EVALUATION OF THE ANT-INFLAMMATORY AND ANTI-NOCICEPTIVE ACTIVITIES OF METHANOLIC STEM BARK EXTRACT OF *Lochocarpus eriocaxylx* (*Harms.*)

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DECLARATION

I declare that this research project is my original work and has not been presented by anybody in any institution for a ward of diploma or degree.

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SUPERVISOR

This research project has been conducted and submitted with my approval as the student supervisor.

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DEDICATION

I dedicate this project to my mother Judith Chemutai. and cousins ken and Bethwel

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ABSTRACT

Inflammation and pain are characteristic conditions of many diseases. These conditions range from the mild known as acute to the severe one known as chronic. The management of the disorders related to pain and inflammation has been through the use of the conventional drugs such as the NSAIDs, corticosteroids and opioids. Even though they have successfully resulted in the management of the conditions, they have not been devoid of side effects. Additionally, they have been found to be inaccessible and unfordable to the majority of the population that lives below the economic levels. This has triggered the search of the alternatives from the natural sources such as plants, micro-organisms and minerals. Medicinal plants have gained favor among many people due to the low cost, high efficacy and lower side effects associated with them Medicinal plants have as well reported to contain various phytochemicals that have various pharmacological properties such as anti-inflammatory and antinociceptive. The use of plants by man in the management of many ailment dates back many years long ago. Despite of the many years of their usage and efficacy very little information is available that support their therapeutic value scientifically. *Lochocarpus eriocaxylx* has been used traditionally in the management of various diseases such as. However, its analgesic and anti-inflammatory activity of the stem bark remains unexplored. In this study the analgesic and anti-inflammatory activity of the methanolic stem bark extract of *Lochocarpus eriocaxylx* was investigated. The analgesic activity was assessed by the acetic acid writhing test while the anti-inflammatory activity was evaluated using the xylene induced ear edema in the swiss albino mice. Etanercept was used as the positive analgesic and anti-inflammatory drug. The analgesic activity results showed that the positive control recorded significantly higher inhibition of the writhing in the swiss albino mic as compared to the methanolic stem bark extract of the *Lochocarpus eriocaxylx* at all doses ($p<0.05$). However, the administration of the methanolic stem extract of *Lochocarpus eriocaxylx* reduced the effects of the acetic acid in the swiss albino mice in a dose dependent manner. The higher dose and the lower dose recorded significantly higher and lower writhe inhibition respectively ($p<0.05$). The anti-inflammatory results showed that the administration of the methanolic stem bark extract of *Lochocarpus eriocaxylx* inhibited the effects of the xylene by significantly reducing the formation of edema on the left ear. However, etanercept, the positive control drug significantly inhibited the formation of edema more as compared to all the other groups ($p<0.05$). In conclusion, the stem bark of *Lochocarpus eriocaxylx* can be a potential source of natural analgesic and anti-inflammatory agent that is characterized by reduced side effects and with the ability to inhibit the tumor necrosis factor.

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ABBREVIATIONS AND ACRONYMS

CNS	Central nervous system
COX	Cyclooxygenase
GIT	Gastrointestinal tract
IL	Interleukins
NO	Nitric oxide
NSAIDS	Non-steroidal anti-inflammatory drugs
PAF	Platelet activating factor
PGE	prostaglandins
PL	Phospholipase
TGF	Transforming growth factor
TNF	Tumor necrosis factor
VIP	Vasoactive intestinal polypeptide

CHAPTER ONE: INTRODUCTION

1.1Background information

Inflammation is the defense mechanism of the body to noxious stimuli that include microbial infections and injury required in order to the maintain proper health status. However, excessive or prolonged inflammation process is harmful and has been linked to the pathogenesis of a many of diseases like rheumatoid arthritis, asthma, cancer, diabetes, inflammatory bowel disease, atherosclerosis, mental and neurological disorders like Parkinson's disease among others (Buhrmann et al., 2019). The inflammation process is characterized by four major cardinal signs: pain (algesia), swelling (edema), redness, and high temperatures which results due to the vascular changes in vessel calibre. During inflammation process, the endothelial cells are activated which in turn results into vasodilation that is characterized by increased vascular permeability. This increases the leakage of plasma proteins from the circulation and emigration of the leukocytes through the vessel wall into the injured area. The main white blood cells involved in acute inflammation process include the neutrophils and macrophages. On the other hand, in the chronic inflammation process, mononuclear cells like macrophages and lymphocytes are involved (Arulselvan et al., 2016).

The inflammation process is characterized by various pro-inflammatory mediators such as nitric oxide (NO) and prostaglandins like PGE_2, and pro-inflammatory cytokines such as tumor necrosis factor (TNF-α) and interleukins (IL-1) which are secreted from macrophages in response to invading pathogenic microbes. The excessive secretion of these pro-inflammatory mediators' cause tissue damage which is noticed in various inflammatory related disorders (Allam & Anders, 2008). Therefore, search of alternative anti-inflammatory agents with the ability to elicit their mode of action through suppression of the pro-inflammatory mediators is a potential with therapeutic agent that helps to minimize the harmful pro-inflammatory activity of macrophages (Foong & Hamid, 2012).

Many anti-inflammatory drugs and antinociceptive drugs with the ability to manage both acute

and chronic conditions of inflammation and pain are available. However, these drugs have been associated with various side effects that are observed upon their use (Olela et al., 2020). Similarly, the same drugs have been reported to have reduced efficacy and this has initiated the search for ana alternative analgesic and anti-inflammatory agents from natural sources. Plants of medicinal value have been used by man in the management of various chronic diseases. This has been attributed to the many phytochemicals present in these plants, the lower side effects and high efficacy witnessed in many plants in management of various condition (Abdulkhaleq et al., 2018)s. the pricing factor has as well contributed to the increased interests in the use of plants as compared to the conventional drugs which are costly (Sen & Samanta, 2014).

Plants have been the main source of relive for the effects of many ailments both in animals and man for many years. They have been regarded as the main source of primary health care globally with more preferences in Africa countries (Oguntibeju, 2018). Many countries all over the world are turning to use of medicinal plants with about 75% of the population in developing countries in the sub-Saharan Africa relaying entirely on them. The medicinal plants possess various classes of phytochemical agents that have antioxidative, antimutagenic, anticarcinogenic and immunomodulatory properties which can be attributed to their potential value in treating associated diseases (Arulselvan et al., 2016). many plants with anti-inflammatory and analgesic effects are available and have been used to treat the inflammatory related disorders such as rheumatism (Oguntibeju, 2018). Through various studies these plants have shown positive results both in invitro and in vivo models. *Curcuma longa* for instance it has been reported to have anti-inflammatory effects via various clinical trials. Its bioactive compound known as curcumin has been reported to have positive impact on the rheumatoid arthritis as it has been reported to reduce its manifestation such as swelling and pain in joints (Ghasemian et al., 2016). Even though various medicinal plants with anti-inflammatory and antinociceptive activities in most part of the countries Kenya included have been studied and

their ethnobotanical uses document, very many potential anti-inflammatory plants remain unexplored. *Lochocarpus eriocaxylx* is one of such plants whose anti-inflammatory effects have not been studied and later after documented. *Lochocarpus eriocaxylx* is a deciduous small shrub/ tree that grows to height of between 3-12 m. this plant is taxonomically placed in the family Fabaceae and s widely used by the Kenya community as a remedy for various conditions. It has been reported to manage conditions such as fever, headache and diarrhea. It has as well be claimed to be an effective insecticide. The analgesic and anti-inflammatory activity of the leaf extract (chloroform and methanol) and the compounds isolated from the leaves have been studied and shown to be potential anti-inflammatory and analgesic agents (Ochung et al., 2020). However, no reports are available on the analgesic and anti-inflammatory activity of the stem bark.Therefore, this study aimed at investigating the anti-inflammatory and anti-nociceptive activity of *Lochocarpus eriocaxylx* methanolic stem bark extracts to lay a framework for the development of safer, cost effective and efficacious drugs for the management of inflammation and pain.

1.2 Problem statement and justification

Even though the process of inflammation is key as far as the elimination of pathogens and both endogenous and exogenous triggers of inflammation is concerned, prolonged inflammatory process has been linked to various chronic disease. The unchecked inflammation process has been linked to as precursor of various diseases that include hay fever, atherosclerosis and rheumatoid arthritis (Subramaniyan, 2018). These have been later on thought to result into the damage or failure of the body organs. The use of the anti-inflammatory drugs to counter this prolonged process of the inflammation is vital in limiting its cause. Quit number of the synthetic anti-inflammatory drugs that consists of the non-steroidal and glucocorticoids as well as the anti TNF drugs like etanercept have been employed in the management of inflammation exit(Li et al., 2017). However, these drugs have shown to impact adverse side effects such as peptic ulcers, renal dysfunction and cardiovascular problems to the users upon prolonged use

(Ng & Chan, 2010). Additionally, many inflammatory diseases like neurodegenerative diseases like Parkinson, Alzheimer or other inflammatory diseases like Inflammatory Bowel Disease no efficient medicines to treat them is available.This condition has fueled up the continuous search for alternative anti-inflammatory and analgesics from the natural products which are safe than the NSAIDs.

In Kenya and the world at large, various varieties of medicinal plants with many vital phytochemicals have been identified (Oguntibeju, 2018). The therapeutic value of these bioactive compounds has been linked to various pharmacological activities they elicit such anti-inflammatory and antinociceptive. However, a small fraction of all the identified plants have been researched on and their therapeutic values documented (Oguntibeju, 2018). Similarly, of the studied plants, the identified activities have not been proven through clinical trials to confirm the pharmacological properties such as antiinflammation and antinociceptive. Therefore, there is need to conduct more research about the therapeutic values of medicinal plants and their pharmacological activities identified, *Lochocarpus eriocaxylx* included and confirm through research there use in management of inflammation disorders by man since time in memorial. The discovery of more anti-inflammatory agents from plants will only not serve as a replacement of the synthetic ant inflammatory agents but reduce the cost of treatment as well. This is due to the low cost of production and the readily availability of the plant and herbal remedies.

1.3 Objectives

1.3.1 General objective

The general objective of this study was to evaluate the anti-inflammatory activity and the anti-nociceptive activity of the methanol extract of the *Lochocarpus eriocaxylx*.

1.3.2 Specific objective

I. To determine the anti-inflammatory activity of the *Lochocarpus eriocaxylx* methanol extract against the xylene ear induced edema.

II. To determine the anti-nociceptive activity of the *Lochocarpus eriocaxylx* methanol extract against the acetic acid induced pain.

1.4 Research questions

I. Does the *Lochocarpus eriocaxylx* methanolic extract has the anti-inflammatory activity against the xylene ear induced edema?

II. Does the *Lochocarpus eriocaxylx* methanol extract has the anti-nociceptive activity against the acetic acid induced pain.

CHAPTER TWO: LITERATURE REVIEW

2.1 Inflammation

Inflammation is the protective response by the body to remove the noxious stimuli like irritant, damaged cells, infection and other stimulants and later initiate the process of healing of the body tissues (Subramaniyan, 2018). This has been shown through the ability of the body tissues to respond to noxious stimuli such as microbial infections and tissues injury. The inflammation process is characterized by four key features; warmth, redness, pain and swelling(V. Stankov, 2012). Inflammation may occur due to various causes that include both the endogenous and exogeneous causes.

2.1.1 Causes of inflammation

Physical agents; e.g. heat, radiation, mechanical trauma (invasive medical surgical procedures), infections caused by pathogens like bacteria, fungi, toxins and parasites, foreign bodies and chemicals (Arulselvan et al., 2016).

2.1.2 Classification of inflammation

Inflammation is broadly categorized into two groups; acute and chronic inflammation. While the acute inflammation proceeds for short period of time, the chronic inflammation persists for longer period of time. the chronic inflammation is accompanied by tissue granulation and fibrosis.

2.1.2.1 Acute inflammation.

The acute inflammation is the short term and the initial response of the immune system against pathogen and the noxious stimuli and it may last for few hours. Its characterized by the classical signs of inflammation; swelling, redness, pain, warmth and lose of function. The acute inflammation occurs as long as the stimuli is still present and upon completely removal of the injurious stimuli and covering of the injured site by formation of the scar it ceases as well. Its initiated by the eicosanoids and vasoactive amines followed by the vascular and cellular that increases the exudation of the plasma proteins and leukocytes into the surrounding tissues in

the infected site. this increased from of the fluid to the injured tissues results into the swelling, while the increased flow of the blood to the injured site cause the reddening and increased heat.

The increased flow of fluid into the tissue causes the characteristic swelling associated with inflammation (Arulselvan et al., 2016) and the increased blood flow to the area causes the reddened color and increased heat. The blood vessels also alter to permit the extravasation of leukocytes through the

2.2 Mediators of inflammation.

These comprises of the endogenous chemical substances from the circulation system, inflammatory cells and injured tissue that actively contribute to and adjust the inflammatory response. These substances have been characterized with similar properties that include derivation from plasma protein, being released in response to certain stimuli, eliciting their action on different targets, have a variety of actions and characterized with short lifespan upon their release. These mediators are broadly categorized as either cell-derived mediators or plasma protein-derived mediators (plasma protease) (Arulselvan et al., 2016).

2.2.1 Cellular derived mediators.

Various cells that are involved in the anti-inflammatory response are available. These include the mast cells, neutrophils, eosinophils, Macrophages, lymphocytes, endothelial cells and platelets. These cells elicit their inflammatory responses by reacting to various signals, stimuli and irritants. The cell mediators are characterized by redundant activities and comprises of various chemical groups such as vasoactive amines and cytokines.

2.2.1.1 Vasoactive amines;

Histamine and serotonin comprise the vital pharmacologically active amine that are associated with mediation of the acute inflammatory responses. They are produced by the mast cells, platelets and basophils. The histamine is produced in smaller quantities that amount to few pictograms and is tasked with the role of maintaining the acute phase of inflammation. The synthesis of histamine is via the decarboxylation of the amino acid histidine by the catalytic

reaction of the L-histidine decarboxylase enzyme. This takes place in the granules of the mast cells and basophils. Histamine release from basophils, mast cells, and platelets causes vasodilation, increased vascular permeability, itching and pain. Stimulation of mast cells and basophil is responsible for the release of slow-reacting substances of anaphylaxis (*SRS-As),* which consist of several leukotriene's (LTC_4, L TD_4 and LTE_4). Serotonin is synthesized through the decarboxylation of the tryptophan and stored in the secretory granules. In other animal serotonin is derived in the mast cells but in the humans, it's also found in serum upon clotting of the blood. It's as well found in the gastrointestinal tract and the central nervous system where it acts as a neurotransmitter and a local hormone in the peripheral vascular system.

Tissues like chromaffin cells of the GIT, spleen, nervous tissues, mast cells and platelets contain a less active vasoactive amine, serotonin (5-hydroxytryptamine) than histamine although studies suggest its involvement in the carcinoid tumor (serotonin secreting tumor).

Tachykinin neuropeptides produced in the CNS and PNS such as substance P, vasoactive intestinal polypeptide (VIP) and somatostatin exhibit proinflammatory activity; increased vascular permeability, pain stimuli transmission and mast cell degranulation(Arulselvan et al., 2016).

2.2.1.2 Arachidonic acid metabolites.

Eicosanoids are biologically active mediators of inflammation that are synthesized from the arachidonic acid substrate. They include products enzymes such as5-lipoxygenase (leukotriene and 5-hydroxyeicosatetraenoic acid), cyclooxygenases (prostaglandins and thromboxanes), and 12-1ipoxygenase (12-hydroxyeicosatetraenoic acid). Eicosanoids functions as vasodilators, bronchoconstrictors and serves to increase vascular permeability. In addition to these functions TXA_2 has platelet aggregation activity, PGI_2 is an antiplatelet aggregation agent

and Resolvins inhibit pro-inflammatory cytokines. Other examples include; PGD_2, PGF_2, alpha (Kubata *et al.,* 2007).

2.2.1.3 Lysosomal components

Neutrophils and monocytes contain lysosomal granules which on release elaborate a variety of mediators of inflammation. Granules of neutrophils namely; azurophil, secondary or specific and tertiary granules, these granules are composed of enzymes, myeloperoxidase, acid hydrolysis, lysozyme, defensin, phospholipase, cathepsin G, elastase and protease.

2.2.1.4 Platelet activating factor (PAF)

PAF are released by IGE-sensitized basophils, mast cell, leucocytes, endothelium and platelets. They increase vascular permeability, vasodilation, broncho-constriction, adhesion of leukocyte to endothelium and chemotaxis (Chandur *et al.,* 2011).

The PAF is an active lipid molecule that are biologic in nature that elicit its action at very low concentrations. It has been reported to mediate both the acute and chronic allergic reactions. Its biosynthesis is through two steps that involve the action of phospholipase A2 (PLA2) on the acyl-PAF to produce lyso-PAF that is acylated generating the PAF. The PAF is synthesized more in the lungs by many inflammatory cells such as mast cell and alveolar macrophages. PAF produces vasodilatation, increased vascular permeability, and weal formation. PAF is a potent chemotaxin for neutrophils and monocytes and recruit eosinophils into the bronchial mucosa in the late phase of asthma. It can activate PLA 2 and initiates eicosanoid synthesis.

2.2.1.5 Cytokines

These are proteins or polypeptides in nature and are synthesized and released by the cells of the immune system during the inflammation process. They include interleukins (IL), chemokines, interferons, colony stimulating factors, growth factors, and tumor necrosis factors (TNFs). Among the all cytokines, the chemokines elicit their action via targeting the G-proteins while the rest of the cytokines target kinase-linked receptors. The chemokines are the chemoattractant cytokines with the role of controlling the migration of the leukocytes during

the inflammatory and immune reactions.TNF-α and interleukin-1 (IL-1) are primary inflammatory cytokines which participate in acute and chronic inflammatory reactions as well as repair and resolution. Transforming growth factor-β (TGF-β), IL-4, IL-10, and IL-13 are anti-inflammatory cytokines that suppress the release of chemokine (1).

Table 2. 1 cytokines involved in inflammation

Cytokine	**Function**
IL-6, IL-1, IL-8, IL-12, IL-17	Active mediators of acute inflammation.
IL-1 and IL-6	Acute inflammations phase.
IL-12 and IL-17	Chronic inflammation.
IL-8	Is a chemokine for acute inflammatory cells.

2.2.1.6 Plasma proteases.

The kalliklein kininogen system involves Bradykinin which acts in the early phase of inflammation and its effects include; smooth muscle contraction, vasodilation, pain and increased vascular permeability. The clotting system, Fibrinopeptides functions in inflammation include increased vascular permeability, chemotaxis for leucocytes and anticoagulant activity. Others include the complement system and the fibrinolytic system (Harsh, 2015).

2.3 Anti-inflammatory drugs

The management of inflammation and inflammatory disorders involve the use of inflammation drugs. These drugs are grouped as NSAIDs, corticosteroids and opiates. The NSAIDs are widely prescribed anti-inflammatory and analgesic drugs although corticosteroids are used as an adjuvant therapy in management of inflammatory diseases (arthritis)(Olela et al., 2020).

The NSAIDs comprises of drugs such as Aspirin, celecoxibs, diclofenac, ketorolac, diflunisal, fenobrufen, flurbiprofen, ibubrofen, indomethacin, mefenamicacid, meclofenamate, salicylate,

meloxicam, oxaprozin, naproxen, rofecoxib (Gupta, n.d.); (Fokunang, 2018). The Steroids include drugs such as Prednisolone, Prednisone, Dexamethasone, Triamcinolone, Fluticasone, Hydrocortisone.

2.3.1 Mode of action of anti-inflammatory drugs

2.3.1.1 NSAIDS

They major mechanism by which the NSAIDs elicit their therapeutic effects (antipyretic, analgesic and anti-inflammatory) is via the inhibition of the synthesis of prostaglandin (pg.) (Fokunang, 2018). This is usually by inhibiting the activity of cyclooxygenases (COXs), enzymes that is tasked with the role of catalyzing the synthesis of cyclic endoperoxidases from Arachidonic acid to form prostaglandins. The COX enzymes are segregated into two groups: COX-1 and COX-2. The COX-1 is expressed constitutively and is present in many tissues most notably in the platelets, endothelial cells, the GIT, the renal microvasculature, glomerulus, and it's essential for the production of prostaglandin for homeostatic maintenance. Inhibition of this COX iso-enzyme is considered as the major contributor to NSAIDs GIT toxicity. The COX-2 iso-enzyme plays an important role in pain and inflammatory processes. This enzyme is inhibited by COX-2 selective inhibitors (Rofecoxib and Celecoxib) (Fokunang, 2018).

2.3.1.2 Steroids

The Corticosteroids mode of action involves inhibiting all forms of inflammation irrespective of their etiology. With the main anti-inflammatory activity being exhibited through the inhibition of enzyme phospholipase A_2 thus limiting the recruitment of inflammatory cells at the local site of action and production of pro-inflammatory mediators like PGs, LTs, and PAF. Indiscriminate use of corticosteroids is hazardous since they caused suppression of cell mediated immunity via inhibition of t-cell proliferation (Williams, 2018).

2.3.1.2. Tumor Necrosis Factor (TNF) Blockers

TNF is pro-inflammatory cytokine that plays a vital role in the pathogenesis of autoimmune inflammatory diseases (Moudgil & Choubey, 2011). Consequently, anti-TNF biologics, which

are agents that have been designed to block the biological function of TNF, have been developed for the therapy of autoimmune inflammatory diseases (Florian et al., 2013). In the past two decades, five TNF-targeting drugs have been approved for clinical use, including infliximab, etanercept, adalimumab, golimumab, and certolizumab pegol, for the treatment of the autoimmune inflammatory diseases including RA, CD and AS (Monaco et al., 2014).

2.4 Complementary management of inflammation

Herbal medicine has been part of man for many centuries and have been widely accepted in the treatment of many diseases. The natural remedies from plants based on traditional practice represent a huge portion of the pharmaceutical products in modern western countries (Abdulkhaleq et al., 2018). Many concerns have been raised that modern pharmaceutical practice are too costly and produces many side effects (Dhami, 2013). However, many studies have shown that remedies from natural sources such as plants can manage many chronic diseases such as inflammatory disorders with fewer side effects (Abdulkhaleq et al., 2018). Similarly, various studies have shown the positive outcomes associated with the combination of conventional medicines and herbal medicines. Additionally, the pricing factor has immensely contributed to the use of herbal medicines by the majority of the publics in the recent years (Sen & Samanta, 2014).

Many plants with the anti-inflammatory effects are available globally. The products from these plants has been incorporated in various formulations such as ointments. The ointment that contain Aloe vera gel along with cortisone (hydrocortisone-21-acetate) is such a product. This product has been used as anti-inflammatory agent in the skin. Its use suggests that it has an important active carrier for steroids (Subramoniam, 2014). Additionally, Eucerin cream containing 25 % of A. vera and 5 % of the irradiated A. vera extract that contains anthraquinone has been reported to be potent against wounds as it reduces wounds in mice (Reid et al., 2016). Generally the extracts of *A. vera* both oral and topical preparations have been widely prescribed

by podiatric physicians for used to treat inflammation and woods of the foot (Kotsirilos et al., 2011).

2.5 *Lonchorcapus aeriocalyx*

2.5.1 Ethnobotanical description

The *Lonchorcapus aeriocalyx* is deciduous plant or shrub that taxonomically belongs to the family fabaceae. It grows to the height range of 3-12 m tall, very slender and it consists of a crown that is round in shape.

2.5.2 Ethnomedicinal uses

The infusion of the bark is used as a remedy for fever, headache, diarrhea and as an insecticide as well (Ceres et al., 1981; Kokwaro, 2009; Adem et al., 2018). The barks have also been used in the management of blood pressure and reduce the sugar levels among the Embu and Mbeere communities in Kenya (Kareru et al. 2006).

2.5.3 Phytochemistry

The phytochemical studies on the *Lonchocarpus eriocalyx* has shown that the presence of lupeol triterpene which is compound has been reported to have anti-plasmodial activity against plasmodium ovale (Ochung et al., 2020). The phytochemical screening of the chloroform and methanolic leaves extracts resulted into isolation of eight compounds. The phytochemical studies done by Moriasi et al.(2020) revealed the presence of cardenolide glycosides, coumarins, phenols, steroids, saponins, and flavonoids in the aqueous and methanolic stem bark extract. However, alkaloids and tannins were only present in the aqueous extract but absent in the methanolic extract.

CHAPTER THREE: MATERIAL AND METHODS

3.1 Plant collection and preparation

The fresh and disease-free stem barks of *Lochocarpus eriocaxylx* were obtained from Mbeere located in Embu county. The collection procedure involved the identification of *Lochocarpus eriocaxylx* plant by the help of a conversant herbalist. The plant was identified by its local name as Muthigiriri. Further identification was done by taxonomist based at the East African herbarium at the National Museum of Kenya and allotted voucher number MMK/BOT/CTX/2/2. Duplicate voucher specimens were prepared and one deposited at the East African herbarium while the other was deposited at department of pharmacognosy, school of pharmacy, Mount Kenya university. The collected stem bark samples were then transported to the laboratory, sorted to remove any unwanted and then cut into smaller pieces prior to air drying under shade for a period of two weeks. The well dried samples were then grounded into fine powdered by help of plant miller and then transferred in an air tight container and stored in a dry cool place waiting extraction.

3.2 Extract preparation

The methanol extracts used in this study was prepared following the method described by (Moriasi et al., 2020). Basically, this involved soaking about 300 grams of the course grounded dry powder of the *Lochocarpus eriocaxylx* in to 0.6 liters of the methanol (analytical grade) in the extraction glass vessel for a period of two complete days with constant agitation. The plant materials were then filtered via the Whatman No. 1 filter paper and under vacuum created by the vacuum pump. The same process was repeated two more times and all the filtrate concentrated under vacuo in the rotary evaporator with the water bath set at 40 ^{0}C in order to recover the extraction solvent. the solvent free extract was emptied into the clean pre-weighed sample bottle and completely dried into the hot air oven overnight prior to capping and completely sealing with the parafilm. The extract was then stored into the fridge at a temperature of 6 ^{0}C until the study day.

3.3 Experimental animals.

The swiss albino mice of the weight range of 25-30 grams were sourced from KALRO in the late November 2019. They were transported to the Mount Kenya university in their respective cages made of the propylene materials. They were kept in the research center laboratories under the constant conditions of 26 ^{0} C that were maintained for the 12-hour cycle day and night. The animals were allowed to acclimatize to the laboratory conditions for a period of five days prior to the day of the study and during this period they were fed onto the standard rodent pellet and clean water via the drinkers that was freely supplied.

3.4 Preparation of the study plant extract doses

The doses of 500 mg/kg bw,100 mg/kg bw, 20 mg/kg bw, and 4 mg/kg bw of the methanol stem bark extract of the *Lochocarpus eriocaxylx* were prepared in accordance with the OCED guidelines. This involved the weighing of all the study mice and then the volume of the administration dosage calculated and the stock solution preparation according to the OCED (Oghenesuvwe et al., 2014).

3.5 Sorting and marking of the study animals

The study animals were fasted for a period of one hour prior to the start of both the anti-inflammatory and analgesic activity study. This was done by depriving the animals both water and the feed. After the elapse of the one-hour timeline the animals were then sorted into six group with each group having five mice and marked with marker pen of different colors. The marking signified different treatment groups as shown in table 3.1.

Table 3. 1 Randomized dose administration protocol for the antinociceptive and antiinflammatory activity

Treatment group	Dose administered	Identification mark
Treatment group 1	4 mg/kg	Black-tail
Treatment group 2	20 mg/kg	Red-right hind-limb
Treatment group 3	100 mg/kg	Blue-belly

Treatment group 4	500 mg/kg	
Normal control	Normal saline	Blue-back
Negative control	Normal saline	Red-right fore-limb
Positive control	Etanercept (50 mg/kg)	Black- head

3.6 Anti-inflammatory activity of the methanol extract of the *Lochocarpus eriocaxylx*

The anti-inflammatory activity of the *Lochocarpus eriocaxylx* methanol extract was evaluated by adopting the xylene ear induced edema method as illustrated by Olela et al. (2020) with minor modifications. The swiss albino mice used in this study were divided into six groups with five mice per group. The three groups constituted three test groups and the other three groups were the controls. The treatment groups received the plant extract with first group receiving the lowest dose of 4 mg/kg per body weight, the second the middle dose of 20 mg/kg and the third group the highest dose of 100 mg/kg per body weight. The plant extracts were administered orally. The negative control and the normal control groups were administered normal saline orally while the positive control group received the etanercept the positive control drug at the dose of 500ul/25mg. the animals were left for 30 minutes after which the edema was induced in the test groups, negative and the positive control groups by accurately applying a drop of xylene in the inner surface of the right ear. The mice were then left for other 15 minutes after which they were anesthetized with diethyl ether and both the right and the left ear sizable cut and weighed. The anti-inflammatory activity was then expressed as the percentage inhibition of the edema calculated by the formula;

$$\%\ Edema\ inhibition = \frac{Weight\ of\ right\ ear - Weight\ of\ left\ ear}{Weight\ of\ left\ ear} \times 100$$

3.7 Anti-nociceptive activity of the methanol extract of the *Lochocarpus eriocaxylx*

The anti-nociceptive activity of the methanol extract of the *Lochocarpus eriocaxylx* was evaluated by the acetic acid induced writhing test method as described by Aquino et al.(2002)

with minor modifications. The swiss albino mice of mixed gender were grouped into six groups randomly with each group comprising of five mice (n=5). the three treatment groups were administered orally 0.2 ml of the plant extracts at different concentrations. The first treatment group was administered the lowest concentration of 4 mg/kg per body weight, the second treatment group was administered the middle concentration of 20 mg/kg bw, the third treatment group was administered the highest plant extract concentration of 100 mg/kg bw and the fourth treatment group received the higher dose of 500 mg/kg bw. The negative and the normal control group were administered 0.2ml of the normal saline which was the vehicle, while the positive control was orally administered 0.2ml of the 500 ul/25 mg of the etanercept the positive control drug. Mice in all the test groups were left for 30 mins after which 0.6% acetic acid was intraperitonially administered by injecting 0.2ml to the mice in the negative and positive control group and the three test treatment groups while in the normal control only normal saline was administered. The all mice were then put into the open cages and the number of the writhing indicated by the contraction of the abdominal muscles recorded for 15 mins at interval of 5mins. The anti-nociceptive activity was then calculated by determining the percentage inhibition of the writhes using the formula below;

$$\%\ Inhibition = \frac{Mean\ No.of\ writhes\ of\ control - mean\ No.of\ writhes\ of\ test}{Mean\ number\ of\ writhes\ of\ control} \times 100$$

3.8 Data management and statistical analysis

The data obtained from the anti-inflammatory and anti-nociceptive activity were recorded into the excel sheet and the percentage inhibition of the edema and writhes calculated. The percentage inhibition data was then imputed into the Minitab software and the descriptive analysis done and the percentage inhibitions presented as mean ± SEM (standard error of the mean). The t-statics was then done and the level of the significance against the different plant extract doses and the controls identified.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Anti-inflammatory activity of the methanolic stem bark extract of *Lochocarpus eriocaxylx*

The results for the anti-inflammatory activity of the methanolic stem bark extract of *Lochocarpus eriocaxylx* are presented in table 4.1. the results showed that the extract elicited a dose dependent percentage inhibition of edema. The positive control drug significantly recorded higher percentage inhibition of edema as compared to all the other groups (P<0.05). Similarly, the higher dose 500 mg/kg bw recorded a higher percentage inhibition of edema while the lower dose 4 mg/kg bw recorded the least percentage inhibition of edema.

Table 4. 1 Anti-inflammatory activity of the methanolic stem bark extract of *Lochocarpus eriocaxylx* (Harms.)

TREATMENT	**% INHIBITION OF OEDEMA MEAN**±SEM
Etanercept (50mg/ml)	97.5± o.00709^{A}
Extract 500mg/kg	92.1±0.00557^{B}
Extract 100mg/kg	85.5±0.00267^{C}
Extract 20mg/kg	79.7±0.00819^{D}
Extract 4mg/kg	71.6±0.00306^{E}

Values are presented as $\bar{x} \pm SEM$; Means sharing a superscript letter within the same column are not significantly different by One-Way ANOVA followed by Fisher's LSD test (p>0.05), whereas means that share a subscript letter within the same concentration (across the rows) are not significantly different by unpaired student t-test statistic (p>0.05).

4.2 Anti-nociceptive activity of the methanolic stem bark extract *Lochocarpus eriocaxylx*

The antinociceptive activity the methanolic stem bark extract of *Lochocarpus eriocaxylx* are presented in table 4.2. The results showed a significant deference between the percentage writhe inhibition means (p<0.05). the positive control drug (Etarnecept 50mg/Kg) recorded the highest percentage inhibition of the writhe means as compared to the extract at all dose levels (p<0.05). the methanolic stem bark extract of *Lochocarpus eriocaxylx* recorded a dose dependent percentage writhe inhibition with the highest and lowest doses recording significantly higher and least percentage inhibition of writhe mean (p<0.05).

Table 4. 2 Antinociceptive activity of the methanolic stem bark extract of *Lochocarpus eriocaxylx* (Harms.)

TREATMENT	% INHIBITION OF WRITHES **MEAN**±SEM
Etanercept 50mg/Kg	98.3 ± 0.424^{A}
Extract 500mg/kg	86.4 ± 0.847^{B}
Extract 100mg/kg	80.1 ± 1.12^{C}
Extract 20mg/kg	72.0 ± 0.734^{D}
Extract 4mg/kg	55.5 ± 1.47^{E}

Values are presented as $\bar{x} \pm SEM$; Means sharing a superscript letter within the same column are not significantly different by One-Way ANOVA followed by Fisher's LSD test ($p>0.05$), whereas means that share a subscript letter within the same concentration (across the rows) are not significantly different by unpaired student t-test statistic ($p>0.05$).

The many side effects and toxic effects associated with use of the conventional anti-inflammatory and antinociceptive drugs such as NSAIDs and the high cost of drugs such as etanercept has necessitate the exploration of the herbal medicine as the alternatives. Due to the fewer side effects and low cost of herbal medicines has geared the continuous search of the indigenous drugs that can provide relief to the inflammation and pain. In the current study the methanolic stem bark extract of *Lochocarpus eriocaxylx* was investigated for its anti-inflammatory and antinociceptive activity. The xylene induced ear edema method was adopted for the anti-inflammatory activity while the acetic acid induced writhing test was used to assess the analgesic activity. The xylene induced ear edema assesses the acute inflammation phase. This model uses xylene as an agent to induce inflammation. Xylene is topically applicated to the left ear as the right ear serves as the control. Upon application of the xylene, symptoms (severe vasodilation and edematous changes of the skin) characterized to the acute inflammation are observed (Kim et al., 2007). In this study the methanolic stem bark extract of *Lochocarpus eriocaxylx* significantly reduced the edema formation in a dose dependent manner. The high dose of the extract performed better as compared to the other doses. However, the positive control drug (etanercept 50 mg/kg bw) significantly reduced the edema more than all the other groups with the extract included. This edema inhibition ability of the

extract can be an indication of the anti-inflammatory potency that is through reduction of the vasodilation and there after improving the edematous condition.

The analgesic activity was evaluated by use of the acetic acid induced pain test in which the acetic acid was intraperitoneally administered and this caused abdominal writhing indicated by the abdominal muscle constriction and stretching of the hindlimbs of the swiss albino mice. The acetic acid-induced pain is analgesic model that has be frequently used to evaluate the analgesic potential of various new analgesic agents and it also incorporates the peripherally acting analgesic (Adel Moallem et al., 2013); (Arome et al., 2014). According to (Berkenkopf & Weichman, 1988), writhing in the laboratory animals can be are induced by various agents with acetic acid and phenylquinone included. The intraperitoneal injection of acetic acid in the laboratory animals has been thought to cause the constriction of the abdominal muscles, elongation of the body parts and extension of the hind limb as major indicators of pain. These indicators are thought to be mediated via the peritoneal receptors (Bentley et al., 1983). It's a proposal that the acetic acid acts by indirectly release of the endogenous substances that are responsible for exciting the nerve endings and causing pain, but it excites neurons that are sensitive to drugs as well (Adel Moallem et al., 2013). In this study the administration of the methanolic stem bark extract of *Lochocarpus eriocaxylx* showed a dose dependent antinociceptive activity. The extract significantly inhibited the writhing in the swiss albino mice. however, the positive control had a higher antinociceptive activity as compared to the drug. The ability of this extract to inhibit writhing its evident it ca act by inhibiting the tumor necrosis factor.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The finding of this study has indicated the methanolic stem bark extract of *Lochocarpus eriocaxylx* has anti-inflammatory and antinociceptive activity. The plant has the ability to inhibit the tumor necrosis factor as it showed an activity nearly similar to that showed by etanercept.

5.2 Recommendation

Based on the finding of the current study various recommendation can be made

I. The isolation of the active compounds responsible for anti-inflammatory and antinociceptive activity
II. Safety studies of the plant extract to be done

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