IN VIVO ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC EFFECTS OF DICHLOROMETHANE STEM BARK EXTRACT OF *Acacia mellifera* IN MICE AND RAT MODELS

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DECLARATION

I, Sindani Veronica Akumu, declare that the work presented in this thesis is my original work and has not been presented for a degree or any other award in any other university or any other institution

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DEDICATION

This thesis is dedicated to my wonderful family for their love and unyielding support towards my education.

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ABBREVIATIONS AND ACRONYMS

°C	Degree Celsius
ACPA	American Chronic Pain Association
ANOVA	Analysis of variance
ATP	Adenosine Triphosphate
bw	Body weight
CGRP	Calcitonin Gene-Related Peptide
CNS	Central nervous system
COX	Cyclooxygenase
D-amphetamine	Dextroamphetamine
DAMPs	Damage-Associated Molecular Pattern
DCM	Dichloromethane
DMSO	Dimethylsulphoxide
FDA	Food and Drug Administration
IL	Interleukin
LPS	Lipopolysaccharides
NSAIDs	Non-steroidal Anti-inflammatory Drugs
PAG	Periaqueductal gray
PAMPs	Pathogen-Associated Molecular Patterns
PGE 2	Prostaglandin E2
PGs	Prostaglandins
SEM	Standard error of mean
sP	substance P
TLRs	toll-like Receptors
TNF-α	Tumor Necrosis Factor- Alfa
TXA	Thromboxane
WHO	World Health Organization

ABSTRACT

Inflammation is a reaction to irritants that causes an injury to the body. Pain is a physical discomfort caused by injury or illness while pyrexia is an increase in body temperature above the normal range (36.5°C–37.5°C). Commonly used conventional drugs against inflammation, pain and pyrexia are effective but due to long term use, these drugs tend to be costly and are associated with adverse side effects. Traditional medicine has been used as an alternative and complementary drug. This is because it is safe, has a good efficacy and fewer side effects. This study was designed to assess the *in vivo* anti-inflammatory, analgesic and antipyretic effects of *Acacia mellifera* as well as their phytochemical composition. Samples of the plant were sourced from Siakago division, Embu County in Kenya. Its bioactive components were extracted using dichloromethane. Inflammation, pain and pyrexia were induced using carrageenan, formalin and turpentine respectively in animal models. Adult Wistar rats and Swiss albino mice were placed into six groups of five animals each; three control groups (normal, negative and positive) and three experimental groups (50, 100 and 150mg/kg body weight). The anti-inflammatory and analgesic activities of the stem herbal extract were compared to diclofenac (reference drug), while the antipyretic activity was compared to aspirin. The stem bark extract of *A. mellifera* and the reference drugs showed anti-inflammatory, analgesic and antipyretic activities. For anti-inflammatory effect, the extract reduced inflamed hind paw diameter when compared to the control group. The inhibitory rates of paw edema ranged between 1.59% and 11.05%, while diclofenac reduced edema by between 0.1% and 8.78%. For the anti-nociceptive study, the *A. mellifera* extract inhibited paw licking time with inhibitory rates ranging between 1.38% and 48.26% (in the early phase) and by between 28.45% and 83.90% (in the late phase) while diclofenac reduced pain by 12.20% and 80.20% in the early and late phases respectively. The stem bark extract of *A. mellifera* lowered the elevated rectal temperature by between 0.16% and 3.95% while aspirin reduced it by between 1.52% and 3.60%. The phytochemical analysis showed the presence of alkaloids, cardiac glycosides, flavonoid, saponins, steroids, terpenoids, tannins and phenolics associated with anti-inflammatory, analgesic and antipyretic activities. The stem bark extract from *A. mellifera* may be used as an alternative bioresource in development of anti-inflammatory, analgesic and antipyretic agent. This study, therefore, confirms the folklore use of *A. mellifera* by Ameru and Embu communities in the management of inflammation, pain and fever.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Inflammation is a non-specific immune response that occurs as a result of pathogen invasion, injury, allergy or chemical irritation (Stankov, 2012). It is a process that involves; recognition of pathogen or injury, activation and release of enzymes and inflammatory mediators, recruitment and migration of cells, tissue breakdown and finally initiation of the healing process (Maskerey *et al.*, 2011). Inflammation may persist due to the body's inability to eradicate the irritant or deregulation of mechanisms of the resolution phase. This leads to chronic inflammation which is associated with diseases such as atherosclerosis, asthma and rheumatoid arthritis (Punchard, 2004; Maskerey *et al.*, 2011).

Anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are used to treat and manage inflammation and inflammatory diseases. However, they are associated with adverse effects, for example, gastric ulceration (Punchard, 2004). Inflammatory mediators released during the inflammatory process cause pain, redness, swelling and loss of function (Nijkamp and Parnham, 2005).

Pain is a very personal, unpleasant sensation associated with potential or actual tissue damage which is perceived and interpreted in the brain (Cole, 2002). Pain is

not only a symptom used to diagnose several diseases and conditions but also has a protective function. The organism's ability to detect noxious stimuli and engage in appropriate protective behaviors against these stimuli is essential for its survival and wellbeing (Ezeja *et al.,* 2011; Prystupa *et al.,* 2013). Unrelieved pain may cause suffering and inability to perform daily activities hence imposing high health costs and economic losses to the victim and society (Prystupa *et al.,* 2013). Pain is treated and managed by analgesics such as NSAIDs (mild pain) and opioids (severe pain). These analgesics are associated with serious adverse effects such as addictive potential, nausea, respiratory depression, gastrointestinal bleeding and ulceration (Sani *et al.,* 2013).

Fever or Pyrexia is the increase in body temperature above normal (Visundra and Divya, 2013). Fever is caused by microbial infections, trauma, drugs and chemicals which trigger the formation of pro-inflammatory cytokines like interleukin (IL), tumor necrosis factor-alpha (TNF-α) and interferon. These cytokines are released into the blood stream and then migrate to the hypothalamus, where they enhance the synthesis of prostaglandin E_2 (PGE_2) next to the pre-optic hypothalamus region hence elevating the body temperature by promoting heat generation mechanism and decreasing heat loss (Kumar *et al.,* 2012; Anochie and Ifesinachi, 2013).

Fever is usually accompanied by symptoms, such as sweating, chills and sensation of cold. It is exhibited in many illnesses for example; malaria, typhoid and arthritis (Kumar *et al.,* 2012; Anochie and Ifesinachi, 2013). Pyrexia is treated and managed by antipyretic drugs. These drugs act by inhibiting biosynthesis of PGE_2, thus reducing the elevated body temperature. However, they are toxic to the brain cortex, hepatocytes, cardiac muscles and glomeruli (Paschapur *et al.,* 2009).

As alternative and/or complementary interventions, herbs have globally been used to ameliorate pain, fever and inflammation. Herbal medicine entails the use of plants and plant extracts to treat diseases. Herbs exist in many local plant varieties depending on the regional flora and are gradually becoming popular throughout the world (Pant *et al.,* 2012). Many conventional drugs were originally extracted from plant sources and are currently being made synthetically by pharmaceutical companies (Saleheen *et al.,* 2010).

Researchers are now focusing on natural products as alternative and complementary therapy to many diseases. They accomplish these by documenting traditional knowledge relating to medicinal plants, scientifically authenticating them as well as isolating active principles from them (Chowdury *et al.,* 2015). One such plant, *Acacia mellifera*, caught the attention of the researchers because of its pharmacological activities such as antimicrobial (Mutai *et al.,* 2009), anti-

leishmanial (Wambui *et al.,* 2007), hepatoprotective and antiviral activities (Parvez *et al.,* 2015). Medicinal plants are associated with many desirable characteristics including alleged *in vivo* safety, affordability and easy accessibility among others.

Acacia mellifera, also known as the black thorn or hook thorn, is a species of *Acacia* which is widely distributed in Kenya and other parts of Africa. Survey done in Kenya shows that the *Acacia mellifera* has been traditionally used to treat stomach problems, indigestion, enhance digestion (Kiringi *et al.,* 2006), pneumonia, back-ache, joint ache (Kareru *et al.,* 2007) chronic joint pain (Wambugu *et al.,* 2011), sexually transmitted diseases (Kipkore *et al.,* 2014), sterility, malaria, coughs and in circumcision rites among the Maasai community (Kokwaro, 2009).

Acacia mellifera has also been used traditionally to treat and manage inflammation, pain and pyrexia among the Ameru and Embu communities of Kenya. However, empirical scientific documentation on the bioactivity of *A. mellifera* against the three ailments is lacking. It is against this background that this study was designed to bio-screen for the dichloromethane stem bark extract of *A. mellifera* for its efficacy against inflammation, pyrexia and pain. This is an important preliminary step towards the development of a plant-derived pharmacological agent against the three ailments. This study aims at scientifically

justifying and documenting the use of *A. mellifera* as herbal agent for treating and managing inflammation, pain and pyrexia. It also revealed research gaps that needed to be explored further.

1.2 Statement of the problem and justification of the study

Inflammation, pain and pyrexia in most cases are nonspecific manifestations depicted in many ailments afflicting millions of people globally. Analgesics, antipyretic and anti-inflammatory drugs currently available in the market are used to treat and manage these conditions. Despite their effectiveness in curing and managing these conditions, prolonged usage of these conventional drugs are associated with numerous adverse effects such as gastrointestinal disturbances, renal damage, respiratory depression, and possible dependence. In addition, these drugs are unavailable in many rural settings due to inadequate modern health care facilities. Moreover, these conventional drugs are ineffective in a way that each drug treats a single manifestation like pain separately, hence the invalid requires multiple drugs to get well (Hoque *et al.,* 2011; Alamgeer *et al*., 2015).

Herbal medicines have been considered as the preferred alternatives to conventional medicines. They have been used to treat and manage various ailments such as diabetes mellitus which is alleviated by *Scoparia dulcis* L. in India (Zulfiker *et al*., 2010), a toothache is suppressed by *Spilanthes acmella* in Sri Lanka (Peiris *et al.,* 2001), and back-ache and joint- ache is treated by *Landolphia buchananii* and *Acacia ataxacantha* in Kenya (Kareru *et al.,* 2007).

Herbal medicines are preferred to conventional medicine due to them being readily available, affordable, easily consumable raw or as simple medicinal preparation, treat multiple conditions and have less or no adverse effects (Abayomi *et al.,* 2013). An example of such herbal medicine is the stem bark extract of *Acacia mellifera* which has been used to treat inflammation, pain and pyrexia among the Kenyan people.

The study also sought to scientifically confirm and documents the use of *A. mellifera* stem bark as the herbal remedy for treating and managing inflammation, pain and pyrexia among the Ameru and Embu communities in Kenya.

1.3 Research questions

i. Does the dichloromethane stem bark extract of *Acacia mellifera* have anti-inflammatory effects on carrageenan-induced inflammation in mice models?
ii. Does the dichloromethane stem bark extract of *Acacia mellifera* have analgesic effects on formalin-induced pain in mice models?
iii. Does the dichloromethane extract from the stem bark of *Acacia mellifera* have antipyretic effects on turpentine-induced pyrexia in rat models?
iv. What is the phytochemical composition of dichloromethane stem bark extract of *Acacia mellifera*?

1.4 Objectives

1.4.1 General objective

To determine *in vivo* anti-inflammatory, analgesic and antipyretic activities of dichloromethane stem bark extract of *Acacia mellifera* in mice and rat models.

1.4.2 Specific objectives

i. To determine *in vivo* anti-inflammatory effects of the dichloromethane stem bark extract of *Acacia mellifera* on carrageenan-induced inflammation in mice models.

ii. To determine *in vivo* analgesic effects of dichloromethane stem bark extract of *Acacia mellifera* on formalin-induced pain in mice models.

iii. To determine *in vivo* antipyretic effect of dichloromethane extract from the stem bark of *Acacia mellifera* on turpentine-induced pyrexia in rat models.

iv. To determine the qualitative phytochemical composition of DCM extract from the stem bark of *Acacia mellifera.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Inflammation

Inflammation is a bodily protective reaction towards an injury caused by inducers of inflammation that disrupt tissue homeostasis. These inducers can be of endogenous or exogenous origin. Exogenous inducers include pathogens, irritants, allergens, foreign bodies and toxic compounds. Endogenous inducers include signals from stressed and damaged tissues (Medzhitov, 2008). The aim of inflammation, therefore, is to localize and eliminate the injurious stimuli as well as the damaged tissue components and to promote healing (Noah *et al.,* 2012; Akor *et al.*, 2015).

The inflammatory cascade begins by recognition of inflammation inducers by host cells. These cells have receptors such as nucleotide-binding oligomerization domain protein (NOD)-like receptors (NLRs) and Transmembrane Toll-like receptors (TLRs) which can distinguish between self and foreign molecules. These receptors are capable of recognizing molecular patterns expressed on the surface of the pathogens-pathogen associated molecular patterns (PAMPs) or infected or injured host cells-damage associated molecular patterns (DAMPs) (Mogensen *et al.*, 2009; Noah *et al.*, 2012).

Once the receptor engages with the relevant PAMP or DAMP, it triggers several downstream signaling pathways that culminate in the activation of gene expression and synthesis of a wide range of molecules. These molecules include cytokines such as interleukin 1-beta (IL-1β) and interleukin-six (IL-6), TNF-α, and inflammatory mediators such as histamine, serotonin, prostaglandins (PGs), leukotriene, bradykinin and adhesion molecules like selectin. These cytokines and inflammatory mediators are responsible for the cardinal signs of inflammation namely redness, pain, swelling, heat and loss of function (Dhalendra *et al.,* 2013).

In addition, they enable the recruitment and migration of effector cells such as basophils, neutrophils, eosinophils, mast cells, connective tissue fibroblasts, lymphocytes and monocytes that become macrophages upon entering the tissue to the site of injury to act on the pathogen and infected cells and promote tissue repair (Medhitov, 2008; Noah *et al.,* 2012).

Neutrophils act by creating a cytotoxic environment. They do so by releasing noxious chemicals, for example, reactive oxygen species and reactive nitrogen species. These noxious chemicals starve both pathogen and the host cell by consuming glucose and oxygen molecule vital for their survival (Noah *et al.,* 2012). The effector cells produce proteases and enzymes from their granules that aid in destroying the pathogens. In addition, the action of the effector cells nonspecifically harms both the pathogens and hosts cells (Medhitov, 2008).

Resolution is the final stage of inflammation. The function of resolution is to limit the collateral damage done to the host, promote tissue repair and its restoration. Resolution is mainly mediated by macrophages. These macrophages that promote inflammation by enhancing the production of arachidonic acid metabolites example prostaglandins and leukotrienes, subsequently stimulate the production of a different class of arachidonic acid metabolite, lipoxin through lipid mediator class switch. Lipoxin blocks further neutrophil recruitment but then promote the infiltration of monocytes. These monocytes are essential for removal of dead cells and initiating wound healing. Other arachidonic acid metabolites protectins and resolvins as well as Fas ligand and transforming growth factor-β stop further recruitment and migration of leukocytes, reduced cytokine expression, blocked the production of pro-inflammatory mediators and initiate tissue repair (Henderson, 1994; Medzhitov, 2008; Noah *et al.,* 2012).

Inflammation is usually life preserving but can be fatal to individuals born without the major components of the inflammatory process. Leukocyte adhesion deficiency is an immunodeficiency disease where a person is unable to mobilize leukocytes to the site of infection due to lack or defect in adhesive glycoprotein example selectin and integrin attached to the leukocyte (Nathan, 2002). Acute inflammation may fail to eradicate the pathogen hence the inflammatory process persists leading to chronic inflammation that is associated with diseases such as rheumatoid arthritis, asthma, cancer, diabetes, gout, atherosclerosis, multiple

sclerosis and inflammatory bowel disease. Persistent inflammation or over-inflammation leads to tissue damage and the possibility of organ failure (Punchard *et al.*, 2004; Peng *et al.*, 2013; Mansouri *et al.*, 2015).

In summary, inflammation can be classified into two forms; acute and chronic. Acute inflammation is immediate and short-lived (for hours or even days), this is due to an early response to an injurious agent that is quickly resolved. Chronic inflammation begins after the onset of acute inflammation. In this case, the inducer persists or there is ineffective host response that interferes with the healing or regeneration process, hence inflammation and repair occur simultaneously. Chronic inflammation can last for weeks to months or years (Dhalendra *et al.*, 2013).

2.2 Pain

Pain is an unpleasant sensory and emotional experience that is associated with real or perceived tissue damage. It warns a person of actual or potential tissue damage or disease hence, minimize physical harm by eliciting a reflex and behavioral response (Prystupa *et al.*, 2013). The benefit of pain is underscored by persons with genetic neuropathies such as congenital insensitivity to pain that renders them incapable of perceiving pain. As a result, they do not engage appropriate protective behaviors and have curtailed life spans (Dubin and Patapoutian, 2010). Pain is the most common symptom or experience reported by patients. It interferes with appetite, sleep, locomotion, social interaction and work.

In addition, it imposes high medical cost and economic losses to the patient's family and society (Dueñas *et al.,* 2016).

Pain is evoked by toxic molecules, inflammatory mediators and extreme temperature and pressure that lead to tissue damage (Dubin and Patapoutian, 2010). Sometimes pain is influenced by psychological factors such as life experiences, mood, emotions and beliefs about pain (Redii *et al.,* 2013). Humans have devised ways of avoiding pain, for instance, psychological persuasion, diverting attention from the painful stimuli and reflex action which trigger behavior process that protect against current or further tissues damage, but the best way is to remove its cause and allow healing (Burger, 2008; Patel and Kopf, 2010).

Pain occurs when nociceptors (specialized sensory neurons) are stimulated by noxious stimuli. These nociceptors, in turn, send a signal to the spinal cord and brain. The information relayed to the brain specify the site, nature and intensity of the pain sensation (Redii *et al.,* 2013). Nociceptors like other primary somatosensory neuron are pseudounipolar with a nerve cell body positioned in the trigeminal ganglia or the dorsal root ganglia, sending an axon to the periphery and another to synapse on the neuron in the spinal cord or brainstem (Woolf, 2004; D'Mello *et al.,* 2008).

Nociceptors are classified based on the nerve fiber at the terminal end, and there are A-delta (Aδ), A-beta (Aβ) and C fibers. These fibers are present throughout the skin, internal organs, joints, muscles, tendons and blood vessels (Lamont *et al.*, 2000). Aδ fibers have a larger diameter and are thinly myelinated axons that conduct nerve impulses faster. The C fibers are unmyelinated nerve with a small diameter that conducts nerve impulses slowly. The Aβ fibers have special features: highly myelinated with a large diameter, allow rapid signal conduction, low activation threshold, respond to light touch and transmit non-noxious stimuli (Ohara *et al.,* 2005).

The pain process begins when nociceptors detect and respond to actual or potentially damaging noxious stimuli. Mechanical, thermal, chemical and electrical stimuli cause damage or injury to the tissues. Chemical substances are either released from the damaged cells during injury or as a result of a humoral and neural response to the injury. These chemicals substances activate the nociceptors as well as initiate the inflammatory response. Lysed cell membrane release intracellular potassium ions (K^+) and hydrogen ions (H^+), as well as arachidonic acid (AA) (which is converted to prostaglandins by cyclooxygenases). In addition, inflammatory mediators such as histamine, leukotrienes, 5-hydroxytryptamine and bradykinin released following tissue injury enhance the activation of nociceptors besides inducing vasodilation, plasma

extravasation, an attraction of macrophages and degranulation of mast cells (Schaible, 2006; Marchand, 2008).

Application of noxious stimuli to nociceptors beyond their threshold leads to their activation or sensitization. The activated or sensitized nociceptors increase the membrane permeability to sodium ions. Sodium ions present on the outside of nerve fiber pour across the membrane to the inside of axon causing the inside of the fiber to be more positive, this increases the membrane voltage. When the membrane voltage increases beyond its threshold it results in the opening of more ion channels in a positive feedback mechanism, this depolarizes the membrane thus creating an action potential that leads to the generation of pain impulse. Pain impulse is transmitted along the nociceptor fibers (C and Aδ fibers) which terminate in the spine's dorsal horn (Goldstein, 2007; Lu *et al.*, 2008).

In the dorsal horn, the fibers trigger the release of neurotransmitters that are stored in the synaptic vesicles. Glutamate, vasoactive intestinal peptide, adenosine triphosphate (ATP), calcitonin gene-related peptide, cholecystokinin and substance P (sP) are examples of neurotransmitters released important in conducting the pain impulses across the synaptic cleft between the fibers and the primary somatosensory neurons. These primary neuron, in turn, makes synaptic contact with the secondary neuron that project to superior centers (Goldstein, 2007; Marchand, 2008).

The secondary neuron sends pain impulses to superior centers rostrally via two distinct pathways; the spinothalamic tract that transmits the incoming signals to the thalamus that in turn projects to the somatosensory cortex. This pathway relays information related to the sensory aspect of pain. The spinoreticular tract which is the other pathway transmits nociceptive signals to the brain stem reticular before projecting to the thalamus, hypothalamus and various areas of the cortex relaying information of emotional aspect of pain (Scholz and Woolf, 2002).

Information reaches cortical structures from the brainstem and thalamic loci. In the cortex, there are various areas; the reticular system in charge of motivational, autonomic as well as motor responses such as retracting when one touches a hot object and evaluating the injury. Somatosensory cortex assesses and interprets pain sensation, which includes where the pain is, type and intensity. The limbic system is involved with the aspect of pain for example how it is going to be felt (emotion) and acted upon (behavior) (Wood, 2008; Redii *et al.*, 2013).

The body has its own mechanisms of modulating pain both at the brain and spine levels. Segmental and descending inhibitions have been used to describe the endogenous pain modulation. The segmental inhibition also known as gate control theory suggested by Melzack and Wall in 1965 explains pain inhibition at the spinal cord level. Pain signals transmitted via Aδ and C fiber activate interneurons

present in the dorsal horn to ‘open the gate’ and allow the transmission of pain impulses to the brain through the spinal cord. Also, these interneurons inhibit pain signal transmission ‘closes the gate’ when activated by tactile and non-noxious stimuli transmitted by the Aβ fibers, this explains why rubbing an injured area reduces the pain sensation (Kitahata *et al.,* 1993; Motoc *et al.,* 2010; Patel and Kopf, 2010; Reddi *et al.,* 2013).

Descending inhibition involves various connections that originate from several areas of the brain and project back to the spinal cord to decrease the activity of nociceptive neurons. The release of endogenous opioid peptides such as enkephalins, endorphins and dynorphins that bind to opioid receptors delta (δ), mu (μ) and (kappa (κ) results in opening of potassium ions channels, closing of calcium ions channels and the inhibition of adenylyl cyclase that leads to inhibition of neurotransmitters release (Chahl *et al.,* 1996; Goldstein, 2007).

Despite the various ways the body tries to modulate pain, pain may persist. Unrelieved pain can cause both physical and psychological damage. In addition, unrelieved acute pain can proceed to chronic pain (Rajagopal, 2006). Excessive pain can lead to side effects such as sweating, apprehension, nausea and palpitation. Pain is a common and distressing feature in many life-threatening diseases such as fibromyalgia, irritable bowel syndrome and tension-type headache (Basbaum *et al.,* 2009; Dubin and Patapoutian, 2010).

In summary, pain is, therefore, classified based on region of the body involved for example eye, nose, stomach and legs; system whose dysfunction is the cause of pain example nerve and gastrointestinal; duration in term of chronic (last for long time) and acute pain (it is resolved quickly) and intensity (Cole, 2002; Kilic *et al.*, 2012). Types of pain include nociceptive pain resulting from activation of nerve endings by tissue damage or inflammation, physiological pain which is a rapid perceived non-traumatic discomfort caused by potential injurious environmental stimuli such as hot object and pathological pain that is due to nervous system injuries or its defects (Lamont *et al.,* 2000).

2.3 Fever

Fever or pyrexia is an increase in body temperature outside the normal range which is usually from 36.0°C to 37.5°C (Anochie and Ifesinachi, 2013). The normal range provides optimal temperature for enzymatic activity. Almost all of the body's biochemical and metabolic processes are affected by the change in temperature (Miyake, 2013). The normal body temperature varies in individuals based on age, sex, ambient temperature, activity level and time of the day (Adhi *et al.*, 2008).

Body temperature also varies according to the site where the temperature is recorded. These sites include the oral cavity, rectum, tympanic membrane, armpit and even the forehead (Adhi *et al.,* 2008). Normally oral temperature is about 35.5-37.5°C and has long been the standard in clinical practice largely because of

its accessibility and prompt response to the change in the core temperature. Axillary temperature (between 36.5°C to 37.5°C) is taken under the armpit and it is convenient for young children and uncooperative adults. Rectal or tympanic temperatures are internal measurement taken in the rectum (36.6°C -38°C) and the ear (35.8°C -38°C), these temperatures are higher than oral temperature (HealthLinkBC, 2016).

Body temperature results from the difference between heat loss and its production. The center of thermal equilibrium is located in the hypothalamus. This center integrates cold and warm thermal receptors' input found all over the body and in turn, generate output responses leading to conservation or release of body heat. Heat is preserved through increased muscle tone or shivering and vasoconstriction or increases its dissipation through the skin surface by radiation and conduction, and through urine and faeces (Adhi *et al.*, 2008; Anochie and Ifesinachi, 2013; Clayman, 2016). Besides, people engage in voluntary activities that assist in the regulation of body temperature, for instance, the selection of proper clothing, staying in shade and use of heating systems and air conditioning to regulate their surrounding temperature (Miyake, 2013).

Fever is caused or induced by substances called pyrogens. These exist in two types: exogenous pyrogens that come from outside the body and endogenous pyrogens formed by the body's cells attributable to outside stimulus (Anochie and

Ifesinachi, 2013). Exogenous pyrogens may originate from infections of bacterial, viral and parasitic origin, immune reactions, several hormones, medications and synthetic polynucleotides or conditions such as myocardial infarction, pulmonary emboli and neoplasms or from graft rejection or inflammation. The injured or abnormal cells in all of the above cases trigger the production of pyrogens (Hossain *et al.*, 2011; Khaled, 2011).

The pyrogens initiate heat generation and conservation mechanism as well as inhibiting heat loss mechanism, thus a rise in the body temperature that is dependent on the nature and dose of the pyrogen (Anochie and Ifesinachi, 2013). The activity of these pyrogens is indirect since they induce the host cells, such as tissue macrophages and blood leukocytes to synthesize endogenous pyrogens (Alcami and Smith, 1996; Luker *et al.*, 2000). All endogenous pyrogens are circulating cytokines. They include interleukins (IL-1α, IL-1β, IL- 6 and IL- 8), interferons (α and β) and TNF-α. These cytokines are important humoral mediators that induce or maintain fever (Roth and Souza, 2010).

The cytokines can pass the blood-brain barrier by active and saturable transport systems or gain access through areas of the brain which lack a tight blood-brain barrier. These areas that lack a tight blood-brain barrier are termed as circumventricular organs examples organum vasculosum laminae terminalis and the subfornical organ, here the cytokines interact with the local microglial cells or

the endothelial receptors on vessel walls. These interactions, in turn, activate the arachidonic acid pathway which enhances the PGE_2 synthesis next to the pre-optic hypothalamus region thereby resetting a hypothalamic set-point. In addition, it generates a systemic response back to the rest of the body to cause heat creating an effect to match and maintain the new temperature (Roth and Souza, 2001; Shah *et al.,* 2010; Walter *et al.,* 2016).

Fever supports the immune system's attempt to fight infection by creating an unfavorable environment where damaged tissues or infectious agents cannot survive since they are temperature sensitive, hence acts as the body's natural defense (Manivel *et al.,* 2011). Fever is associated with symptoms such as lethargy, depression, anorexia, sleepiness and inability to concentrate (Vasundra and Divya, 2013). Thyroid storm, intracranial haemorrhage, Kawasaki syndrome, serotonin syndrome and sepsis which are considered as medical emergencies are associated with persistent high temperature (Hossain *et al.,* 2011).

2.4 Experimental inflammation induction methods

2.4.1 Carrageenan-induced edema test

This is a standard experimental model for acute and nonimmune inflammatory response used in assessing the anti-inflammatory activities of natural products as well as synthetic chemical compounds (Muhammad *et al.,* 2012; Mansouri *et al.,* 2015). Carrageenan is a sulphated polysaccharide made up of repeating units of galactose-related monomers obtained from seaweeds (Rhodophyceae) (Hafeez *et*

al., 2013). There are three type's lambda, kappa and iota, but the lambda form does not gel strongly at room temperature. Also, carrageenan is not known to be antigenic. In addition, it has no systemic side effects (Hussain *et al.,* 2015).

Acute inflammation is induced when 0.05-0.1ml of 1% carrageenan is administered to the sub-plantar region in the right hind paw of the animal, with peak effect occurring 3-5 hours after induction (Pareek *et al.,* 2011; Dhalendra *et al.,* 2013). Carrageenan-induced inflammation is bi-phasic in which various mediators operate to produce the inflammatory response. The initial phase is characterized by the release of serotonin, histamine, kinins and prostaglandins (in smaller amount) in the first hour; while the following phase (the late phase) that occurs from the second to the fourth hour after the administration of the irritant is ascribed to the release of prostaglandins, oxygen and nitrogen-derived free radicals, proinflammatory cytokines such as TNF-α and IL-1β and the infiltration of neutrophils. This second phase is sensitive to most anti-inflammatory drugs that are clinically valid (Hafeez *et al.,* 2013; Asfar *et al.,* 2015; Mansouri *et al.,* 2015; Mondal *et al.,* 2016; Samriti *et al.,* 2016).

2.4.2 Egg albumin-induced inflammation test

A quantity of 0.1ml of egg albumin is injected sub-plantarly to the animals' left hind paw. The paw's circumference is determined using a Vernier calliper at intervals of 30 minutes post induction of inflammation (Salawu *et al*., 2008; Kolawole and Dapper, 2016). When egg albumin is presented to the intercellular

space, it induces acute inflammation that is mediated through the release of 5-hydroxytryptamine (Perianayagam *et al.,* 2012). Edema formation is regarded as a biphasic event. The first phase of edema starts immediately after the irritant is administered and lasts up to 2 hours while the late phase, occurs from 3 to 5 hours after the administration of the irritant (Anosike *et al.,* 2012).

2.4.3 Croton oil-induced mouse ear edema

This is a handy model for testing topical anti-inflammatory processes. Croton oil is a highly irritant agent which induces cutaneous inflammation characterized by vasodilation, edema and leukocytic migration, as well as the local liberation of inflammatory mediators such as histamine, 5-hydroxytryptamine, bradykinin and prostaglandins (Araujo *et al.*, 2004). Croton oil of about 5% is applied on the left ear's inner surface of the mouse while the right ear is left untreated. The target drug is administered topically on the left ear's inner surface for approximately 30 min after the administration of croton oil. The mice are then euthanized after six hours. Using a dermatologic punch, a six-millimeter plug in diameter is removed from the treated and untreated ears. The weight difference between the two plugs is measured as the edematous response (Oliveira *et al.,* 2013).

2.5 Experimental pain induction methods

2.5.1 Tail-flick model

The test involves the application of radiant heat to a small portion of the tail or the tail is immersed in hot water at about 55^{o}C or cold water, these evokes a nociceptive response that is stable and spinally mediated. This response occurs in

the form of a jerk or a flick of the tail away from the source of heat or by a brief, vigorous movement of the body. The reaction time of this body movement is what is recorded (often referred to as "tail-flick latency") (Keefe *et al.,* 1991; Milind *et al.*, 2013).

2.5.2 The hot plate test

This is commonly used to study thermal (anti) nociception in rats and mice. It is based on the fact that the paws of the animals are very sensitive to heat. In addition, this test is suitable for evaluating analgesic of central origin hence, peripherally acting analgesic show little or no activity. Hot-plate test involves placing a mouse or rat into an open-ended cylindrical region with a metallic floor. These metallic floor is heated using a thermode or boiling liquid to a constant temperature of 55°C. Two behavioral response that is paw licking and jumping are produced when the animal's footpad is in contact with the heated floor. These response occurs in seconds and is termed as reaction time (Le Bar *et al.,* 2001; Paschapur *et al.*, 2009).

2.5.3 Acetic acid-induced writhing test

The mouse writhing test is very handy in the evaluation of peripherally acting drugs such as non-steroidal anti-inflammatory drugs. The test involves administering acetic acid to the animal's peritoneal cavity. The animal responds by stretching (writhing). It is characterized by abdominal contraction which is recorded for thirty minutes (Mishra *et al.,* 2011). The introduction of acetic acid into the peritoneal cavity results to sensitization of the peripheral receptor system

present in the cell lining the peritoneal cavity by prostaglandins such as PGE_2 and $PGF_{2\alpha}$. In addition, the endogenous substances example serotonin, bradykinins and histamine released further stimulates the sensory nervous endings (Akindele *et al.*, 2012; Bhattacharya *et al.*, 2014).

2.5.4 Formalin-induced writhing test

Formalin test is a well-established *in vivo* model used to illustrate nociceptive processes and analgesic drug effects (Chang *et al.*, 2011; Arzi *et al.*, 2013). This test shows a continuous rather than a transient process with two temporary distinct phases; the early and late phase of the nociceptive response. The two phases are based on duration and underlying mechanisms. The early phase is short-lived (last for 5-10 minutes), there is intense pain. It starts immediately after formalin injection and is as a result of direct nerve stimulation by formalin (Sufka *et al.*, 1998; Jeong and Lee, 2002).

After a short period of very little or no nociceptive behavior, a continuous prolonged late phase commences, that last between 15-60 minutes after the formalin injection. It involves moderate pain that is believed to be due to inflammation which involves the inflammatory mediators' release that influences functional change in the dorsal horn of the spine. These mediators include prostaglandin, histamine, bradykinin and serotonin. Different analgesics may act differently in the two-phase (Sharma *et al*., 2013; Gong *et al*., 2014). Normally, 2.5% formalin is subcutaneously administered to the animal to induce

nociception. Sufka *et al.,* (1998) suggested that formalin concentrations higher than 5% should be avoided since they evoked unnecessary and unquantifiable pain that may hinder the primary behavior. Also, the use of low concentration of formalin increases the sensitivity of the test (Tjolsen *et al*., 1992).

The advantage of the formalin model in comparison with other pain causing tests is that the induced pain is more continuous rather than being transient; hence bears resemblance to most clinical pain. It could discriminate between central and peripheral pain components, and the animals used are lightly restrained or not at all while under observation (Svendsen and Hau *et al.*, 1994; Gong *et al.*, 2014).

2.6 Experimental pyrexia induction methods

2.6.1 Brewer's yeast-induced test

Brewer's yeast is an exogenous pyrogen consisting of lipopolysaccharides from the cell wall of gram-negative bacteria that induces pyrexia by causing an increase in prostaglandins' synthesis (Akpan *et al.,* 2012). This test involves recording the basal temperature of each animal before pyrexia is induced; 15-20% of brewer's yeast suspension is injected subcutaneously. In 17-20 hours after the administration of brewer's yeast, the rectal temperature of each animal is taken and those with a 0.5-1^{o}C increase in temperature are used for the test and the rest are rejected. After that, the rectal temperature is monitored every hour (Kumar *et al.*, 2015).

2.6.2 D-amphetamine-induced pyrexia test

Amphetamine action in the brain causes the biogenic amines' release from the nerve ends. This action results in increased cAMP level and subsequent prostaglandins synthesis from the arachidonic acid made in the neuron by receptor-mediated hydrolysis of phospholipids (Akpan *et al.,* 2012). This test involves the measuring of the basal temperature for each animal rectally using a clinical digital thermometer. Fever is induced by administering D-amphetamine intraperitoneally. One hour after D-amphetamine induction, the temperature of the rectum is taken and recorded. After that, they are observed keenly after each hour for consequent five hours until the temperature rises appreciably (Agbaje and Ajidahum, 2011).

2.6.3 Turpentine oil-induced test

Turpentine is oil that is refined from the *Pinus palustris* mill. Turpentine irritates the tissue leading to fever, local inflammation, loss of body weight, abscess formation, anorexia, cytokine production and changes in acute phase protein levels (Renckens *et al.,* 2005). Turpentine is an exogenous pyrogen that induces pyrexia by stimulating the synthesis and release of endogenous pyrogens such as cytokine from the host' immune cells example the phagocytic cells. These pyrogens in turn work on the thermoregulatory center in the hypothalamus to cause a rise in temperature when the concentration of prostaglandins E_2 increases (Vasundra and Divya, 2013). Turpentine (20%) is used to induce pyrexia intraperitoneally, whose quantity is dependant on the body weight of the animals.

Induction of fever takes about 1 hour after which animals whose temperature rise by 0.7-1.0^{0}C are noted as pyretic hence used for the test (Gitahi *et al.*, 2015).

2.7 Conventional modulation of inflammation, pain and pyrexia

Steroidal and non-steroidal anti-inflammatory drugs are used to treat and manage inflammation. Steroidal drugs are also known as corticosteroids include mineralocorticoids and glucocorticoids which are natural hormones generated by adrenal glands. They provide an immediate, short-lived and powerful relief from inflammation, though they have many side effects (Dewick, 2009; Becker, 2013).

Glucocorticoids act on carbohydrates, protein and fat metabolism and have both anti-inflammatory and anti-rheumatic effect. They act by inhibiting phospholipase enzymes, thus prevents the release of phospholipids from storage needed for PG formation. Adrenal gland malfunction leads to diseases such as Addison's disease, acne, hypertension, overweight and decreased resistance to infection. Mineralocorticoids such as aldosterone (the only natural mineralocorticoid) and flourocortisone regulate the electrolyte balance by promoting readsorption of sodium, chloride and hydrogen carbonate in the kidney with minor analgesic properties (Dewick, 2009; Becker, 2013).

The non-steroidal anti-inflammatory drug such as aspirin, diclofenac, indomethacin, celecoxib, ibuprofen, naproxen, ketorolac, piroxicam and diflunisal is used to treat mild pain, reduce fever and suppress inflammation (Burger, 2008;

Deghrigue *et al.,* 2015). These drugs act by inhibiting the synthesis of pro-inflammatory prostaglandins by blocking both COX-1 and COX-2 enzymes to a variable extent. This blockage reduces sensitization of the peripheral nervous tissue resulting in less nerve stimulation and ultimately less pain (Mworia *et al.,* 2015). The enzymes (COX-1 and COX-2) oxygenate arachidonic acid to generate prostanoids (Francischi *et al.,* 2002; Malkowski *et al.,* 2016).

Cyclooxygenase enzyme is bifunctional homodimeric enzymes that contribute to a variety of physiological and pathophysiological functions. COX-1 is constitutively expressed in most tissues while COX-2 is rapidly induced in inflammatory cells. The prostanoids produced via COX-1 are involved in platelet aggregation and maintaining renal functions. In addition, they preserve the integrity of the gastrointestinal tract by stimulating mucus formation in both the stomach and small intestine, ensure mucosal blood flow and the protection of the gastric mucosa by lowering acid secretion while enhancing bicarbonate secretion (Reanmongkol and Songkram, 2013).

Indomethacin and ibuprofen are the reversible inhibitors of COX enzymes; they compete with arachidonic acid for the active site. Paracetamol also known as acetaminophen is the most used analgesic. Paracetamol is classified under NSAIDs though it has minimum anti-inflammatory activity. Its ability to prevent fever and pain is attributed to COX-1 enzyme inhibition. Also, it can be safely

given to children with fever caused by viral illnesses (Dewick, 2009). Paracetamol is metabolized by cytochrome P_{450} and a number of electron transfer reaction result in the formation of superoxide and hydrogen peroxide that injure the liver cells (Clayman, 2016).

Salicylates such as aspirin (acetylsalicylic acid), salsalate, choline magnesium salicylate and methyl salicylate were isolated from willow bark which is used in the treatment of pain and fever. Aspirin was synthesized in 1899 during the search for a salicylate derivative that would be less irritating to the stomach than salicylic acid. Since then aspirin has been widely used as an analgesic, anti-inflammatory and antipyretic drug in adult but should be avoided in children because it has been associated with Reye's syndrome (Greenstein *et al.,* 2007).

Aspirin is weak organic acid that nonspecifically and irreversibly inhibits COX enzymes. It does so by covalently acetylating the hydroxyl groups of serine 530 within the cyclooxygenase active site in the peripheral tissues and the central nervous system thereby reducing the synthesis of PG and thromboxane. It lowers the fever by targeting PG synthesis in the temperature control area of the hypothalamus and has no effect on normal body temperature. Moreover, aspirin has an antiplatelet effect through the inhibition of thromboxane A2 leading to bleeding (Vane and Botting, 2003; Brenner and Stevens, 2013; Malkowski *et al.,* 2016).

Diclofenac is a phenylacetic acid derivative usually formulated as a sodium or potassium salt (Fotopoulos *et al.,* 2011). It is a commonly used NSAIDs with analgesic, anti-inflammatory and antipyretic activities. It has a higher potency than indomethacin and naproxen. It acts by inhibiting prostaglandin synthesis (Brunton *et al.,* 2011; Brenner and Stevens, 2013). Diclofenac is also reported to produce its analgesic effects through interacting with endogenous opioids, serotonin, noradrenalin, the L-arginine-nitric oxide-cGMP pathway and the activation of potassium channels (Flores-Ramos and Díaz-reval, 2013). These popular NSAIDs do not cause respiratory depression or sedation and are less addictive. Despite this, they are not without adverse effect as they tend to irritate the stomach lining, cause ulcers and bleeding (Lawton and Witty, 2008).

Analgesics are used to relieve both acute and chronic pain without altering consciousness (Milind *et al.,* 2013). They act by blocking pain sensitivity of the nervous system or inhibit the formation of mediators of pain such as bradykinin, histamine and prostaglandins (Food and Drug Administration (FDA), 2009; Bhattacharya *et al.,* 2014). There are two types of analgesics; opioids (are derived from opium, they are morphine, codeine, oxycodone, fentanyl and hydrocodone) and nonsteroidal anti-inflammatory drugs (such as paracetamol, diclofenac and aspirin) (ACPA, 2015).

Opioids are the most powerful analgesics for treating and managing severe acute and chronic pain caused by cancer and serious disorders. Opioids such as morphine, fentanyl and oxycodone mimic the effects of endorphins by binding to the analgesic or opioid receptors in the central nervous system which leads to a reduction in the transmission of pain signals to the brain. The side effects of opioids are constipation, drowsiness, addiction, hallucination, nausea, vomiting and confusion especially in older people (Macor, 2010; Clayman, 2016). In addition, these centrally acting analgesic drugs inhibit both phases of the formalin test (Kammathy *et al.,* 2014).

Antipyretic drugs act by inhibiting the enzyme cyclooxygenase that leads to the reduction in the levels of PGE2 within the hypothalamus hence lowering the elevated body temperature (Bherji *et al.,* 2016). These drugs also act peripherally, through vasodilation and heat dissipation though they are toxic to the glomeruli, heart muscles, hepatic cells and the cortex of the brain. Antipyretic drugs include paracetamol, aspirin and indomethacin (Khan *et al.,* 2008; Paschapur *et al.,* 2009; Shah *et al*., 2010). Many hormones such as the melanocyte-stimulating hormone, arginine vasopressin, and glucocorticoids are endogenous antipyretics (Leon *et al.,* 1999; Shah *et al*., 2010).

With limitation in the current spectrum of therapies available for inflammation, pain and pyrexia, there is an unmet need for the development of more efficacious

and better new drugs in treatment and management of inflammation, pain and pyrexia (Lawton and Witty, 2008).

2.8 Herbal management of inflammation, pain and pyrexia

The World Health Organization (2002) describes folklore medicine as a compound consisting of two aspects; medical therapies, for example, herbal medicines and non-medicated such as acupuncture. Herbal medicine is defined as, use of herbs, herbal materials, herbal preparations and finished herbal products to treat and manage diseases. Herbal medicine has been employed by man for centuries as a remedy for various diseases and is thought to be due to the history of qualified success, reasonable science, its affordability, its accessibility and long-standing cultural and spiritual values (Wambugu *et al.*, 2011; Sani *et al*., 2013).

Plants found in the genus *Acacia* are traditionally used in the management of inflammation, pain and fever. *Acacia nilotica* pods, bark and roots have been useful in treating wounds, malaria, sore throat and toothache (Elhefian *et al*., 2012). *Acacia pennata* is used as a remedy for fever, headache and rheumatism (Dongmo *et al*. 2005). The bark of *Acacia arabica* is used to treat cough, syphilis, gonorrhea, diarrhea, sore throat and leprosy (Mohammad *et al*., 2014). *Acacia modesta* plant is used to treat an oral toothache, dysentery, venereal diseases, trachoma wounds and leprosy (Bukhari *et al*., 2010). *Acacia ataxacantha* leaf decoction is used in febrile convulsions, its bark is used for tooth decay,

bronchitis and cough, pods and seeds are used against dysentery and the roots are used in the treatment of joints and back pain (Lagnika *et al.*, 2014).

Some studies have been done on herbal plants and evidence used to support their ethnomedicinal use in the treatment of inflammation, pain and pyrexia. *Crossopteryx febrifuga* Benth (family Rubiaceae) preparations have been used traditionally for treating septic wounds, respiratory infections, fever, dysentery, pain and malaria. A study done on the methanolic extracts of *C. febrifuga* showed significant and dose-dependent analgesic activities on the two models employed; acetic acid and mechanically induced pain using an analgesymeter. The methanolic extract reduced the number of writhes and increased pain threshold; it also had significant anti-inflammatory and antipyretic activities as it inhibited albumin-induced edema and yeast-induced pyrexia respectively in a dose-dependent manner (Salawu *et al*. 2008).

Acacia hydaspica is used traditionally to treat diarrhoea, arresting secretion or bleeding, removing phlegm from the bronchial tubes and catarrhal matter, and soothing the genito-urinary organ's, alimentary canal's and pharynx’s inflamed membranes. Studies were done on the *Acacia hydaspica* methanol extract (AHM) and *Acacia hydaspica* ethyl acetate (AHE). In yeast induced pyrexia, the extracts exhibited a decrease in body temperature in a time and dose-dependent manner. These extracts also inhibited pain in the hot plate test and the acetic acid induced

writhing. It also had anti-inflammatory effects in both carrageenan-induced inflammation and prostaglandin E_2 (PGE_2) induced edema in rats (Afsar *et al.*, 2015).

Thymus serphyllum is commonly used locally for various purposes such as antiseptic, anti-helminthic, carminative and expectorant. Alamgeer *et al*., (2015) did a study on analgesic, anti-inflammatory and antipyretic actions of *T. serphyllum*. Three extracts (aqueous, ether and methanolic) were used and all the three extracts produced significant anti-inflammatory, analgesic and antipyretic activities. However, ether extract was more effective.

A study carried out by Safari *et al. (*2016a) on the analgesic, anti-inflammatory and antipyretic actions of the *Acacia nilotica* aqueous bark extract depicted a non-dose dependent reduction in paw licking time in both phases of the formalin-induced test, with most effects shown at 50mg/kg body weight dose. It also exhibited anti-inflammatory and antipyretic activities with the highest effect at doses of 100mg/kg and 150mg/kg body weight respectively.

Medicinal plants are widely distributed throughout the world and due to geographical and regional difference, their composition is known to be very diverse and consists of various phytochemical substances which can act singularly, additively or synergistically for the improvement of health and

treatment of various diseases (Pant *et al.,* 2012; Gurib *et al.,* 2013). Phytochemicals are classified either as primary compounds (Common sugars, proteins and chlorophyll) or secondary compounds (phenolic compounds, alkaloids and terpenoids) (Erharuyi *et al*., 2014).

Phytochemicals are deposited in the roots, stems, leaves, flowers, fruits and seeds. They are produced by the plant for protection against environmental hazards such as drought, pathogenic attack, pollution, UV exposure as well as contributing to the plant's colors (Dai and Mumper, 2010; Saxena *et al.,* 2013). In addition, they show a variety of biological activities which depend on the nature of the plant, geographic location of the plant, the system used to isolate these agents, weather conditions and method used to evaluate a particular character. These biological activities include; antioxidant, anti-inflammatory, antifungal, antimicrobial, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anticancer and immunomodulatory properties (Wadood *et al.,* 2013; Akhtar *et al.,* 2015).

Knowledge of the phytochemicals is desirable for the discovery of healthcare products, for example, some alkaloids are analgesics e.g. morphine; Tannins and flavonoids have also been reported to possess antimicrobial properties; Some glycosides like digoxin and digitoxigenin, are used to treat and manage cardiac disease and several terpenoids are in artemisinin and taxol used as malaria and

cancer medicines (Goto *et al.,* 2010; Jigam *et al.,* 2011). These compounds are also new sources of economic materials: alkaloids as anaesthetic agents, terpenoids as a pesticide, tannins as caustics for cationic dyes, in the production of inks, to clarify wine, beer, and fruit juices and as coagulants in rubber production (Kumara *et al.,* 2011; Saxena *et al.,* 2013; Wadood *et al.,* 2013).

Secondary metabolites present in *Acacia* species include amines, cyanogenic glycosides, cyclitols, alkaloids, fatty acids, seed oils, fluoroacetate, gums, nonprotein amino acids, terpenes (including essential oils, diterpenes, phytosterol, triterpene genins and saponins), flavonoids, hydrolyzable and condensed tannins (Malviya *et al.*, 2011).

2.8.1 *Acacia mellifera* (Vahl) Benth

2.8.1.1 Description and distribution

Acacia name was first adopted in 1754 by English botanist and gardener Phillip Miller (Stearn, 1974). They are shrub or small trees belonging to the family *Fabaceae* and subfamily *Mimosoideae* which, contain more than 1250 species, about 170 of these species are native to Africa. Acacia thrives in diverse habitats including tropical rain forest, coastal dunes, arid and semi-arid. All species are good sources of wood and non-wood products such as fodder, gums, resin and pharmaceuticals. Moreover, many species are; tolerant to fire and resistant to attack by termite (Hayward, 2004).

Acacia mellifera is a species of acacia. Some of its common names include *black thorn*/*hook thorn* (English), *Katogwa* (Ndebele) *Muthigira* (Embu), *Muthiia/Kithia* (Kamba), *Oiti* (Maasai), *Kikwata* (Swahili), K*itr* (Sudan) *Lanen* (Somali). *Kezia* (Taveta), *Ebunyu* (Turkana), *Sabansa-Gurach* (Boran) (Orwa *et al.*, 2009; Kokwaro, 2009). It is a perennial non-climbing tree which is widely distributed in Kenya and other parts of Africa such as Tanzania, Angola, Namibia, Botswana, Zambia, Zimbabwe, Mozambique and South Africa (Schimdt and Mbora, 2008; Quattrocchi, 2012).

Acacia mellifera is described by Orwa (2009) as a shrub or small branched tree with a more or less spherical crown, the bark stem is black but becomes ash-grey to light brown on the branches, the branches bear pairs of small, short and sharp hooked spines which are black in older plant but green in younger plants, the bluish-green leaves are bipinnate. The flowers are sweet-scented with creamy-white color. The fruits are papery pods with two to three seeds. It has an extensive root system that is shallow as it rarely penetrate more than one meter.

Figure 2.1: *Acacia mellifera* picture captured Embu County, Kenya

2.8.1.2 Cultural and medicinal uses

Acacia mellifera are used as fodder for both domestic and wild ruminants in arid and semi-arid areas, they form good live hedge or fence and provide shade for animals when pruned regularly. They also produce edible gum which may be mixed with clay to make floors. The flowers are good sources of nectar for bees, which produce high-quality honey, wood as charcoal source and the sap when mixed with powdered grub is used on poison arrows by the Khoisan people of Botswana. It is also used in circumcision rites among the Maasai community of Kenya (Kokwaro, 2009; Orwa *et al.*, 2009; Nonyane, 2013). *Acacia mellifera* has

nitrogen-fixing ability that enrich soil fertility and improves herbage quality (Nzehengwa, 2013). Also, it can be used to make pulp with good papermaking properties (Khider *et al.,* 2012).

Acacia mellifera has been used for medicinal purposes in humans. The roots are boiled and taken with goat soup to treat back-ache and joint ache while the decoction of the bark is used to treat pneumonia by the Mbeere and Aembu communities in Embu County (Kareru *et al.,* 2007). The roots are boiled to treat sexually transmitted diseases by the Marakwet community in the rift valley region (Kipkore *et al.,* 2014). The bark is boiled and used to treat stomach, indigestion and enhance digestion among the Amasaai people of Kajiado (Kiringi *et al.,* 2006). The Akamba people in Machakos and Makueni use boiled roots and bark to treat chronic joint pain (Wambugu *et al.,* 2011).

Other studies that have been done on *A. mellifera* include anti-leishmanial activities (Wambui *et al.,* 2007), whose finding indicated that the methanol stem bark extract of *Acacia mellifera* had inhibitory activities against *Leishmania promastigotes in vitro,* but the same extract had no effect on the transformation of amastigotes to the promastigote forms. Study conducted by Mutai *et al*. (2009) on the antimicrobial effect of methanolic extract of the stem bark of *A. mellifera,* reported that it contains triterpenoids ((20*S*)–oxolupane–30–al, (20*R*)–oxolupane–30–al and betulinic acid) active against *Staphylococcus aureus*, with the

triterpenoid (20*S*)–oxolupane–30–al exhibiting good antifungal activity. The total ethanol leaf extract of *Acacia mellifera* has promising hepatoprotective and antiviral activities against hepatitis B virus in both *ex vivo* and *in vivo* experimental conditions (Parvez *et al.*, 2015). This study, therefore, aims at providing information on the anti-inflammatory, analgesic and antipyretic properties of *Acacia mellifera* in laboratory animal models in attempts to scientifically justify its use as Kenyan traditional medicine.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection and preparation

Fresh stem barks of *Acacia mellifera* were collected from Siakago division, in Mbeere North Sub County, Embu County, Kenya. The local traditional medicinal practitioners assisted in identifying the plant. The sample was collected from its natural habitat with acceptable bio-conservation methods, sorted out, cleaned, and transported in burlap bags to the Biochemistry and Biotechnology laboratories of Kenyatta University for study. This process was conducted during the month of February 2016, a season in which the local herbalist believed that the medicinal plant had its maximum medicinal activity. The plant sample was provided to an acknowledged taxonomist for botanical authentication and a voucher specimen (001/04/2016) deposited at the Kenyatta University Herbarium. The collected stem barks were chopped into small pieces and air dried in the shade for two weeks until they properly dried. They were then ground into fine powder using an electric mill (Zhengzhou Yize machinery co., Ltd. Henan, China).

3.2 Extraction

The powdered sample material weighing 400g was soaked in one liter of dichloromethane for 24 hours with occasional swirling to facilitate the extraction process. The mixture was then filtered using a Whatman's filter paper No.1 and the filtrate concentrated using a rotary evaporator (Buchi Rotavapor R-200,

Labequip Ltd, Ontario, Canada) at about 40°C and stored in stoppered containers at 4°C until use.

3.3 Experimental design

3.3.1 Experimental animals

The male and female Swiss albino mice aged 5–6 weeks old (weighing 18–25 g) were used for both anti-inflammatory and analgesic test while adult Wistar albino rats of either sex and aged 2-4 months (115-120g body weight) were used for the antipyretic test. All experimental animals were housed in the animal house department of Biochemistry and Biotechnology of Kenyatta University. Ethical guidelines and procedures for handling experimental animals were adhered to according to the guidelines of Vogel, (2002). The animals were kept in cages at room temperature under standard laboratory conditions. They were fed on the standard pellet diet and water *ad libitum*. All the tests were carried out during daytime in a quiet laboratory setting with ambient illumination.

3.3.2 Determination of anti-inflammatory effects

Adult albino mice were grouped into six groups comprising 5 animals each. The Group I mice (normal control) was administered with 10% dimethyl sulfoxide (DMSO) (vehicle). Group II mice (negative control) was administered with 10% DMSO. Group III mice (positive control) received the standard drug (diclofenac sodium) at a dose of 15 mg/kg body weight. Groups IV, V and VI mice (experimental groups) were treated with DCM stem bark extract of *A. mellifera* at dose levels of 50, 100 and 150 mg/kg body weight respectively. Thirty minutes

post-treatment, inflammation was induced by injecting 0.05 ml of 1% (w/v) carrageenan into the sub-plantar region of the right hind paw of the mice in all groups except normal control group mice. This design is summarized in Table 3.1.

Table 3. 1: Treatment protocol for evaluation of anti-inflammatory activity of dichloromethane stem bark extract of *Acacia mellifera* in mice model

Animal groups	**Treatment**
I (Normal control)	10% DMSO
II (Negative control)	10% DMSO+ Carrageenan
III (Positive control)	Diclofenac sodium (15mg/kg bw) + Carrageenan
IV (Experimental A)	*A. mellifera* extract (50mg/kg bw) + Carrageenan
V (Experimental B)	*A. mellifera* extract (100mg/kg bw) +1 Carrageenan
VI (Experimental C)	*A. mellifera* extract (150mg/kg bw) + Carrageenan

Key: bw- body weight; 1% carrageenan (0.5ml)

The paw diameter of the rats was measured in mm using 27 SDC041 digital vernier calipers (Xuzhou Smile Trading Company Ltd., Jiangsu, China) and recorded before inflammation induction and at 1, 2, 3 and 4 hours following carrageenan administration. Paw edema Inhibitory activity was calculated according to the formula described by Jia *et al*. (2003).

$$\%\ \text{paw edema inhibition} = \frac{Ct - Tt}{Ct} \times 100$$

Where; Ct = Paw diameter at 1 hour after carrageenan administration (control)

Tt = Paw diameter after Treatment

3.3.3 Determination of the analgesic effect

Adult albino mice were divided into six groups of five animals each. Group I mice (normal control) was administered with 10% dimethyl sulfoxide (DMSO) only. Group II mice (negative control) was treated with 10% DMSO, while Group III mice (positive control) received the standard drug (diclofenac sodium) at a dose of 15mg/kg body weight. Groups IV, V, and VI mice were given DCM stem bark extract of *A. mellifera* at dose levels of 50, 100 and 150 mg/kg body weight respectively. Thirty minutes post-treatment, nociception was induced in mice by administration of 0.05 ml of 2.5% formalin into the left dorsal hind paw of mice in all groups except normal control group mice. This design is summarized in

Table 3.2. Treatment protocol for evaluation of antinoceptive activities of dichloromethane stem bark extract of *Acacia mellifera* in mice model

Animal groups	**Treatment**
I (Normal control)	10% DMSO
II (Negative control)	Formalin
III (Positive control)	Diclofenac sodium (15mg/kg bw) + Formalin
IV (Experimental A)	*A. mellifera* extract (50mg/kg bw) + Formalin
V (Experimental B)	*A. mellifera* extract (100mg/kg bw) + Formalin
VI (Experimental C)	*A. mellifera* extract (150mg/kg bw)+ Formalin

Key: bw-body weight; 2.5% formalin (0.5ml)

A mouse was individually placed in transparent glass chamber for 30 minutes before the experiment. The mice were then gently held, injected with formalin and returned to the glass chamber for observation. The duration the mice spent licking

or biting the injected paw was recorded according to the protocol described by Tjolsen *et al*. (1992). Two distinct periods (early and late phases) of intensive licking/biting activity were identified and recorded separately. The early phase was recorded 1-5 minutes after administering formalin and the late phase recorded 15-30 minutes after formalin injection. The percentage licking time inhibition was then calculated using the formula described by Dubuisson and Dennis (1977).

$$\%\ licking\ time\ Inhibition = \frac{C - T}{C} X100$$

Where; C–the vehicle-treated control group value for each phase

T–the treated group value for each phase

3.3.4 Determination of antipyretic activity

The experimental rats were grouped into six groups of five rats each. Group I (normal control) was administered with 10% DMSO only. Group II (negative control) had induced pyrexia and was administered with 10% DMSO. Group III (Positive control) had induced pyrexia and was administered with aspirin (100 mg/kg body weight). Groups IV, V and VI had induced pyrexia and received DCM stem bark extract of *A. mellifera* at dose levels of 50, 100 and 150 mg/kg body weight respectively. Pyrexia was induced in rats by administration of 20% turpentine. All the treatments were administered intraperitoneally. This design is summarized in Table 3.3.

Table 3.2: Treatment protocol for evaluation of antipyretic activities of dichloromethane stem bark extract of *Acacia mellifera* in rat model

Animal groups	Treatment
I (Normal control)	10% DMSO
II (Negative control)	Turpentine+10% DMSO
III (Positive control)	Turpentine +Aspirin (100mg/kg bw)
IV (Experimental A)	Turpentine +*A. mellifera* extract (50mg/kg bw)
V (Experimental B)	Turpentine +*A. mellifera* extract (100mg/kg bw)
VI (Experimental C)	Turpentine +*A. mellifera* extract (150mg/kg bw)

Key: bw- body weight; 20% turpentine

The rats had their body temperatures measured using a digital thermometer (Mode YB-009, Shenzhen Osykyoo Technology Co., Ltd, Guangdong, China) by inserting a well lubricated digital thermometer about 3cm into the rectum of the animals. Rats that showed an increase in rectal temperature of at least 0.8 ^{0}C were considered pyretic. One hour post-treatment, the body temperature for each rat in groups II-VI was recorded. The mean body temperature of Wistar albino rats was recorded at 20 minutes intervals over the first hour before turpentine injection and at hourly intervals up to the fourth hour after turpentine injection.

Rectal temperature before and after treatment was compared, the percentage change in rectal temperature was calculated using the formula described by Hukkeri *et al. (*2006) and Ray (2006).

$$\%\ change\ in\ rectal\ temperature = \frac{B - Cn}{B} \times 100$$

Where; B - Rectal temperature at 1 hour after turpentine administration

C_n - Rectal temperature after drug administration

3.4 Qualitative Phytochemical Screening

Preliminary qualitative phytochemical screening of the dichloromethane stem bark extract of *A. mellifera* was carried out to determine the class of secondary metabolites present using standard procedure according to Harbone (1998) and Kotake (2000). Active principles tested included flavonoids, phenolics, saponins, alkaloids, cardiac glycosides, steroids and terpenoids, as they are associated with anti-inflammatory, analgesic and antipyretic effects.

3.4.1 Alkaloids

Alkaloids were tested by acidification of 5 ml of the extract with 1M hydrochloric acid. The acidic medium was heated and a few drops of Dragendroff‘s reagent added. The development of an orange or reddish-brown precipitate was regarded as positive for the presence of alkaloids.

3.4.2 Saponins (Froth test)

The extract was tested for saponins by mixing 2ml of the extract with a few drops of sodium bicarbonate solution and shaken vigorously. The extract was then allowed to stand for 15 minutes. Froth formation indicated the presence of saponin.

3.4.3 Terpenoids (Salkowski test)

To test for terpenoids, 1 ml of ethyl acetate was added to 0.5 g of the extract, followed by 2 ml of chloroform and then shaken vigorously. Three ml of concentrated sulphuric acid was carefully added alongside to underlayer the mixture. The presence of a reddish brown coloration at the interface indicated the presence of terpenoids.

3.4.4 Flavonoids (Sodium hydroxide test)

The extract was screened for flavonoids by mixing 2 ml of the extract with 2 ml of diluted sodium hydroxide. An intense/golden yellow precipitate indicated positive results for flavonoids.

3.4.5 Cardiac glycosides (Keller-Kilian test)

Cardiac glycosides were tested by dissolving 0.5 g of the extract in 2 ml glacial acetic acid containing 2 drops of 10% ferric chloride solution. One ml of concentrated sulphuric acid was carefully added alongside to underlayer the mixture. A brown, violet or greenish ring at the interphase indicated the presence of cardiac glycoside.

3.4.6 Steroids

Presence of steroids was tested by dissolving 0.5 g of the extract in 2 ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added alongside the test tube to form an under layer. A reddish brown colour at the interface indicated the presence of a steroidal ring.

3.4.7 Phenols

The extract was screened for phenols by adding 1 ml of ferric chloride solution to 2 ml of the extract. Formation of blue to green colour indicated the presence of phenols.

3.5 Data collection and statistical analysis

Quantitative data on the licking time/latency of pain response, change in the diameter of the paw and change in rectal temperatures were obtained, recorded and tabulated on a broadsheet using Micro Soft excel. The raw data were subjected to descriptive statistics and expressed as the mean ± standard error of the mean (SEM). The quantitative data was then analyzed by Analysis of Variance (ANOVA) followed by Tukey's post hoc test for pairwise comparison and separation of means. Statistical significance was set at 95% confidence level ($P \leq 0.05$). Minitab statistical software package, version 17.0 (Minitab Inc., 2007) was used for data analysis. The results were presented in tables and graphs. The qualitative data on phytochemical screening was tabulated and presented in a table.

CHAPTER FOUR

RESULTS

4.1 Anti-inflammatory effects of DCM stem bark extract of *Acacia mellifera*

The anti-inflammatory activity of *A. mellifera* was determined on carrageenan-induced paw edema test in mice. The administration of the plant extract and diclofenac significantly reduced the paw edema as shown in Table 4.1 and Figure 4.1.

In the first hour of the test period, the extract doses of 50 and 100mg/kg body weight showed anti-inflammatory effects by reducing inflamed hind paw diameter by 1.59% and 3.72% respectively. However, the dose level of 150mg/kg body weight did not mount any anti-inflammatory effects at this hour (Figure 4.1; Table 4.1). The results indicated that there were no significant anti-inflammatory effects in the groups treated with the extract when compared to the control groups ($p>0.05$; Table 4.1).

Conversely, the three experimental doses of DCM stem bark extract of *A. mellifera* (50, 100 and 150mg/kg body weight) demonstrated anti-inflammatory activities by reducing the inflamed paw diameter by 4.39%, 5.40% and 2.76% respectively in the second hour (Figure 4.1 and Table 4.1). However, the anti-inflammatory effects exhibited by the three dose levels of the stem bark extract

were not significantly different from each other as well as to the normal and positive controls (p>0.05; Table 4.1).

Inflammation decreased in a reverse dose-dependent manner in the third hour. This trend persisted through to the fourth hour of the test period (Table 4.1; Figure 4.1). Treatment of the mice models with DCM stem bark extract of *A. mellifera*, at the doses of 50, 100 and 150 mg/kg body weight, reduced the paw edema diameter by 10.56%, 8.95% and 4.57% respectively (Table 4.1; Figure 4.1). Notably, the anti-inflammatory effects of the three dose levels did not vary from each other significantly and were comparable to the standard conventional drug (Diclofenac), which decreased the carrageenan-induced paw edema by 5.231% (p>0.05). The DCM stem bark extract of *A. mellifera*, at the dose level of 150 mg/kg body weight, reduced the paw edema diameter as to normal control group with comparable efficacy with Diclofenac (p>0.05; Table 4.1).

Results show that in the fourth hour, the DCM stem bark extract, at the three dose levels (50, 100 and 150 mg/kg body weight), reduced the paw diameter by 11.05%, 9.91% and 8.27% respectively (Table 4.1; Figure 4.1). Indeed, the three extract doses were as effective as the standard drug (p>0.05; Table 4.1).

Table 4. 1: Anti-inflammatory effects of DCM stem bark extract of *Acacia mellifera* on carrageenan-induced inflammation in mice models

Group	Treatment	1 hr	2 hr	3 hr	4 hr
Normal control	DMSO	99.91±0.09^{a} (0.09)	99.92±0.16^{ab} (0.08)	99.91±0.16^{bc} (0.09)	99.91±0.28^{b} (0.09)
Negative control	DMSO+ Carrageenan	101.72±0.69^{a} (-1.72)	102.81±0.61^{b} (-2.81)	103.25±0.91^{c} (-3.25)	104.68±1.40^{b} (-4.68)
Positive control	diclofenac + Carrageenan	99.91±0.63^{a} (0.10)	98.40±0.84^{ab} (1.61)	94.77±0.78^{ab} (5.23)	91.22±0.79^{a} (8.78)
Experimental A	*A. mellifera* 50mg/kg bw + Carrageenan	98.41±0.32^{a} (1.59)	95.61±1.61^{a} (4.39)	89.44±2.67^{a} (10.56)	88.95±2.74^{a} (11.05)
Experimental B	*A. mellifera* 100mg/kg bw + Carrageenan	96.28±2.28^{a} (3.72)	94.60±2.29^{a} (5.40)	91.05±1.82^{a} (8.95)	90.09±1.93^{a} (9.91)
Experimental C	*A. mellifera* 150mg/kg bw + Carrageenan	100.97±2.11^{a} (-0.97)	97.24±2.47^{ab} (2.76)	95.43±2.62^{ab} (4.57)	91.73±2.05^{a} (8.27)

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison was made within a column and values with different superscript are significantly different by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Figures in parenthesis indicate percentage paw edema inhibition. Carrageenan = 1%; DMSO acted as the vehicle; Diclofenac = 15 mg/kg.

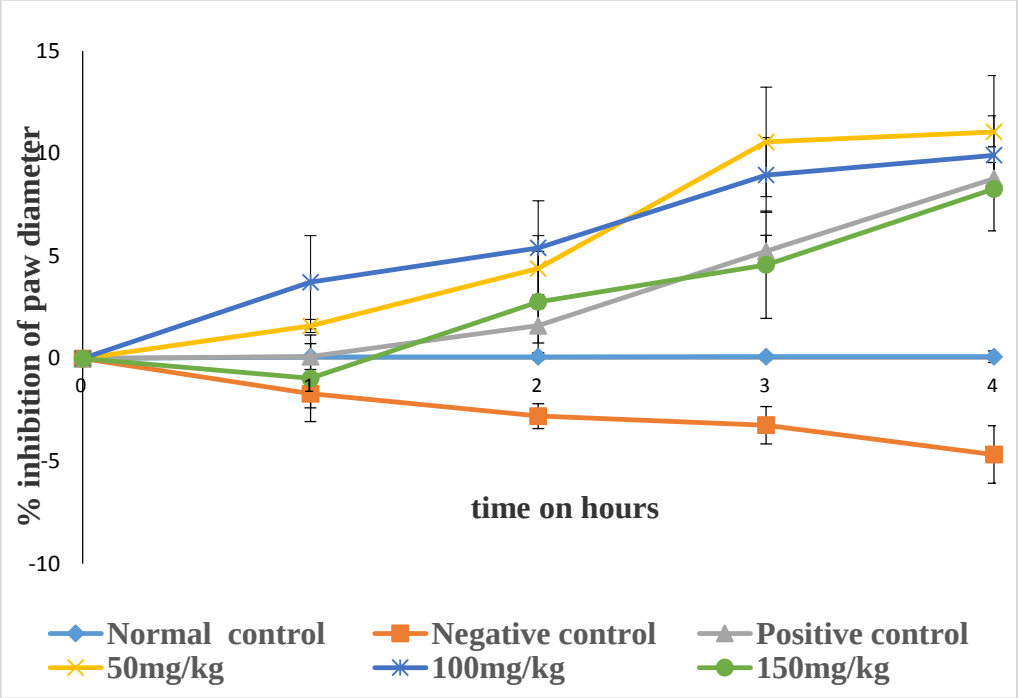

Figure 4.1: Percent paw edema inhibition by DCM stem bark extract of *Acacia mellifera* in carrageenan-induced inflammation in mice

4.2 Analgesic activity of dichloromethane stem bark extracts of *Acacia mellifera*

The formalin-induced paw licking model was used to study the analgesic effects of dichloromethane (DCM) stem bark extract of *A. mellifera* during the early and late phases. After inducing pain, the control group mice experienced pain throughout the experiment. The administration of the standard drug (diclofenac sodium) and the herbal extracts successfully reduced the formalin-induced pain in mice in both phases (Table 4.2).

In the early phase, the percentage inhibition of paw licking time upon administration of DCM stem bark extracts of *A. mellifera* at the three dose levels (50, 100 and 150 mg/kg body weight) was 48.26%, 1.38% and 22.96% respectively (Table 4.2; Figure 4.2). The analgesic effectiveness of the DCM stem bark extract at the dose levels of 100 and 150mg/kg body weight was statistically insignificant compared to the reference drug (diclofenac) *(p* >0.05; Table 4.2). In addition, the antinociceptive effectiveness of the two dose levels (100 and 150 mg/kg body weight) were significantly different compared to the extract at the dose of 50mg/kg body weight ($p < 0.05$; Table 4.2).

In the late phase, the DCM stem bark extracts of *A. mellifera,* at dose levels of 50, 100 and 150 mg/kg body weight, reduced formalin-induced pain in mice by 28.45%, 57.37% and 83.90% respectively (Table 4.2; Figure 4.2). The effectiveness of stem bark extract of *A. mellifera,* at the three dose levels (50, 100 and 150 mg/kg body weight), were all significantly different from each other ($p < 0.05$), with the highest tested dose (150 mg/kg bw) being as effective as the reference analgesic (Diclofenac) ($p < 0.05$; Table 4.2).

Table 4. 2: Analgesic effects of DCM stem bark extract of *Acacia mellifera* on formalin-induced pain in mice model

Group	Treatment	1 st phase	2nd phase
Normal control	DMSO	0.0±0.0[a] (100)	0.0±0.0[a] (100)
Negative control	DMSDO+ Formalin	100±0.0[a] (0.0)	100±0.0[e] (0.0)
Positive control	Diclofenac+ Formalin	87.80±7.43[c] (12.20)	19.80±2.27[b] (80.20)
Experimental	*A. mellifera* extract (50mg/kg bw)+ Formalin	51.74±3.33[b] (48.26)	71.55±1.72[d] (28.45)
Experimental	*A. mellifera* extract (100mg/kg bw)+ Formalin	98.62±7.91[c] (1.38)	42.63±2.52[c] (57.37)
Experimental	*A. mellifera* extract (150mg/kg bw)+ Formalin	77.04±6.13[c] (22.96)	16.10±1.93[b] (83.90)

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison was made within a column and values with different superscript are significantly different by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Figures in parenthesis indicate percent paw licking inhibition. Formalin = 2.5%; DMSO acted as the vehicle; Diclofenac = 15 mg/kg.

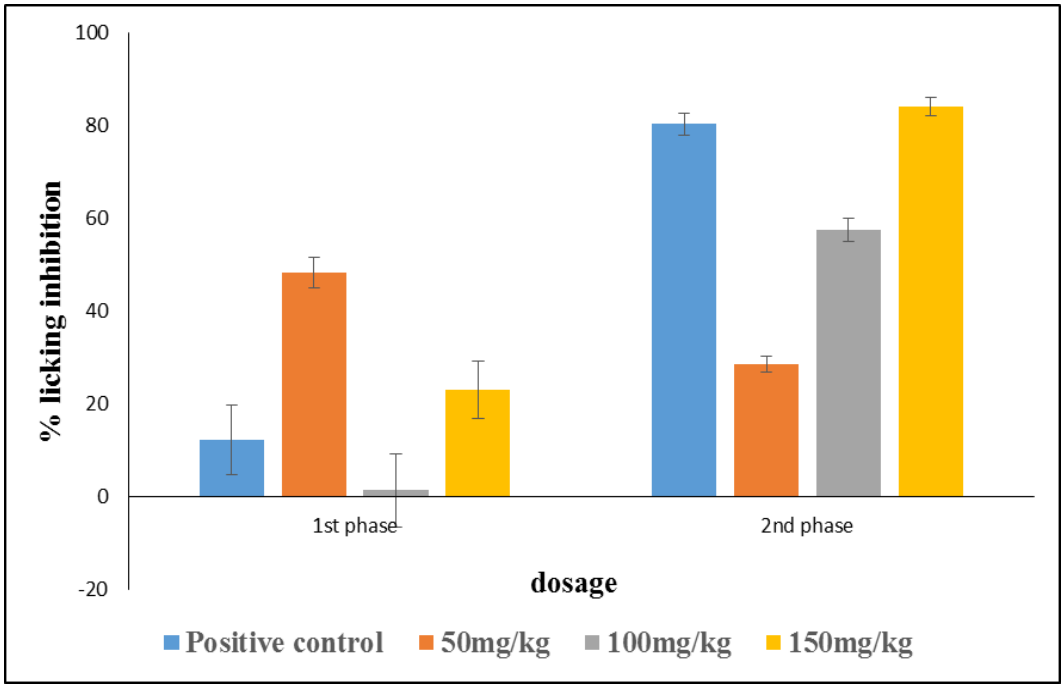

Figure 4.2: Percent paw licking inhibition by DCM stem bark extracts of *Acacia mellifera* in formalin-induced pain in mice.

4.3 Antipyretic activity of DCM stem bark extracts of *Acacia mellifera*

Antipyretic activity of DCM stem bark extract of *A. mellifera* was evaluated by turpentine-induced fever in rat model. Administration of the standard drug (aspirin) and the herbal extracts significantly reduced the rectal temperature in rats (Table 4.3).

During the first hour, the DCM stem bark extracts of *A. mellifera*, at a dose level of 100mg/kg body weight, decreased the elevated rectal temperature by 0.16% (Table 4.3; Figure 4.3). However, the other two doses of the extract did not exhibit any antipyretic activity at this hour (Table 4.3). The antipyretic effect of

the three doses of DCM stem bark extract of *A. mellifera* was not significantly different when compared to the three control groups ($p > 0.05$). Besides, the positive control was highly antipyretic at this hour (Table 4.3; Figure 4.3).

In the second hour, the DCM extract of *A. mellifera,* at all the three dose levels, demonstrated antipyretic action by decreasing the elevated rectal temperature by 1.58%, 1.03% and 2.38% respectively (Table 4.3; Figure 4.3). The antipyretic effectiveness of the herbal extract at the three dose levels was not significantly different from the positive control group ($p > 0.05$; Table 4.3). However, the antipyretic action of the extract at the doses of 50 and 100 mg/kg body weight was not significantly different from the normal control group ($p > 0.05$; Table 4.3).

In the third hour of the bioscreening period, the three doses of the extract lowered the turpentine-induced fever by 2.05%, 1.65% and 2.99% respectively (Table 4.3; Figure 4.3). The antipyretic activity of the three doses of the herbal extract was comparable to that of the positive control ($p >0.05$; Table 4.3). However, the antipyretic effectiveness of the herbal extract, at the dose level of 100 mg/kg body weight, was not significant compared to the normal control group ($p > 0.05$; Table 4.3).

A similar pattern was observed in the fourth hour with the three extract doses showing antipyretic action by decreasing the increased temperature by 2.61%, 3.24% and 3.95% respectively (Table 4.3; Figure 4.3). However, the antipyretic activities of the three doses of the extracts and aspirin were not significantly different (p <0.05; Table 4.3).

Table 4. 3: Effects of intraperitoneal administration of DCM stem bark extract of *Acacia mellifera* in turpentine-induced pyrexia in rat model

Groups	Treatment	1hr	2hr	3hr	4hr
Normal control	DMSO	100.06+0.22^{ab} (-0.06)	100.11+0.25^{bc} (-0.11)	100+0.1^{b} (-0.002)	99.90+0.14^{b} (0.11)
Negative control	Turpentine+ DMSO	101.53+0.84^{b} (-1.53)	102.02+0.73^{c} (-2.05)	102.25+0.49^{c} (-2.25)	101.42+0.76^{b} (-1.42)
Positive control	Turpentine+ Aspirin	98.49+0.19^{a} (1.52)	97.08+0.59^{a} (2.92)	96.61+0.41^{a} (3.39)	96.41+0.35^{a} (3.60)
Experimental A	Turpentine+ *A. mellifera* 50mg/kg bw	100.21+0.15^{ab} (-0.21)	98.418+0.37^{ab} (1.58)	97.96+0.36^{a} (2.05)	97.39+0.31^{a} (2.61)
Experimental B	Turpentine+ *A. mellifera* 100mg/kg bw	99.84+0.3^{ab} (0.16)	98.97+0.51^{ab} (1.03)	98.36+0.47^{ab} (1.65)	96.76+0.45^{a} (3.24)
Experimental C	Turpentine+ *A. mellifera* 150mg/kg bw	100.41+0.19^{b} (-0.41)	97.62+0.42^{a} (2.38)	97.01+0.57^{a} (2.99)	96.05+0.86^{a} (3.95)

Values are expressed as Mean ± SEM for five animals per group. Statistical comparison were made within a column and values with different superscript are significantly different by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Turpentine =20%; Aspirin = 100 mg/kg body weight and DMSO used as the vehicle.

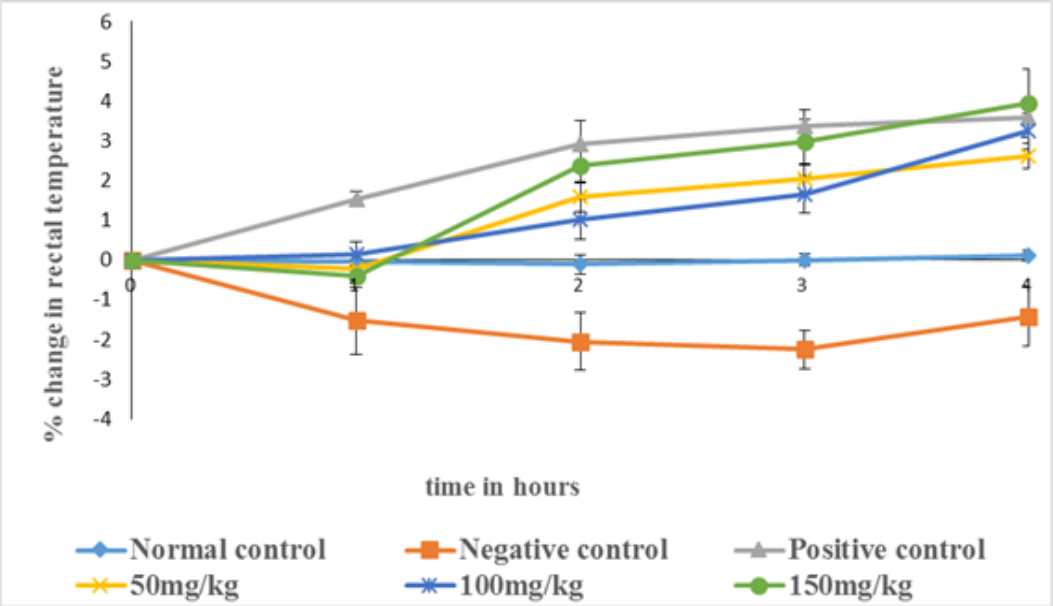

Figure 4. 3: The percent change in rectal temperature by DCM stem bark extracts of *Acacia mellifera* in turpentine-induced pyretic rats

4.4 Qualitative phytochemical screening

The secondary metabolites screening of the DCM stem bark extracts of *A. mellifera* revealed the presence of phytochemical compounds like alkaloids, cardiac glycosides, saponins, flavonoids, phenolics, terpenoids, and steroids (table 4.4).

Table 4. 4: Phytochemical composition of DCM stem bark extracts of Acacia mellifera

Phytochemicals	Presence/absence
Phenolics	+
Cardiac Glycosides	+
Alkaloids	+
Steroids	+
Terpenoids	+
Tannins	+
Saponins	+
Flavonoids	+

Key: Presence of phytochemical is denoted by (+) sign and absent of phytochemical is denoted by (-) sign

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Presently, drugs that are in use for the management of inflammation, pain and pyrexia are effective but possess many known side and toxic effects (Deghrigue *et al.*, 2015), hence, effort is ongoing to find alternative and complementary medicine for managing these condition. Herbal medicines have been extensively used to treat and manage various diseases globally due to its natural origin, availability, safety, cultural acceptability and stronger therapeutic activities with lesser side effects (Malviya *et al.*, 2011). This study was principally designed to scientifically support the traditional use of the stem bark of *Acacia mellifera* in the treatment of inflammation, pain and pyrexia.

The present study assessed the *in vivo* anti-inflammatory, analgesic and antipyretic effects of dichloromethane stem bark extract of *Acacia mellifera*. The anti-inflammatory activity of the plant extract was evaluated using carrageenan-induced paw edema test. Carrageenan-induced edema occurs in two phases (early and late phase). The early phase begins after the administration of the irritant and lasts for an hour. It is characterized by the release of serotonin, histamine, and cytokinins. The late phase occurs from the second to the fourth hour after the administration of the irritant and is ascribed to the release of prostaglandins,

oxygen-derived free radicals, lysosome enzymes and proteases (Asfar *et al.,* 2015; Samriti *et al.,* 2016).

The results showed that *A. mellifera* slightly inhibited inflammation in the early phase and significantly inhibited it in the late phase, with the highest anti-inflammatory effect of 3.72% and 11.05% respectively. The herbal extract's ability to reduce paw edema in both phases of the carrageenan-induced edema in mice suggests the involvement of the herbal extract's active principles in inhibiting the release or action of the early and late phase mediators of inflammation thereby suppressing edema.

The active principles with good anti-inflammatory potential include flavonoids, terpenoids, saponins, phenolics and tannins. Flavonoids and saponins act synergistically to reduce inflammation by inhibiting key enzymes such as cyclooxygenase, lipoxygenase and nitric oxide synthase important in the production of inflammatory mediators and metabolism of arachidonic acid (Ashfaq *et al.,* 2016). Moreover, the inflammatory process is accompanied by the production of free radicals (Samriti *et al.,* 2016) especially in the late phase of carrageenan test (Samanta *et al.,* 2013). Flavonoids, terpenoids, tannins and phenolics are antioxidant substances that scavenge these free radicals. In addition, tannins is involved in promoting wound healing (Abayomi *et al.,* 2013; Ghasemzadeh and Ghasemzadeh, 2011).

The extract in the first and second hour was less effective as compared to the third and fourth hour where it mounted pronounced anti-inflammatory activity. This suggests that the active components in the extract had to undergo biotransformation to have the anti-inflammatory activity or it took time for bioactive compounds to be absorbed across the peritoneum cavity.

Acacia mellifera demonstrated reverse dose-dependent anti-inflammatory response especially from the third hour, with the highest anti-inflammatory effect at the dose of 50 mg/kg body weight. The reverse dose-dependent activities may be due to saturation of the receptors with the active principles in the higher dose level or it takes a longer time to deliver the high dose across the peritoneum hence delaying its activity.

The reverse dose-dependent anti-inflammatory activity was observed in the methanolic extracts of *Sargassum swartzii* at 175 and 350 mg/kg body weight doses on carrageenan-induced edema in rats (Hong *et al.,* 2011). Moreover, dichloromethane: methanolic leaf extracts of *Maytenus obscura* at three dose levels (50, 100 and 150mg/kg body weight) demonstrated significant reverse dose-dependent anti-inflammatory effects on carrageenan-induced edema in mice (Mwangi *et al.,* 2015). Similar results were observed in work done by Anosike *et al*. (2012) on the methanolic extract of garden egg (*Solanum aethiopicum*) at dose

levels of 100, 200 and 400mg/kg body weight on egg albumin-induced edema in rats.

The dose levels of the extract used in the present study to evaluate the anti-inflammatory, analgesic and antipyretic activities of DCM stem bark extract of *Acacia mellifera* were 50, 100 and 150mg/kg bw and were similar to the dose levels used by Kamau *et al*. (2016), Mwangi *et al.* (2015), safari *et al.* (2016a), Afsar *et al*. (2015) and Mworia *et al.* (2015) These dose levels were chosen after carrying out a pilot study on a number of dosages.

Pretreatment used in carrageenan-induced edema test and formalin-induced paw licking test evaluated the prophylactic role of DCM stem bark extract of *A. mellifera* in the prevention of induced pain and inflammation (Bolegave *et al.,* 2015). It was also used to observe the effect of the extract on the phases of carrageenan-induced edema test and formalin-induced paw licking test though, post-treatment resembles a natural clinical condition (Chang *et al.*, 2012).

In this study, the analgesic properties were investigated on DCM stem bark extract of *A. mellifera* using formalin-induced paw licking test. Pain induced by formalin takes place in two distinct phases (Rezaee-Als *et al.*, 2014). The early phase begins immediately after formalin injection and last for five minutes, and is probably due to the activation of nerve fibers (nociceptors). The late phase is

initiated 15–30 minutes after formalin administration and is mainly mediated by histamine, prostaglandins, bradykinins and serotonin. Centrally acting analgesics inhibit both the early and late phases while peripheral drugs such as NSAIDs and steroids have the ability to interfere with the pain mediators in the late phase (Paschapur *et al.,* 2009; Adebayo *et al.,* 2014).

The DCM stem bark extract of *A. mellifera* exhibited the antinociceptive effect as it reduced formalin-induced paw licking time in both phases, with significant activity in the second phase. This phenomenon could be attributed to inhibition of either the synthesis and/or release of pain mediators or through nociceptor blockage by analgesic principles (Mahdi and Vihid-reza, 2008; Vyas *et al.,* 2016).

Plants containing organic acids, terpenoids, alkaloids, saponins and flavonoids are known to show significant analgesic activities. Flavonoids, saponins and alkaloids inhibit prostaglandins involved in pain perception (Salawu *et al.,* 2008; Sani *et al.,* 2013; Ashfaq *et al.,* 2016). Alkaloids also are known as the mood enhancer and can promote a sense of wellbeing (Gurib *et al.,* 2013). Moreover, terpenoids interfere with several steps of signal transduction mechanisms at the intracellular level and inhibiting platelet aggregation (Mworia *et al.,* 2015)

In the early phase, the stem bark extract of *A. mellifera* exhibited a non-dose dependent behavior and a dose-dependent effect in the late phase of the formalin-

induced paw licking test. The dose-dependent behavior could be due fast metabolism and clearance of the bioactive component in the lower dose or the lower dose had an insufficient concentration of the bioactive constituents. The non-dose dependent behavior may be due to the fact that some drugs exert their therapeutic effect over a limited range of drug dose or drug plasma concentration and if this dose range is exceeded, its activity reduces (Nthiga *et al.,* 2016b; Koech *et al.,* 2017).

A study carried out by Safari *et al.* (2016b) on the aqueous stem extract of *Cynanchum viminale* (L) showed that the extract had a dose-independent analgesic activity in both phases of formalin-induce pain in mice. However, a study done by Nthiga *et al.* (2016a) on antinociceptive activity of methanolic stem bark extract of *Harrisonia abyssinica* showed dose-dependent activity in both acute and chronic phases of the formalin-induced pain model in mice.

The stem bark extract of *A. mellifera* was also investigated for antipyretic effects using turpentine-induced pyrexia in rats. Turpentine is an exogenous pyrogen that induces fever indirectly by initiating the synthesis and release of endogenous pyrogens such as pro-inflammatory cytokines from the host phagocytic cells. These pyrogens, in turn, act by increasing the concentration of prostaglandins E_2 on the thermoregulatory center in the hypothalamus thus raising the core body temperature (Gitahi *et al.,* 2015).

The DCM stem bark extract of *A. mellifera* showed appreciable antipyretic activity after four hours of the test period, with 150 mg/kg body weight dose demonstrating the highest reduction in rectal temperature. The ability of the studied extract to reduce the elevated temperature suggests the ability of the bioactive principles in the extract to cross the brain blood barrier and inhibit prostaglandins biosynthesis or the active principles' ability to stimulate the body to produce its own antipyretic substances such as arginine vasopressin and glucocorticoids (Leon *et al.,* 1999; Nthiga *et al.,* 2016b).

Steroids, tannins, alkaloids, flavonoids, saponins and terpenoids are associated with a good antipyretic activity. Steroids, tannins, alkaloids and terpenoids are predominant inhibitors of PG synthase while flavonoids inhibits arachidonic acid peroxidation, it also inhibit the production of tumor necrosis factor-α, which stimulates the synthesis of PGE_2 necessary for fever induction. In addition, saponins inhibit the enzymes cyclooxygenase and phospholipase A_2 which are involved in the development of pyrexia (Kumar *et al.,* 2015; Ashfaq *et al.,* 2016; Kamau *et al.,* 2016).

The stem bark extract of *A. mellifera* reduced the elevated temperature in a time and dose-dependent manner. These observations could be due to the fact the lower dose had an insufficient concentration of the bioactive constituents. In

addition, the time-dependent activity could be attributed to the fact that it took time for bioactive compounds to be absorbed across the peritoneum cavity thereby causing antipyretic activities (Koech *et al.,* 2017).

The dose and time-dependent activity exhibited by the stem bark extract of *A. mellifera* are consistent with a study on the methanolic and ethyl acetate extracts of *Acacia hydaspica* on brewer's yeast-induced pyrexia in Sprague Dawley rats (Afsar *et al.,* 2015). The dichloromethane: methanolic root bark extracts of *Carissa edulis* showed a dose-dependent antipyretic activity on turpentine-induced pyrexia in rats (Gitahi *et al.*, 2015). Similarly, dose-dependent antipyretic activities were observed in methanolic extracts of *Kigelia africana* and *Acacia hockii* on turpentine-induced pyrexia in rats (Kamau *et al.,* 2016).

Non-steroidal anti-inflammatory drugs such as aspirin are used to treat and manage fever in routine practice. Aspirin exerts its antipyretic activity by inhibiting both cyclooxygenase and prostaglandin synthase enzyme involved in PGE2 biosynthesis within the hypothalamus (Gege-Adebayo *et al.,* 2013). The DCM stem bark extract of *A. mellifera* was as effective as aspirin in this study, thus suggesting mimicry of aspirin action by the bioactive components of the extract. A study done by Elgorashi *et al.* (2009) on some *acacia* species showed that the DCM stem bark extract of *A. nubica, A. nilotica* and *A. senegal* had high selective COX-2 inhibition properties. Therefore, it is possible that the action of

the DCM extract of *A. mellifera* may be due to the inhibition of cyclooxygenase and/or prostaglandin synthase, but other possible mechanisms for blocking fever cannot be ruled out.

The therapeutic benefits of the traditional medicine are believed to be due to a combination of active principles (Agbaje and Ajidahum, 2011). The qualitative phytochemical screening of dichloromethane stem bark extract of *A. mellifera* indicated the presence of flavonoids, steroids, alkaloids, saponins, cardiac glycosides, phenolics, terpenoids and tannins. These phytochemicals are associated with good anti-inflammatory, analgesic and antipyretic potential (Salawu *et al.*, 2008; Ghasemzadeh and Ghasemzadeh, 2011; Sani *et al.*, 2013; Kumar *et al.*, 2015; Ashfaq *et al.*, 2016; Mworia *et al.*, 2015;). Therefore, the anti-inflammatory, analgesic and antipyretic activities of the stem bark extract of *A. mellifera* could be due to the overall effect of the plant constituents. Indeed, it is not a wonder that the stem bark extract of *A. mellifera* is used traditionally by the Embu and Mbeere communities to treat and manage inflammation, pain and pyrexia.

5.2 Conclusions

In conclusion, the dichloromethane (DCM) stem bark extract of *Acacia mellifera* showed anti-inflammatory, antinociceptive, and antipyretic activities in animal models used, thereby establishing a pharmacological basis for its use in pain, inflammation and pyrexia treatment in folk medicine. In all the three doses used,

the stem bark extract of *A. mellifera* displayed significant anti-inflammatory effect in acute inflammation and significantly inhibited pain sensation through both peripheral and central mechanisms. Moreover, the herbal extract also exhibited appreciable antipyretic effects.

On the other hand, the classes of phytochemicals found in *A. mellifera* stem bark extract are known to contribute to anti-inflammatory, antinociceptive and antipyretic activities. It may also serve as a more effective alternative and complementary treatment strategy to the conventional interventions. Therefore, this study scientifically supports the traditional use of *Acacia mellifera* for management of inflammation, pain and fever. The research questions in the present study have affirmatively been answered.

5.3.1 Recommendations from the study

The extract of *Acacia mellifera* may be used as an alternative and complementary candidate for the development of effective anti-inflammatory, analgesic and antipyretic agent.

5.3.2 Suggestions/recommendations for further research

i. Bioscreening of *Acacia mellifera* plant to isolate and identify the compounds with anti-inflammatory, analgesic and antipyretic activities. This may lead to the discovery of compounds that might be used as lead compounds in the discovery of anti-inflammatory, analgesic and antipyretic agents.

ii. Elucidate the possible mechanism of anti-inflammatory, antinociceptive and antipyretic actions of stem bark extracts of *A. mellifera*. This will create a major break-through in the management of inflammation, pain and fever.

iii. Bioscreening of aqueous extracts of *A. mellifera* to establish how they compare with organic extracts in terms of anti-inflammatory, analgesic and antipyretic activities.

iv. Use of an alternative route in the administration of the extract other than intraperitoneally. The data from both routes of the administration can be compared in the management of inflammation, pain and fever. In addition, this will relieve the animal of the pain and inflammation associated with parenteral administration of the extracts.

v. Evaluation of acute and chronic toxicity to determine the safety of the extract in animal models.

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APPENDICES

Appendix I Effects of DCM stem bark extract of Acacia mellifera on carrageenan-induced inflammation in mice

paw diameter (mm)						
Group	**Treatment**	**0hr**	**1hr**	**2hr**	**3hr**	**4hr**
Normal Control	DMSO	2.42+0.04^{c}	2.42+0.04^{b}	2.42+0.04^{b}	2.42+0.04^{c}	2.42+0.05^{c}
Negative Control	DMSO+ Carrageenan	3.45+0.05^{ab}	3.51+0.04^{a}	3.54+0.05^{a}	3.56+0.06^{a}	3.61+0.05^{a}
Positive Control	diclofenac + Carrageenan	3.23+0.1^{b}	3.23+0.09^{a}	3.18+0.08^{a}	3.06+0.09^{b}	2.95+0.09^{b}
Experimental group A	*A. mellifera* extract (50mg/kg bw)+ Carrageenan	3.31+0.14^{ab}	3.25+0.15^{a}	3.16+0.15^{a}	2.964+0.19^{b}	2.95+0.19^{b}
Experimental group B	*A. mellifera* extract (100mg/kg bw)+ Carrageenan	3.68+0.08^{a}	3.54+0.13^{a}	3.48+0.13^{a}	3.35+0.11^{ab}	3.31+0.10^{ab}
Experimental group C	*A. mellifera* extract (150mg/kg bw)+ Carrageenan	3.54+0.157^{ab}	3.56+0.08^{a}	3.43+0.06^{a}	3.37+0.06^{ab}	3.24+0.08^{ab}

Appendix II Effects of DCM stem bark extract of Acacia mellifera on formalin-induced pain in mice

Paw licking time in seconds			
Group	Treatment	1st phase	2nd phase
Normal Control	DMSO	0.00+0.00[d]	0.00+0.00[e]
Negative Control	DMSO+ Formalin	115.0+6.24[a]	186.2+3.44[a]
Positive Control	Diclofenac+ Formalin	99.2+3.67[ab]	86.8+4.04[d]
Experimental group A	*A. mellifera* extract (50mg/kg bw)+ Formalin	59.4+4.70[c]	133.2+3.75[b]
Experimental group B	*A. mellifera* extract (100mg/kg bw)+ Formalin	111.6+3.80[a]	79.4+5.09[c]
Experimental group C	*A. mellifera* extract (150mg/kg bw)+ Formalin	87.4+4.01[b]	30.0+3.74[d]

Appendix III Effects of DCM stem bark extract of Acacia mellifera on turpentine-induced pyrexia in rats

Rectal temperature in ^{o}C						
Group	Treatment	0hr	1hr	2hr	3hr	4hr
Normal Control	DMSO	37.22+0.21^{c}	37.24+0.19^{c}	37.26+0.18^{b}	37.22+0.19^{c}	37.18+0.17^{bc}
Negative Control	Turpentine+DMSO	38.38+0.28^{b}	38.96+0.23^{ab}	39.16+0.20^{a}	39.24+0.17^{a}	38.92+0.27^{a}
Positive Control	Turpentine+ Aspirin	38.38+0.13^{b}	37.80+0.16^{c}	37.26+0.23^{b}	37.08+0.17^{c}	37.0+0.17^{c}
Experimental group A	Turpentine+ *A. mellifera* extract (50mg/kg bw)	39.40+0.19^{a}	39.56+0.13^{a}	38.46+0.19^{a}	38.22+0.18^{b}	37.84+0.29^{bc}
Experimental group B	Turpentine+ *A. mellifera* extract (100mg/kg bw)	38.86+0.08^{ab}	38.80+0.17^{b}	38.46+0.15^{a}	38.22+0.17^{b}	37.60+0.1^{bc}
Experimental group C	Turpentine+ *A. mellifera* extract (150mg/kg bw)	39.06+0.16^{ab}	39.14+0.14^{ab}	38.44+0.10^{a}	38.26+0.16^{b}	38.04+0.13^{ab}

Appendix IV Analysis of anti-inflammatory effects of DCM stem bark extract of Acacia mellifera on carrageenan-induced inflammation in mice

Descriptive Statistics: 0 hr, 1st hr, 2nd hr, 3rd hr, 4th hr

Variable	group	Mean	SE Mean
0 hr	100mg/kg	3.6760	0.0792
	150 mg/kg	3.536	0.105
	50mg/kg	3.306	0.146
	negative control	3.4480	0.0524
	normal control	2.4180	0.0427
	positive control	3.2320	0.0964
1st hr	100mg/kg	3.542	0.133
	150 mg/kg	3.5640	0.0770
	50mg/kg	3.254	0.147
	negative control	3.5060	0.0363
	normal control	2.4160	0.0435
	positive control	3.2280	0.0904
2nd hr	100mg/kg	3.480	0.131
	150 mg/kg	3.4300	0.0594
	50mg/kg	3.162	0.154
	negative control	3.5440	0.0451
	normal control	2.4160	0.0425
	positive control	3.1780	0.0796
3rd hr	100mg/kg	3.348	0.105
	150 mg/kg	3.3660	0.0647
	50mg/kg	2.964	0.188
	negative control	3.5600	0.0620
	normal control	2.4160	0.0448
	positive control	3.0620	0.0865

4th hr	100mg/kg	3.312	0.103
	150 mg/kg	3.2380	0.0752
	50mg/kg	2.948	0.189
	negative control	3.6080	0.0526
	normal control	2.4160	0.0458

One-way ANOVA: 0 hr versus group

Factor Information

Factor	Levels	Values
group	6	100mg/kg, 150 mg/kg, 50mg/kg, negative control, normal control, positive control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
group	5	4.980	0.99592	22.69	0.000
Error	24	1.053	0.04389		
Total	29	6.033			

Means

group	N	Mean	StDev	95% CI
100mg/kg	5	3.6760	0.1770	(3.4826, 3.8694)
150 mg/kg	5	3.536	0.235	(3.343, 3.729)
50mg/kg	5	3.306	0.328	(3.113, 3.499)
negative control	5	3.4480	0.1171	(3.2546, 3.6414)
normal control	5	2.4180	0.0955	(2.2246, 2.6114)
positive control	5	3.2320	0.2156	(3.0386, 3.4254)

Pooled StDev = 0.209503

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

group	N	Mean	Grouping
100mg/kg	5	3.6760	A
150 mg/kg	5	3.536	A B
negative control	5	3.4480	A B
50mg/kg	5	3.306	A B
positive control	5	3.2320	B
normal control	5	2.4180	C

Means that do not share a letter are significantly different.

One-way ANOVA: 1st hr versus group

```
Factor Information

Factor  Levels  Values
group        6  100mg/kg, 150 mg/kg, 50mg/kg, negative control, normal
control, positive
                control


Analysis of Variance

Source  DF  Adj SS   Adj MS  F-Value  P-Value
group    5   4.727  0.94544    20.03    0.000
Error   24   1.133  0.04721
Total   29   5.860


Means

group             N    Mean   StDev       95% CI
100mg/kg          5   3.542   0.298  ( 3.341,  3.743)
150 mg/kg         5  3.5640  0.1723  (3.3635, 3.7645)
50mg/kg           5   3.254   0.329  ( 3.053,  3.455)
negative control  5  3.5060  0.0811  (3.3055, 3.7065)
normal control    5  2.4160  0.0974  (2.2155, 2.6165)
positive control  5  3.2280  0.2020  (3.0275, 3.4285)

Pooled StDev = 0.217279
```

Tukey Pairwise Comparisons

```
Grouping Information Using the Tukey Method and 95% Confidence

group             N    Mean  Grouping
150 mg/kg         5  3.5640  A
100mg/kg          5   3.542  A
negative control  5  3.5060  A
50mg/kg           5   3.254  A
positive control  5  3.2280  A
normal control    5  2.4160    B

Means that do not share a letter are significantly different.
```

One-way ANOVA: 2nd hr versus group

```
Factor Information

Factor  Levels  Values
group        6  100mg/kg, 150 mg/kg, 50mg/kg, negative control, normal
control, positive
                control
```

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
group	5	4.331	0.86620	19.02	0.000
Error	24	1.093	0.04554		
Total	29	5.424			

Means

group	N	Mean	StDev	95% CI
100mg/kg	5	3.480	0.294	(3.283, 3.677)
150 mg/kg	5	3.4300	0.1329	(3.2330, 3.6270)
50mg/kg	5	3.162	0.344	(2.965, 3.359)
negative control	5	3.5440	0.1009	(3.3470, 3.7410)
normal control	5	2.4160	0.0950	(2.2190, 2.6130)
positive control	5	3.1780	0.1781	(2.9810, 3.3750)

Pooled StDev = 0.213405

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

group	N	Mean	Grouping
negative control	5	3.5440	A
100mg/kg	5	3.480	A
150 mg/kg	5	3.4300	A
positive control	5	3.1780	A
50mg/kg	5	3.162	A
normal control	5	2.4160	B

Means that do not share a letter are significantly different.

One-way ANOVA: 3rd hr versus group

Factor Information

Factor	Levels	Values
group	6	100mg/kg, 150 mg/kg, 50mg/kg, negative control, normal control, positive control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
group	5	4.147	0.82941	15.57	0.000
Error	24	1.278	0.05326		
Total	29	5.425			

Means

group	N	Mean	StDev	95% CI
100mg/kg	5	3.348	0.236	(3.135, 3.561)
150 mg/kg	5	3.3660	0.1447	(3.1530, 3.5790)
50mg/kg	5	2.964	0.420	(2.751, 3.177)
negative control	5	3.5600	0.1387	(3.3470, 3.7730)
normal control	5	2.4160	0.1001	(2.2030, 2.6290)
positive control	5	3.0620	0.1933	(2.8490, 3.2750)

Pooled StDev = 0.230770

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

group	N	Mean	Grouping
negative control	5	3.5600	A
150 mg/kg	5	3.3660	A B
100mg/kg	5	3.348	A B
positive control	5	3.0620	B
50mg/kg	5	2.964	B
normal control	5	2.4160	C

Means that do not share a letter are significantly different.

One-way ANOVA: 4th hr versus group

Factor Information

Factor	Levels	Values
group	6	100mg/kg, 150 mg/kg, 50mg/kg, negative control, normal control, positive control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
group	5	4.166	0.83330	15.45	0.000
Error	24	1.294	0.05392		
Total	29	5.461			

Means

group	N	Mean	StDev	95% CI
100mg/kg	5	3.312	0.229	(3.098, 3.526)
150 mg/kg	5	3.2380	0.1681	(3.0237, 3.4523)
50mg/kg	5	2.948	0.423	(2.734, 3.162)
negative control	5	3.6080	0.1176	(3.3937, 3.8223)
normal control	5	2.4160	0.1024	(2.2017, 2.6303)
positive control	5	2.9480	0.1985	(2.7337, 3.1623)

Pooled StDev = 0.232210

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

group	N	Mean	Grouping
negative control	5	3.6080	A
100mg/kg	5	3.312	A B
150 mg/kg	5	3.2380	A B
positive control	5	2.9480	B
50mg/kg	5	2.948	B
normal control	5	2.4160	C

Means that do not share a letter are significantly different.

Appendix V Analysis of antinociceptive effects of DCM stem bark extract of Acacia mellifera on formalin-induced pain in mice

Descriptive Statistics: phase 1

Variable	group	Mean	SE Mean
phase 1	100mg/kg	111.60	3.80
	150 mg/kg	87.40	4.01
	50mg/kg	59.40	4.70
	negative control	115.00	6.24
	normal control	0.000000	0.000000
	positive control	99.20	3.67

Descriptive Statistics: phase2

Variable	group	Mean	SE Mean
phase2	100mg/kg	79.40	5.09
	150 mg/kg	30.00	3.74
	50mg/kg	133.20	3.75
	negative control	186.20	3.44
	normal control	0.000000	0.000000
	positive control	36.80	4.04

One-way ANOVA: phase 1 versus group

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
group	5	47311	9462.19	108.12	0.000
Error	24	2100	87.52		
Total	29	49411			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
9.35503	95.75%	94.86%	93.36%

Means

group	N	Mean	StDev	95% CI
100mg/kg	5	111.60	8.50	(102.97, 120.23)
150 mg/kg	5	87.40	8.96	(78.77, 96.03)
50mg/kg	5	59.40	10.50	(50.77, 68.03)
negative control	5	115.00	13.96	(106.37, 123.63)
normal control	5	0.000000	0.000000	(-8.634729, 8.634729)
positive control	5	99.20	8.20	(90.57, 107.83)

Pooled StDev = 9.35503

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

group	N	Mean	Grouping
negative control	5	115.00	A
100mg/kg	5	111.60	A
positive control	5	99.20	A B
150 mg/kg	5	87.40	B
50mg/kg	5	59.40	C
normal control	5	0.000000	D

Means that do not share a letter are significantly different.

One-way ANOVA: phase2 versus group

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
group	5	124204	24840.7	363.17	0.000
Error	24	1642	68.4		
Total	29	125845			

Means

group	N	Mean	StDev	95% CI
100mg/kg	5	79.40	11.37	(71.77, 87.03)
150 mg/kg	5	30.00	8.37	(22.37, 37.63)
50mg/kg	5	133.20	8.38	(125.57, 140.83)
negative control	5	186.20	7.69	(178.57, 193.83)
normal control	5	0.000000	0.000000	(-7.633635, 7.633635)
positive control	5	36.80	9.04	(29.17, 44.43)

Pooled StDev = 8.27043

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

group	N	Mean	Grouping
negative control	5	186.20	A
50mg/kg	5	133.20	B
100mg/kg	5	79.40	C
positive control	5	36.80	D
150 mg/kg	5	30.00	D
normal control	5	0.000000	E

Means that do not share a letter are significantly different.

Appendix VI Analysis of antipyretic effects of DCM stem bark of Acacia mellifera on turpentine-induced pyrexia in rats

Descriptive Statistics: 0hr, 1st hr, 2nd hr, 3rd hr, 4th hr,

Variable	group	Mean	SE Mean	StDev
0hr	100mg/kg	38.860	0.0812	0.182
	150 mg/kg	39.060	0.163	0.365
	50mg/kg	39.400	0.192	0.430
	negative control	38.380	0.282	0.630
	normal control	37.220	0.211	0.471
	positive control	38.380	0.128	0.286
1st hr	100mg/kg	38.800	0.170	0.381
	150 mg/kg	39.140	0.144	0.321
	50mg/kg	39.560	0.129	0.288
	negative control	38.960	0.229	0.513
	normal control	37.240	0.194	0.434
	positive control	37.800	0.158	0.354
2nd hr	100mg/kg	38.460	0.154	0.344
	150 mg/kg	38.440	0.103	0.230
	50mg/kg	38.460	0.189	0.422
	negative control	39.160	0.204	0.456
	normal control	37.260	0.175	0.391
	positive control	37.260	0.229	0.513
3rd hr	100mg/kg	38.220	0.166	0.370
	150 mg/kg	38.260	0.157	0.351
	50mg/kg	38.220	0.180	0.402
	negative control	39.240	0.172	0.385
	normal control	37.220	0.185	0.415
	positive control	37.080	0.171	0.383
4th hr	100mg/kg	37.600	0.100	0.224
	150 mg/kg	38.040	0.133	0.297
	50mg/kg	37.840	0.294	0.658
	negative control	38.920	0.273	0.610
	normal control	37.180	0.169	0.377
	positive control	37.000	0.170	0.381

One-way ANOVA: 0hr versus group

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
group	5	14.527	2.9054	16.57	0.000
Error	24	4.208	0.1753		
Total	29	18.735			

Means

group	N	Mean	StDev	95% CI
100mg/kg	5	38.8600	0.1817	(38.4735, 39.2465)
150 mg/kg	5	39.060	0.365	(38.674, 39.446)
50mg/kg	5	39.400	0.430	(39.014, 39.786)
negative control	5	38.380	0.630	(37.994, 38.766)
normal control	5	37.220	0.471	(36.834, 37.606)
positive control	5	38.380	0.286	(37.994, 38.766)

Pooled StDev = 0.418728

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

group	N	Mean	Grouping
50mg/kg	5	39.400	A
150 mg/kg	5	39.060	A B
100mg/kg	5	38.8600	A B
positive control	5	38.380	B
negative control	5	38.380	B
normal control	5	37.220	C

Means that do not share a letter are significantly different.

One-way ANOVA: 1st hr versus group

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
group	5	19.354	3.8707	25.61	0.000
Error	24	3.628	0.1512		
Total	29	22.982			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.388802	84.21%	80.92%	75.33%

Means

group	N	Mean	StDev	95% CI
100mg/kg	5	38.800	0.381	(38.441, 39.159)
150 mg/kg	5	39.140	0.321	(38.781, 39.499)
50mg/kg	5	39.560	0.288	(39.201, 39.919)
negative control	5	38.960	0.513	(38.601, 39.319)
normal control	5	37.240	0.434	(36.881, 37.599)
positive control	5	37.800	0.354	(37.441, 38.159)

Pooled StDev = 0.388802

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

group	N	Mean	Grouping
50mg/kg	5	39.560	A
150 mg/kg	5	39.140	A B
negative control	5	38.960	A B
100mg/kg	5	38.800	B
positive control	5	37.800	C
normal control	5	37.240	C

Means that do not share a letter are significantly different.

One-way ANOVA: 2nd hr versus group

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
group	5	14.387	2.8773	17.74	0.000
Error	24	3.892	0.1622		
Total	29	18.279			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.402699	78.71%	74.27%	66.73%

Means

group	N	Mean	StDev	95% CI
100mg/kg	5	38.460	0.344	(38.088, 38.832)
150 mg/kg	5	38.440	0.230	(38.068, 38.812)
50mg/kg	5	38.460	0.422	(38.088, 38.832)
negative control	5	39.160	0.456	(38.788, 39.532)
normal control	5	37.260	0.391	(36.888, 37.632)
positive control	5	37.260	0.513	(36.888, 37.632)

Pooled StDev = 0.402699

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

group	N	Mean	Grouping
negative control	5	39.160	A
50mg/kg	5	38.460	A
100mg/kg	5	38.460	A
150 mg/kg	5	38.440	A
positive control	5	37.260	B
normal control	5	37.260	B

Means that do not share a letter are significantly different.

One-way ANOVA: 3rd hr versus group

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
group	5	15.736	3.1472	21.24	0.000
Error	24	3.556	0.1482		
Total	29	19.292			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.384924	81.57%	77.73%	71.20%

Means

group	N	Mean	StDev	95% CI
100mg/kg	5	38.220	0.370	(37.865, 38.575)
150 mg/kg	5	38.260	0.351	(37.905, 38.615)
50mg/kg	5	38.220	0.402	(37.865, 38.575)
negative control	5	39.240	0.385	(38.885, 39.595)
normal control	5	37.220	0.415	(36.865, 37.575)
positive control	5	37.080	0.383	(36.725, 37.435)

Pooled StDev = 0.384924

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

group	N	Mean	Grouping
negative control	5	39.240	A
150 mg/kg	5	38.260	B
100mg/kg	5	38.220	B
50mg/kg	5	38.220	B
normal control	5	37.220	C
positive control	5	37.080	C

Means that do not share a letter are significantly different.

One-way ANOVA: 4th hr versus group

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
group	5	11.850	2.3699	11.56	0.000
Error	24	4.920	0.2050		
Total	29	16.770			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.452769	70.66%	64.55%	54.16%

Means

group	N	Mean	StDev	95% CI
100mg/kg	5	37.600	0.224	(37.182, 38.018)
150 mg/kg	5	38.040	0.297	(37.622, 38.458)
50mg/kg	5	37.840	0.658	(37.422, 38.258)
negative control	5	38.920	0.610	(38.502, 39.338)
normal control	5	37.180	0.377	(36.762, 37.598)
positive control	5	37.000	0.381	(36.582, 37.418)

Pooled StDev = 0.452769

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

group	N	Mean	Grouping
negative control	5	38.920	A
150 mg/kg	5	38.040	A B
50mg/kg	5	37.840	B C
100mg/kg	5	37.600	B C
normal control	5	37.180	B C
positive control	5	37.000	C

Means that do not share a letter are significantly different.

Appendix: V Research Clearance Permit

THIS IS TO CERTIFY THAT:
MISS. VERONICA AKUMU SINDANI
of KENYATTA UNIVERSITY, 63036-200
nairobi,has been permitted to conduct
research in *Nairobi County*

on the topic: *IN VIVO ANTIINFLAMMATORY, ANALGESIC AND ANTIPYRETIC EFFECTS OF DICHLOROMETHANE STEM BARK EXTRACT OF ACACIA MELLIFERA(VAHL) BENTH*

for the period ending:
15th December,2017

.....
Applicant's Signature

Permit No : NACOSTI/P/16/07046/14136
Date Of Issue : 16th December,2016
Fee Recieved :Ksh 1000

.....
Director General
National Commission for Science, Technology & Innovation

CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit.
2. Government Officer will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one (1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

REPUBLIC OF KENYA

National Commission for Science, Technology and Innovation

RESEACH CLEARANCE PERMIT

Serial No.A 12398

CONDITIONS: see back page

Appendix: VI Research Permit Letter

NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION

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when replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/16/07046/14136**

Date:
16th December, 2016

Veronica Akumu Sindani
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"In vivo antiinflammatory, analgesic and antipyretic effects of dichloromethane stem bark extract of acacia mellifera(vahl) benth,"*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi County** for the period ending **15th December, 2017.**

You are advised to report to **the County Commissioner and the County Director of Education, Nairobi County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nairobi County.

The County Director of Education
Nairobi County.