effectively. Moreover, a few of these hormones includes sex hormones.

Analgesics and anti-inflammatory drugs (antinociceptive agents) acts by targeting and modifying both peripheral and central nociceptive pathways to supress pain as shown in figure 2.1 (Dinakar and Stillman, 2016).

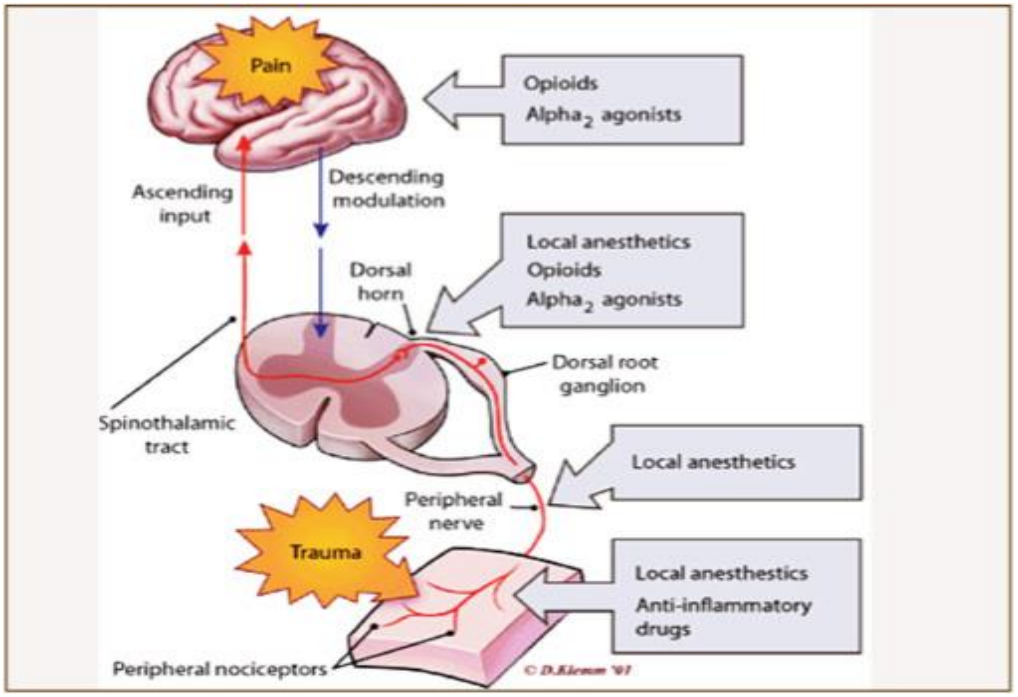


Figure 2.1: Structures of Pain Process, adopted from Dinakar and Stillman, 2016. Sciencedirect.com:https://doi.org/10.1016/j.spen.2016.10.003

For instance, NSAIDs targets prostanoids with the aim of alleviating inflammatory component of pain. Some agents act by blocking hyper-excitability and elicit their consequences on sodium channels, G-protein coupled membrane nociceptors, Ca^{2+}channels and mechanisms of monoamine uptake (Dinakar and Stillman, 2016). Sodium channel blockers (e.g. carbamazepine or lidocaine) blocks the abnormal firing of sodium channels (Dinakar and Stillman, 2016). Nerve injury leads to expression of more calcium channels with eventual discharge of numerous neurotransmitters like substance P and glutamic acid salt (Dinakar and Stillman, 2016).

Medication therapies that inhibits excitability are effective just like ones that increases inhibition within the spinal cord, for example, Ketamine elicits this effect by modulating the NMDA-receptor–driven excitation while Gabapentin lowers excitatory input; it achieves this by binding to calcium channels and eventually disrupting the channel's trafficking to the synaptic membrane, thus limiting the release of the neurotransmitter (Dinakar and Stillman, 2016). SNRIs inhibits re-uptake of serotonin and noradrenaline by interacting with spinal cord-brain- spinal cord loop which incorporates central areas that are vital in control of aversive and emotional responses towards pain (Dinakar and Stillman, 2016).

2.4. Pain management

2.4.1 Conventional management of pain

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 (Dinakar and Stillman, 2016). Currently, a wide range of analgesics and anti-inflammatory drugs are being utilized to manage inflammation, alleviate pain and to reduce or stop tissue injury

(Andreicut *et al.,* 2018). Acute and chronic pain may be treated by different pharmacologic agents that targets different sites of peripheral and central nervous system (Dinakar and Stillman, 2016). Analgesics and inflammatory antagonists generally fall under four classes namely, para-aminophenols (acetaminophen), non-steroidal anti-inflammatory drugs (NSAIDs) e.g. Ketoprofen and diclofenac), corticosteroids, disease modifying anti-rheumatic drugs (DMARDs) and opioids such as dihydrocodeine (Lundberg and Howatson, 2018; True *et al.,* 2013). Furthermore, research has revealed that some anticonvulsant and antidepressant drugs raise the threshold in patients, thereby preventing hyperalgesia and general pain (Lundberg and Howatson, 2018; True *et al.,* 2013).

Neuropathic pain responds best to SNRIs (Serotonin and Norepinephrine Reuptake Inhibitors) as well as TCAs (Tricyclic Antidepressants) or anticonvulsant while osteoarthritic pain responds best to para-amino phenols (e.g. acetaminophen), mild opioids (e.g. tramadol) and nonsteroidal anti-inflammatory drugs (Dinakar and Stillman, 2016; Schaible, 2004). Fibromyalgia is best relieved by muscle relaxants, TCAs, selective serotonin re-uptake inhibitors (SSRIs) or/and SNRIs synergistically with either anticonvulsants or opioids (Dinakar and Stillman, 2016). Para-amino phenols, NSAIDs, muscle relaxants and/or mild opioids offers best relief to Lower back pain. Sodium channel blockers (e.g. carbamazepine or lidocaine-local anaesthetics) blocks the abnormal firing of sodium channels (Dinakar and Stillman, 2016).

Paracetamol acts through the cyclooxygenase pathway just like NSAIDS whereby they cause blockage in production of prostaglandins (e.g. PGE2, PGI2, and PGF2a). On the other hand, Opioids acts by mimicking the action of the endogenous opioids peptides by interacting with mu,

delta or kappa opioid receptors. Despite the remarkable potencies of the conventional drugs in managing pain and inflammation in modern medicine, numerous shortcomings restraining their clinical utilization have been reported (Carter *et al.,* 2014).

2.4.2 Drawbacks of current pain medication

Despite the current analgesics and anti-inflammatories exhibiting marked pain relief capabilities, they are not universally accessible, affordable, and are associated with mild to severe side effects and sometimes adverse reactions, including constipation, drowsiness, dizziness, stomach upset, skin itching or rash and dry mouth (Maund *et al.,* 2011; Cazacu *et al.,* 2015). Generally, NSAIDs such as aspirin (acetylsalicylic acid) and diclofenac sodium are implicated in bringing about complications in the gastro-intestinal tract such as indigestion, stomach/bleeding ulcers, kidney damage, hepatotoxicity, stroke, cerebral haemorrhage, Reye's syndrome and gastric perforations/obstructions among others (Carter *et al.,* 2014; Lundberg and Howatson, 2018). Besides, ibuprofen and Para-aminophenols i.e. acetaminophen are also not left out since they are associated with stomach and kidney problems. On the other hand, Naproxen is associated with Indigestion, stomach ulcers, kidney damage, stroke, brain bleeding and Reye's syndrome. Conversely, corticosteroids such as prednisolone , cortisone and methylprednisolone has been linked to osteoporosis, fluid retention (edema), weight gain (obesity), hypertension, and delayed wound healing process (Activation and Britain,1987; Carter *et al.,* 2014; Steinmeyer, 2000). Besides, opioids among them dihydrocodeine, morphine sulphate, pethidine and codeine on their part have led to unpleasant behavior, addiction, cause constipation, weakened immune system, nausea, drowsiness, sweating, depression, itching, euphoria as well as respiratory suppression to the patients using them. (Cazacu *et al*., 2015). Furthermore, DMARDs comprising methotrexate,

penicillamine and sulfasalazine are reported to give rise to disorders of the liver, integument allergic reactions, gastrointestinal disorders and in the worst case renal failure (Activation and Britain,1987; Carter *et al.*, 2014; Steinmeyer, 2000 and True *et al.*, 2013).

Therefore, owing to the high prevalence rates of pain across the world and the bottlenecks including the many associated side effects above, arguably unaffordable costs and inaccessibility of the conventional drugs, there is a dire need for the exploration of complementary agents and alternatives which are safe, accessible, effective and affordable for pain management. Consequently approaches have been intensified in the scientific arena (Ghasemian *et al.,* 2016; Shakya, 2016). Of the many alternatives, medicinal plants has the best chances coming up with inexpensive, accessible, and potent analgesic products due to their rich history of use, claimed efficacy and safety (Shakya, 2016). Indeed, the WHO has demonstrated that ,globally more than 80 %, particularly, the developing nations utilize plants with medicinal value to cater for their primary healthcare requirements (WHO, 2005).

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 (Mückter, 2003). 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 (Jothy *et al.,* 2011; Mückter, 2003; Saleem *et al.,* 2017a). 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 (Mückter, 2003). Toxicological evaluation of medicinally potent plants thus elevates potentiality of the said plants as origin of safer, easy to access in addition to efficiently tolerated drugs to compensate for the insufficiencies of synthetic

agents (Mishra and Tiwari, 2011; Saad *et al.,* 2006 ; Sciences, 2015; Shakya, 2016; Thukham-mee and Wattanathorn, 2012).

2.5 Herbal medicinal management of pain

Herbal medicinal plant therapy as an origin for medication is as old as mankind; they have a rich history of folklore usage, are relatively safer, inexpensive, and readily available (Abdullahi, 2011; Kigen *et al.,* 2013; Mahomoodally *et al.,* 2013; Omwenga *et al.,* 2015). Based on the study that was done by WHO (1988), it was revealed that many people use herbal medication from plants in pain management. In fact, over 40% of the global human population in developed continents and over 80% of individuals from developing continents including in Africa, use traditional drugs majorly derived from plant origin for their primary healthcare needs, including pain management (WHO, 2018). Moreover, herbal medicines have secondary metabolites which are pharmacologically active against various diseases and conditions including pain in addition to dietary and health promoting benefits (Moriasi *et al.,* 2020).

Anti-nociceptive activity has previously been reported in many medicinal plants such as *Xanthium indicum* J. Koenig ex Roxby. (Asteraceae*), Xanthosoma violaceum* Schott (Araceae), *Cyperus rotundus* L. *(*Cyperaceae*), Artemisia annua* L. (Asteraceae), *Muntingia calabura* L. (Muntingiaceae) and *Curcuma zedoaria* (Christm.) Roscoe (Zingiberaceae.) (Faisal *et al.,* 2014); (Favero *et al.,* 2014); (Imam and Sum, 2014); (Ullah *et al.,* 2014) and (Haque *et al.,* 2013). Among other plants reported to have anti-nociceptive activity include *Monotheca buxifolia* (Falc.) A.DC. (Sapotaceae), *Clinacanthus nutans* (Burm. F.) Lindau (Acanthaceae*)* and *Geranium bellum* Rose (Cyperaceae) (Ullah *et al.,* 2016) and (Velázquez-González *et al.,* 2014). The mechanisms attributed to the anti-nociceptive activity in alleviation of pain included either

activation of opioidergic receptors , Nitric Oxide-mediated/cGMP, adenosinergic α-2-noradrenergic receptors (Abubakar *et al.*, 2019 and Zakaria *et al.*, 2019) , β-adrenergic receptors (Zakaria *et al.*, 2014), ATP-sensitive K+ channel (Abubakar *et al.*, 2019) and blockade of bradykinin and protein kinase C actions (Zakaria *et al.*, 2014).

Murang'a County is not left out in the use of various herbal medicinal plants in managing different types of pain (Githinji and Maina, 2018). Like many other communities in the world, Murang'a county residents use widely *Solanum incanum* (A. Rich.) Abedin. (Solanaceae), *Plecranthus barbatus* Andrews. (Lamiaceae), *Vernonia auriculiferas* Hiern. (Asteraceae), *Ficus sycamorus* L. (Moraceae), *Warburgia ugadensis* Verdc. (Canellaceae), *Caesalpinia volkensii (*Harms.) L. (Fabaceae), *Mondia whitei* (Hook. F.) Skeels. (Apocynaceae), *Cuscuta kilimanjari* Oliv. (Convolvulaceae), *Erythrina abyssinica* (Lam.) (Fabaceae) and *Ajuga remota* Wall. ex Benth (Lamiaceae) for pain management especially stomach-ache relief (Githinji and Maina, 2018). Other plants that have found use in analgesia includes *Acacia mallifera* (Vahl) Benth (Leguminoceae), *Prunus africana* (Hook. F.) Kalkman (Rosaceae), and *Piliostigma thonningii (*Schumach.) Milen-redh. (Fabaceae) (Githinji and Maina, 2018; Olela *et al.*, 2020). Additionally, *Zanthoxylum usambarense* (Engl.) Kokwaro (Rutaceae), *Cassia spectabilis* DC. (Fabaceae), *Croton megalocarpus* Hutch. (Eufobiaceae), *Urtica massaica* Mildbr. (Ulticaceae), *Warburgia ugadensis* Verdc. (Canellaceae), *Solanum aculeastrum* Dunal, (Solanaceae), *Azadirachta indica* A. Juss. (Meliaceae), *Withania somnifera* (L.) Dunal (Solanaceae), *Prunus africana* (Hook. F.) *Kalkman (Rosaceae), Strychnos henningsii* Gilg. (Loganiaceae), *Coredendrum myricoides* (Hochst.) Vatke (Lamiaceae), *Hibiscus crenatus* Vell. *(Malvaceae), Maytenus obscura* (A. Rich) Cufod. *(Celastraceae)* and *Mystroxylon aethiopicum (*Thunb.)Loes. (Celastraceae) are also

largely used in management of back pain as well as bone and joint pain (Githinji and Maina, 2018). In spite of the popularity and widespread usage of *Mystroxylon aethiopicum* (Thunb.) Loes. (Celastraceae) in pain management among the Agikuyu community of Kenya; it has not been scientifically evaluated to ascertain its pharmacologic efficacy, toxicity, and safety profiles. 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 (Nasri and Shirzad, 2013). This has been worsened by lack of government regulation of traditional medicine leading to resurgences of unscrupulous practitioners (Ekor, 2014 ; George, 2011). Furthermore, there are no clearly defined dosages, modes of preparation, modes of action and administration of herbal preparations (Chanda *et al.,* 2015; Subramanian and Sankaramourthy, 2018).

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 (Chanda *et al.,* 2015; George, 2011). Consequently, medicinal plants have been suspected to be toxic despite their longstanding use by many communities (Subramanian and Sankaramourthy, 2018). It is, therefore, important to perform evaluation of toxicity and safety profile of medicinal plants, claimed to alleviate several diseases as framework to determining safe dose regimens and side effects if they exist.

2.6 *Mystroxylon aethiopicum* (Thunb.) Loes. (Celastraceae)

2.6.1 Botanical description and morphology

Mystroxylon aethiopicum (Thunb.) Loes. (Celastraceae) is a small to medium in size attractive evergreen tree of Celastraceae family found in bushveld and forests, more so in rocky ridges. The tree is planted as a hedge, in solitary or groups. It has multiple stems that grows up to 12 metres

in height. The tree branches are thickly covered with conspicuous, short hairs which often confers greyish colouration. The leaves appear leathery in texture, darkish-green glossy underneath and pale green on the upper surface; the leaves vary in shape. It has blackish to dark brown bark with a rough texture. It has small flowers which are yellowish green in colour and usually borne in clusters in leaf axils. It has small fleshy yellowish green fruits while unripe or bright red while ripe which are edible to both animals and man since they are sweet tasting.

Mystroxylon aethiopicum is known by several common names such as Murigi (Kikuyu), kooboo-berry, spoon-wood (Eng.); koeboebessie, lepelboom, lepelhout (mukwatikwati), mungugunu (Venda) umbomvane (Xhosa), monamane (Northern Sotho), Mukawa (Rift valley Kikuyus), mukongoo/Kikongoo (Kamba) and olodong'anaiyoi in Maasai (Trees, Liana and shrubs of Africa, 2004).

2.6.2 Ethnomedical indications/uses of *Mystroxylon aethiopicum*

Stem Bark extract of *Mystroxylon aethiopicum* is believed to be useful in treatment of worm infestation in cattle i.e. anthelmintic as well as analgesia/antinociception in management of back, bone and joint pain. It's also useful as a haematinic in management of anaemia in animals and as a cough remedy in humans (Githinji *et al.*, 2018; Mhuji *et al.*, 2018). Additionally, the plant is also used in the management of haemorrhagic diarrhoea, stomach-ache, hypertension and gonorrhoea (Mhuji *et al.*, 2018).

2.6.3 Phytochemistry

The presence of antioxidant phytochemical compounds in medicinal plants have been considered to play crucial functions in mitigating pain and inflammation (Arulselvan *et al.,* 2016) . 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 (Liu *et al.,* 2018). A previous study involving gas chromatographic screening of the chloroform root bark extracts of *Mystroxylon aethiopicum* reported presence of Caryophyllene, Cubenol and Elexine, which have anti-inflammatory properties, among other antioxidant phytochemical compounds (Mhuji *et al.*, 2017). Another study via GC-MS revealed presence of sesquiterpenes, diterpenes , monoterpenes and fatty acids (Mhuji *et al.*, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant materials

Figure 3.1 Map of Sample Collection Area in Murang'a County – Source: Google Maps

Figure 3.2**:** Photo of *Mystroxylon aethiopicum* (Thunb.) Loes. (Celastraceae) courtesy of Dr. John K. Muchonjo, (2020).

About 1500g of freshly harvested barks of *Mystroxylon aethiopicum* plant was collected from Kaharati area, kamahuha location in Murang'a south sub county in Murang'a county map ref: -0.8489900, 37.1401285 (UTM) or 0°51'04.8"S 37°06.11.9"E which is 66km East of the capital Nairobi in their native habitat where they grew naturally, with the assistance of a local herbalist with reputation. The plant specimen was collected by peeling off the bark, prepared by putting them between newspapers and pressing between sheets of corrugated cardboard. They were then transported in a carton to the East African herbarium where it was identified and authenticated by a seasoned plant taxonomist. After the plant was successfully identified and authenticated, it was assigned a unique reference identity REF: NMK/BOT/CTX/1/5. Subsequently, the voucher specimens were professionally prepared and deposited at the East African herbarium for future referencing. The plant samples (stem barks) were collected from their source, cut into tiny fragments, evenly spread on a wooden bench top and shade/air-dried in a properly aerified room for two weeks with regular grabbling for proper aeration as well as even drying in the

Pharmacognosy laboratory, at the school of pharmacy, Mount Kenya University. After the plant material was evenly dried at room temperature, it was eventually granulated into a coarse powder using an electric mill. The resultant powdered plant material (about 500g) then was packaged in a properly labelled plastic container tightly sealed and stored to ensure they were free off moisture pending extraction.

3.2 Extraction methods/Preparation of the aqueous and methanolic stem bark extracts

3.2.1 Methanolic extract

Methanolic extraction was done in accordance with a method described by Harborne (1998) with slight modification as described by Anwar *et al.,* (2013). Briefly, about 300g of powdered dried material was soaked (macerated) in 750ml of 99.8% methanol (analytical grade) in a two-liter conical flask which was then covered with an aluminium foil and shaken occasionally at room temperature for 72 hours. The menstruum was then drawn off and filtered out through Whatman's filter paper No. 1 via a Buchner funnel apparatus. This procedure was replicated thrice to ensure extraction has been exhausted fully. The filtrates collected were put together and concentrated in vacuo at 40^{o}C using a rotary evaporator to remove residual solvent. The extract obtained was then put in a thoroughly cleaned and dried universal glass bottle for further drying. The dried filtrate from the rotary evaporator was then carefully put in a hot air oven adjusted to 40^{o}C for 48 hours to ensure it's completely dried. This was then followed by sand bathing for 24 hours into a dry powder. The extract was then transferred into a well closed, light resistant glass bottle and kept in a fridge at 4°C awaiting biological studies (Ismail *et al.,* 2012).

The percentage yield of the dried product was then calculated as percentage weight by

Weight (%w/w) as shown below,

$$\% \text{ Yield Crude extract} = \frac{(M_2-M_1)}{M_0} \text{ X } 100$$

Where;

M_2 = mass of container +extract (g)

M_1 = mass of empty container (g)

M_0 = mass of the initial bark powder sample (g)

3.2.2 Aqueous extract

To obtain the aqueous extract, the standard method described by Harborne (1998) was followed. Briefly, about 100 g of dried *stem* bark powder of *Mystroxylon aethiopicum* was marinated in 500 ml of distilled universal solvent (water) and boiled at a constant temperature of 58^{o}C for five minutes. Thereafter, the mix was allowed to cool down until it acquired the desired optimal room temperature and subsequently filtered out via Whatman's filter paper No. 1. The cooled filtrate was poured into a freeze-dryer flasks whose surfaces had dry CO_2 coated/augmented mixed with acetone. The freeze-dryer flasks connected to the freeze-dryer and lyophilized *in vacuo* for a duration of two (2) days. Afterwards, the extract obtained from lyophilization process was placed in a pre-weighed universal glass bottles, weighed (via analytical balance) and recorded for eventual calculation of yield. The extract was then preserved in a fridge at 4°C pending the intended biologic studies (Harborne ,1998).

The average yield of the aqueous extract was expressed as a percentage weight (%w/w) as shown below;

$$\% \text{ Yield of Crude extract} = \frac{(M_2 - M_1)}{M_0} \times 100$$

Where;

M_2 = mass of container +extract (g)

M_1 = mass of empty container (g)

M_0 = mass of the initial bark powder sample (g)

3.3 Experimental animals

3.3.1 Sourcing of experimental animals

In this study, nulliparous, healthy, non-pregnant female Swiss albino mice aged between 4-5 weeks old, weighing between 20-25grams were collected from animal breeding section hosted by Department of Public Health, Pharmacology and Toxicology, College of Veterinary and Agricultural Science, Kabete Campus, University of Nairobi for use in the present study. Their weight data was recorded.

3.3.2 Housing and feeding of the animals

The mice models for the experiment were accommodated in cages made from polypropylene measuring 35 centimeter (L) × 25 centimeter (W) × 18 centimeter (H) and they were kept in strict standard laboratory conditions laid out. Their bedding comprised of comfy wood shavings that were uniformly overspread on the floor of the cages to give warmth to the mice and avoid dumping of the cages. They were housed and adapted to standard laboratory conditions (23± 2^{o}C room temperature; 55-65 % Relative humidity; twelve (12)-hour daytime/night time cycle). The mice were fed with recommended standard mice pellets. The mice were also supplied with clean drinking tap water *ad libitum* as specified in OECD (2008) guidelines. The mice underwent

adequate acclimatization to laboratory conditions prior to experimentation. Ethical clearance to undertake the studies was granted by the Faculty of Veterinary Medicine's Biosafety, Animal Care and Use Committee (FVM-BACUC), while the national ethical regulatory body, National Commission for Science, Technology and Innovation (NACOSTI) gave the licence to conduct the study.

3.3.3 Preparation of animals

The selection of the laboratory animals (mice) was carried out randomly. An individual mouse was uniquely marked on the tail for the purposes of maintaining their identity. The mice were then accommodated inside cages made from polypropylene for a duration of 10 (ten) days preceding dosing to enable adaptation to the prevailing standard laboratory conditions.

3.3.4 Preparation of Doses for Administration

After successful pilot study, graded doses (i.e. 50 mg/kg bwt, 100 mg/kg bwt and 200 mg/kg bwt) of methanolic and aqueous stem bark extracts of *M.aethiopicum* were chosen.

Preparation of suitable dosages to administer to the mice followed the standard protocol as per the OECD (2008) guidelines, Document No. 425 well outlined by Erhierhie *et al.,* (2014). Briefly, preparation of stock solution containing 200 mg/kg bwt; dose level, intended for oral administration to mouse weighing 25 g, the formula modified by Erhierhie *et al.,* (2014) was applied as elaborated below:

$$\text{Animal dose (mg/kg bwt)} = \frac{\text{Animal weight (g)}}{1000\text{g}} \text{ X Selected dose}$$

So for a mice weighing 25g,

$$\text{Animal dose (mg/kg. bwt)} = \frac{25\ \text{g}}{1000\text{g}} \text{ X } 200 \text{ mg/kg bwt}$$

$$= 5 \text{ mg}$$

In accordance to OECD (2008) guidelines, 5 mg was dissolved in 0.2 ml of the vehicle; sodium chloride or (0.9% normal saline). In this case, 10ml stock solutions containing 200 mg/kg bwt of respective extract (aqueous and methanolic) of *M.aethiopicum* was compounded and diluted serially with the vehicle, that is, normal saline to obtain other lower doses i.e. 100 mg/kg bwt and 50 mg/kg bwt. A similar procedure was applied to the standard drug (Diclofenac 50 mg).

3.3.5 Occupational Health and Personal Protection equipment

Protective clothes, Latex gloves and protective masks were donned throughout the experiments.

3.4 Determination of analgesic/anti-nociceptive) Activity/Efficacy of the Aqueous and Methanolic Stem Bark Extracts of *M.aethiopicum*

To determine the peripheral anti-nociceptive/analgesic activity of *M. aethiopicum* bark extracts (methanol/aqueous) in mice models, acetic acid-induced writhing procedure described by Koster *et al.,* (1959) with modification by Thorat *et al.,* (2019) was adopted. Complete randomised design of study was fully embraced. Briefly, mice for the experiment were randomised and grouped into 6 (six) treatment groupings (1, 2, 3, 4, 5 and 6) comprising five (5) mice each and treated as shown in Table 3.1

Table 3.1: Experimental design for the determination of the analgesic activity of the methanol and aqueous stem bark extracts of M. aethiopicum

Group	**Treatment Administered**
1: Normal Control	Normal Saline (10 ml/Kg bwt; *p.o.*) only
2: Negative Control	Normal Saline (10ml/Kg bwt; *p.o.*) + 0.6 % w/v acetic acid (*i.p.*)
3: Positive Control	Diclofenac (50 mg/Kg bwt; *p.o.*) + 0.6 % w/v acetic acid (*i.p.*)
4: Experimental [A]	Extract (50 mg/Kg bwt; *p.o.*) + 0.6 % w/v acetic acid (*i.p.*)
5: Experimental [B]	Extract (100 mg/Kg bwt; *p.o.*) + 0.6 % w/v acetic acid (*i.p.*)
6: Experimental [C]	Extract (200 mg/Kg bwt; *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 adminstration was 200 µl or 0.2 ml.; Extract: Aqueous or methanolic bark extract of *M. aethiopicum* prepared in normal saline; Acetic acid was injected 30 minutes post adminstration of respective treatements.

Group 1 (Normal control grouping) mice took 10 ml/kg bwt; 0.9% normal saline alone via oral route (p.o.). Groups 2 (Negative control grouping) and Groups 3 (positive control grouping) acquired 0.9% normal saline 10 ml/kg. bwt; orally and diclofenac sodium 50 mg (50 mg/kg bwt; p.o.) respectively. Groups 4, 5 and 6 acquired graded doses of 50, 100 and 200 mg/kg bwt; p.o. methanol and aqueous extract of *M.aethiopicum* respectively.

Thirty (30) minutes post treatment, all the mice were intraperitoneally injected with 200 µl (0.2 ml) 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 as follows;

$$\% \text{ writhing inhibition} = \frac{\text{Mean No. Writhes (ctl group)} - \text{Mean No. Writhes (test)}}{\text{Mean No. of Writhes (ctl groups)}} \times 100$$

Where **ctl** is the control group.

It's worth noting that any decline in the counts of writhes in comparison with the control group mice conferred evidence of anti-nociception/analgesia.

3.5 Evaluation of Acute Oral Toxicity Effect of Methanol and Aqueous Bark Extracts of *Mystroxylon aethiopicum*.

To perform evaluation, appraisal and safety profile of *M.aethiopicum* extracts, Up-and- Down technique as descriptively outlined in OECD [425] guidelines was embraced in totality on this study of acute oral toxicity (OECD,2008). Briefly, nine (9) experimental mice (per extract) were randomized, chosen and marked with an indelible marker pen on different parts of the tails e.g. tip , mid and base of the tail .

The mice models were placed singly in polypropylene cages for forty (48) hours for acclimatization before being subjected to the treatments. The mice were then categorized into groups of 3 (i.e. the control and experimental groups) and fasted for four hours prior to dosing. Upon acclimatization, a dose of Normal saline (10 ml/Kg bwt) was administered per oral into the control grouping comprising three (3) mice. On the other hand, 175 mg/kg bwt; as the starting dosage, of crude extracts (methanol and aqueous) of *M. aethiopicum* were orally administered into the respective experimental groups comprising three (3) mice. After that, wellness parameters including water and food intake, appearance of the skin, fur, mucous

membranes and eyes; rate of respiration and circulation, neuronal responses; Somato-motor activities and behavioral pattern such as diarrhoea, trembling, fits, excessive salivation, lethargy, drowsiness and induced coma, body weight variations and mortality were monitored and recorded down on the lapse of half an hour, 4 hrs, 24 hrs and 48 hrs in that order post treatment. Monitoring of wellness parameters continued once daily. On the 7^{th} and 14^{th} day in that order post treatment, their weights were recorded. Upon absence of death or any observable signs of toxicity after 48 hours, new sets of 6 mice received orally the next higher treatment dose of 550 mg/Kg bwt. Again they were closely observed for death or any sign of toxicity as in the previous dose. Since there were no deaths or any sign of toxicity observed, another set of 6 mice (3 for aqueous, 3 Methanolic) received per oral administration of the cut off dose, 2000 mg/Kg bwt then monitored and recorded accordingly. In each case, at the lapse of the 7 days the mice were weighed and recorded. Consequently, at the lapse of 14 days observation period, the mice were weighed and sacrificed through cervical dislocation and their kidneys, spleen, heart and liver were dissected out and weighed/organ weights (OECD,2008). The LD_{50}values of both methanol and aqueous extracts of *Mystroxylon aethiopicum* were determined as per the OECD guidelines (OECD,2008) document no.425.

3. 6 Disposal of rodent carcasses

The carcasses were thrown into a designated non- polyvinylchloride, sealable transparent plastic bags and eventually incinerated.

3.7 Qualitative screening of the phytochemicals present in aqueous and methanolic crude extracts of *Mystroxylon aethiopicum*

Qualitative methods by Evans and Trease, 2009; Harborne, 1998; Savithrama *et al.*, (2011) ; Sultana *et al.*, (2014) were used with slight modifications by Moriasi *et al.*, (2020) to establish the phytochemical composition contained therein the prepared extracts of *Mystroxylon aethiopicum* which included alkaloids, flavonoids, Terpenes/Terpenoids, Saponins, Tannins, sugars/saccharides, proteins and phenolic.

The following qualitative tests were carried out:

3.7.1 Test for alkaloids (Drageundorff's test)

0.6 g of crude plant extracts of the *Mystroxylon aethiopicum* was blend with 8ml of 1 % HCl, warmed and filtered with Whatman's paper no.1. 2 ml of the resultant filtrate were then mixed with Mayer's reagent and observations made. The formation of creamy coloured precipitous conferred the existence of alkaloids. Analogous to the above method, 2 ml of the extract obtained after filtration was blend with Dragendorff's reagent and observed for colour change. The formation of a reddish-brown precipitate conferred to alkaloids' presence in the affirmative (Evans, 2009; Savithrama *et al.*, 2012).

3.7.2 Test for glycosides- Keller-killiani test

Chloroform was used in extraction of the crude phytocomponent. The extract was then evaporated until it was completely dry. 0.4 ml of glacial acetic acid with $FeCl_3$ traces amour was introduced into test tube. Further, 0.5 ml of concentrated H_2SO_4 was meticulously poured into the test tube (containing acetic acid with traces of $FeCl_3$) via the side and observed. A blue-coloured acetic acid layer was formed which conferred an affirmative test for presence of

cardiac glycosides (Evans and Trease, 2009; Savithrama *et al.,* 2012).

3.7.3 Alkaline reagent Test for flavonoids

Five (5) drops of NaOH were introduced into 1ml of methanol extracts of the plant material. Thereafter, few drops of 2M hydrochloric acid was added. Abrupt formation of a red coloration conferred existence of flavonoids. (Evans, 2009; Savithrama *et al.,* 2012).

3.7.4 Ferric chloride test for phenolics

Approximately 0.5 g of powdered *Mystroxylon aethiopicum* extract was added to 10 ml of 70 % ethanol in a test tube and boiled on a water bath for a duration of five minutes. The resultant decoction was immediately filtered out whilst hot and then left to cool down to room temperature. 2ml of cool filtrate was blend with 5% $FeCl_3$ in each test tube. Presence of a greenish precipitate indicated existence of phenols (Evans, 2009; Savithrama *et al.,* 2012).

3.7.5 Test for Saponins (Foam test)

Frothing Test was embraced. Briefly, 0.5 g crude extract of *Mystroxylon aethiopicum* was introduced into a test tube already containing boiling water. It was then given adequate time to cool down completely, followed by thorough shaking of the content. Appearance of froth/foam conferred the existence of saponin (Evans, 2009; Savithrama *et al.,* 2012).

3.7.6 Ferric chloride test for tannins

0.5 g of plant-derived decoction was introduced into 20 ml of distilled water contained in a test tube .The mixture was boiled, cooled down and eventually filtered. To the filtrate, 5% $FeCl_3$ was added and then agitated whilst observations were made. Formation of a bluish- green precipitous conferred existence of tannins in affirmative (Evans, 2009; Savithrama *et al.,* 2012).

3.7.7 Salkowski test for terpenes/terpenoids

Acetic acid anhydride was carefully placed in a test tube. This was followed by addition of 2 ml of *M.aethiopicum* extract to it (test tube containing Acetic acid anhydride) and mixed thoroughly. Concentrated H_2SO_4 was meticulously introduced into mixture content on the test tube by the side. The development of bluish-green ring indicated existence of terpenoids (Evans, 2009; Savithrama *et al.,* 2012).

3.8 Data management and statistical analysis

Quantitative data generated from analgesic activity study were tabulated on Microsoft Excel (Microsoft 365) spreadsheet and transferred to GraphPad Prism software (version 8.4.3) for statistical data analysis. Data were descriptively analyzed and results were presented as $\tilde{x}$±SEM. Thereafter, One-Way ANOVA was performed to establish significant differentials amid treatment groups followed by Tukey's *post hoc* test to allow pairwise comparison and separation of means. Also applied, was unpaired student *t*-test statistic to achieve comparison between the effects of methanol and aqueous extract of *M.aethiopicum*. In both instances, $P<0.05$ was considered significant.

The data obtained from acute oral toxicity study was tabulated in a table and analyzed according to the OECD guidelines (OECD, 2008). Qualitative phytochemical screening results were just tabulated.

3.9 Ethical consideration and approval

Ethical approval was granted by the Faculty of Veterinary Medicine's FVM-BACUC (Biosafety, Animal Care and Use Committee) of the University of Nairobi and referenced as REF: FVM BAUEC/2020/257(Appendix 3). Furthermore, the NACOSTI (National Commission for Science,

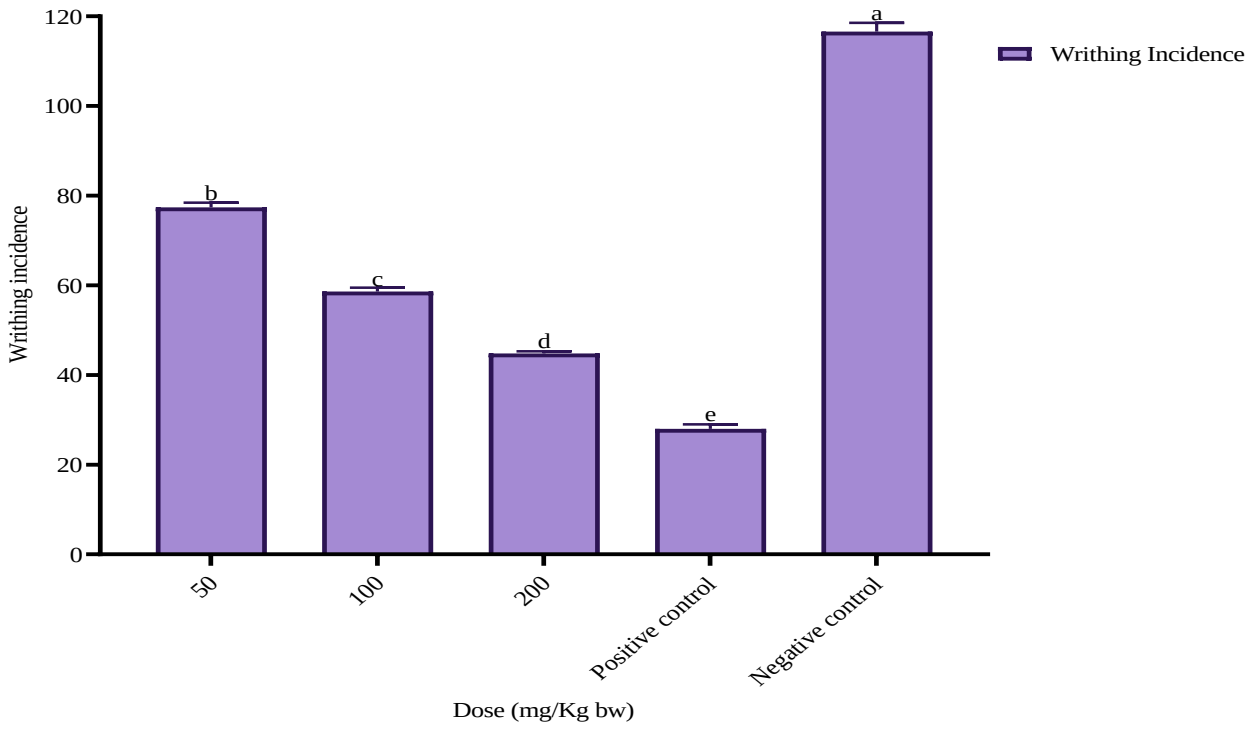
Technology and Innovation) under the ministry of education approved this study via license No: 688114 (Appendix 4). Approval to proceed with the study was granted by the director of Graduate School as referenced in REF: J56/12647/2018 (Appendix 2).

CHAPTER FOUR

RESULTS

4.1 Effect of the methanolic and aqueous stem bark extracts of *Mystroxylon aethiopicum* on acetic acid-induced writhing in mice.

In this study, the results unveiled that aqueous stem bark extract of *M. aethiopicum* inhibited the writhing frequency in experimental mice significantly in a dose dependent manner ($p<0.05$; Figure 4.1)

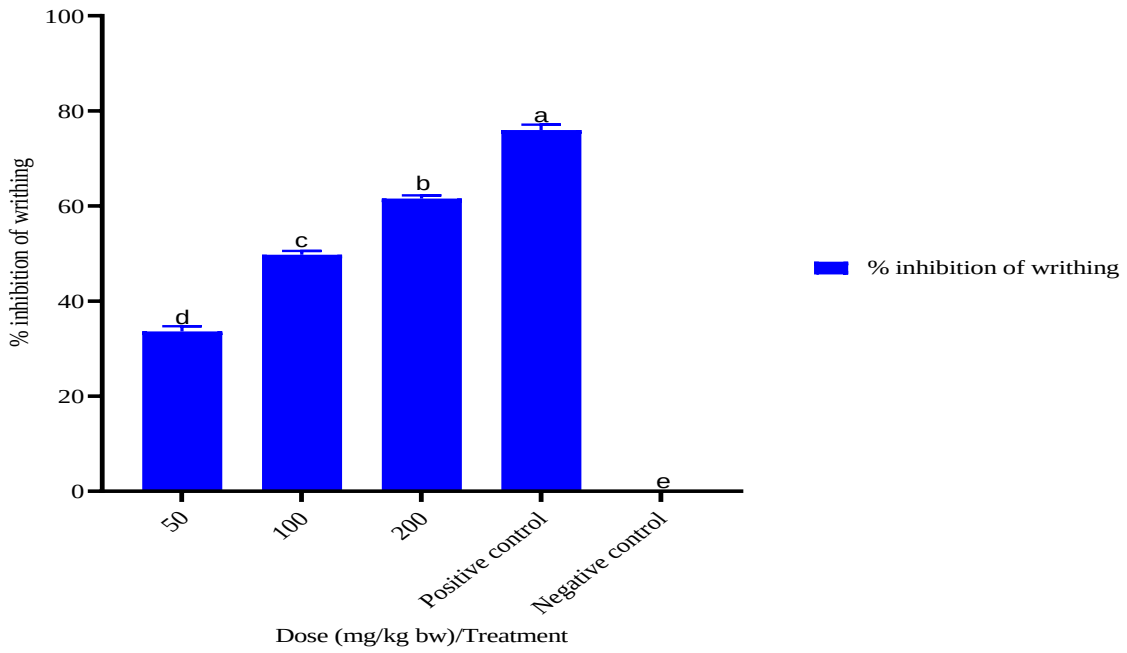


Bars with dissimilar lowercase alphabet are significantly different by one-Way ANOVA followed by Tukey's test ($p<0.05$).

Figure 4.1: Effects of aqueous stem bark extract of M aethiopicum in acetic acid-induced writhing in mice.

Those mice models that received aqueous concentrate; p.o. at all the studied dosage levels had significantly lower writhing incidences as compared to the writhing incidences on the negative control set of mice at a significance level ($p<0.05$). Nevertheless, writhing incidences recorded on the positive control grouping of mice were significantly reduced as compared to writhing incidences exhibited by all mice in all the other treatment groupings used in the experiment at the significance level of $p<0.05$ (Figure 4.1).

Further, percentage inhibition of acetic acid-induced writhing in laboratory animals was determined. Ultimately, the outcome unveiled that aqueous crude bark extract of *M. aethiopicum* significantly inhibited frequency of writhes in experimental mice (Figure 4.2).

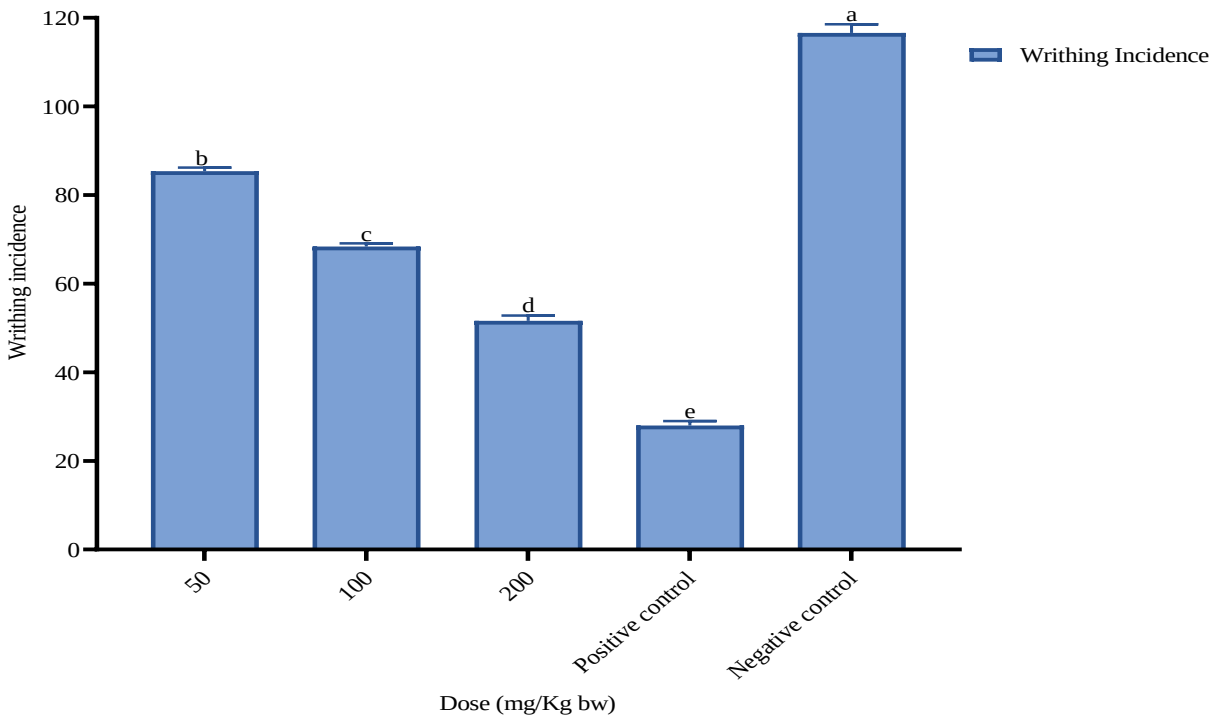


Bars with dissimilar lowercase alphabet are significantly different by one-Way ANOVA followed by Tukey's test ($p<0.05$)

Figure 4.2: percentage inhibitions of acetic acid -induced writhing by aqueous crude extract of M. aethiopicum in mice

The percentage inhibitions of writhes ranged between 33.62±1.10 % at a dosage of 50 mg/Kg bwt and 61.58±0.67 % at 200 mg/Kg bwt (cut off) dosage level (Figure 4.2). Notably, the positive control (diclofenac sodium 50 mg/Kg bwt) showcased up-scaled percentage inhibition of acetic acid-induced writhing in mice as compared to percentage inhibitions exhibited by the studied extract at all dose levels ($p<0.05$; Figure 4.2).

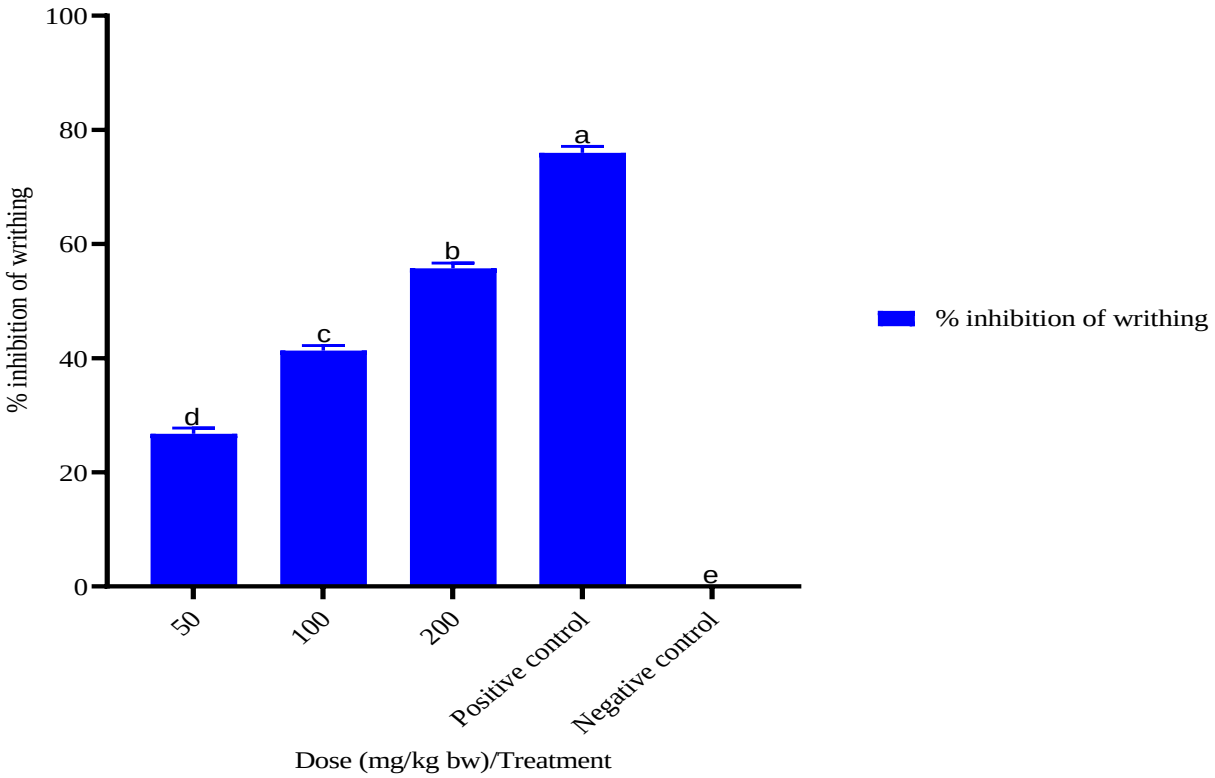
Conversely, methanolic crude extract of *M. aethiopicum* significantly decreased acetic acid-induced writhing in mice in a dosage-dependent way ($p<0.05$; Figure 4.3).



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

Figure 4.3: Effects of methanolic crude extract of M. aethiopicum in acetic acid induced writhing in mice

Additionally, results obtained unveiled that writhing incidences recorded in experimental mice into which studied graded dose levels of methanol bark extract of *M. aethiopicum* were administered were significantly lower than the writhing incidences recorded in negative control grouping of mice ($p<0.05$). Generally, positive control grouping of mice presented significantly lower writhing incidences than those recorded in mice models in the rest of the experimental groups ($p<0.05$;Figure 4.3). Moreover, the percentage inhibition of acetic acid-induced writhing in mice by the studied methanol stem bark extract was determined (Table 4.2; Figure 4.4).

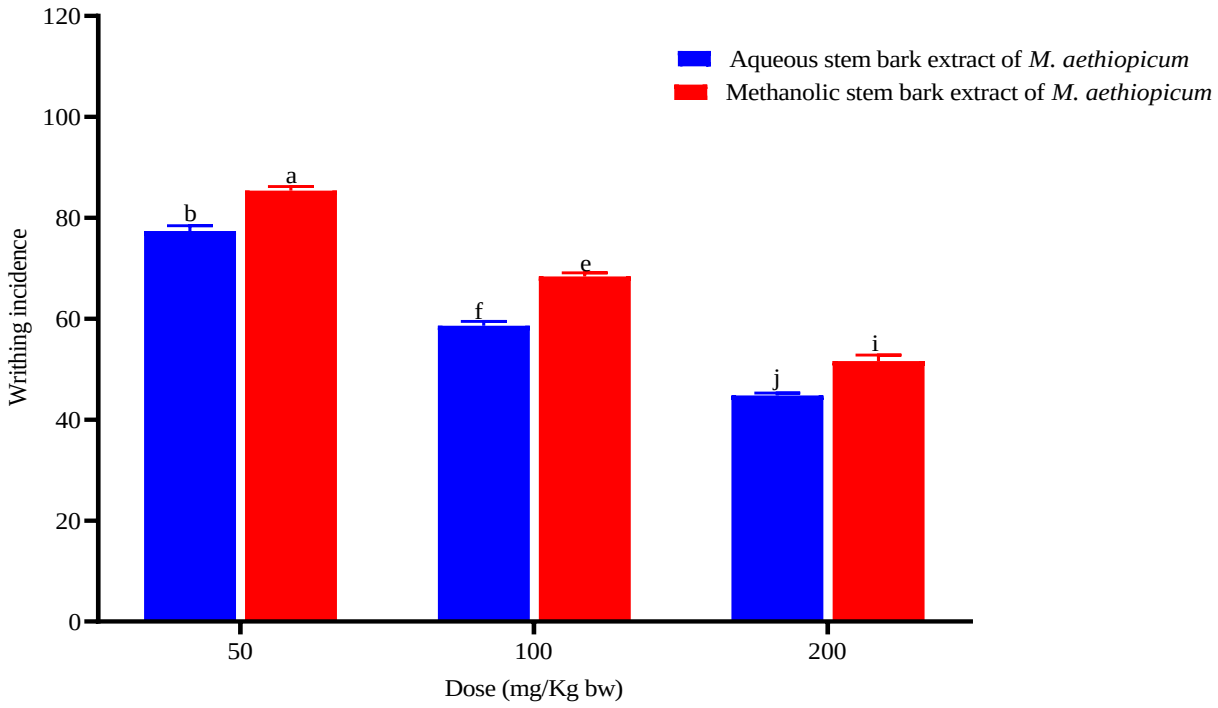


Bars with dissimilar lowercase alphabet are significantly different by one-Way ANOVA followed by Tukey's post hoc test ($p<0.05$)

Figure 4.4: Percentage inhibitions of acetic acid induced writhing by the methanolic stem bark extract of M aethiopicum in mice

It was observed that the percentage inhibitions of writhing ranged from 26.75±1.00 % on the set of mice that ingested 50 mg/Kg bwt; p.o. to 55.75±0.93 % on the mice that ingested 200 mg/Kg bwt; p.o. of the studied methanolic extract (Table 4.2; Figure 4.4). Overall, the reference drug caused the highest inhibition of writhing (75.99±1.12 %) in experimental mice.

Furthermore, a comparison of writhing incidences in mice that received the aqueous stem bark extract of *M.aethiopicum* were compared to those in mice that ingested methanolic stem bark extract of the same plant (Figure 4.5).

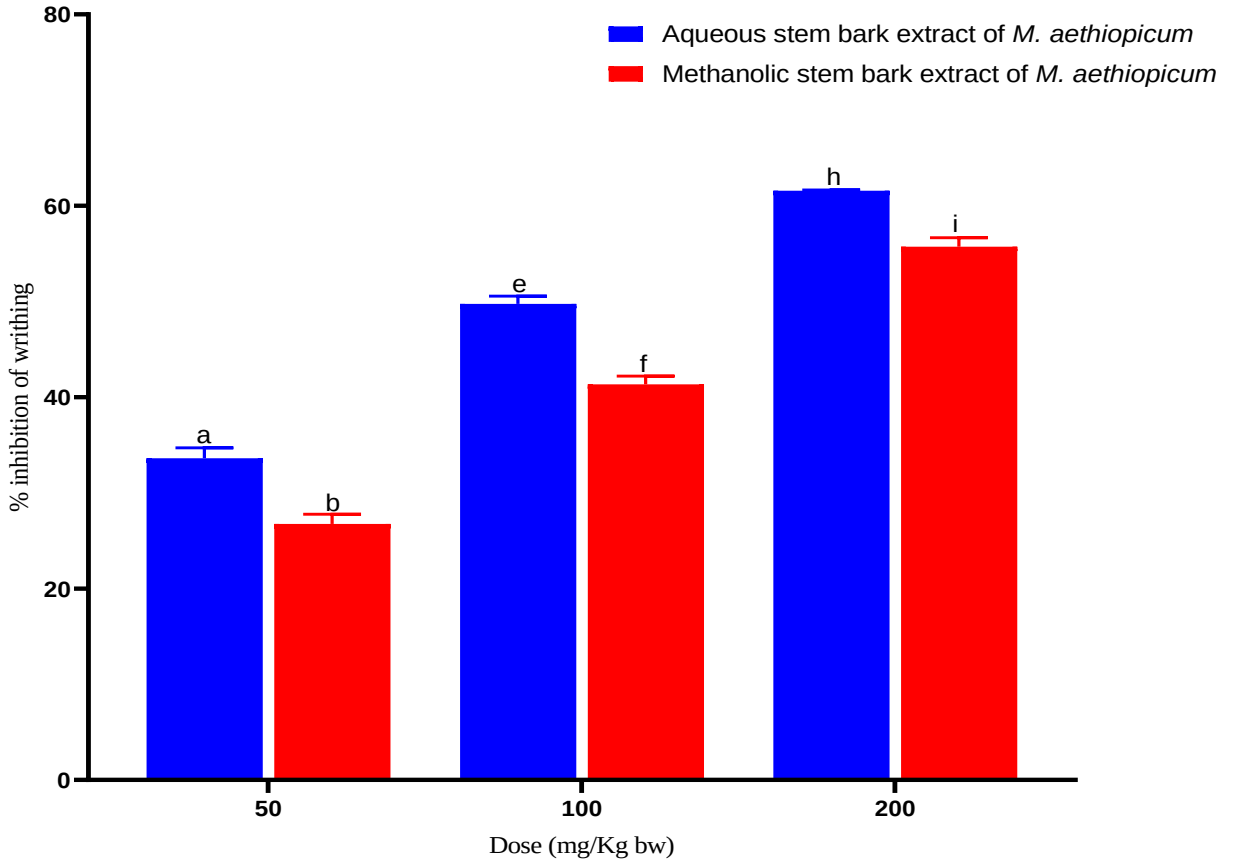


Bars with different alphabet letter within the same dose level are significantly different by t-test ($p<0.05$).

Figure 4.5: Comparison of effects of M. aethiopicum in acetic acid induced writhing in mice.

The results unveiled that, the mice that received the aqueous stem bark extract of *M. aethiopicum,* at all the three graded dose levels, had significantly lower writhing incidences compared with the writhing incidences in mice that orally ingested methanolic stem bark ($p<0.05$; Figure 4.5).

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 4.6).



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

Figure 4.6: Comparison of percentage inhibitions of writhing by M. aethiopicum extract in mice

The results further unveiled that, at all the studied graded dose levels, the aqueous extract produced significantly higher percentage inhibitions of writhing in mice than those caused by the methanolic extract at the same dose levels ($p<0.05$; Figure 4.6).

4.2 Acute oral toxicity effects of the aqueous and methanolic crude extracts of *Mystroxylon aethiopicum* in mice.

4.2.1 Acute Oral Toxicity effects of the aqueous and methanolic stem bark extracts of Mystroxylon aethiopicum in mice

Both methanolic and aqueous stem bark extracts *M. aethiopicum* were found to be non-toxic based on the OECD 425 (2008) guidelines. The behavioural observations which included grooming, vocalization and restlessness were recorded. In addition, neurological symptoms of tremors, convulsions, urination, diarrhoea and coma were documented. The observations were similar at all the tested doses (0 mg/kg, 175 mg/kg, 550 mg/kg and 2000 mg/kg bwt respectively). Consequently, no mortality was recorded in all the experimental mice that were involved in this study and hence the LD_{50} was considered to be >2000 mg/Kg bwt.

4.2.2 Effects of the methanolic and aqueous stem bark extracts of *Mystroxylon aethiopicum* on the weight of selected organs of mice

Following acute oral toxicity investigation, the experimental mice were stupefied, decapitated and select organ were dissected and their weights measured out. The results obtained unveiled no differences on the average weights of the liver, spleen and right kidney organs among all the experimental mice ($p>0.05$; Table 4.1).

Table 4.1: Effects of M. aethiopicum extracts on selected organ weights in oral acute toxicity model in mice

Treatment		Organ weights				
	Dose (mg/Kg bwt)	Liver	Heart	Spleen	Left Kidney	Right Kidney
Control		1.33±0.10[a]	0.15±0.01[b]	0.19±0.02[a]	0.18±0.0[2ab]	0.18±0.01[a]
Aqueous extract	175	1.23±0.09[a]	0.17±0.02[b]	0.26±0.01[a]	0.20±0.01[ab]	0.21±0.01[a]
	550	1.19±0.10[a]	0.45±0.35[b]	0.26±0.03[a]	0.13±0.02[b]	0.17±0.03[a]
	2000	1.36±0.12[a]	1.16±0.01[a]	0.57±0.30a	0.21±0.02	0.20±0.01a
Methanolic extract	175	1.28±0.01[a]	0.22±0.01[b]	0.28±0.01[a]	0.21±0.01[a]	0.22±0.01a
	550	0.98±0.43[a]	0.13±0.02[b]	0.26±0.03 [a]	0.22±0.02 [a]	0.18±0.02[a]
	2000	1.70±0.12[a]	0.18±0.02[b]	0.28±0.02[a]	0.22±0.01[a]	0.21±0.01[a]

Values are expressed as x̃±SEM; means with similar superscripted lowercase alphabet on similar column are not significantly different by one-Way ANOVA followed by Tukey's test ($p>0.05$)

Similarly, no significant difference on average weight of the heart among the mice models that received the aqueous stem bark extract of the studied plant at doses of 175 mg/Kg bwt and 550 mg/Kg bwt and those administered with the methanolic stem bark extract at all the three dose levels compared with the control group mice ($p>0.05$; Table 4.1). Nevertheless, the average weight of the heart recorded for the mice which received a 2000 mg/Kg bwt dose of the aqueous stem bark extract of the studied plant was slightly higher compared with those of other groups of mice though not significant ($p<0.05$; Table 4.1).

Besides, the average left kidney organ weights recorded for the group of mice that received aqueous stem bark extract of the studied plant at a dose of 2000 mg/Kg bwt and those that received the methanol stem bark extract at all the three doses were not significantly different ($p>0.05$; Table 4.1). Likewise, the weights of the left kidneys recorded for mice treated with 175 mg/Kg bwt and 550 mg/Kg bwt doses of the aqueous stem bark extract were not significantly different from those obtained for the control mice ($p>0.05$; Table 4.1).

4.3 Qualitative screening of phytochemicals of methanolic and aqueous 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 4.2). However proteins were absent in both extracts studied (Table 4.2).

Table 4.2: Qualitative Phytochemical profile of crude extracts of M. aethiopicum

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

Key: + Present

- Absent

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Both stem bark extracts (aqueous and methanol) of *Mystroxylon aethiopicum* have shown statistically significant analgesic efficacy by inhibiting acetic-acid induced writhes compared to control group of mice models. However, aqueous extracts had more efficacy than methanolic which gave percentage inhibitions of writhes that ranged between 33.62±1.10 % at a dosage of 50 mg/Kg bwt and 61.58±0.67 % at 200 mg/Kg bwt (cut off) dosage level (Figure 4.2).Thus, the results implicated the aqueous crude extract of *M. aethiopicum* as more potent inhibitor for acetic acid inducing in experimental animals compared with the methanol extracted ones. Conversely, methanolic crude extract of *M. aethiopicum* significantly decreased acetic acid-induced writhing that ranged between 26.7515±1.00 % at a dosage of 50 mg/Kg bwt and 55.74614±0.93 % at 200 mg/Kg bwt (cut off) dosage level ($p<0.05$; Figure 4.3). This could be attributable to specific phytochemical compounds that are in high concentration in the aqueous extract than in the methanolic extract (Sdayria *et al.,* 2018). Perhaps, this could be the reason behind the usage and claimed healing properties of the aqueous extract of this plant in traditional medicine. There are huge consistency between these findings and those of previous reports on antinociceptive efficacy of extracts from plants as shown in mice models (Jakaria *et al.,* 2015; Kaushik *et al.,* 2012; Rahman *et al.,* 2019; Subedi *et al.,* 2016) which indicated that both the stem bark and root extracts have antinociceptive activity. Notably, the positive control (diclofenac sodium 50 mg/Kg bwt; 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 (Gan, 2010) showcased up-scaled percentage inhibition of acetic acid-induced writhing in mice as compared to percentage inhibitions exhibited by the studied extract at all dose levels ($p<0.05$; Figure 4.2). Its mode of action is through the inhibition of prostaglandin synthesis through the cyclooxygenase (COX) pathway which terminate pain signal transduction (Gan, 2010). It is suggestive, partly, that extracts from *Mystroxylon aethiopicum* maybe inhibiting prostaglandin synthesis in the peripheral system thus ameliorating pain. Furthermore, these extracts could be modifying pain messages and averting the damage caused by the pathogens thereby ameliorating pain (Miya *et al.*, 2016).

Deriving from the reported results in the just concluded study therefore imply analgesic efficacy of *Mystroxylon aethiopicum* crude extracts by inhibiting acetic acid-induced writhing in experimental animal models could be replicable to the effects conferred by this plant's extracts in the gastrointestinal tract (Hakansson and Molin, 2011; Kilonzo and Ndakidemi, 2016; Lundberg and Howatson, 2018). Nevertheless, the studied plant extracts appear to have active principles which have analgesic activity as evidenced by the obtained results.

From the study, orally administered methanol and aqueous crude extracts of *Mystroxylon aethiopicum* did not evoke any noticeable signs of toxicity or death throughout the fourteen-day experimentation period in all graded dosage levels up to the cut off dosage of 2000 mg/Kg bwt hence $LD_{50} > 2000$ mg/Kg bwt. There was no expectation of toxicity sign or death, probably due to the shorter duration of study. This therefore, stipulate that *Mystroxylon aethiopicum* crude extracts offers compounds with well tolerated analgesia when taken orally and with least or without adverse side effects (OECD, 2008; Saleem *et al.*, 2017).

In cognisance of organ weight (which is an important indicator of physiologic and pathologic state of the animal), it was observed that, there were no significant differences on the weights of the liver, heart, spleen and kidneys of mice that received the extracts compared with those of the control (Table 4.1). This shows that the studied plant extracts did not negatively affect the organ functioning and the overall body processes (Jothy *et al.*, 2011; OECD, 2008; Pariyani *et al.*, 2015). It is therefore anticipated that if these extracts are taken orally by human beings, no signs of toxicity would show. However, this data cannot confirm whether there would be weight changes if duration of study was prolonged in other studies. Nevertheless, these inferences could be attributable to the low toxicities of the studied plant extracts as they have been utilized traditionally for ages in the management of various ailments (Kilonzo and Ndakidemi, 2016; Mhuji *et al.*, 2017). Additionally, non-toxicity of these extracts, could be associated to absence of or least abundance of toxic phytochemical constituents making them pharmacologically viable.

From the qualitative phytochemical study of crude extracts of *M. aethiopicum*, alkaloids, saponins, tannins, glycosides, phenols, flavonoids, terpenoids and saccharides phytochemicals were detected. Nonetheless, proteins were not detected. These results contributes to knowledge since no other study has been done on the stem bark. 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 (Mhuji *et al.*, 2017). This observation compares well with those of earlier investigations by other authors who established that not all phytochemicals may be present in plant parts (Ayinde, 2007).

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

5.2 Conclusions

It was deduced that:

1. Methanolic and aqueous crude extracts of *Mystroxylon aethiopicum* have peripheral antinociceptive activity in acetic acid induced writhing in experimental mice.

2. Methanolic and aqueous crude extract of *Mystroxylon aethiopicum* are safe with LD_{50} of > 2000 mg/kg bwt.

3. Both Methanolic and aqueous crude extracts of *Mystroxylon aethiopicum* were found to contain alkaloids, flavonoids, saponins, glycosides, phenols, terpenoids and saccharides as active phytochemicals.

5.3 Recommendations

1. Herbalists to continue administering *Mystroxylon aethiopicum* extracts in management of pain.
2. Further toxicity studies (sub-acute and chronic toxicity) to be conducted on the plant extract to fully assure the safety of the studied plant extracts.
4. Further studies on characterization, elucidation and isolation of specific analgesic compounds from the extracts of studied plant and their actual mechanism of action.
5. Further research to quantitatively determine the phytochemical constituents of the active plant extracts of *Mystroxylon aethiopicum.*

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APPENDICES

APPENDIX I: PUBLICATION ASSOCIATED WITH THIS THESIS: Analgesic efficacy and phytochemical composition of the aqueous and methanolic stem bark extract of *Mystroxylon aethiopicum* (Thunb.) Loes. (Celastraceae)

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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, Raye'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].

~ 48 ~

APPENDIX 2: GRADUATE SCHOOL APPROVAL

UNIVERSITY OF NAIROBI
GRADUATE SCHOOL

Telephone: 3318262
Fax Number: 243626
Telegrams:"Varsity of Nairobi"
E-mail: gs@uonbi.ac.ke
P. O. Box 30197 - 00100
NAIROBI, KENYA

Our Ref: J56/12647/2018

10th December, 2019

John Karanja Muchonjo
C/o Department of PHPT
FACULTY OF VETERINARY MEDICINE, CAVS

Dear Mr. Muchonjo,

RESEARCH PROPOSAL AND SUPERVISORS

This is to inform you that the Director, Graduate School has approved your MSc. research proposal titled **"Phytochemical Screening, Acute Toxicity Study and Anitociceptive Efficacy of Mystroxylon Aethiopicum Extract in Mice."**

She has also approved **Prof. James M. Mbaria** and **Dr. Joseph Mwanzia Nguta** as the supervisors of your thesis.

You should therefore begin consulting them and ensure that you submit your thesis for examination in **May, 2020**. The Guidelines on Postgraduate Supervision can be accessed on our website (www.gs.uonbi.ac.ke) while the Research Notebook is available at the University Bookstore.

Yours sincerely,

JANET OMBWAYO (MS.)
FOR: DIRECTOR, GRADUATE SCHOOL

cc Dean – Faculty of Veterinary Medicine
Chairman – Department of PHPT
Prof. James M. Mbaria – Department of PHPT
Dr. Joseph Mwanzia Nguta – C/o Department of PHPT

JO/lwk

APPENDIX 3: ETHICAL APPROVAL

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2020/257

Dr. John Karanja Muchonjo
University of Nairobi
Dept. PHP & T
08/01/2020

Dear Dr. Muchonjo

RE: Approval of proposal by Faculty of Veterinary Medicine, Biosafety, Animal use and Ethics committee

Phytochemistry screening, acute oral toxicity study and antinociceptive efficacy of Mystroxylon aethiopicum extract in mice.

Dr. John Karanja J56/ 12647/2018.

We refer to your MS.c proposal submitted to our committee for review and your application letter dated December 2019. We have reviewed your application for ethical clearance for the study.

The number of mice to be used in the acute oral toxicity and antinociceptive protocols meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.

We have also noted that registered veterinary surgeons will supervise the work.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi.

APPENDIX 4: APPROVAL BY NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 688114

Date of Issue: 19/February/2020

RESEARCH LICENSE

This is to Certify that Dr., John Karanja Muchonjo of University of Nairobi, has been licensed to conduct research in Muranga on the topic: STUDIES ON ANTINOCICEPTIVE ACTIVITY, TOXICITY AND PHYTOCHEMICAL COMPOSITION OF Mystroxylon aethiopicum(Thunb.)Loes. (Celastraceae). for the period ending : 19/February/2021.

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APPENDIX 5: CERTIFICATE OF PLANT IDENTIFICATION

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John Karanja Muchonjo

Tel. 0720-677361

Thika.

Dear Sir,

PLANT IDENTIFICATION

The plant specimen you brought to us for identification has been determined as follows:

E003J - Mystroxylon aethiopicum (Thunb.) Loes **(Family: Celastraceae)**

Venacular name: Murigi (Kikuyu)

Thank you for consulting the EAH for plant identification and confirmation.

Yours Sincerely

Mathias Mbale

For: Head, Botany Department.

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APPENDIX 6: APPROVAL BY MOUNT KENYA UNIVERSITY TO USE THEIR CENTRE LABORATORIES

Mount Kenya University

Head of Research Centre,
Mount Kenya University,
P O Box 342 – 01000.
THIKA.

Through,

Director, Research and Innovation

January 21, 2020

Attention; Prof. Mbaria M. J.

REF: MUCHONJO JOHN KARANJA (J56/12647/2018)

.....

The above-mentioned student has requested to use Mount Kenya University Research Centre laboratories during his research project as a requirement for partial fulfilment of a ward of Msc. degree in pharmacology and toxicology of University of Nairobi.

The student will be facilitated to achieve his objectives by being provided with necessary assistance and 30% sponsorship of consumables.

The role of Mount Kenya Research Centre will be to:

(a) Facilitate collection and identification of ***Mystroxylon aethiopicum***
(b) Ensure access to working bench together with appropriate equipment, apparatus and reagents pertaining to preparation of extracts and phytochemical screening

The student will be required to:

i. Acknowledge Mount Kenya University in all publications or workshops that will bear results acquired at the Research Centre

Thank you.

Mount Kenya University
Head, Research Centre
P. O. Box 342 - 01000,
Thika

Dr. Jared Misonge Onyancha
HEAD, RESEARCH CENTRE
MOUNT KENYA UNIVERSITY

APPENDIX 7: ANTI-PLAGIARISM REPORT

ANALGESIC ACTIVITY, ACUTE ORAL TOXICITY AND PHYTOCHEMICAL SCREENING OF CRUDE EXTRACTS OF Mystroxylon aethiopicum (Thunb.) Loes. (Celastraceae.)

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(1) Prof. James Mbaná 02/11/2021 Supervisor

(2) PROF. J.D. MANDE
DEAN, FVM
3/11/2021

Dean
Faculty of Veterinary Medicine
University of Nairobi
P.O. Box 29053-00625, NAIROBI