ACUTE ORAL-TOXICITY, ANTI-INFLAMMATORYAND ANALGESIC EFFECTS OF AQUEOUS AND METHANOLIC BARK EXTRACTS OF *PILIOSTIGMA THONNINGII* (SCHUM) IN MICE

BEN OTIENO OLELA (BPharm)
REG: J56/11961/2018

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DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY
FACULTY OF VETERINARY MEDICINE
UNIVERSITY OF NAIROBI

AUGUST 2021

DECLARATION

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

Signature_____ **Date** 24th/8/2021

Dr. Ben Otieno Olela

Reg. No: J56/11961/2018

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

Signature_____ **Date** 24/8/2021

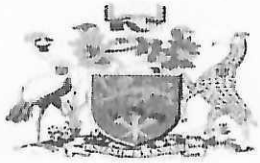
Prof. James M. Mbaria (BVM, MSc, PhD)

Department of Public Health, Pharmacology and Toxicology

Signature_____ **Date** 24/8/2021

Dr. Timothy Wachira (BVM, MVSc, PhD)

Department of Public Health, Pharmacology and Toxicology



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UNIVERSITY OF NAIROBI

NAME OF STUDENT: DR. BEN OTIENO OLELA

REGISTRATION NUMBER: J56/11961/2018

COLLEGE: COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES

FACULTY/SCHOOL/INSTITUTE: VETERINARY MEDICINE

DEPARTMENT: DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY

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DEDICATION

I dedicate this thesis to my wife and family members for their invaluable support and encouragement throughout the journey of my study.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
BW	Body Weight
Cox	Cyclooxygenase
IL	Interleukin
IL-1 α	Interleukin 1-alpha
IL-1β	Interleukin -1 beta
KEMRI	Kenya Medical Research Institute
LD_{50}	Median lethal dose
LOX	Lipoxygenase
NACOSTI	National council of science, technology and innovation
NOS	Nitric oxide synthase
NSAIDs	Non-steroidal anti-inflammatory drugs
OECD	Organization for economic co-operation and development
PG	Prostaglandin
PG-E_2	Prostaglandin E_2
p.o	*'Per os'*
SEM	Standard error of the mean
TNF-α	Tumor necrosis factor alpha
UDP	Up-and-Down-Procedure

ABSTRACT

Aqueous and methanolic extracts of some medicinal plants in African have been shown to have anti-inflammatory and analgesic effects. Previous studies have documented diverse ethno-medical applications of various plants, including *Piliostigma thonningii,* a leguminous herb in the family *Caesalpiniacea.* As a way of establishing the scientific justification for the ethno-medical use of *Piliostigma thonningii* (Schum) bark extracts, the current study was designed to evaluate acute oral toxicity, anti-inflammatory and analgesic effects of aqueous and methanolic stem bark extracts of *P. thonningii*. In this study, fresh stem barks of *P. thonningii* were obtained from Kiangombe forest in Embu County, where the plant grew naturally with the help of a renowned herbalist. Voucher specimen was prepared and authenticated by a taxonomist at the East Africa herbaria at the National Museums of Kenya. Methanolic extracts were prepared by cold maceration using analytical grade methanol whereas aqueous extracts were obtained through the freeze-drying process according to standard phytochemical methods. The extracts were stored in refrigerator set at 4^{o}C and retrieved only during use. Acute oral toxicity was investigated according to the Up-and-Down-Procedure described by the OECD/OCDE document number 425 guidelines. Anti-inflammatory effects of the aqueous and methanolic stem bark extracts of *P. thonningii* were done using the xylene-induced ear-oedema in Swiss albino mice. Peripheral analgesic effects of the aqueous and methanolic stem bark extracts of *P. thonningii* were performed using the acetic acid induced writhing technique on mice. Acute oral toxicity data was analyzed and interpreted according to the OECD guidelines. Anti-inflammatory and analgesic assay data were descriptively analysed and the expressed as Mean±SEM. One-way ANOVA was done to determine differences among means followed by Tukey's *post hoc* test, or un-paired student t-test were performed appropriately for pairwise comparison and separation of means at α=0.05. The acute oral toxicity assay results revealed that all the studied plant extracts were safe with LD_{50} value of >2000 mg/Kg BW. Anti-inflammatory results showed that, the aqueous extract at a dose levels of 100 mg/Kg BW and 500 mg/Kg BW and the methanolic extract at a dose of 500 mg/Kg BW caused significantly higher percentages of xylene-induced oedema in mice than that caused by the reference drug, dexamethasone ($p<0.05$). A comparison between the effects of the two studied plant extracts on xylene-induced ear oedema in mice showed that the aqueous stem bark extract of *P. thonningii* at all the studied dose levels produced significantly higher percentage inhibition of oedema as compared with the percentage inhibitions caused by the methanolic extract at the same dose levels ($p<0.05$). On the other hand, analgesic activity data revealed that the aqueous extract at the highest dose of 500 mg/Kg BW reduced acetic acid induced writhing frequency significantly more than the standard drug, Acetylsalicylic acid ($p<0.05$). A comparison between the percentage inhibitions of writhing frequencies in mice treated with the studied plant extracts was done. The aqueous stem bark extract treated mice, at all doses exhibited significantly lower writhing frequencies compared with the frequencies recorded by mice that received the

methanolic stem extract ($p<0.05$). The remarkable anti-inflammatory and analgesic effects of these extracts could be attributed to the presence of active phytochemicals, which target various pathways leading to mitigation and modulation of these conditions. Furthermore, the safety of these extracts could be due to low concentration/absence of toxic phytocompounds. This in part explains the use of this plant in the traditional management of pain and inflammatory disorders. From the obtained results, the aqueous and methanolic stem bark extracts of *P. thonningii* possess anti-inflammatory and analgesic properties and are safe. Further studies aimed at isolating and characterizing active compounds responsible for the reported activities are recommended.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Inflammation is a physiological response to infection, mediated by signaling molecules that are secreted by leucocytes. The activation of complement cascade causes oedema due to consolidation of proteins and fluids. It also causes the gathering of white blood cells at the site of infection (Azab *et al*., 2016; Foster and Herring, 2010; Ghasemian *et al*., 2016).

Pain is a condition characterized by unpleasant, subjective, and multidimensional state that results from damage of tissues. It includes sensory experiences, such as motivation, cognition, emotion, space, intensity, and time (Rahman *et al*., 2019; Yasmen *et al*., 2018b). When an individual feels pain, it signals that there is something wrong in the body. It, therefore, brings attention to tissue injury as a way of protecting the affected part (Alemu *et al*., 2018; Gou *et al*., 2017). Although pain is beneficial to the immune system, it causes a lot of discomfort and suffering and lowers the quality of life for the victim. Hence, there is a need for its management.

Ordinarily, treatment of algesia and inflammation depends on the use of non-steroidal anti-inflammatory drugs (NSAIDs), adjuvants, and opioids. As noted by Donkor *et al*. (2013), most of the drugs are either expensive or inaccessible, and they often lead to adverse effects. Conventional drugs only provide a symptomatic relief, and they are often toxic to body tissues and organs including the liver, kidney among others (Donkor *et al*., 2013). In this regard, plant-based medicines, such as extracts from *Piliostigma thonningii* (Schum.), can be used for treatment of pain and inflammation (Fürst and Zündorf, 2014; Oguntibeju, 2018). This has necessitated the research into safer and more effective alternatives. Natural products are more potent, and they are relatively less toxic.

For a long time, herbal medicine has been exploited by various cultures for management of various human and animal illnesses. Many African communities, especially in the rural areas, herbs are still used in healthcare because they are readily available and relatively less expensive than western medicine (Awhin *et al*., 2013). In developing countries such as Kenya, traditional medicine is relied on, although orthodox medicine is acceptable and preferred. Globally, over 80 % of the human population utilize traditional medicines out of which 85% are based on plant extracts (Ighodaro *and* Omole, 2012).

Generally, aqueous and methanolic extracts of some medicinal plants in African traditional medicine have analgesic effects, including *Leonotis Ocymifolia* (Alemu *et al*., 2018) and *Rhodiola rosa* (Chatterjee *et al*., 2015). Various other plants serve as analgesic and anti-inflammatory remedies, as evidenced in many regions, like in India, the dried leaves of *Murraya koenigii* Linn are used for this purpose (Singh *et al*., 2016). In Europe, *Hypochaeris radicata*, the flat weed, is used for pain relief, in addition to treating dyspepsia, jaundice, constipation, and renal problems (Abu-Izneid *et al*., 2018).

Previous studies have documented diverse ethno-medical applications of various plants, including *Piliostigma thonningii*, a leguminous herb in the family *Caesalpiniacea*. According to Ighodaro and Omole (2012), the under-explored plant is naturally perennial and produces white to pink petals between November and April. Various parts of *P. thonningii* including seeds, roots, barks, and fruits, are traditionally used for management of many diseases including gingivitis, wounds, ulcers, gastric pain, and pyrexia (Dasofunjo *et al*., 2013). In some countries, such as Zimbabwe and Tanzania, extracts from the root back have been used to treat coughs. Bark extracts have also been shown to have analgesic/ anti-inflammatory activity (Ighodaro and Omole, 2012).

As a way of establishing the scientific justification for the ethno-medical use of *P. thonningii* (Schum) bark extracts, the current study was designed to investigate acute oral toxicity, anti-inflammatory and analgesic effects of aqueous and methanolic stem bark extracts of *P. thonningii*.

1.2 Problem statement and justification of the study

Human diseases, both acute and chronic require the patients to be on pain/inflammation medication for extended periods of time and in higher doses over time due to pain habituation. The mostly prescribed anti-inflammatory drugs (NSAIDs) are associated with adverse side effects such as increased bleeding, hepatotoxicity, nephrotoxicity, GIT disorders among others (Harirforoosh *et al*., 2013).

On the other hand, administration of analgesic medications cause drowsiness, anorexia, dizziness, constipation, headache, nausea, vomiting among others. NSAIDs also cause oedema of the arms and legs due to fluid retention in the body which is associated with renal failure (Harirforoosh *et al*., 2013).

Although many medicinal plants have been traditionally used for alleviation of pain, fever, and inflammation, they have for a long time been excluded from conventional healthcare plans despite their curative properties and the few associated side effects. Novel phytochemical substances of plant origin can be used for therapeutic purposes. Most synthetic drugs used for analgesic, anti-inflammation, antipyretic, and among other conditions have been derived from plants (Fürst and Zündorf, 2014; Ghasemian *et al*., 2016; Oguntibeju, 2018; Sun *et al*., 2018).

Despite the phenomenal advances in medical sciences, the treatment of inflammation, fever, pain, and other indicators of ill health is still complex and problematic. *Piliostigma thonningii* is commonly used in many countries for various therapeutic functions, including

pain and inflammation management (Kareru *et al*., 2007). However, there is no adequate scientific evidence to back its ethno-medicinal applications.

The efficacy of *P. thonningii* as an analgesic should be explored since herbal medicines are relatively safer, with fewer side effects, and more culturally accepted than synthetic drugs. Although the plant is widely used in different countries across Africa, there is little documented research data about its toxicity, anti-inflammatory and analgesic effects.

Therefore, the present study aimed at investigating acute oral toxicity, anti-inflammatory and analgesic effects of aqueous and methanolic stem bark extracts of *P. thonningii* to provide empirical data towards discovery of well tolerable, affordable, safer, and accessible anti-inflammatory and analgesic drugs.

1.3 Research questions

i. Do the aqueous and methanolic bark extracts of *P. thonningii* cause acute oral toxicity in mice models?
ii. Do the aqueous and methanolic stem bark extracts of *P. thonningii* have anti-inflammatory activity in xylene-induced ear oedema in Swiss-albino mice?
iii. Do the aqueous and methanolic stem bark extracts of *P. thonningii* have analgesic activity in acetic acid-induced writhing in mice models?

1.4 Objectives

1.4.1 General objective

To investigate acute oral toxicity, anti-inflammatory and analgesic effects of the aqueous and methanolic stem bark extracts of *P. thonningii* Schum.

1.4.2 Specific objectives

i. To evaluate acute oral toxicity effects of aqueous and methanolic stem bark extracts of *P. thonningii*.

ii. To investigate anti-inflammatory effects of aqueous and methanolic stem bark extracts of *Piliostigma thonningii* on xylene-induced ear oedema in mice models.

iii. To evaluate the analgesic activity of aqueous and methanolic stem bark extracts of *P. thonningii* on acetic acid-induced writhing in mice models.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Inflammation

Inflammation is defined as a reaction to infection, irritation, or injury to tissues. It is characterized by five cardinal features which include tumor (swelling/oedema), color (redness), dolor (pain), fever (warmth) and *functio laesa* (organ/tissue dysfunction) (Stankov, 2015). These responses are indispensable in successful maintenance of body's homeostasis and pathogen eradication (Gou *et al*., 2017). The underlying goal of inflammatory events is localization and elimination of harmful assaults, and, to remove damaged tissues and their components culminating in healing of injured tissues (Gou *et al*., 2017).

Inflammatory responses involve neutrophils and macrophages which secrete various mediators (Azab *et al*., 2016). The inflammatory mediators, which drive initiation, progression, persistence, regulation all geared towards resolution of inflammatory state. Resolution entails a concerted effort by both anti-inflammatory mediators and monocytes recruited to clear damaged tissue debris. If acute inflammation is not resolved, chronic inflammation ensues (Azab *et al*., 2016).

Chronic inflammation is among the major contributors of pathologic conditions burdening both the developed and the developing nations especially those in Africa (Oguntibeju, 2018). For instance, chronic inflammation is associated with the emergency and persistence of obesity-associated diabetes mellitus after insulin resistance among a continuum of other complex human diseases (Lalrinzuali *et al*., 2016; Alemu *et al*., 2018; Oguntibeju, 2018).

Research has shown that both innate and adaptive immune responses play critical roles in inflammation (Wa *et al*., 2010; Azab *et al*., 2016). Innate immunity forms the first line of

defense against intruding microbes and cancer cells. This is achieved via recruitment of mast cells, macrophages as well as dendritic cells which work to identify and phagocytose the invaders and facilitate their removal from the body (Azab *et al*., 2016; Alemu *et al*., 2018; Yasmen *et al*., 2018). On the other hand, adaptive immune responses utilize specialized B and T cells to eradicate invading microbes and cancer cells through specific receptors and antibodies secreted by these cells (Azab *et al*., 2016; Alemu *et al*., 2018; Yasmen *et al*., 2018).

Many pro- and anti-inflammatory mediators are secreted during inflammatory events in during assault (Ghasemian *et al*., 2016; Gou *et al*., 2017; Oguntibeju, 2018). However, some of these mediators including interleukin-12 (IL-12) exhibit both pro-inflammatory and anti-inflammatory features (Azab *et al*., 2016). Many of these mediators have been extensively demonstrated to play integral functions in human pathologic conditions. They include eicosanoids (prostaglandins and leukotrienes), cytokines (interferons, tumor necrosis-α and interleukins), chemokines (chemoattractant protein-1 and monocytes) and the nuclear factor kB transcription factor which is a potent modulator of inflammation (Azab *et al*., 2016; Oguntibeju, 2018).

The tumor necrosis factor α (TNF- α), is a crucial pro-inflammatory cytokine secreted by different cells, and causes a diverse array of cellular effects (Azab *et al*., 2016; Sun *et al*., 2018). For instance, TNF- α has been linked to a myriad of disease states in humans ranging from immune and inflammatory disorders, cancer, neuropsychiatric diseases among other complications (Azab *et al*., 2016; Yasmen *et al*., 2018b). Besides, another pro-inflammatory cytokine, the interleukin 1-β (IL-1 β) exerts anti-inflammatory activity. Similarly, IL-1 α and IL-6 have both pro- and anti-inflammatory properties (Azab *et al*., 2016).

On the other hand, prostaglandin E ($PG\text{-}E_2$) is the most considered among other prostaglandins regarding human pathological and biological molecules (Azab *et al*., 2016; Lalrinzuali *et al*., 2016). Under normal circumstances, $PG\text{-}E_2$ regulates body temperature, maintains gastric mucosal integrity, modulates renal blood flow and proper functioning of the female reproductive system (Fürst and Zündorf, 2014; Azab *et al*., 2016). Conversely, modifications of $PG\text{-}E_2$ functioning body temperature, drives inflammatory disorders, causes colorectal cancer and many more diseases (Fürst and Zündorf, 2014; Azab *et al*., 2016).

Research has established that, the synthesis of PGs is initiated by production of arachidonic acid from biological membrane phospholipids in a phospholipase A_2 (PLA_2) mediated mechanism (Fürst and Zündorf, 2014; Azab *et al*., 2016; Gou *et al*., 2017). This is followed by the action of cyclooxygenase (COX) enzymes which convert arachidonic acid to PGs. Of the three recognized COX enzyme isoforms, COX-2, an inducible enzyme, is considered the most active mediator of inflammation (Azab *et al*., 2016). Similarly, inducible nitric oxide synthase (iNOS), is the prominent pro-inflammatory NOS isoform tightly linked with human inflammatory syndromes (Azab *et al*., 2016).

Furthermore, leukotrienes including leukotriene B_4 are associated to various human diseases including depression, inflammation, asthma, among others. The enzyme 5-lipooxygenase (5-LOX) has been identified as the main producer of leukotrienes in the body (Azab *et al*., 2016; Gou *et al*., 2017; Oguntibeju, 2018).

Singh *et al*., (2013) noted that pyrexia (fever) is a natural defense mechanism aimed at creating a hostile environment to discourage the survival of an invading pathogen. Various studies have shown that pyrexia is initiated by the pyrogens, which are produced by

microorganisms, and act on leucocytes producing endogenous toxins that stimulate increase of body temperature (Cheruiyot, 2017).

Although the inflammatory response is beneficial to the individual, it can sometimes lead to detrimental effects and discomfort (Mbiri *et al*., 2016). Apart from infections, there are other conditions associated with inflammatory process. They include degenerative diseases such as rheumatoid arthritis, olymyalgiarheumatica, shoulder tendonitis, asthma, and heart disease (Maina *et al*., 2015).

2.2 Pain

Pain is an unpleasant sensory and emotional experience associated with potential/actual tissue damage (Raja *et al*., 2020). It is a localized sensation ranging from mild discomfort to agonizing experience (Raja *et al*., 2020). Stimulation of nociceptors in the skin transmits pain messages to the brain for interpretation and response. Some nociceptors only respond to severe stimulations while others respond to innocuous and warning stimuli (Raja *et al*., 2020).

Noxious stimuli caused by thermal, chemical and mechanical injuries sensitize nociceptors via various mediators which mediate pain sensation (Foster and Herring, 2010; Azab *et al*., 2016) . Pain sensation starts from peripheral nociceptors which then generate pain impulses which are conducted to the central nervous system by appropriate nerves (Omoigui, 2007; Foster and Herring, 2010). Thereafter, the brain interprets the pain messages and effector mechanisms are instituted to avert the harmful effects.

Research has recognized two major nerve fibers, the slow and fast pain fibers respectively, which mediate pain message transmission (Foster and Herring, 2010). Fast pain is transduced via A delta fibers (Aδ fibres) to the spinal cord. Eventually, this communication

is terminated at the luminal region of the spinal cord (Foster and Herring, 2010; Azab *et al*., 2016). Then, the second neuron is activated and transmits via the neospinothalamic and eventually terminate in the brain stem (Omoigui, 2007). Fast pain nerve dendrites secrete glutamate neurotransmitter which transfer fast pain signals in the brain's cortex. Research has established that key mediators of fast pain include histamine, prostaglandins, serotonins and bradykinins which work in a coordinated manner to ensure survival of the organism by wading off pain (Foster and Herring, 2010; Wang *et al*., 2014; Azab *et al*., 2016).

Conversely, slow pain sensed immediately after stimulation, lasts for some minutes and if not appropriately mitigated can translate to chronic pain which can persist for months after noxious stimuli. The C-fibers transmit slow pain messages at speed of between 0.5 to 2 m/s to the brain for processing (Omoigui, 2007; Foster and Herring, 2010). Other recognized forms of pain are neuropathic, visceral and somatic pain (Omoigui, 2007).

2.3 Conventional Management of Pain and Inflammation

The treatment of pain is done conventionally using over the counter analgesics such as aspirin, paracetamol, anti-depressants, opioids, and NSAIDs (Ibuprofen, Naproxen Sodium, and Diclofenac Sodium (Mbiri *et al*., 2016). NSAIDs form the most widely used category of drugs for analgesia. Although they serve well for acute pain management, there is a need for long-term solution to chronic pain. Most conventional drugs have severe side effects. For instance, ibuprofen affects the gastrointestinal tract, the coagulation system, and the kidneys. Diclofenac is toxic to the liver, and it leads to deposition of urates crystals in the spleen, heart, and kidneys (Gan, 2010; Cazacu *et al*., 2015).

For management of fever, many antipyretic drugs are used. They work by inhibiting PGE_2 synthesis, consequently reducing the elevated body temperature (Subedi *et al*., 2016). Preventing the activity of cyclooxygenase enzyme causes the levels of PGE_2 to reduce in

the hypothalamus, consequently lowering the body temperature (Subedi *et al*., 2016). NSAIDs work by inhibiting thrombolytic aggregation and therefore work to manage not only fever, but also pain and inflammation. However, they too have many side effects, including causing stomach ulceration leadinf to bleeding (Moriasi *et al*., 2021).

Inflammation management is conventionally done using NSAIDS. For instance, Diclofenac works by inhibiting prostaglandins synthesis and production. Aspirin, the most widely used drug, is the most commonly known anti-inflammation medicine; however, the use of these drugs has been shown to lead to duodenal lesions and peptic ulcers (Holstege, 2016; G. A. Moriasi *et al*., 2021).

2.4 Herbal management of Pain and Inflammation

Traditional medicine is the practice of healing that has been used even before the advent of modern medicine, and it is being utilized even today. However, little information is available on the use of traditional medicine in Kenya as noted by (Kigen *et al*., 2013). Most traditional medicine depends on barks, leaves, and flowers of plants. Nature provides numerous remedies to heal most diseases, and the search for new medication has led scientists to herbal remedies. Consequently, there is a renewed focus on medicinal plants such as *P. thonningii*.

Various research studies have reported on various plants that can be used to manage pain, inflammation, and fever (Chatterjee *et al*., 2015; Bukhari *et al*., 2016; Mbiri *et al*., 2016; Rastogi *et al*., 2018; Yasmen *et al*., 2018; Moriasi *et al*., 2020; Moriasi *et al*., 2021). Various herbs have been researched on, including *Carissa edulis, Annona vepretorum*, *Acacia nilotica* and *Solanum incanum* (Maina *et al*., 2015; Mbiri *et al*., 2016; Cheruiyot, 2017; Abu-Izneid *et al*., 2018). Herbs are safer because they are natural; however, some of them have been shown to have adverse side effects, including allergic reactions, direct

toxicity, and interaction with other drugs (George, 2011; Nasri and Shirzad, 2013; Mensah *et al*., 2019).

As part of alternative and complementary interventions, plants are used to ameliorate fever, pain, and inflammation. Herbal medicine involves the application of plant extracts in basic healthcare. The herbs differ from one region to another depending on the local climate and regional flora. Many conventional drugs are based on herbal extracts. Researchers who focus on natural products have documented traditional knowledge of these plants, and they use scientific experiments to authenticate their use, in addition to isolating the active principles (Piana *et al*., 2013; Moriasi *et al*., 2021). One such plant is *P. thonningii*, which is valuable to herbalists and researchers, owing to its broad pharmacological properties (Igbe *et al*., 2013; Afolayan *et al*., 2018; Moriasi *et al*., 2020).

2.5 *Piliostigma thonningii*

2.5.1 Botanical Classification

The botanical classification of Schum is as follows: It belongs to the Kingdom Plantae, Division Spermatophyta, Sub-division, Angiospermatophyta, Class Eudicots, Order Fabales, Family Fabaceae, Genus *Piliostigma*, and Species *thonningii*. Its common names include Kalgo, Camel's foot, monkey bread and Mchekeche (Swahili) (Orwa *et al*., 2009). Plate 2.1 shows a photograph of *P. thonningii* taken *in situ*.

Plate 2.1: Photograph of *P. thonningii* taken *in situ*

2.5.2 Morphology of *P. thonningii*

Pilistigma thonningii is a woody plant that grows up to 15 meters high. It has a rounded crown and a crooked bole, which is often short. The plant has hairy twigs, a rough bark of creamy brown color, and leathery leaves. The flowers have five petals, white to pink in color. Also, the flowers are unisexual, and in most cases the stamen pistil parts are on different trees. Seeds are borne on pods which are surrounded by an edible pulp. Moreover, the plants have deep roots which help them to survive strong winds and droughts. The tree is mainly found in wooded grasslands such as those in sub humid regions of Africa, more especially to the western side of the continent. In Kenya, *P. thonningii* is mostly found in semi arid reagions of eastern and central Kenya regions (Kareru *et al*., 2007; Moriasi *et al*., 2020).

2.5.3 Ethno-medicinal use of *P. thonningii*

Most of the species in the genus have been widely used by mankind for food and medicinal purposes (Kareru *et al*., 2007; Keter and Mutiso, 2012; Moriasi *et al*., 2020a). Some of the medicinal uses include the treatment of teething children, ulcers, worms' infestation, wound dressing, inflammations, bacterial infections, gonorrhea, headache, and stomachache (Moriasi *et al*., 2020a).

Kwaji *et al*. (2010) reported the local use of twigs in Nigeria to treat fever, dysentery, snake bites, respiratory ailments, and skin infections. Other studies have reported its efficacy in treating malaria. Apart from medical uses, the plant products can also be used to make ropes, tanning of leather, roofing tiles, for fencing, and building bridges. They also serve as soil erosion control agents due to their deep roots. In Kenya, the leaves, stem barks and roots of *P. thonningii* are used to manage chronic joint pains, diabetes, among other inflammatory and pain related disorders (Kareru *et al.*, 2007; Wambugu *et al*., 2011).

2.5.4 Phytochemistry

Phytochemical investigations on *P. thonningii* showed that the plant has a wide range of compounds including anthraquinones, alkaloids, saponins, flavonoids, glycosides, tannins, and sterols (Bello *et al.*, 2013). Other csompounds that have been isolated include Piliostigmin (XXI), 16α - hydroxy - (-) - kauran-18-oic acid (XXII) (Echu, 2014). Some of the isolated compounds that have shown anti-inflammatory activity include 6, 8 – di – C – methyl quercetin 3 - methyl ether (XXIII), 6 – C - methyl quercetin 3, 7- dimethyl ether (XXIV) and 6, 8 – di – C - methyl quercetin3, 7 – dimethyl ether (XXV). Flavonoids have been recognized as effective anti-inflammatory agents (Afolayan *et al*., 2018).

2.6 Toxic Effects of *P. thonningii*

A study by Adjene *et al*. (2013) demonstrated the toxic effects on liver tissues of laboratory rats following long term use of *P. thonningii* bark. Another study by Ukwuani *et al.* (2012), investigated the toxic response of male and female rat models following sub-acute and acute exposure to this plant. They found out that the acute toxicity was very low, and that it was practically non-toxic at oral doses. Therefore, there is no adequate data about the toxicity of the plant extracts.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant materials

Fresh barks of *P. thonningii,* were obtained from Embu County, Kiang'ombe forest in the natural habitat where the plant grew with the help of a reputable herbalist. Identification and authentication were done by a taxonomist at the East Africa Herbaria (National Museums of Kenya) and the plant assigned voucher reference number: NMK/BOT/CTX/1/2. Voucher specimens were prepared and deposited at the school of biological sciences, Chiromo Campus, University of Nairobi. The collected plant samples were transported to the Department of Public Health, Pharmacology, and Toxicology laboratory for processing and analysis. The plant materials were cut into small fragments, air-dried in a well aerated room with regular grabbling for 14 days and ground into a coarsely powdered material using an electric mill. The powder was kept in a well labeled manila bag and kept in a dry place awaiting extraction.

3.2 Extraction methods

3.2.1 Methanolic extract

Extraction was performed according to the method described by Harborne, (1976) and modified by Bibi *et al*. (2012). Briefly, 500 g of the powered material was macerated in 1 litre of analytical grade methanol in a 2-liter conical flask and then covered with a foil paper with constant shaking for 48 hours. The menstruum was decanted and filtered using a filter paper (Whatman Number 1). This procedure was repeated three times to exhaust extraction. The resultant filtrates were combined and reduced *in vacuo* at 55 ^{0}C using a rotary evaporator. Thereafter, the extract was transferred into a clean, dry universal glass

bottle and placed in a hot-air oven set at 35 ^{0}C for complete drying. The extract was stored at 4^o C in a refrigerator awaiting bioassay.

3.2.2 Aqueous extract

Approximately 100 g of the powdered plant materials was boiled in 750 ml of distilled water for a period of five minutes. The mixture was filtered through a filter paper (Whatman No. 1), cooled and then lyophilized using a freeze-dryer *in vacuo*. The actual weight of the dried extract was measured using an analytical balance and recorded before it was stored in a refrigerator at 4^o C awaiting biological assay (Bibi *et al*., 2012).

3.3 Experimental animals

In this study, both male and female Swiss-Albino mice (4-5 weeks old, weighing 24±2 g) were obtained from the animal breeding facility of the Department of Public Health, Pharmacology and Toxicology, College of Veterinary and Agricultural Science, Kabete Campus of the University of Nairobi. The experimental animals were housed in polypropylene cages measuring 30 cm × 20 cm × 13 cm in standard laboratory conditions. The beddings comprised of soft wood shavings that were evenly spread in the holding cages to provide warmth to the housed animals and to deter dumping. Standard laboratory animal pellets and tap water were provided *ad-libitum.* Animal use and care guidelines set out by the Ethical Review committee and the National Council for Science, Technology and Innovation (NACOSTI) were followed in this study.

3.4 Preparation of administration doses

To prepare appropriate dosages for administration to experimental mice, the OECD (2008) guidelines described by (Erhierhie *et al*., 2014) were adopted. Briefly, to prepare a stock solution of dose level 500 mg/Kg BW to be administered to a mouse weighing 20 g body weight, the formula posited by Erhierhie *et al*. (2014) was followed as demonstrated.

Animal dose (mg/Kg BW)= $\frac{\text{Body weight of the animal(g)}}{1000\ \text{g}} \times$ selected dose

(Erhierhie *et al.,* 2014).

Therefore; Animal dose (mg/Kg BW)= $\frac{20(\text{g})}{1000\ \text{g}} \times 500$ mg = 10 mg

According to the OECD (2008) guidelines, 10 mg was to be reconstituted in 0.2 ml of the vehicle (Normal saline). To prepare enough stock solution for 5 mice, 50 mg of the respective studied plant extracts was weighed and reconstituted in 1 ml of Normal saline.

The same procedure was followed for all the other doses of the studied plant extracts and the standard drugs.

3.5 Acute oral toxicity effects of the aqueous and the methanolic stem bark extracts of *P. thonningii*

To evaluate and appraise safety of the studied plant extracts, the Up-and -down procedure (UDP) for acute oral toxicity described by OECD (2008) was adopted. The experimental mice were randomly selected and labeled with a permanent marker pen on their tails.

The mice were housed individually in polypropylene cages for 48 hrs for acclimatiation before being subjected to the experiments. In the experimentation day, food was withdrawn for four hours before recording their body weights. Afterwards, an initial dose of 175 mg/Kg BW was orally administered to the experimental group consisting of three (3) mice and 10 ml/Kg BW of normal saline to the control group (3 mice).

After oral adminstration or respective drugs, wellness parameters including appearance of mucous membrane, eyes, skin fur, salivation, convulsions, lethargy, coma, sleep, diarrhea, tremors, body weight deviation and mortality were monitored and recorded after 30 minutes, 1 hour, 4 hours, 24 hours, 48 hours, 7 days and 14 days (OECD /OCDE, 2008).

The same procedure was adopted for a 550 mg/Kg BW dose and for the cutoff dose of 2000 mg/Kg BW (OECD, 2008).

3.6 *In vivo* anti-inflammatory effects of the aqueous and methanolic stem bark extracts of *P. thonningii*

A completely randomized study design was employed in this study from which the experimental design was derived. The Xylene-induced ear oedema technique described by Igbe *et al*., (2010) was followed in this study. Briefly, the experimental mice were randomly placed into six groups (A, B, C, D, E and F), each group having five (5) mice. Mice in groups A, B, C and D respectively were orally administered with 200 µl of 4 mg/Kg BW, 20 mg/Kg BW, 100 mg/Kg BW and 500 mg/Kg BW of the studied plant extracts *p.o* and 1 drop of xylene topically on the inner pinna of the right ear. The control groups (E and F respectively) received 1 mg/Kg BW of dexamethasone as positive control and 10 ml/Kg BW of distilled water as negative control respectively *p.o* and 1 drop of xylene topically on the inner pinna of the right ear. Table 3.1 summarizes this design.

Table 3.1: Experimental design for anti-inflammatory activity

Treatment Groups		Treatment
A	Experimental group 1	Extract (4 mg/Kg BW) + 1 drop of Xylene
B	Experimental group 2	Extract (20 mg/Kg BW) + 1 drop of Xylene
C	Experimental group 3	Extract (100 mg/Kg BW) + 1 drop of Xylene
D	Experimental group 4	Extract (500 mg/Kg BW) + 1 drop of Xylene
E	Positive Control	Dexamethasone (1 mg/Kg BW) + 1 drop of Xylene
F	Negative Control	Normal saline (10 ml/Kg BW) + 1 drop of Xylene

n= 5 mice in each treatment group

After 60 minutes, oedema in each mouse was induced by smearing 1 drop of xylene on the inner pinna of the right ear and left for 15 minutes, after which the experimental mice were anesthetized using diethyl ether and both the right (oedematous) and left ears were dissected (6 mm diameter sections) and accurately weighed using an analytical balance. The respective weights were recorded and used to calculate the anti-inflammatory effects of the extracts and expressed as the percentage inhibition of oedema according to the formula described by Igbe *et al*., (2010).

$$\%\ inhibition\ of\ edema = \frac{A - B}{A} \times 100$$

Where A= Difference in ear weight in the negative control

B= Difference in ear weight in the experimental/ positive control mice.

3.7 Determination of the analgesic (antinociceptive) activity of the aqueous and methanolic stem bark extracts of *P. thonningii*

Peripheral analgesic effects of the aqueous and methanolic stem bark extracts of *P. thonningii* were evaluated using the Acetic acid-induced writhing method of Rashid *et al.* (2015) in Swiss albino mice, using a completely controlled randomized experimental design. Briefly, experimental mice were randomly assigned to six groups (I, II, III, IV, V and VI), each consisting of 5 animals. Groups I, II, III and IV received an oral treatment of 4 mg/Kg BW, 20 mg/Kg BW, 100 mg/Kg BW and 500 mg/Kg BW respectively of the studied plant extracts *p.o*. On the other hand, groups V and VI received 75 mg/Kg BW of acetylsalicylic (Asprin) and 10 ml/Kg BW of distilled water orally as positive and negative controls, respectively. After 30 minutes, writhing was induced in each experimental mouse with an intraperitoneal injection of 0.6 % v/v acetic acid. All the drugs were administered at a volume of 200 µl. Table 3.2 is a summary this experimental approach.

Table 3.2: Experimental design for analgesic activity

Treatment Groups		Treatment
I	Experimental group 1	Extract (4 mg/Kg BW) + 0.6 % Acetic acid
II	Experimental group 2	Extract (20 mg/Kg BW) + 0.6 % Acetic acid
III	Experimental group 3	Extract (100 mg/Kg BW) + 0.6 % Acetic acid
IV	Experimental group 4	Extract (500 mg/Kg BW) + 0.6 % Acetic acid
V	Positive Control	Acetylsalicylic Acid (75 mg/Kg BW) + 0.6 % Acetic acid
VI	Negative Control	Normal saline (10 ml/Kg BW) + 0.6 % Acetic acid

Each treatment had 5 experimental mice: All drugs were administered orally except for Acetic acid which was injected intraperitoneally

Thereafter, experimental mice were monitored individually, and the number of writhes counted after 5 minutes of writhing induction for 30 minutes and recorded. The average number of writhes and the percentage inhibition of writhing were calculated as an indicator of analgesic activity following the equation as described by Rashid *et al.,* (2015).

$$\%\ Writhing\ inhibition = \frac{Wc - W}{Wc} X100$$

Where;

Wc is the mean number of writhes in the control; and W is the mean number of writhes in the experimental group (Extracts/Standard).

3.8. Statistical data management and analysis

The obtained data from anti-inflammatory and analgesic activities were tabulated on MS Excel spreadsheet (2016) and exported to GraphPad Prism statistical software version 8.3.0.538. The data were subjected to descriptive statistics and expressed as mean ± standard error of the mean (SEM) of replicate experiments.

One-way ANOVA was done to compare differences among means followed by Tukey's *post hoc* test for pairwise comparison and separation of means at $\alpha=0.05$. Values with $p \leq 0.05$ were considered statistically significant. Acute oral toxicity data were analyzed according to OECD guideline document No. 425 (OECD /OCDE, 2008) and LD_{50} values recorded.

3.9. Ethical Consideration

3.9.1. Disposal of experimental animals

The disposal of used mice followed guidelines set out by the University of Nairobi, ethical committee, OECD, (2008) and NACOSTI.

3.9.2. Research approvals

Permission to conduct this study was obtained from University of Nairobi Ethical committee and the National Council of Science Technology and Innovation (NACOSTI) under licence number: NACOSTI/P/19/2448.

CHAPTER FOUR

4.0 RESULTS

4.1 Acute oral toxicity effects of the studied plant extracts

The acute oral toxicity results demonstrated no observable signs of toxicity and lethal effects in experimental groups of mice at the three dose levels (175 mg/Kg BW, 550 mg/Kg BW, and 2000 mg/Kg BW). The LD_{50} values for each of the studied plant extracts were thus envisaged to be above 2000 mg/Kg BW.

4.2 *In vivo* Anti-inflammatory effects of the aqueous and methanolic stem bark extracts of *P. thonningii*

The obtained results showed significant reductions in xylene-induced ear oedema in mice. The experimental mice that received 4 mg/Kg BW of the aqueous stem bark extract of *P. thonningii* showed a significantly lower percentage inhibition of xylene-induced ear oedema in mice compared with the percentage inhibition produced by the standard drug (Dexamethasone) ($p<.05$; Figure 4.1).

Generally, a dose dependent increase in percentage inhibition of xylene-induced ear oedema caused by this extract was noted among the tested doses. Remarkably, the aqueous stem bark extract of *P. thonningii* at a dose of 500 mg/Kg BW revealed the highest percentage inhibition compared with the other dose levels of the same extract and the standard drug ($p<.05$; Figure 4.1).

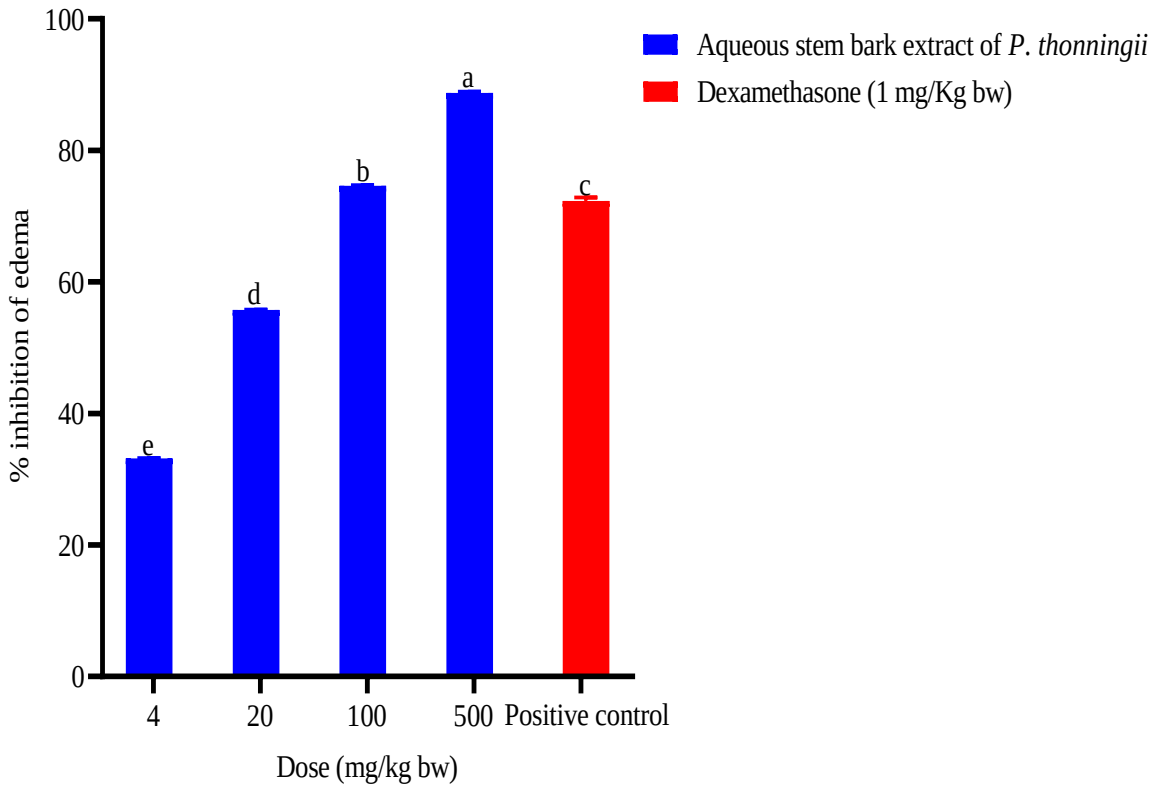

Figure 4.1: Effect of the aqueous stem bark extract of *P. thonningii* on xylene-induced ear oedema in mice

Values are plotted as Mean±SEM; Bars with same superscript letter within the same dose level are not significantly different (one-way ANOVA followed by Tukey's test; p>0.05)

Similarly, the results revealed that, the methanolic stem bark extract of *P. thonningii* at dose levels of 4 mg/Kg BW, 20 mg/Kg BW and 100 mg/Kg BW imparted significantly lower percentage inhibition of xylene-induced ear oedema in experimental mice compared with the percentage inhibition in mice treated with Dexamethasone at a dose of 1 mg/Kg BW ($p<.05$; Figure 4.2).

However, the results showed that, the methanolic stem bark extract of the studied plant at a dose of 500 mg/Kg BW significantly inhibited xylene-induced ear oedema in mice, more

than the inhibitions caused by all the other dose levels of the same extract and dexamethasone ($p<.05$; Figure 4.2). Overall, a positive dose-dependent increase in percentage inhibition of oedema was observed in the experimental that received the studied plant extract (Figure 4.2).

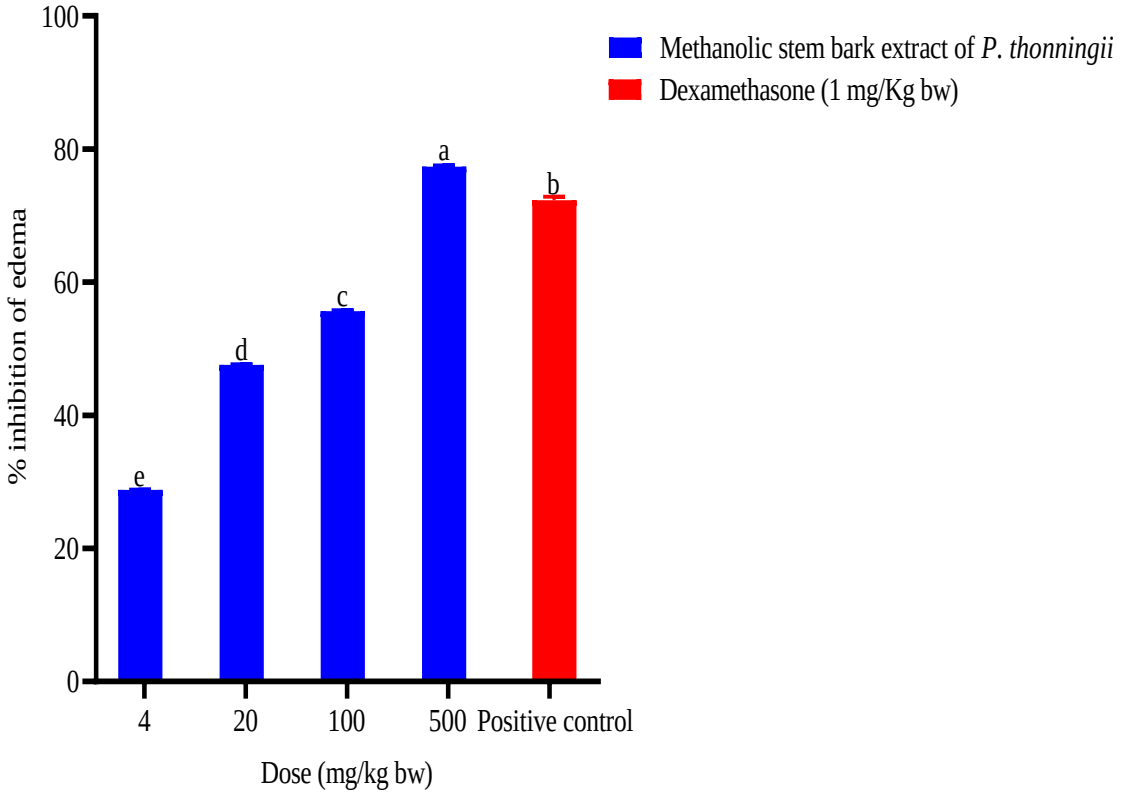

Figure 4.2: Effect of the methanolic stem bark extract of *P. thonningii* on xylene-induced ear oedema in mice

Values are plotted as Mean±SEM; Bars with same superscript letter within the same dose level are not significantly different (one-way ANOVA followed by Tukey's test; $p>0.05$)

Moreover, a comparison between the effects of the aqueous and methanolic stem bark extracts of *P. thonningii* in xylene-induced ear oedema was done. The results showed that, the experimental mice into which the aqueous stem bark extract of this plant was

administered at all the tested dose levels imparted significantly higher percentage inhibitions of oedema compared with the inhibitions in mice that received the methanolic extract at all the studied dose levels ($p<.05$; Figure 4.3).

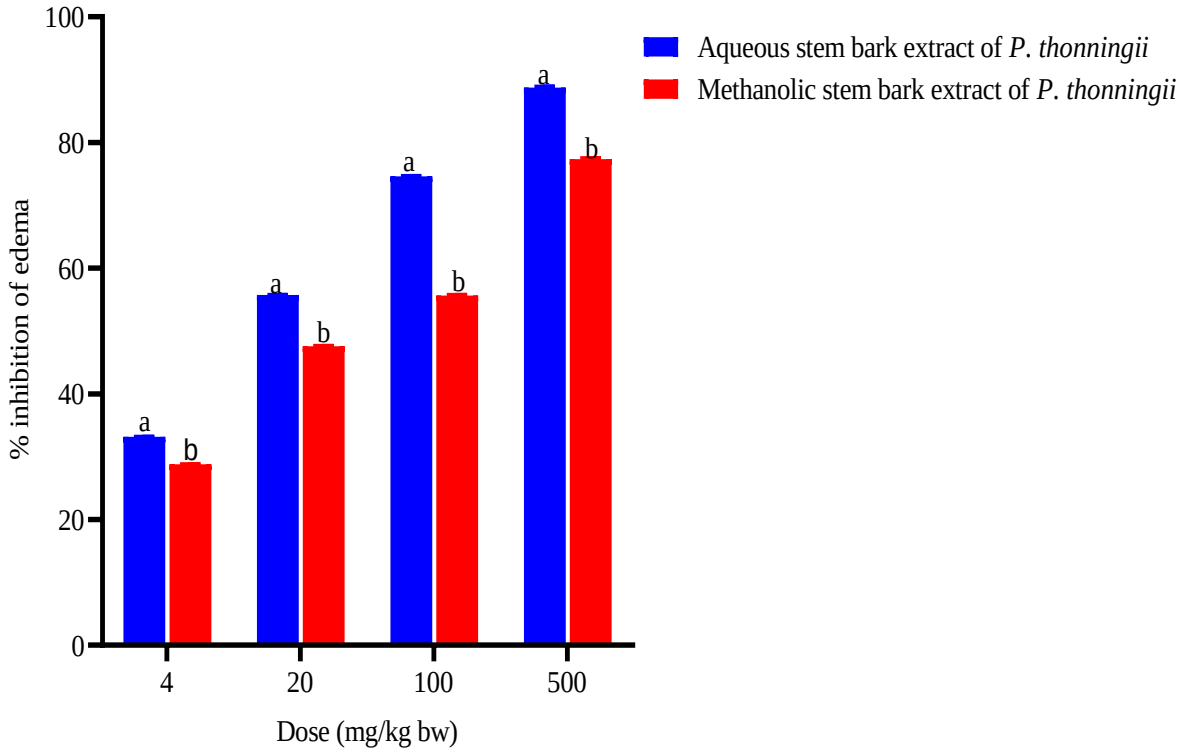

Figure 4.3: Comparison between the effects of the studied plant extracts on xylene-induced ear oedema

Values are plotted as Mean±SEM; Bars with same superscript letter within the same dose level are not significantly different (un-paired t-test; $p>0.05$)

4.3 *In vivo* analgesic effects of the aqueous and methanolic stem bark extracts of *P. thonningii*

In this study, the results revealed a dose dependent reduction in writhing corresponding to an increase in percentage inhibition of acetic acid induced writhing in mice (Table 4.1). Upon administration of the aqueous stem bark extract of *P. thonningii* to mice, the percentage inhibition of writhing significantly increased in a dose dependent manner ($p<.05$; Table 4.1).

Notably, at a dose level of 500 mg/Kg BW, the mice that received the aqueous stem bark extract of *P. thonningii* showed significantly reduced writhing compared to that recorded for mice in all the other experimental groups including those in the positive control group ($p<.05$; Table 4.1).

Table 4.0.1: Effects of the aqueous stem bark extract of *P. thonningii* on acetic acid induced writhing in mice

Dose (mg/Kg BW)	**Aqueous stem bark extract of *P. thonningii***	
	Writhing frequency	**% Inhibition**
4	66.20 ± 1.16^{b}	29.72
20	56.40 ± 2.04^{c}	40.13
100	24.80 ± 1.86^{d}	73.67
500	14.00 ± 1.14^{e}	85.14
Acetylsalicylic Acid (75 mg/Kg BW)	15.40 ± 1.21^{e}	83.65
Negative control	94.20 ± 3.97^{a}	0

Values are expressed as Mean±SEM; Values with the same superscript letter along the column are not significantly different (one-way ANOVA followed by Tukey's test; $p>0.05$).

On the other hand, the mice that received the methanolic stem bark extract demonstrated significantly reduced writhing in a dose dependent fashion ($p<.05$; Table 4.2). The results

indicated significantly higher writhing frequency in mice treated with 4 mg/Kg BW compared with the writhes in mice that received the other studied extract doses and the standard drug ($p<.05$; Table 4.2).

Conversely, the experimental mice that were administered with this extract at a dose level of 500 mg/Kg BW demonstrated significant lower writhing frequency compared with the writhing frequencies recorded for mice in all the other experimental groups ($p<.05$; Figure 4.2). Interestingly, at a dose level of 500 mg/Kg BW of the methanolic stem bark extract of *P. thonningii*, the recorded number of abdominal writhes was significantly lower than the writhes in the positive control ($p<.05$; Table 4.2).

Table 4.2: Effect of the methanolic stem bark extract of *P. thonningii* on acetic acid induced writhing in mice

Dose (mg/Kg BW)	**Methanolic stem bark extract of *P. thonningii***	
	Writhing frequency	**% Inhibition**
4	83.80 ± 3.14^{b}	11.04
20	68.60 ± 1.44^{c}	27.18
100	40.00 ± 1.41^{d}	57.54
500	25.40 ± 1.12^{e}	73.04
Acetylsalicylic Acid (75 mg/Kg BW)	15.40 ± 1.21^{f}	83.65
Negative control	94.20 ± 3.97^{a}	0

Values are expressed as Mean±SEM; Values with the same superscript letter along the column are not significantly different (one-way ANOVA followed by Tukey's test; $p>0.05$)

A comparison of the effects of the studied plant extracts on acetic acid-induced writhing in mice was also done in this study (Figure 4.4). The results revealed that, at all the studied dose levels, the aqueous stem bark extract of *P. thonningii* conferred significantly higher percentage inhibition (less writhing frequency) of acetic acid induced writhing compared to its methanolic counterpart at the same dose levels ($p<.05$, Figure 4.4).

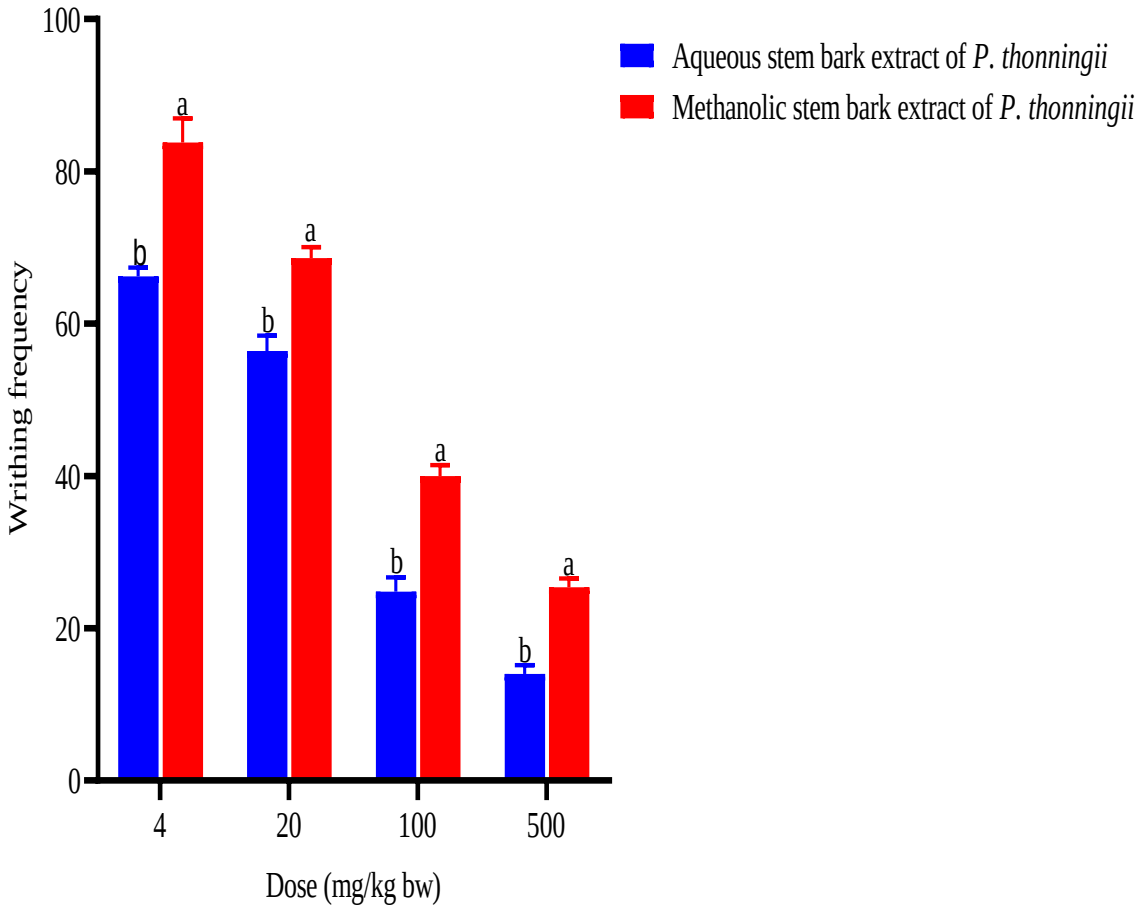

Figure 4.4: Comparison between the effects of the studied plant extracts on acetic acid induced writhing in mice

Values are plotted as Mean±SEM; Bars with same superscript letter within the same dose level are not significantly different (un-paired t-test; p>0.05)

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1 Discussion

Inflammation is the wellspring of symptoms elicited by maladies affecting the body. It is a multifaceted biological retort by vascular tissues to injurious stimuli like pathogens, damaged cells, physical and chemical assaults as well as immunological responses (Rahman *et al*., 2019). As far as humankind and health are concerned, understanding of inflammation and associated processes has been a major conundrum (Mbiri *et al.*, 2016).

The cardinal signs which characterize inflammation include increased cellular metabolism, release of cellular soluble inflammatory mediators, increased blood flow, vasodilation, extravasation of fluids and cellular influx, formation of abnormal granulations, necrosis, excessive tissue degeneration and exudation. All these lead to varied degrees of tissue injuries and can even lead to death (Stankov, 2012).

The existence of an inflammatory agent, on or in the body triggers cellular membranes which in turn induce activation of phospholipase A_2 (Yasmen *et al*., 2018; Rahman *et al*., 2019). This leads to release of arachidonic acid among other mediators of inflammation like histamine, cytokines, prostaglandins, serotonin, and leukotrienes. These mediators increase cellular membrane permeability thereby aiding migration of leukotrienes and other agents to the inflamed site (Rahman *et al*., 2019). This causes broad spectra of tissue alterations observed in pathology of many maladies (Rahman *et al*., 2019).

In this study, acute inflammation was evaluated using the xylene-induced ear oedema technique. Xylene is a chemical inflammatory trigger which causes release of inflammatory mediators including histamine, bradykinin and serotonin (Yasmen *et al*., 2018). Consequently, there is greater permeability in the vasculature with elevated vasodilation.

The result is the accumulation of fluid at the inflamed/induced site, as observed in the xylene-induced ear oedema in mice (Yasmen *et al*., 2018a).

A drug agent capable of inhibiting fluid accumulation in this technique is considered as having anti-inflammatory activity (Yasmen *et al*., 2018a). The results reported herein suggest remarkable anti-inflammatory effects of the aqueous and methanolic stem bark extracts of *P. thonningii* as evidenced by their ability to inhibit/reduce xylene-induced ear-oedema in experimental mice. Particularly, the aqueous stem bark extract of *P. thonningii* proved to be more potent than the methanolic extract of the same plant at all the studied dose levels. The current medications used to avert inflammation are topical steroids and non-steroidal anti-inflammatories which work by inhibiting the activity of phospholipase A_2 (Monteiro and Steagall, 2019).It is partly suggested the studied plant extracts could be working through this mechanism.

Generally, acetic acid induced writhing test serves as a standard technique for evaluating antinociceptive/analgesic efficacy of natural products. In this method, acetic acid induces the release of various endogenous noxious mediators such as histamine, serotonin, and bradykinin (Moriasi *et al*., 2021).

The pain caused by the acetic acid is evidenced in the contraction of abdominal muscles and the expansion of forelimbs, as well as body elongation generally regarded as writhing whose frequency is quantified in 30 minutes (Yasmen *et al*., 2018b). This elongation is thought to be caused by local peritoneal receptors and prostaglandin pathways in the experimental model animal.

Upon injection of acetic acid intraperitoneally into experimental mice, it causes the release of inflammatory mediators which excites pain receptors (nociceptors) which in turn send pain messages to the central nervous system through the prostaglandin system (Mansouri *et*

al., 2015). Furthermore, other pain mediators like bradykinins and histamine are released from cells lining the peritoneal cavity and further help stimulate nociceptors (Zhao *et al*., 2012). Agents which reduce/inhibit the acetic acid induced writhing frequency are considered as having analgesic effects.

In this study, the studied stem bark extracts of *P. thonningii* plant showed significant inhibition of the acetic acid induced writhing in a manner that suggests blockade/inhibition of the prostaglandin pathway in the pain perception cascade. Previous studies have shown that drugs which inhibit the cyclooxygenase enzyme pathway inhibit writhing, an indicator of pain in experimental animal models (Moriasi *et al*., 2021). The findings of this study agree with those previously reported that NSAIDs reduce the number of writhes by inhibiting cyclooxygenase in peripheral tissues. The aqueous and methanolic stem bark extracts of the studied plant may be acting through a similar mechanism in averting pain in this study. Indeed, previous studies indicate that *P. thonningii* possess demonstratable analgesic activities (Igbe *et al*., 2013). The peripheral action differs from the one by centrally acting drugs such as opioids which inhibit both phases of nociception (Early and late phases of nociception) equally.

The findings of this study suggest that the aqueous and methanolic extract have anti-inflammatory and analgesic activity and that the mechanism of action is mediated through the inhibition of local peritoneal receptors due to the potential of cyclooxygenase inhibition. The analgesic activity, therefore, could be due to the way the phytoactive principles interfere with the release of pain mediators (Maina *et al*., 2015; Moriasi *et al*., 2021). Besides, the aqueous stem bark extract of *P. thonningii* exhibited higher analgesic efficacy than the methanolic extract, probably due to higher concentration of analgesic phytochemicals.

Besides, the association of anti-inflammatory and antinociceptive activity is also observed in non-steroidal anti-inflammatory drugs because they also inhibit the action of cyclooxygenase. Also, NSAIDs produce antipyretic actions by inhibiting the prostaglandin synthetase in the hypothalamus (van Rensburg and Reuter, 2019). Therefore, it is possible that the action of the aqueous and methanolic extracts of *P. thonningii* is also due to the inhibition of prostaglandin synthesis. However, other actions for managing pain and inflammation cannot be completely ruled out.

According to Ashfaq *et al*. (2016), tannins, phenolics, saponins, terpenoids, and flavonoids are some of the active principles that have good anti-inflammatory properties. Previous studies have shown that saponins and flavonoids act synergistically to reduce inflammatory reactions by inhibiting the enzymes lipoxygenase, cyclooxygenase, and nitric oxide synthase, which are all vital in the production of mediators and arachidonic acid metabolism (Radhika and Begum, 2017). Perhaps, the higher anti-inflammatory efficacy of the aqueous extract of *P. thonningii* could be due to high concentration of anti-inflammatory associated phytochemicals.

On the other hand, phenolics, tannins, terpenoids, and flavonoids serve as antioxidant substances that scavenge free radicals. Moreover, research shows that tannins, alkaloids, saponins, phenolic compounds, proteins, and tannins have analgesic and anti-inflammatory activity (Dzoyem *et al*., 2017).

Although most of the drugs presently used to manage pain and inflammation are effective, they have been associated with toxic effects and adverse side effects (Gan, 2010; Maund *et al*., 2011; Cazacu *et al*., 2015; Holstege, 2016; Fokunang, 2018). As a result, there is a continuous search for alternative treatments that can alleviate these conditions with

minimum adverse effects. Herbal medicines have been established to be a safe alternative due to their natural origin, cultural adaptability, availability, and safety (WHO, 2013).

In this study, acute oral toxicity effects of both the methanolic and aqueous stem bark extracts of *P. thonningii* were investigated according to the OECD/OCDE (2008) guidelines. There were no observable signs of toxicity in the experimental groups at the various dose levels up to the cutoff dose of 2000 mg/Kg BW, consistently with previous findings (Adjene *et al*., 2013), which had found acute toxicity to be very low, and that the extract is practically non-toxic at oral doses.

It was necessary to evaluate the toxicity, because, although plant extracts are natural, they contain some bioactive principles that can cause adverse effects. Considering that there was no death in the test animals and there was no sign of toxicity, it suggests that the LD_{50} is greater than 2000 mg/Kg, and thus, all the studied plant extracts were deemed safe.

5.2 Conclusions

Based on the results of this study, the following conclusions were drawn.

i. The aqueous and methanolic stem bark extracts of *P. thonningii* have acute anti-inflammatory effects by reducing/inhibiting xylene-induced ear oedema in Swiss albino mice.
ii. The aqueous and methanolic stem bark extracts of *P. thonningii* have analgesic activity in acetic acid induced writhing in Swiss albino mice.
iii. The aqueous and methanolic stem bark extracts of *P. thonningii* are safe with LD_{50} of >2000 mg/Kg BW.

Therefore, all the research questions formulated were all answered in affirmative

5.3 Recommendations from the Study

From this study, the following recommendations were made:

i. The aqueous and methanolic stem bark extracts of *P. thonningii* may be utilized as alternatives in the management of inflammatory disorders as claimed to treat in traditional medicine.

ii. The studied plant extracts may be used as analgesic alternatives as claimed by traditional practioners.

5.4 Suggestions/recommendations for further Study

i. There is a need for bio-screening the *P. thonningii* plant to identify and isolate the specific compounds with analgesic and anti-inflammatory activities. This way, new compounds might be discovered which will be used for treatment of the two conditions.

ii. Future studies should elucidate the possible mechanism(s) for analgesic and anti-inflammatory actions of the aqueous and methanolic stem bark extracts.

iii. In addition to acute toxicity, there is a need to evaluate chronic toxicity to determine the safety of the bark extracts in animal models.

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APPENDICES

Appendix i: Research Proposal Approval

UNIVERSITY OF NAIROBI
GRADUATE SCHOOL

Telephone: 020 491-0000/3129
Email: gs@uonbi.ac.ke
Our Ref: J56/11961/2018

P. O. Box 30197 00100
NAIROBI, KENYA
14th October 2019

Mr. Ben Otieno Olela
C/o Chairman,
Department of PHPT,
Faculty of Veterinary Medicine, CAVS

Dear Mr. Olela,

RESEARCH PROPOSAL AND SUPERVISORS

This is to inform you that the Director, Graduate School has approved your MSc. research proposal titled; **"Anti-inflammatory, Analgesic, and Toxic Effects of *Piliostigma thonningii* Aqueous and Methanolic Bark Extracts"**.

She has also approved **Prof. James M. Mbaria** and **Dr. Timothy Wachira** as the supervisors of your thesis.

You should therefore begin consulting them and ensure that you submit your thesis for examination **in April 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, CAVS
Chairman, Department of PHPT,
Prof. James M. Mbaria (Supervisor) - C/o Department of PHPT
Dr. Timothy Wachira (Supervisor) – C/o Department of PHPT

JO/mv

Appendix ii: Approval by biosafety, Animal use and Ethics Committee

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/2019/245

Dr. Ben Otieno Olela
University of Nairobi
Dept. PHP & T
25/10/2019

Dear Dr. Olela

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Anti-inflammatory, Analgesic and Toxic effects of *Piliostigma thonningii* aqueous and methanolic bark extracts.

Dr. Ben Otieno Olela J56/11961/2018

We refer to your MS.c proposal submitted to our committee for review and your application letter dated 7th October 2019. We have reviewed your application for ethical clearance for the study on Anti-inflammatory, Analgesic and toxic effects of *Piliostigma thonningii* aqueous and methanolic bark extracts using laboratory mice model.

The proposed acute oral toxicity, anti-inflammatory and analgesic protocols and numbers of mice to be used in the study meets minimum standards of the Faculty 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

Appendix iii: Research Authorization by NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **746977**

Date of Issue: **29/November/2019**

RESEARCH LICENSE

This is to Certify that Dr.. Ben Olela of University of Nairobi, has been licensed to conduct research in Kiambu, Nairobi on the topic: ANTI-INFLAMMATORY, ANALGESIC, AND TOXIC EFFECTS OF PILIOSTIGMA THONNINGII AQUEOUS AND METHANOLIC BARK EXTRACTS for the period ending : 29/November/2020.

License No: **NACOSTI/P/19/2448**

746977

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix iv: Authorization for biological sample material collection

KENYA
Forest Service

Kenya Forest Service Hqs
Karura, Off Kiambu Rd
P. O. Box 30513 - 00100
Nairobi, Kenya.

Ref: No. RESEA/1/KFS/VOL. V (33)

Date: 20th November 2019

Prof. James M. Mbaria
Chairman of Public Health, Pharmacology and Toxicology
College of Agriculture and Veterinary Sciences
University of Nairobi
P. O. Box 29053-00625,
NAIROBI.

RE: AUTHORIZATION FOR BIOLOGICAL SAMPLE MATERIAL COLLECTION

Reference is made to your letter dated 9th November 2019, in which you requested access for Mr. Ben Otieno Olela, to Kiangombe Forest to undertake research in: ***"Anti-inflammatory analgesic and toxic effects of Piliostigma thonningii aqueous and methanolic bark extracts".***

Permission is hereby granted for Mr.Otieno Olela to undertake the study. The permit is valid from 22nd November 2019 to 21st November 2020. As part of the requirements of this permit you shall be required to provide a copy of your final report including publications arising out of your work to the Chief Conservator of Forests.

By a copy of this permit, the respective Ecosystem Conservatoris hereby instructed to facilitate access.

Julius Kamau
Chief Conservator of Forests

Copy to: Ecosystem Conservator- Embu County

OMM/ia

Trees for better lives

Appendix v: Plant Identification by the National Museums of Kenya

NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

8th Nov. 2019

REF: NMK/BOT/CTX/1/2

Mr. Ben Otieno Olela

0723-635-222

Dear Sir,

PLANT IDENTIFICATION

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

Piliostigma thonningii (Fabaceae family)

Thank you for consulting the East African Herbarium for plant identification and confirmation.

Yours Sincerely,

NATIONAL MUSEUMS OF KENYA * HERBARIUM NAIROBI *

14 NOV 2019

Dr. Beth Kamaara

For; Head, Botany Department