

**ANTINOCICEPTIVE ACTIVITIES OF ACETONE LEAVES EXTRACTS
OF *Carissa spinarum* AND *Caesalpinia volkensii* IN MICE**

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Declaration

I, Joseph Kiambi Mworira, duly 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 wife Esther kinya, my Mum Zipporah Mworira and my children Gakii Neema and Victor Munene for their sacrifice towards my education.

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Table of contents

DECLARATION	II
DEDICATION	III
ACKNOWLEDGEMENT	IV
TABLE OF CONTENTS	V
LIST OF FIGURES	VII
LIST OF TABLES	VIII
LIST OF APPENDICES	IX
ABBREVIATIONS AND ACRONYMS	X
ABSTRACT	XI
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background information.....	1
1.2 Problem statement	3
1.3 Justification.....	4
1.4 Null hypothesis	5
1.5 Objectives	5
1.5.1 Main objective	5
1.5.2 Specific objectives	5
CHAPTER TWO	6
LITERATURE REVIEW	6
2.1 Description of pain	6
2.2 Biochemical and physiological basis of pain	7
2.3 Modulation of pain	8
2.3.1 The Gate Control Theory	8
2.3.2 Experimental pain induction	10
2.4 Conventional management of pain	12
2.5 Role of herbal and complementary medicine in pain management.	15
2.6 Plants used in this study	17
2.6.1 <i>Carissa spinarum</i> (Linn) and <i>Caesalpinia volkensii</i> (Harms)	17
CHAPTER THREE	21
MATERIALS AND METHODS	21
3.1 Collection and preparation of plant materials	21
3.2 Extraction	21
3.3 Experimental animals	22
3.4 Experimental design	22
3.4.1 Formalin-induced antinociceptive assay.....	22
3.4.2 Acetic-acid induced antinociceptive assay	24
3.5 Qualitative phytochemical screening	24
3.5.1 Test for alkaloids	25
3.5.2 Test for flavonoids	25

3.5.3 Test for steroids	25
3.5.4 Test for saponins	25
3.5.5 Test for cardiac glycosides	26
3.5.6 Test for phenolics.....	26
3.5.7 Test for terpenoids	26
3.6 Data management and statistical analysis	26
CHAPTER FOUR	28
RESULTS.....	28
4.1 Antinociceptive activities of acetone extracts on formalin induced pain.....	28
4.2 Antinociceptive effects of acetone extracts on acetic acid induced pain	32
4.3 Phytochemical screening	35
CHAPTER FIVE	37
DISCUSSION, CONCLUSION AND RECOMMENDATIONS	37
5.1 Discussion.....	37
5.2 Conclusions	40
5.3 Recommendation	41
5.4 Suggestions for further studies	41
REFERENCES	43
APPENDICES.....	53

List of figures

Figure 2.1: <i>Carissa spinarum</i> (Linn) fruits, stems, leaves and flowers	18
Figure 2.2: <i>Caesalpinia volkensii</i> (Harms) stems and leaves.....	19
Figure 4.1: Phase I antinociceptive activities of acetone leaves extracts on formalin induced pain	31
Figure 4.2: Phase II antinociceptive activities of acetone leaves extracts on formalin induced pain	32
Figure 4.3: Antinociceptive effects of acetone leaves extracts on acetic acid induced pain.	35

List of tables

Table 4.1: Antinociceptive activities of acetone leaves extracts of <i>C. spinarum</i> (Linn) on formalin induced pain in mice	29
Table 4.2: Antinociceptive activities of acetone leaves extracts of <i>C.volkensii</i> on formalin induced pain in mice	30
Table 4.3: Antinociceptive properties of <i>C. spinarum</i> (Linn) in Acetic acid-induced pain in mice	33
Table 4.4: Antinociceptive properties of <i>C.volkensii</i> (Harms) in Acetic acid-induced pain in mice	34
Table 4.5: Phytochemical composition of acetone leaves extracts.	36

List of appendices

- Appendix I: Comparison of antinociceptive activities of *C.volkensii* (Harms) and *C.spinorum* (Linn) acetone Leaves extracts phase1 on formalin induced pain in mice.....57
- Appendix II: Comparison of antinociceptive activities of *Carissa spinorum* (Linn) and *Caesalpinia volkensii* (Harms) acetone leaves extracts phase II on formalin induced pain in mice.....58
- Appendix III: Comparison of antinociceptive activities of *C.volkensii* and *C.spinorum* acetone Leaves extracts on acetic acid induced pain in mice.....59

Abbreviations and acronyms

AAIN	Acetic Acid Induced Writhing
ACC	Anterior Cingulate Cortex
ANOVA	Analysis of Variance.
BW	Body weight
CB1	Cannabinoid Receptor Type 1
CNS	Central Nervous System
COX	Cyclooxygenase Enzyme
DMSO	Dimethyl sulphoxide
FIP	Formalin Induced Pain
IASP	International Association for the Study of Pain
IP	Intraperitoneal
IV	Intravenous
KG	Kilogram
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
PAG	Periaqueductal Gray
PG	Prostaglandin
PNS	peripheral nervous system
SEM	Standard Error of Mean
THC	Tetrahydrocannabinols
TRPA 1	Transient Receptor Potential Ankyrin Subtype 1 receptors
TRPV 1	Transient Receptor Potential Vanilloid Receptors
WHO	World Health Organization
TAHM	Traditional African Herbal Medicine
TENS	Transcutaneous Electrical Nerve Stimulation

Abstract

Despite the progress that has occurred in recent years in the development of therapy, there is still a need for effective and potent analgesics for pain. Pain is defined as unpleasant feeling essential for body's defense system. Pain is managed using analgesics such as aspirin, paracetamol, diclofenac, morphine, opioids, among others. Conventional antinociceptives are expensive and have many side effects. Continued use of these drugs may lead to tolerance. Medicinal plants have been used to relieve pain and form a better alternative. Herbal antinociceptives are affordable and have arguably fewer side effects. *Carissa spinarum* (Linn) is used to treat rheumatoid pain, fever and inflammation related disorders. *Caesalpinia volkensii* (Harms) has pharmacological activities that include antimicrobial, immune modulatory properties and antimalarial. These two plants are used locally by people in Embu County as analgesics. This study was designed to bioscreen the acetone leaves extracts of *C. volkensii* (Harms) and *C. spinarum* (Linn) for anti-nociceptive potential. The plant parts were collected from Siakago-Mbeere north sub-county, Embu County, Kenya. The samples were prepared and extraction of the active compounds carried out using organic solvent acetone in the ratio 1:2. Swiss albino mice were divided into five groups of five mice each: Normal, negative, reference and experimental group. Pain was induced experimentally using formalin and acetic acid. The experimental groups were treated with 50 and 100 mg/kg dose quantities of each plant extracts prepared. The acetone leaves extracts of the two plants were evaluated for antinociceptive properties in mice compared to the reference drug diclofenac sodium. Mice were injected intraperitoneally with doses of the herbs, diclofenac and the vehicle. Thirty minutes later the animals were injected with 0.01 ml of 2.5 % formalin in the sub-planter region of the left hind paw and the other set with 0.4 ml of 5% acetic acid. The total time spent lifting; biting, licking the paw and writhing were counted and scored. The acetone leaves extracts lowered paw licking time in a dose dependant manner, The leaf extracts of *C. volkensii* at the dose levels of 50 and 100 mg/kg body weight reduced the formalin-induced pain in mice by 72.74% and 99.38 % respectively and acetic acid writhing by levels of 50 mg/kg body weight reduced the number of writhes by 81.40%, 100 mg/kg body weight did not reduce writhing. *C. spinarum* at the dose levels of 50 mg/kg and 100 mg/kg body weight reduced formalin induced pain by 3.47% and 34.46 and 20.2% and 95.50% respectively. Acetic acid induced pain in mice by 73.77 % and 86.89 % respectively. Diclofenac reduced the pain by 15.34 in early phase and 98.02% in late phase. Further, the phytochemical screening results showed that the acetone leaves extracts of *C. volkensii* (Harms) and *C. spinarum* (Linn) have phytochemicals associated with anti-nociceptive activities. The study has established that the acetone leaves extracts of *C. spinarum* (Linn) and *C. volkensii* (Harms) are effective in management of pain. It is therefore recommended that further fractionation of the metabolites of the two plant extracts be carried out with a view to identifying the most active compounds for further development into drugs for management of pain and inflammation.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Pain is an unpleasant injury that is essential to the body's defense system. It provides a rapid warning to the nervous system to initiate a motor response to minimize physical harm. Lack of the ability to experience pain, as in congenital insensitivity to pain with anhidrosis (inability to sweat normally), can cause very serious health problems such as self mutilation, autoamputation and corneal scarring (Axelrod and Hilz, 2003).

Inadequate pain relief is a known problem worldwide. Surveys show that many patients still suffer from moderate to severe pain (Marks and Sachar, 1973; Donovan, 1983; Wilder-Smith and Schuler, 1992; Carr and Goudas, 1999; Svensson *et al.*, 2000; Dolin *et al.*, 2002), despite an increased focus on pain and the development of new standards for pain management (Apfelbaum *et al.*, 2003).

Studies have revealed that electrical stimulation of the periaqueductal gray (PAG) produces analgesia, demonstrating the presence of analgesic circuit in the brain (Mayer *et al.*, 1971). When elicited from the ventral portion of PAG, this electrical stimulation produces analgesia which is mediated by the release of endogenous opiates (Akil *et al.*, 1978).

However, when elicited from the dorsal or lateral part of the PAG, the analgesic effect of stimulation is mediated by unidentified non-opiate substances among the prime candidates for these unknown pain modulatory substances are endogenous cannabinoid compounds which produce analgesia (Buxbaum, 1972). Pain involves multiple mechanisms that ideally require a multimodal or balanced analgesic technique with the aim of improving analgesia by combining analgesics with additive or synergistic effects (Paul and Henrik, 2010).

Conventional analgesics available over the counter include aspirin, paracetamol and ibuprofen. Others are diclofenac and morphine based pain killers such as codeine phosphate or tramadol, opioids and antidepressants. The management of pain is a daily challenge in modern medicine despite the currently available wide range of analgesics. Conventional analgesics are expensive, have arguably many side effects such as gastric disorders, kidney, liver and heart failure, prolonged bleeding after injury and diabetes and continued use may lead to addiction and drug resistance. Almost all pharmacological treatments may produce side effects (Goldberg, 1984).

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects against pain (Girish and Farnsworth, 1989; Gupta *et al.*, 2006). Traditional African herbal medicine (TAHM) is among the most ancient natural therapies and perhaps the oldest folk medicine currently practiced (Tariq, 2012; Brendler *et al.*, 2010). Alternative medicines are thought to possess many safe and effective phytochemicals useful in treating various disorders including pain. Several medicinal plants have been tested for antinociceptive

activities. *Gunnera perpensa* (Gunneraceae) rhizome water and methanol extracts have been found to significantly reduce writhing (Mpumelelo *et al.*, 2010). Mangroves have been analyzed for antinociceptive activity using hexane, carbon tetrachloride and aqueous extracts. The results showed that antinociceptive properties appeared to be wide spread among mangrove species (Shilpi *et al.*, 2012). *Solanum incanum* (Linn) has also been analysed for antinociceptive properties using dichloromethane root extracts. The results showed that the roots contain secondary metabolites which have antinociceptive properties (Mwonjoria *et al.*, 2014). It is against this background that this study was designed to bioscreen antinociceptive properties of the *Carissa spinarum* (Linn) and *Caesapinia volkensii* (Harms) in mice. These plants have been used traditionally in Embu County in the management of pain.

1.2 Problem statement

Pain being a disturbing condition which affects a significant proportion of the population is the main reason which prompts patients to consult their physicians. Acute pain is useful for the organism in case of danger but chronic pain is harmful to the body. None treated can lead to many complications. They include impaired recovery that can potentially progress to a chronic pain condition. Non treated chronic pain can reduce daily activities, increase disability, negatively affect the quality of living, create suffering, cause anxiety, depression, anger, fear and increase the risk for suicide. Pain is managed using conventional and herbal medicines. Conventional pharmacological antinociceptives in the market are not sufficient in treating pain. These drugs have arguably many side effects, they are expensive and

continued use may lead to resistance. Herbal medicines have arguably fewer side effects; they are cheap and are readily available. *Carissa Spinorum* (Linn) and *caesalpinia volkensii* (Harms) are used in treating ailments including pain, but there is no scientific authentication of these claims.

1.3 Justification

Pain management requires wide variety of antinociceptives compounds. Plant derived herbal treatment are believed to have fewer side effects, are cheap and are readily available. Interestingly some conventional drugs used as antinociceptives today are plant derived, for example, morphine comes from opium poppy plant while salicylic acid comes from Salicaceae family (willow bark) including, *Salix alba*, *Salix purpurea*, *Salix fragilis*, and other species. This study will avail information regarding antinociceptive activity of acetone leaf extracts of *C. spinorum* (Linn) and *C. volkensii* (Harms) in mice. The preliminary information of this study is a vital first step for development of cheaper and easily accessible antinociceptive drugs. Besides, this study will provide information regarding phytochemical composition of the acetone leaf extracts of *C. spinorum* (Linn) and *C. volkensii* (Harms). This will inform the efforts towards bioassay-guided fractionation of antinociceptive secondary metabolites of acetone leaves extracts of *C. spinorum* (Linn) and *C. volkensii* (Harms), which will help in developing plant derived antinociceptive drugs.

1.4 Null hypothesis

The acetone leaf extracts of *C.spinarum* (Linn) and *C.volkensii* (Harms) have no antinociceptive effects on formalin and acetic acid induced pain in mice.

1.5 Objectives

1.5.1 Main objective

To bioscreen acetone leaf extract of *C. spinarum* (Linn) and *C. volkensii* (Harms) for antinociceptive activities in mice models.

1.5.2 Specific objectives

- i. To determine antinociceptive activities of acetone leaf extracts of *C. spinarum* (Linn) and *C. volkensii* (Harms) on formalin induced pain in mice.
- ii. To determine antinociceptive activities of acetone leaf extracts of *C. spinarum* (Linn) and *C. volkensii* (Harms) on acetic acid induced pain in mice.
- iii. To determine the phytochemical composition of the acetone leaf extracts of *C. spinarum* (Linn) and *C. volkensii* (Harms).

CHAPTER TWO

LITERATURE REVIEW

2.1 Description of pain

Several attempts have been made to define pain. McCafferey (1972) states pain is whatever the experiencing person says it is. The word “pain” comes from the Greek word “*pione*”, which means penalty. Physiologically, nociception refers to signals arriving in the CNS resulting from activation of specialized sensory receptors called nociceptors that provide information about tissue damage. Pain is one of the common symptoms in medicine it is the main cause of one third of all first consultations (Axelrod and Hilz, 2003). It is the most common reason for patients to seek advice from health practitioners. It presents frequently as a symptom of different pathologies and represents important medical and economic costs for the community (Jageroyic *et al.*, 2002).

Pain is a complex experience consisting of physiological responses to noxious stimulus which in some cases is followed by emotional response. The International Association for the Study of Pain (IASP) defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Merskey *et al.*, 1986). Pain is an essential component of the body’s defense system. It provides a rapid warning to the nervous system to initiate motor responses to minimize physical harm. Pain is a common and distressing feature of many diseases such as tumour, surgical procedures, physical trauma, noxious chemical stimulation among others (Aliu, 2007). Pain is also a

warning signal and primarily protective. However excessive pain can lead to other side effects such as sweating, apprehension, nausea and palpitation (Raquibul *et al.*, 2010).

The amount of pain perceived is merely directly proportional to the extent of injury (Melzack *et al.*, 2001). The problems associated with pain have of particular interest to mankind's religious and medical thinking. Pain is a powerful and insistent sensations and a force behind much of man's behavior. This has made it a power plethora of interpretations and a focal point among religious group around philosophers as a culture (Jaros, 1991).

2.2 Biochemical and physiological basis of pain

Pain is generally seen as nociceptive, inflammatory or neuropathic, giving pain a pathophysiological correlate (Kehlet *et al.*, 2006). Nociceptive pain results from activation of high thresholds peripheral sensory neurons (nociceptors) by intense mechanical, chemical or thermal noxious stimuli. Signals from these nociceptors travel primarily along small myelinated A-delta and unmyelated C- sensory afferent fibres to the dorsal horn of the spinal cord where they make synaptic contact with second order neurons. The signals travel post-synaptic mainly along the spinothalamic tract of the spinal cord to the thalamus and sensory cortex (Gottschalk and Smith, 2001). This spino-cerebral signalling continues also partly to the hypothalamus and the limbic system, the loci being important in determining the individual's emotional reactions to pain (Woolf, 1994).

The nociceptive input and rostral transmission signaling is under the influence of both local and bulbo-spinal neural activity. These can be either inhibiting or facilitating. There are a numerous pharmacologically identified transmitters that can act as modulators in this circuitry of nociceptive input. Inflammatory pain is the heightened pain that occurs in response to tissue injury and inflammation. It results from the release of sensitizing inflammatory mediators that lead to a reduction in the threshold of nociceptors that innervates the inflamed tissue (peripheral sensitization). The peripheral sensitization is augmented by important biological processes that result in central sensitization of the spinal cord and rostral medulla sites (Willis and Coggeshall, 1991).

2.3 Modulation of pain

2.3.1 The Gate Control Theory

This theory was developed by Melzack and Wall (1965). It postulated that in each dorsal horn of the spinal cord, there is a gate-like mechanism which inhibits or facilitates the flow of afferent impulses into the spinal cord before it evokes pain perception and response. This theory was proposed as an alternative to the specificity theory of pain, which holds that pain is a specific modality with its own specialized sensors, neuronal pathways and centers and the pattern theory which maintains that stimulus intensity of non-specific receptors and central summation were the critical determinants of pain. The theory, originally stated that the opening or closing of the 'gate' is dependent on the relative activity in the large diameter (A-b) and small diameter fibers (A-d and C), with activity in the large diameter fibers

tending to close the 'gate', and activity in the small diameter fibers tending to open it (Melzack and Wall, 1967). It was suggested in this study the dorsal horn neurons can potentially transmit noxious information to supra spinal levels, which eventually decreases their cell activity during transcutaneous electrical nerve stimulation (TENS) application to somatic receptive fields. These findings are consistent with the concept of the 'gate control theory of pain' in which the less noxious information is involved in the pain perception (Fein, 2012).

The gate control theory proposes that the substantia gelatinosa, which caps the grey matter of the spinal horn in the spinal cord, is the essential site of control. The control mechanism is referred to as a 'gate' and is operated by external and internal influences. Pain impulses can only pass through when the gate is open, and not when it is closed (Jensen, 2009). Therefore, if nociceptive input exceeds a-b fiber input, then the gate is open and the pain impulse ascends the spinal cord to the brain. If A-b fiber input exceeds nociceptive input then the gate is closed and the pain impulse is stopped or diminished due to the action of the inhibitory neurotransmitters and, therefore, does not pass up the spinal cord (Jensen, 2009).

An essential part of the theory is that the position of the 'gate' is in addition influenced by the brain's descending inhibitory system (Jensen, 2009). Nearly a century ago, Sherrington proposed the existence of the nociceptor, a primary sensory neuron that is activated by stimuli capable of causing tissue damage (Sherrington, 1906). According to this model, nociceptors have characteristic thresholds or sensitivities that distinguish them from other sensory nerve fibres

Electrophysiological studies have in fact, shown the existence of primary sensory neurons that can be excited by noxious heat, intense pressure or irritant chemicals, but not by innocuous stimuli such as warming or light touch(Burgess and Perl, 1967).

Analgesics decrease pain sensation by increasing pain threshold to external stimuli. Noxious pain stimuli can be developed by thermal, chemicals and physical pressure (Tripathi, 2003). Two neurotransmitters are released by the nociceptive afferent fibers in the dorsal horn of the spinal cord. These neurotransmitters, which stimulate the second-order sensory neurons, include: Glutamate and Substance P. The amino acid glutamate is the major neurotransmitter released by A-delta fibers and C- fibers. Glutamate binds to the AMPA-type glutamate receptor on the second-order sensory neuron to elicit action potentials and continue transmission (Chauhan, 1988).

2.3.2 Experimental pain induction

Generally antinociceptive activity of various drugs can be tested using several methods namely; Formalin test, tail flick, tail immersion, hot plate, acetic acid writhing test, among others.

2.3.2.1 Acetic acid writhing test

Writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principles like phenylquinone, acetic acid in mice. Analgesic activity of the test compound is inferred from decrease in the frequency of writhing. The method involves injecting the rodent with 20mg/kgbw acetic acid

intraperitoneally and thirty minutes later pain is induced by injecting the rodent with 0.1ml of 2% acetic acid. The number of abdominal constrictions are counted and scored (Dannerman, 1977).

2.3.2.2 Formalin test

This is a reliable model of nociception on pain acting centrally. The method involves administering a known dosage antinociceptive intraperitoneally and thirty minutes later pain is induced by injecting a known amount of formalin for example 0.01ml of 1% formalin in the left paw. The analgesic activity of test compound is inferred from decrease in number of lifting, biting or licking the injected part (Hunskar *et al.*, 1987).

2.3.2.3 Hot-Plate and Electrical Stimulation test

This test has been used in many investigations and is suitable for evaluation of centrally but not of peripherally acting analgesics. Hot plate involves placing a rodent on an enclosed hot plate at a 52.5 °C. The latency to lick a hindpaw or jump when the rat was placed on plate is measured. The reaction time on pain is recorded. Low intensity hot plates are suitable and sensitive to analgesic drugs (Ankier, 1974).

Electrical stimulation is the application of electrical current through electrodes placed on the skin. It is applied with varying frequencies from low (<10Hz) to high frequencies (>50 Hz). Low frequencies measure peripherally acting analgesic while high frequencies measure centrally acting analgesics (King *et al.*, 2005).

2.3.2.4 Tail flick and tail immersion test

The tail flick test involves placing the tail of the rodent on a tail flick device and a 375-w movie light beam is focused onto the rat's tail by means of condenser lens. The time taken by the rodent to withdraw the tail is noted and recorded (Jensen and Yaksh, 1986). The tail immersion model is used to test for acute pain. The tail-withdrawal response of mice is predominantly considered to be selective for centrally acting analgesics, whereas the peripherally acting drugs are known to be inactive on such heat-induced pain response (Srinivasan *et al.*, 2003). In this study pain was experimentally induced in mice by intraperitoneal injection of formalin and acetic acid.

2.4 Conventional management of pain

Conventionally pain is managed using analgesics. Analgesics are classified as opioid and non-opioid drugs. The opioid drugs bind to specific opioid receptors located throughout the central nervous system and other tissues the specific non opioid drugs include paracetamol and non-steroidal anti inflammatory drugs (NSAIDs) which act as cyclooxygenase inhibitors (Paul and Chauhan 2005).

The non-specific non opioid drug group consists of drugs with analgesic properties that are commonly used for other purposes, for example in treatment of depression or epilepsy (Katz *et al.*, 2005). Opioids are used in treatment of moderate to strong pain. They can be classified as naturally occurring, for instance morphine, or as synthetic substances, for instance fentanyl, pentazocine and methadone (Miller,

2010). They bind to several types of opioid receptors in the body which belong to a family of G-protein-coupled receptors (Pasternak, 1988). Three major types of opioid receptor have been identified; μ (mu), κ (kappa) and δ (delta). Opioid-like receptor has been identified, which was first named ORL1-receptor (Trescot *et al.*, 2008).

Generally, most opioids exert their analgesic effects through μ -receptor binding in the central nervous system. Opioids may also bind to opioid receptors in the periphery, when they are present (Stein and Lang, 2009). Binding to the different opioid receptors causes the different opioids drug effects. Long-term opioid therapy may be ineffective or not well tolerated by one-third of chronic pain patients who are put on strong opioids. The benefits of analgesic opioid use are weighed by related costs, risk for abuse in susceptible individuals and negative side effects which include constipation, nausea, sedation, respiratory depression, and death (Dunn *et al.*, 2010). There is also a possibility of opioid-induced hyperalgesia (Angst and Clark, 2006). The changes may contribute to abnormal behaviors in some individuals during the course of chronic opioid therapy, evidence further indicates that patients who remain on opioids tend to escalate dosages sometimes dramatically, creating a potential conundrum while clinicians pursue an ever-moving target for adequate pain relief (Schneider and Kirsh, 2010).

The NSAIDs inhibit the activity of both cyclooxygenase-1(COX-1) and cyclooxygenase-2 (COX-2), and thereby, the synthesis of prostaglandins and thromboxanes. It is thought that inhibiting COX-2 leads to the anti-inflammatory,

analgesic and antipyretic effects and that those NSAIDs also inhibits COX-1, particularly aspirin, may cause gastrointestinal bleeding and ulcers (Clive *et al.*, 1998). Meta-analysis has confirmed that paracetamol is an effective post operative analgesic and the addition of 60mg codeine to paracetamol produces worthwhile additional pain relief with single oral doses (Moore and Collins, 1997). Conventional drugs may lead to tolerance and resistance especially when used constantly.

Diclofenac is a commercially available as oral NSAID, intravenous, suppository, transdermal patch or gel formulations. Conditions effectively treated by diclofenac include arthritis, musculoskeletal injuries, migraines and postsurgical analgesia and inflammation (Morton and Obrian, 1999). The pharmacological efficacy of diclofenac stems from its potent inhibition of prostaglandin synthesis as well as other pro-nociceptive actions at peripheral and spinal sites (Ramirez-Alcantara *et al.*, 2005). Although classified as a nonspecific COX inhibitor, diclofenac is regarded as a relatively specific inhibitor of the COX-2 isoform. Additionally, many other mechanisms of action have been discovered for diclofenac and are classified as established, putative and emerging mechanisms.

Acetaminophen is a pain reliever (analgesic) widely available without prescription, and there is considerable evidence that its site of action is central. Metabolites of acetaminophen have been demonstrated to activate human TRPA1 receptors and intrathecal (within the sheath of the spinal cord) administration of these metabolites in mice produced analgesia that was lost in TRPA1 knockout mice (Andersson and

Gentry, 2011). These findings suggest that metabolites of acetomenophen produce a TRPA1 mediated spinal analgesia in mice and by extension in humans.

2.5 Role of herbal and complementary medicine in pain management.

Complementary medicine refers to non-conventional medical treatment that is used in conjunction with standard medical interventions, whereas alternative medicine comprises treatment interventions that are used in place of standard medical care (Kim *et al.*, 2002). Several medicinal plants have been used in treatment and management of pain. Devil's Claw (*Harpagophytum procumbens* DC) has been used for thousands of years in Africa for the treatment of fever, rheumatoid arthritis, and skin conditions, and is currently available as an alternative treatment for pain and osteoarthritis (McGregor, 2005). Harpagoside, one of the major components of the plant, has been shown to suppress lipopolysaccharide-induced inducible nitric oxide synthase and cyclooxygenase (COX-2) expression through inhibition of nuclear factor κ B activation (Krentz, 2000). Other sources of antipain remedies include *S. incanum* (Linn) (Mwonjoria *et al.*, 2014) and mangroves (Shilpi *et al.*, 2012).

The *Cannabis* plant is also used as pain killer. It contains a complex mixture of substances that include at least 60 different cannabinoids, most of which have been shown to present pharmacological bio-activities (Burstein, 1997). The major active constituent of *Cannabis*, THC, has been shown to possess antinociceptive properties when assessed in several experimental models, and this effect is attenuated by a Cannabinoid receptor type1 (CB1) receptor antagonist. The ethanol extract of the

herb and the remulacin, are present in the bark of White willow plant (Robbers and Tyler, 1999). In 1829, Leroux isolated the active ingredient, salicin, from the willow bark, and in 1838 salicylic acid was obtained (Hedner and Everts, 1998).

The search for new pharmacologically active agents obtained from plants has led to the discovery of many clinically useful drugs that play a major role in the treatment of human disease. About 25% of all available modern drugs are derived directly or indirectly from higher plants (Farnsworth *et al.*, 1985). In spite of the progress that has taken place in recent years in the development of therapy, the medical community still urgently needs effective and potent analgesics, especially for chronic pain. Thousands of patients with intense and unrelenting pain, such as that resulting from cancer or injury, rely on morphine, despite its well-known side effects. This has renewed the interest of the major pharmaceutical companies in higher plant-derived secondary metabolites as part of the search for new clinically useful drugs (Shu, 1998).

Modern drugs still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health. Bio-prospecting of new plant-derived drugs is an alternative solution to this problem (Lucy and Edgar, 1999). The medicinal properties of plants could be based on the

antioxidant, antimicrobial antipyretic effects of the phytochemicals in them (Cowman, 1999; Adesokan *et al.*, 2008).

Traditionally, herbs have been considered to be less toxic and have been used for treating various problems by the general public “and/or” traditional medicine doctors worldwide (Oduola *et al.*, 2007). The aim of this study is to determine the formalin and acetic acid induced antinociceptive activity of *Carissa spinarum* (Linn) and *Caesalpinia volkensii* (Harms) in mice.

2.6 Plants used in this study

2.6.1 *Carissa spinarum* (Linn) and *Caesalpinia volkensii* (Harms)

2.6.1.1 Plants description

Carissa spinarum (Linn) is an erect thorny shrub with forked branches 2-3 meters in height. It is woody very hard with a bark that is light brown to green. Thorns are 3.2 cm long. The leaves are ovate 4.5 cm long, broad and exude white latex after they are plucked from the stem. The flowers are short-stalked, sweetly scented, bisexual and complete. The inflorescence has 10 flowers; calyx, polysepalous, with 5 sepals which are green. The fruit are ovoid berry, 9 mm in length, 6 mm in diameter, and 642 mg in weight (Patrick and Bo Tengnas, 2005).

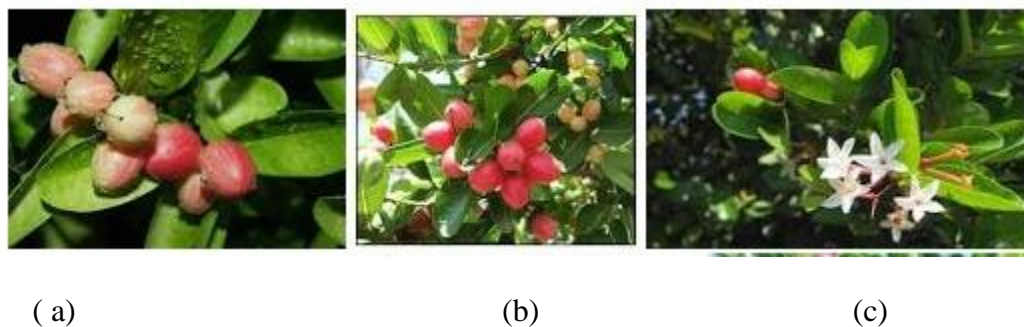


Figure 2.1: *Carissa spinarum* (Linn) fruits, stems, leaves and flowers

Caesalpinia volkensii (Harms) is an evergreen plant found growing in lowland forests (figure 2.1). In Kenya, it is mostly a home grown plant in parts of Central, Rift Valley, Coast and Eastern province. The plant is commonly known as Mkomew (Taita), Mkomwe (Swahili), Msoo Miba (Shambaa), Mubuthi (Gikuyu), Muvuthi (Kamba), Mburuga (Digo), Olnkulankula (Maasai) (Kokwaro, 1976). The plant belongs to the family Caesalpinaceae. It is a woody climber 1.8-4 m high. The stems have deflexed prickles to 5 mm. Its bipinnate, leaves are in 3-6 pairs. Leaflets appear in 3-6 pairs which are ovated (Figure 2.2). It has yellow flowers; petals are about 16 mm by 3.5-4.5 mm. The fruit is densely prickly, beaked 7-13 cm by 3.5-6.5 cm. The fruits seeds are smooth, shiny and hard to crack (Evans *et al.*, 2002). Its fruits are edible ripe and unripe. It is used as fodder, live fence among others. Propagation is mainly through seedlings or direct sowing at site (Patrick and BoTengnas, 2005).



Figure 2.2: *Caesalpinia volkensii* (Harms) stems and leaves

2.6.1.2 Ethnopharmacological properties of *Carissa spinarum* (Linn) and *Caesalpinia volkensii* (Harms)

The roots and leaves of *Carissa spinarum* (Linn) are rich in tannins, carissone, palmitic acid, farnesene, stigmasterol, ursolic acid, lupeol campesterol, 17-11-oxo – nor- β amyron and urs-12-ene-3 β , 22 β -diol-17-carboxylic acid (Hegde *et al.*, 2012). The plant has many medicinal properties. The roots are antihemetic and are used to treat stomachache, bleeding after delivery and muscle cramps. The roots have long been prescribed in the indigenous system of medicine as purgative for the treatment of inflammation related disorders such as rheumatism and pain, cleaning worm infected wounds of animals and treatment of snake bite (Fatima *et al.*, 2013). The leaves are used to treat fever, jaundice, hepatitis and chest pain (Saghir *et al.*, 2001). Earlier studies have shown that the extract of the plant possesses cardiogenic (Hegde *et al.*, 2009), anticonvulsant (Hegde *et al.*, 2011), antibacterial (Sanwal and Chaudhary, 2011), potent, antioxidant and CNS depressant activities (Irum *et al.*, 2011).

Caesalpinia volkensii (Harms) is native to Ethiopia, Kenya, Uganda and Tanzania and is used in Kenya and Tanzania in treatment of malaria. Around Nairobi (Kenya), over 60% of the herbalists prescribe its leave extracts to cure malaria, sometimes alone, but mostly mixed with other plants extracts (Kokwaro, 2009). Roots and seeds can be eaten raw or cooked for their aphrodisiac properties, pain management in pregnant mothers and treating eye problems (Kokwaro, 2009). The roots are used in curing gonorrhoea and bilharzias. The dye extracted from flower buds is used in treatment of eye. The roots have antiplasmodial properties (Irungu *et al.*, 2011; Ochieng *et al.*, 2011). *C. volkensii* has been shown to possess hypoglycemic properties (Njagi *et al.*, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and preparation of plant materials

Fresh leaves of *C. spinarum* (Linn) and *C. volkensii* (Harms) were collected from their natural habitats on the basis of ethnobotanical information with the help of local herbalist from Siakago division, Mbeere North Subcounty in Embu County, Kenya. The samples were cleaned to ensure they are free of dust and other contaminants and transported to the department of Biochemistry and Biotechnology of Kenyatta University for studies. The samples were taxonomically identified and authenticated by an acknowledged taxonomist and a voucher specimen deposited at the Kenyatta University herbarium for future reference. The leaves were air dried at room temperature for two weeks until completely dry. The dried leaves were then crushed by use of an electric mill to obtain fine powder which was stored at room temperature in appropriately labeled air tight containers until used in extraction.

3.2 Extraction

A mass of 200gms of the powder of the two plants were weighed and put into separate conical flasks and labeled. Then 750 ml of acetone were added into each conical flask and corked. The mixture was allowed to stand overnight. The following day, the extract was filtered into separate containers using Whatman No.1 filter papers. The

containers were then labeled. Then 250ml of acetone was added to the remnant of each and allowed to stand for four hours. Second filtering was then done. The procedure was repeated until the solvent remained clear. The extracts were then concentrated using rotary evaporator at 56⁰C for five hours. The extracts were then placed in open beakers for two weeks to allow any remaining acetone to evaporate until a sticky solid was obtained. This was stored at room temperature until use in bioassays.

3.3 Experimental animals

One hundred Swiss albino mice were used in this study. The mice breeding colony was acquired and bred in the animal breeding and experimentation facility of the department of Biochemistry and Biotechnology, of Kenyatta University. The animals were kept in standard cages and maintained under the standard laboratory conditions at room temperature and with 12 hours dark and 12 hours light cycle. They were fed on rodent pellets diet and supplied with water *ad libitum*. The ethical guidelines and procedures for handling animals were followed in the study.

3.4 Experimental design

3.4.1 Formalin-induced antinociceptive assay

The formalin induced antinociceptive assay was carried out as described by Hunskar *et al.* (1987). Swiss albino male and female mice weighing between 20-25gms were divided into five groups of 5 animals each. Group I was intraperitoneally administered

with 0.1ml normal saline (negative control). Group II was administered with 0.01ml of 2.5% formalin which was injected in the left hind paw (positive control). Group III was split into two groups of five animals each. Each group received treatment as follows; Group IIIa was intraperitoneally administered with 50mg/kg body weight dose of each plant extract, group IIIb was intraperitoneally administered with 100mg/kg body weight dose of each plant extract. Group IV was intraperitoneally administered with the standard drug, diclofenac (the reference drug) at a dose of 15 mg/kg body weight. Thirty minutes after each dose administration, pain was induced by injecting 0.01 ml of 2.5% formalin in the left hind paw. The mice were placed in the prexi- glass box for observation. The time that the mice spent lifting, licking or biting the injected paw was recorded according to the pattern described by Tjolsen *et al.* (1992). The two distinct periods of intensive licking, biting or lifting activity were identified and scored separately. The first period (early phase) was recorded between 1-5 minutes and the second period (late phase) was recorded between 15-30 minutes.

The percentage inhibition of the licking was calculated using the following formula

$$\frac{C - T}{C} \times 100$$

Where,

C = the vehicle control group value for the each phase,

T = the treated group value for the each phase.

3.4.2 Acetic-acid induced antinociceptive assay

This was carried out as described by Koster *et al.* (1959). Swiss male and female mice weighing between 20-25 grams were divided into five groups of 5 animals each. The groups will receive treatment as follows

Group 1 was intraperitoneally administered 0.1 ml of the vehicle, (DMSO). Group II was intraperitoneally administered with 0.4 ml of 5% acetic acid solution. Group III was divided into two subgroups of five animals each. Each subgroup received treatment as follows; Group IIIa was intraperitoneally administered with 50 mg/kg body weight of each plant extract. Group IIIb was intraperitoneally administered with 100mg/kg body weight of each plant extract. Group IV was intraperitoneally administered with standard drug, diclofenac sodium (the reference drug) at a dose of 15 mg/kg body weight. Thirty minutes after administration of the each treatments pain was induced by injection of 20 ml/kgbw of 5% acetic acid in the right side of the belly intraperitoneally. The mice under experiments were then placed in prexi-glass box for observation. The number of abdominal constrictions (writhes) was counted 5 to 15 minutes after acetic acid injection. The percentage inhibition of the writhing was calculated using the following formula described in section 3.3.1.

3.5 Qualitative phytochemical screening

Freshly prepared acetone leaf extracts of *C. Volkensii* and *C. Spinarum* were subjected to qualitative phytochemical screening to identify presence or absence of major

secondary metabolites using the protocol described by Harborne (1998) and Kotake (2000). The classes of secondary metabolites, screened included alkaloids, saponins, terpenoids, sterols, flavonoids, cardiac glycosides and phenolics,

3.5.1 Test for alkaloids

To 5ml of 0.5g of the plant extract were measured and put in a test tube. 2 ml of 1% of hydrochloric acid were then added and the mixture was heated in a water bath. This was followed by addition of 1ml of Dragendoff's reagent. An orange or red precipitate produced immediately indicated presence of alkaloids.

3.5.2 Test for flavonoids

To 2ml of 0.5g of the plant extract were measured in a test tube and a few drops of dilute sodium hydroxide were added. This was followed by addition of a few drops of hydrochloric acid. An intense yellow colour indicated presence of flavonoids.

3.5.3 Test for steroids

To 1ml of 0.5g of the extract was measured, to which 10ml of chloroform was added and an equal volume of concentrated sulphuric acid added to the sides of the test tube. Yellow upper layer with green fluorescence indicates presence of steroids.

3.5.4 Test for saponins

To 2ml of 0.5g of the extract were put in a test tube to which a few drops of sodium bicarbonate solution were added. Froth production indicated presence of saponins.

3.5.5 Test for cardiac glycosides

To 1ml of 0.5g of plant extract was put in a test tube after which 1ml of glacial acetic acid was added, which was followed by addition of 2drops of iron chloride. 1ml of concentrated sulphuric acid was then added. A brown ring at the interface indicated presence of cardiac glycosides.

3.5.6 Test for phenolics

To 2ml of 0.5g of the plant extract were put in a test tube and 1ml of ferric chloride solution added. Blue to green colour indicates presence of phenols.

3.5.7 Test for terpenoids

To 1ml of 0.5g of the extract was put in a test tube, equal amount of petroleum ether or ethyl acetate were added. 2ml of chloroform was added to the mixture followed by addition of 2 ml of concentrated sulphuric acid alongside. Formation of reddish brown colour indicated presence of terpenoids.

3.6 Data management and statistical analysis

The data on latency of pain response was obtained for all the animals in different groups, recorded and tabulated on a broad sheet using MS Excel. The results were expressed as mean \pm standard error of mean (SEM). Statistical significance of differences among group were analyzed using one-way analysis of variance (ANOVA)

followed by Tukey's post *hoc* analysis to separate the means and obtain specific significant differences. $P \leq 0.05$ was considered significant. Analysis was done using Minitab statistical software.

CHAPTER FOUR

RESULTS

4.1 Antinociceptive activities of acetone extracts on formalin induced pain

Formalin induced pain in two phases; early phase, which lasted between 1 and 5 minutes and the late phase, which lasted between 15-30 minutes after formalin injection. Generally, the administration of acetone leaf extract of *C.spinarum* significantly reduced the formalin-induced pain in the early and late phase. This was indicated by reduction in paw licking time (Table 4.1).

In the early phase, the treatment of mice with acetone leaf extracts of *C.spinarum* at the dose levels of 50 and 100 mg/kg body weight reduced paw licking by 3.47% and 20.27 % respectively (Table 4.1). The antinociceptive effectiveness of the two extract dose levels was not significantly different from each other as well as the controls ($p>0.05$; Table 4.1).

In the late phase, the treatment of mice with acetone leaf extracts of *C.spinarum* at the dose levels of 50 and 100 mg/kg body weight reduced paw licking by 34.46 % and 95.50% respectively (Table 4.1). The antinociceptive effectiveness of the acetone leaf extracts of *C.spinarum* at the dose level of 100 mg/kg body weight was comparable to the reference drug (Diclofenac) which reduced the paw licking time by 96.09%.

Although the antinociceptive effectiveness of the acetone leaves extracts of *C. spinarum* at the dose level of 50 mg/kg body weight was not significantly different from the baseline and negative controls ($p>0.05$; Table 4.1), it showed a slight but significant antinociceptive effect compared to the positive control group and the group treated with the extract at the dose level of 100 mg/kg body weight ($p< 0.05$; Table 4.1).

Table 4.1: Antinociceptive activities of acetone leaves extracts of *C. spinarum* (Linn) on formalin induced pain in mice

ANIMAL GROUPS (N=5)	TREATMENT	PHASE1 (1-5 MIN)	PHASE2 (15-30 MIN)
Baseline	Formalin (2.5%)	300.40±20.8 ^a (-21.16)	287.20±3.84 ^c (-24,89)
Negative Control	DMSO (30%)	247.60±3.19 ^a (0.00)	271.60±2.98 ^a (0.00)
Positive Control	Diclofenac (15mg/kgbw)	209.60±3.53 ^a (15.34)	10.600±1.54 ^b (96.09)
Acetone Extract	50mg/kg bw	239.00±3.11 ^a (3.47)	178.00±2.77 ^{ac} (34.46)
	100mg/kg bw	197.40±8.08 ^a (20.27)	12.20±0.80 ^b (95.50)

Values are expressed as Mean ± SEM for five animals per group. Values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p>0.05$). The figures in brackets represent % inhibition.

Treatment of mice with acetone leaf extracts of *C. volkensii* at the dose levels of 50 and 100mg/kg body weight reduced paw licking in the early phase by 14.59 % and 14.73% respectively (Table 4.2). The antinociceptive effectiveness of the two extract dose levels was not significantly different from each other as well as the controls ($p>0.05$; Table 4.2).

On the other hand, in the late phase, administration of acetone leaf extracts of *C.volkensii* at the dose levels of 50 and 100 mg/kg body weight successfully reduced the formalin-induced pain in mice by 72.74% and 99.38 % respectively (Table 4.2).

The antinociceptive effectiveness of the two extracts dose levels was not significantly different from each other and was comparable to the Diclofenac (reference drug) which reduced the formalin induced pain by 98.02% .This shows that the plant extracts was as effective as the reference drug ($p > 0.05$; Table 4.2).

Table 4.2: Antinociceptive activities of acetone leaves extracts of *C.volkensii* on formalin induced pain in mice

ANIMAL GROUPS (N=5)	TREATMENT	PHASE 1 (1-5 MIN)	PHASE 2 (15-30 MIN)
Baseline	Formalin (2.5%)	258.60±10.9 ^a (2.78)	803.80±1.74 ^a (11.88)
Negative Control	DMSO (30%)	266.00±8.14 ^a (0.00)	718.40±2.66 ^a (0.00)
Positive Control	Diclofenac (15mg/kgbw)	259.40±3.44 ^a (2.63)	14.20±1.20 ^b (98.02)
Acetone Extract	50mg/kgbw	227.20±3.89 ^a (14.59)	195.80±2.60 ^b (72.74)
	100mg/kgbw	226.80±4.28 ^a (14.73)	4.00±0.71 ^{ac} (99.38)

Values are expressed as Mean ± SEM for five animals per group. Values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p > 0.05$). The figures in brackets represent % inhibition.

In comparison, the administration of acetone leaf extracts of *C.volkensii* at the dose levels of 50 and mg/kg body weight had a higher antinociceptive effectiveness in phase one than the acetone leaf extract of *C.spinarum*. However, at the dose level of 100

mg/kg body weight, *C.spinarum* exhibited a higher antinociceptive effectiveness in the same phase (Figure 4.1).

In the late phase, the acetone leaf extract of *C.volkensii* exhibited a higher antinociceptive activity than the acetone leaf extracts of *C.spinarum* at both dose levels, However the difference between the two plant leaf extracts was not significantly different from each other (Figure 4.2).

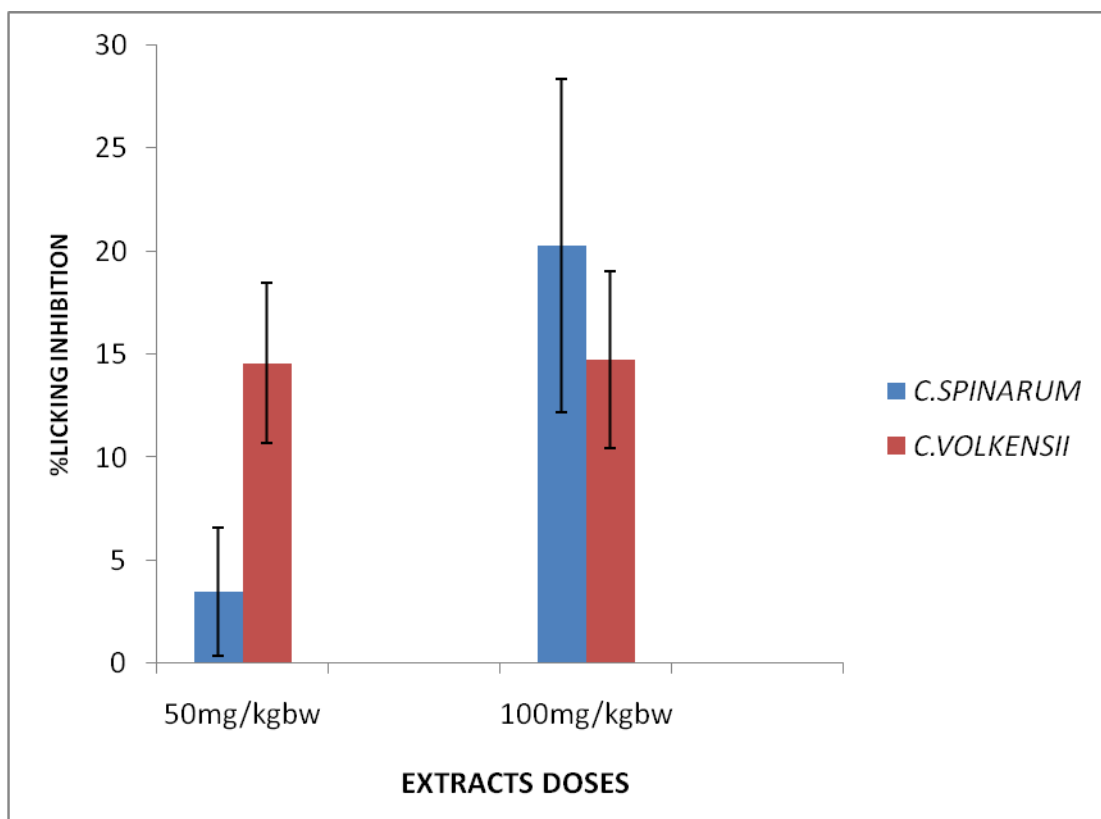


Figure 4.1: Phase I antinociceptive activities of acetone leaves extracts on formalin induced pain

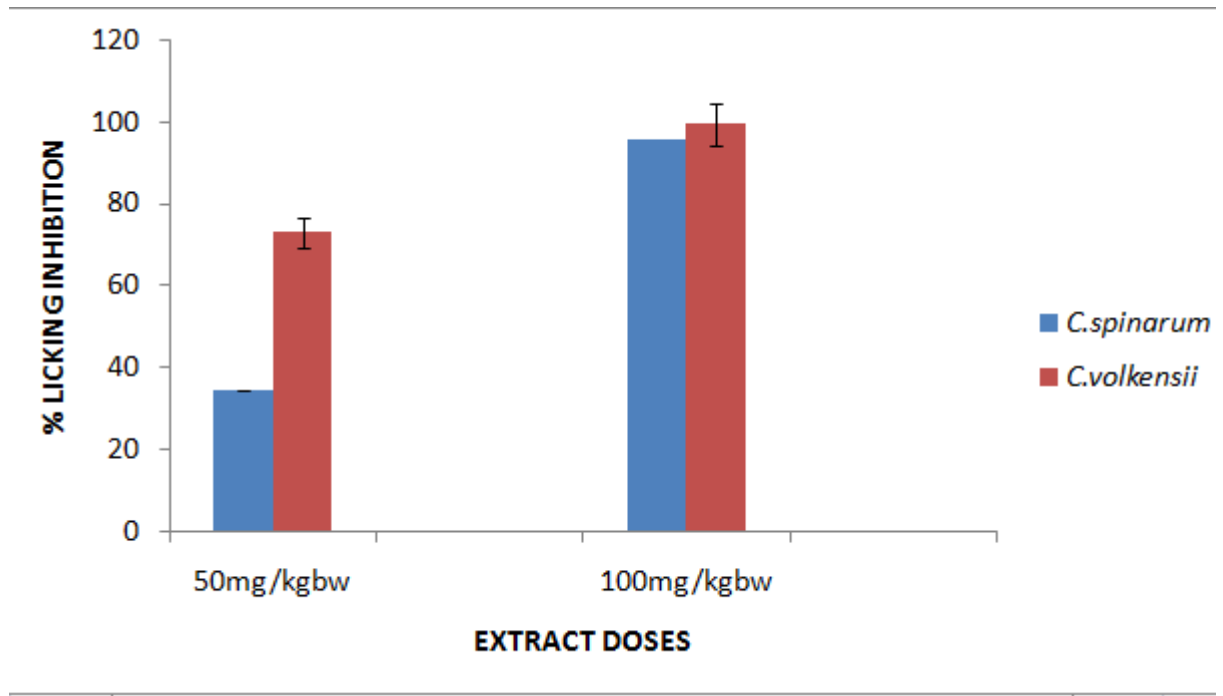


Figure 4.2: Phase II antinociceptive activities of acetone leaves extracts on formalin induced pain

4.2 Antinociceptive effects of acetone extracts on acetic acid induced pain

Generally, the administration of acetone leaf extract of *C.spinarum* successfully reduced the acetic acid-induced pain (Table 4.3). This was indicated by reduction in the number of writhing movement.

The acetone leaf extracts of *C.spinarum* at the dose levels of 50 mg/kg and 100 mg/kg body weight reduced acetic acid induced pain in mice by 73.77% and 86.89% respectively (Table 4.3). However, the antinociceptive effectiveness of the two dose levels was not significantly different from each other but it was comparable to

Diclofenac (reference drug), which reduced the number of writhing by 70.49% ($p > 0.05$; Table 4.3).

Table 4.3: Antinociceptive properties of *C. spinarum*(Linn) in Acetic acid-induced pain in mice

ANIMAL GROUPS (N=5)	TREATMENT	NUMBER OF WRITHINGS (5-20 MIN)	% INHIBITION
Baseline	Acetic acid 20ml/kgbw	19.60±3.70 ^a	-60.66
Negative Control	DMSO (30%)	12.20±1.50 ^a	0.00
Positive Control	Diclofenac(15mg/kgbw)	3.60±1.50 ^b	70.49
Acetone Extract	50 mg/kgbw	3.20±1.02 ^b	73.77
	100 mg/kgbw	1.60±0.40 ^b	86.89

Values are expressed as Mean ± SEM Values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p > 0.05$)

On the other hand treatment of mice with acetone leaf extracts of *C.volkensii* at dose levels of 50 mg/kg body weight reduced the number of writhes by 81.40%, which was comparable to the positive control (83.72%) ($p > 0.05$; Table 4.4). However, the administration of 100 mg/kg body weight acetone leaf extracts of *C.volkensii* did not reduce the number of writhings (-80.23%) highly concentrated extract molecules took longer to be absorbed across the membranes via filtration so that lower concentration of the extract in the 50 mg/kg body weight dose level was absorbed faster than 100 mg/kg body weight and caused antinociceptive activity in mice (Table 4.4).

Table 4.4: Antinociceptive properties of *C.volkensii* (Harms) in Acetic acid-induced pain in mice

ANIMAL GROUPS (N=5)	TREATMENT	NUMBER OF WRITHINGS (5-20 MIN)	% INHIBITION
Baseline	Aceticacid (20ml/kgbw)	20.2±1.66 ^a	-17.44
Negative Control	DMSO (30%)	17.2±2.18 ^a	0.00
Positive Control	Diclofenac (15mg/kgbw)	2.80±0.97 ^b	83.72
Acetone Extract	50mg/kg bw	3.20±1.16 ^b	81.40
	100mg/kg bw	31.0±2.53 ^c	-80.23

Values are expressed as Mean ± SEM Values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p>0.05$)

In comparison, the acetone leaf extract of *C.volkensii* exhibited more effective antinociceptive activity than the acetone leaf extracts of *C.spinarum* at dose of 50 mg/kg body weight (Figure 4.3). However, at the dose level of 100 mg/kg body weight the acetone leaf extract of *C.spinarum* exhibited better antinociceptive activity than *C.volkensii* (Figure 4.3).

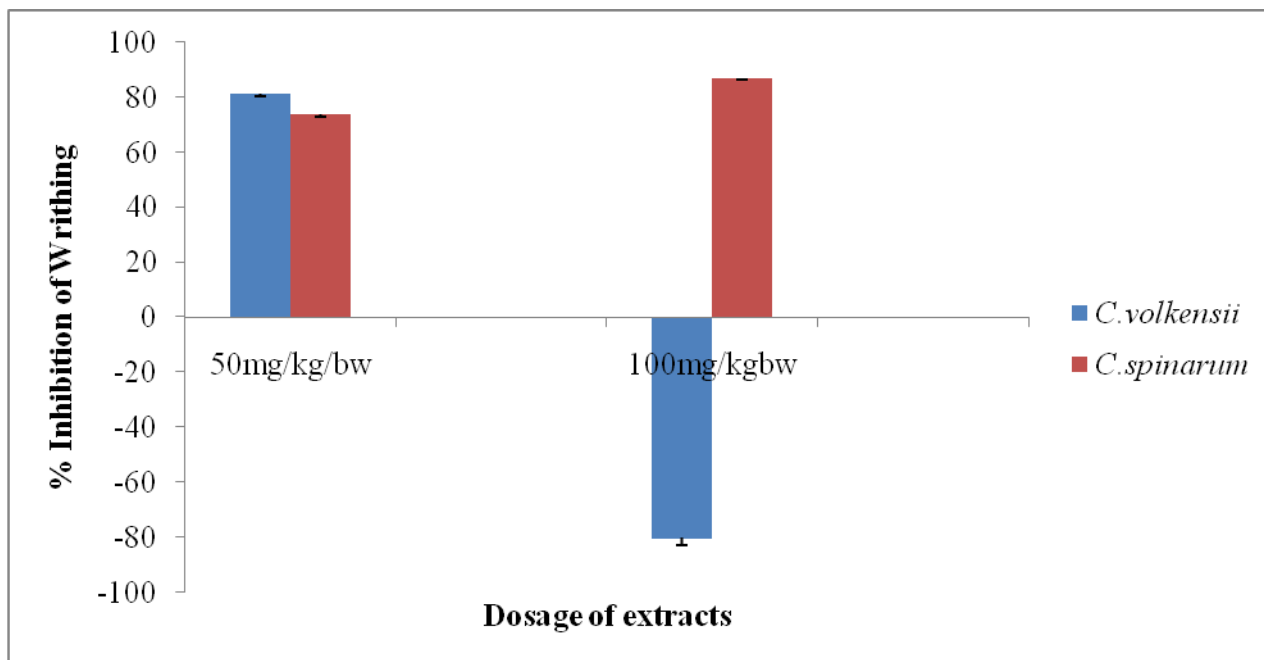


Figure 4.3: Antinociceptive effects of acetone leaves extracts on acetic acid induced pain.

4.3 Phytochemical screening

Upon qualitative phytochemical screening, the acetone leaf extracts of *C.spinorum* was found to contain alkaloids, flavonoids, steroids, phenolics and terpenoids while saponins and cardiac glycoside were absent. The acetone leaf extracts of *C.volkensii* tested positive for flavonoids, steroids and phenolics while alkaloids, saponins, cardiac glycosides and terpenoids were absent (Table 4.5).

Table 4.5: Phytochemical composition of acetone leaves extracts.

PHYTOCHEMICALS	<i>C.spinarum</i>	<i>C.volkensii</i>
Alkaloids	+	-
Flavonoids	+	+
Steroids	+	+
Saponins	-	-
Cardiac glycosides	-	-
Phenolics	+	+
Terpenoids	+	-

Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (-) sign.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

In this study, the acetone leaf extract of *C.volkensii* (Harms) and *C.spinorum* (Linn) showed a significant antinociceptive effect by reducing formalin paw-licking time in early and late phases and acetic acid induced writhing, with a more potent activity in the second phase. This suggests both central and peripheral antinociceptive effects (Idris *et al.*, 2014). This central antinociceptive effect could have been caused possibly by inhibition of the nociceptive effects of serotonin, adrenaline, noradrenaline, prostaglandins, bradykinin, acetylcholine and adenosine and peripherally by inhibiting the release of endogenous mediators such as PGE₂ prostaglandins E₂ and PGE₂ α in perionetal fluids as well as lipooxygenase which stimulates the nociceptive neurons (Tjolsen *et al.*, 1992).

The significant pain reduction by the plant extracts in mice might be due to the presence of analgesic principles acting through the prostaglandin pathways. The mechanism of action of these two plant extracts can be postulated to be similar to that of non-steroidal anti-inflammatory agents, such as ibuprofen, aspirin and diclofenac. The NSAIDs, block the production of prostaglandins by truncating the COX1 pathway (Lethaby *et al.*, 2000). This blockage reduces sensitization of the peripheral nervous tissue resulting in less nerve stimulation and ultimately less pain (Lethaby *et al.*, 2000).

The results of this study are similar to other previous studies on evaluation of antinociceptive activities of medicinal plant extracts (Gitahi *et al.*, 2015), and (Hesseinzadez *et al.*,2005) . That the acetone leaf extract of *C.volkensii* (Harms) and *C.spinarum* (Linn) showed significant antinociceptive effect by reducing formalin paw-licking and acetic acid writhings time in both phases is consistent with Gitahi *et al.* (2015) and Hesseinzadez *et al.* (2005) who worked on DCM methanolic leaf of *C.edulis*(Forssk) Vahl in laboratory animals and *M.officinalis* agnaist pain respectively.

The dose ranges used in this study were within the dose ranges used by Onzago *et al.* (2013), Norma *et al.* (2013), Ishola *et al.* (2014), Mohammed *et al.*.(2014), Gitahi *et al.* (2015). Gitahi *et al.* (2015) used dose ranges of 50, 100 and 150 mg/kg body weight while evaluating antinociceptive activity of DCM: methanolic leaf and root bark extracts of *C. edulis* (Forssk.) Vahl in laboratory animals.

The acetone leaf extract of *C.spinarum* (Linn) at the lower dose level of 50 mg/kg body weight was not as effective as the higher dose of 100 mg/kg body weight in both tests (Tables 4.1 and 4.3) This may mean that the effect is dose related or on the other hand there could have been fast metabolism, clearance and inactivation of the lower concentration of the active principle. Dose dependent antinociceptive effects were reported by Peter *et al.* (2012) working with ethanolic extracts of *Ocimum kilim*,

scharicum baker ex gürke and *ocimum kenyense ayob. ex a.j. paton* leaves and Gitahi *et al.* (2015) working with DCM: methanolic leaf and root bark extracts of *C. edulis* (Forssk.)Vahl in laboratory animals.

In this study, high concentration (100mg/kg body weight) of the acetone leaf extracts of *C. volkensii* failed to reduce the writhes. This possibly may be explained by the lower dissociation in higher concentration which may have impeded filtration across the mucosal lining. This is consisted with the observations of Christine *et al.* (1998) who worked on formalin test to assess the analgesic activity of diflunisal in the rat. The antinociceptive effect of the acetone leaf extract of *C. volkensii* (Harms) and *C. spinarum* (Linn) can be attributed to one or more groups of the phytoconstituents detected in the extracts.

The flavanoids too from *M. officinalis* have been associated with anti-nociceptive effects according to Miladi-Gorgi *et al.* (2005), Flavanoids have been shown to cause anti-nociceptive effects by widely targeting prostaglandins which are involved in the pain perception through moderating opioidergic mechanism (Manjunatha *et al.*, 2006). the anti-nociceptive effects of *M. officinalis* are attributed to the flavonoids present in the extracts. On the other hand studies, have shown that flavonoids widely target prostaglandins which are involved in the pain perception through moderating opioidergic mechanism (Manjunatha *et al.*, 2006).

Reanmongkol *et al.* (2005) reported that alkaloids present in *K. macrophylla* showed analgesic actions while Mei *et al.* (2011) asserted that the aqueous extracts of *Radix Aconiti Carmichaeli* exhibited antinociceptive effect probably due to the presence of high content of mesaconitine alkaloids.

Among other actions, naturally occurring terpenoids present antinociceptive properties by inhibiting platelet aggregation, and interfering at the intracellular level, with several steps of signal transduction mechanisms (Juma *et al.*, 2015)

5.2 Conclusions

In conclusion, the present study has demonstrated antinociceptive potential of acetone leaves extracts of *C.spinarum* (Linn) and *C.volkensii* (Harms) in mice models.

- The significant reduction in formalin induced pain when treated with standard drugs as well as different doses of extracts reflects that *C.spinarum* (Linn) and *C.volkensii*(Harms) acetone leaves extracts at 100 mg/kg body weight were almost similar to the standard drug diclofenac sodium.
- For acetic acid induced pain, there was significant reduction of pain when treated with the standard drug diclofenac as well as *C.spinarum*(Linn) and *C.volkensii* (Harms) at doses 100 mg/kgbw and 50 mg/kgbw respectively. The acetone leaves extracts of *C.spinarum* and *C.volkensii* contain different

phytochemicals (secondary metabolites) which are responsible for antinociceptive activity.

- The acetone leaves extracts of *C.volkensii* (Harms) and *C.spnarum* (Linn) demonstrated antinociceptive properties and acts possibly centrally and peripherally.

5.3 Recommendation

The acetone leaf extracts of both plants have significant antinociceptive activity hence this study recommends continued use of these plants in treating pain especially at dose level of 100 mg/kg body weight.

5.4 Suggestions for further studies

- i. This study recommends further elucidation of the mechanisms of action for the two plant acetone extracts.
- ii. The study also recommends evaluation of other plant parts for antinociceptive effects
- iii. Isolation of pure compounds for substructure analysis for antinociceptive effects is also recommended.
- iv. A thorough toxicological study of the effective plant fractions is also recommended.

- v. Other routes of extracts administration need to be explored to determine if the effects would be replicated.

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APPENDICES

Appendix I: Phase I antinociceptive activities of acetone extracts on formalin induced pain

ANIMALGROUPS N=5	<i>C.volkensii</i>	<i>C.spinarum</i>
Baseline formalin	258.60±10.9	300.40±20.80
Negative control(DMSO)	266.00±8.14	247.60±3.19
Positive control	295.40±3.44	209.60±3.53
diclofenac		
50mg/kgbw	227.20±3.89	239.00±3.11
100mg/kgbw	226.80±4.28	197.40±8.08

Values expressed as Mean \pm SEM for five animals per group. *P<0.05 versus leaf extract at dose of 50mg/kgbw by unpaired Student's t-test; ^aP<0.05 versus leaf extract at dose of 100mg/kgbw by unpaired Student's t-test;

Appendix II: Phase II antinociceptive activities of acetone extracts on formalin induced pain

Animal Groups (N=5)	<i>C.volkensii</i> (Harms)	<i>C.spinarum</i>(Linn)
Baseline (Formalin)	803.80±1.74	287.20±3.84
Negative control (DMSO)	718.4±2.66	271.60±2.98
Positive control (Diclofenac)	259.40±3.44	10.60±1.54
50mg/kgbw	195.80±2.60	178.0±2.77
100mg/kgbw	4.00±0.707	12.20±0.80

Values expressed as Mean ± SEM for five animals per group. *P<0.05 versus leaf extract at dose of 50mg/kgbw by unpaired Student's t-test; ^aP<0.05 versus leaf extract at dose of 100mg/kgbw by unpaired Student's t-test;

Appendix III: Antinociceptive activities of acetone extracts on acetic acid induced pain.

Treatment Groups (N=5)	<i>C.volkensii</i>(Harms)	<i>C.spinarum</i>(Linn)
Baseline (Formalin)	20.20 ± 1.66	19.60 ± 3.70
Negative control (DMSO)	17.20 ± 2.18	12.20 ± 1.50
Positive control (Diclofenac)	2.80 ± 1.66	3.60 ± 1.50
50mg/kgbw	3.20 ± 1.16	3.20 ± 1.02
100mg/kgbw	31.0 ± 2.53	1.60 ± 0.40

Values expressed as Mean ± SEM for five animals per group. *P<0.05 versus leaf extract at dose of 50mg/kgbw by unpaired Student's t-test; ^aP<0.05 versus leaf extract at dose of 100mg/kg bw by unpaired Student's t-test;



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Ref: No.

Date: **15th June, 2015**

NACOSTI/P/15/6765/5819

Kiambi Joseph Mworira
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on "*Evaluation of antinociceptive activity of acetone leaf extracts of carissa spinarum and caesalpinia volkensii in mice,*" I am pleased to inform you that you have been authorized to undertake research in **Nairobi County** for a period ending **30th September, 2015.**

You are advised to report **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.

Said Hussein
SAID HUSSEIN
FOR: DIRECTOR-GENERAL/CEO

Copy to

The County Commissioner
Nairobi County.

The County Director of Education
Nairobi County.

COUNTY COMMISSIONER
NAIROBI COUNTY
P. O. Box 30124-00100, NBI
TEL: 341666



