EVALUATION OF THE ANTINOCICEPTIVE, ANTIPYRETIC AND ANTI-INFLAMMATORY PROPERTIES OF METHANOLIC BARK EXTRACTS OF *Terminalia brownii* IN WISTAR RATS

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DECLARATION

DECLARATION

I, Mbiri Jane Wanja, declare that this thesis is my original work and has not been presented for the award of a degree in any other university or any other award.

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We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as University supervisors.

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DEDICATION

This thesis is dedicated to my late father Reuben Mbiri Kamanda, late grandfather James Kamanda, grandmother Bertha Kamanda and my sister Beth Mbiri for their immeasurable support, guidance and sacrifice towards my education.

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ABSTRACT

Pain, pyrexia and inflammation cause unnecessary discomfort, suffering and also lower productivity of the victims. Conventional drugs for these conditions are expensive, not easily available and have adverse side effects. There is therefore need to develop alternative therapeutic agents, such as medicinal plant derivatives, that are cheaper and have lesser side effects. *Terminalia brownii* is used in traditional medicine to treat pain, pyrexia, inflammation but there is no scientific evidence to confirm these ethno-medicinal claims. The present study therefore tested for the anti-nociceptive, antipyretic and anti-inflammatory properties of methanolic bark extracts of *T. brownii* in *Rattus novegicus*. The plant samples sourced from Kitui County, Kenya were dried and milled at Kenyatta University Biochemistry department laboratory. Adult male Wistar rats (*R. novegicus*), 2-3 months old, weighing 140-180g were divided into six groups of 5 rats each scheduled for different treatments; normal, negative and positive controls and three experimental groups (50, 100 and 150mg/kg bw extract). Formalin-induced pain, turpentine oil-induced pyrexia and carrageenan-induced paw edema were used to assess the antinociceptive, antipyretic and anti-inflammatory properties of the extract, respectively. The antinociceptive and anti-inflammatory activities of the extract were compared to those of diclofenac while the antipyretic activity of the extract was compared to that of aspirin. The phytochemical secondary metabolites tested for include alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids and terpenoids. *T. brownii* methanolic bark extract demonstrated significant antinociceptive, antipyretic and anti-inflammatory effects in a dose-dependent manner. The extract at the dose level of 150mg/kg bw exhibited the highest antinociceptive, antipyretic and anti-inflammatory activities and its activities were comparable to those of the respective reference drugs. The methanolic bark extracts of *T. brownii* reduced the paw licking time by between 4.62%-44.96% ($p \leq 0.05$) in the early phase and 35.77%-58.89% ($p \leq 0.05$) in the late phase. Diclofenac on the other hand reduced the paw licking time by 44.79% in the early phase and 55.33% in the late phase. The extract reduced the elevated rectal temperatures by between 1.15%-4.38% ($p \leq 0.05$) while aspirin reduced by between 0.00%-4.85%. The extract reduced the inflamed paw diameter by between 1.57%-20.41% ($p \leq 0.05$) while diclofenac reduced by between 11.12%-25.33%. Phytochemical screening of the extract indicated the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids and terpenoids. The present study therefore demonstrated the antinociceptive, antipyretic and anti-inflammatory properties of methanolic bark extracts of *T. brownii* hence providing a basis for further research that may result in pure compounds that can be advanced into drug discovery.

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ABBREVIATIONS AND ACRONYMS

°C	Degrees Celsius
ANOVA	Analysis of variance
COX	Cyclooxygenase
CM	Centimeter
CNS	Central Nervous System
DMSO	Dimethylsulphoxide
$FeCl_3$	Ferric Chloride
HCL	Hydrochloric Acid
H_2SO_4	Sulphuric Acid
IL	Interleukin
KU	Kenyatta University
LBP	Lipopolysaccharides Binding Proteins
LPS	Lipopolysaccharides
NAOH	Sodium Hydroxide
NSAIDs	Non-steroidal ant-inflammatory drugs
PGE 2	Prostaglandin E2
SEKU	South Eastern Kenya University
SEM	Standard Error of the Mean
NSAIPs	Non-steroidal ant-inflammatory drugs
TM	Traditional Medicine
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Traditional medicine refers to therapeutic practices that have been in existence before the development and spread of modern medicine and are still in use today (Pal and Shukla, 2003). About 75-80% of the world populations rely on herbal medicine for primary health care (Kambo, 2000). According to Pal and Shukla, (2003), the use of herbal medicine exceeds that of conventional drugs by 2-3 times. However, there is little information regarding the literature on medicinal plants use in Kenya (Kigen *et al.,* 2013). Most communities in Kenya, especially from the poor rural areas still rely on herbal remedies (Kigen *et al.,* 2013).

Pain, pyrexia and inflammation act as a warning of external noxious stimuli and microbial invasion to the body. However, they are viewed as sources of discomfort and are commonly suppressed with analgesics, antipyretics and anti-inflammatory medications respectively (Shah and Seth, 2010). These conventional drugs may have various severe side effects. The major adverse reactions of ibuprofen, an analgesic, include the effects on the kidney, the gastrointestinal tract and the coagulation system (Rocca *et al.,* 2005). Diclofenac, an analgesic and anti-inflammatory drug, is a known hepatotoxic drug in certain individuals and it also causes deposition of urate crystals in kidneys, liver, heart and spleen (Modi *et al.,* 2012). Sulindac causes serious gastrointestinal (GI)

adverse effects including inflammation, ulceration, bleeding, stomach perforations, large and small intestines perforations, which can be fatal (Modi *et al.*, 2012). In addition to having the above side effects, the conventional drugs are expensive and have low efficacy (Amaral *et al*., 2007). This therefore calls for continuous research to discover new compounds as therapeutic alternatives. Bordgers *et al*. (2013) showed that naturally occurring agents, such as *T. brownii* derivatives are best alternatives.

Terminalia brownii is used in traditional medicine to suppress conditions like ulcers, malaria, cough, stomach ache, arthritis, jaundice, sexually transmitted diseases and diarrhea (Machumi *et al.,* 2013). *T. brownii* has medicinal properties of the three conditions under investigation but this lacks scientific validation since its use has been based on traditional knowledge. This study is therefore aimed at evaluating the antinociceptive, antipyretic and anti-inflammatory properties of the methanolic bark extracts of *Terminalia brownii.*

1.2 Problem statement

Terminalia brownii is usually used in traditional medicine to treat pain, pyrexia and inflammation but there is of scientific evidence of the anti-nociceptive, antipyretic and anti-inflammatory properties of *T. brownii* to confirm these ethno medicinal uses.

1.3 Justification

Pain, pyrexia and inflammation cause unnecessary suffering and discomfort (Kim *et al.,* 2004). The use of conventional drugs has been ineffective due to side effects and their expensive nature (Maina *et al.,* 2015). Thus, there is need for cheaper and effective alternative therapeutic agents such as medicinal plants derivatives to manage the conditions. This study provides scientific evidence based on the ethno-medicinal claims of the use of *T. brownii* on pain, pyrexia and inflammation. The study also provides crucial ethno pharmacological lead towards the discovery of plant-based antipyretic, anti-inflammatory and anti-nociceptive drugs with fewer side effects.

1.4 Hypotheses

i. The methanolic bark extracts of *T. brownii* have no antinociceptive properties

ii. The methanolic bark extracts of *T. brownii* have no antipyretic properties

iii. The methanolic bark extracts of *T. brownii* have no anti-inflammatory properties

iv. The methanolic bark extracts of *T. brownii* have no phytochemical secondary metabolites

1.5 Objectives of the study

1.5.1 Broad Objective

To determine the anti-nociceptive, antipyretic and anti-inflammatory properties of methanolic bark extracts of *T. brownii* in *Rattus norvegicus*

1.5.2 Specific Objectives

i. To determine the anti-nociceptive properties of methanolic bark extracts of *T. brownii.*

ii. To evaluate the antipyretic properties of methanolic bark extracts of *T. brownii*

iii. To determine the anti-inflammatory properties of methanolic bark extracts of *T. brownii*

iv. To identify the major phytochemical secondary metabolites in methanolic bark extracts of *T. brownii*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biochemical and physiological basis of pain, pyrexia and inflammation

Pain is defined as a displeasing sensory and emotional feeling associated with actual or potential tissue damage or described in terms of such damage (Maina *et al.,* 2015). Pyrexia is a normal tissue response to invasion of the body by microorganisms and is part of the defense against infection (Watson *et al.,* 2003). Inflammation is the basic way in which the body reacts to irritation, infection, or other tissue injuries, the key features being pain, swelling, redness and warmth (Stankov, 2012).

There are two basic types of pain; acute and chronic pain depending on the duration (Hudspith *et al.,* 2006). Acute pain can be as a result of tissue injury, inflammation or disease. This type of pain generally comes on suddenly and especially after a trauma or surgery and may be accompanied by anxiety or emotional distress. Clinically, acute pain lasts less than 30 days, chronic pain last more than six months, and sub acute pain lasts from 1-6 months. A popular alternative definition of chronic pain, involving no arbitrarily fixed durations is pain that exceeds the period within which it is supposed to heal (Turk and Akiko, 2000).

Pyrexia, also known as fever, is a natural defense mechanism by the body that creates an environment where infectious agent or damaged tissue cannot survive (Watson *et al.,* 2003). Studies by Watson *et al.* (2003) have also shown that pyrexia generally occurs due to tissue injury or infection by micro-organisms that produce pyrogens. These pyrogens act on white blood cell, which in turn produce endogenous toxins that act on the anterior hypothalamus raising the body temperature to above 37.5^{o}C, a phenomenon known as fever. As a person's temperature increases, there is, in general, a feeling of cold and a warm feeling is experienced upon reaching the new temperature (Singh *et al*., 2013).

The endogenous pyrogens involved in the production of a highly regulated inflammatory response to infections and tissue injury are polypeptide cytokines (Shah and Seth, 2010). Pyrogenic cytokines, such as interleukin-1b (IL-1b), (TNF), and IL-6, are those that act on the hypothalamus directly leading to the production of a fever response (Luheshi, 1998). The inflammatory response is the body's natural defense strategy as a result of damage to body tissues and most of the body defense elements are found in the blood (Patil *et al*., 2015). Inflammation occurs in response to physical injuries, intense irritating chemicals, intense heat and infection by viruses and bacteria (Ali and Siddiqui, 2013).

Five cardinal signs were used by the ancients to characterize inflammation; heat (*calor),* redness (*rubor*), pain (*dolor*), swelling (*tumour*), and loss of function

(*functiolaesa*), based on visual observation (Punchard *et al.,* 2004). Celsus named the first four signs in ancient Rome (30–38 B.C.) while the last sign was named by Galen (A.D 130–200) (Punchard *et al.,* 2004). The heat sensation is usually as a result of the increased blood movement into the environmentally cooled extremities through the dilated blood vessels and this also results to the increased redness; due to the increased erythrocytes passing through the inflamed area. The edema is the result of infiltration of cells into the damaged area, increased passage of fluid from dilated and permeable blood vessels into the surrounding tissues, and deposition of connective tissue in prolonged inflammatory responses. Pain is due to the direct effects of the pain mediators involved and the stretching of sensory nerves due to edema. The loss of function may either refer to the situation where functional cells are replaced with a scar tissue or loss of mobility in a joint mainly due to the edema and pain.

The inflammatory response is important because it; disposes of pathogens and cell debris, prevents damaging agents from spreading to the nearby tissues, and sets the stage for the repair process. Inflammation is diverse as it ranges from the acute inflammation which is associated with the infection of the skin by *S. aureus* (the humble boil), to chronic inflammation that results in remodeling of the artery wall in atherosclerosis and the bronchial wall in chronic bronchitis and asthma. The immune system cells involved in these processes include; basophils, T-cells, neutrophils, mast cells and B-cells (Punchard, 2004).

According to Punchard *et al.* 2004, mediators and regulators like leukotrines, peptides, cytokines, growth factors, prostaglandins, complement and are all involved in the domineering of these events. The mediators and cells that come into play depends on; what stage the process of inflammation is at; the initiating event, i.e. chemical or physical injury, pathogen type, auto-immune etc.; whether the inflammation is of acute or chronic, type; the organ or tissue involved; whether there is granuloma formation.

Many degenerative diseases such as olymyalgiarheumatica, rheumatoid arthritis, shoulder tendonitis, inflammatory bowel disease, heart disease, gouty arthritis and asthma are often associated with the inflammatory process (Iwalewa *et al*., 2007; Mwangi *et al*., 2015). Furthermore, the oxidative and inflammatory processes are some of the pathological features that are associated with the CNS in Alzheimer's disease according to Howes and Houghton, (2003).

2.2 Conventional Management of Pain

Conventionally, over the counter, analgesics such as anti-depressants, opioids (Morphine Sulphate, Oxycodone, and Tramadol hydrochloride), simple analgesics (aspirin and paracetamol) and non-steroidal anti-inflammatory drugs (NSAIDS) (Ibuprofen, Diclofenac Sodium and Naproxen Sodium), are used for treatment of pain (Abbott *et al*., 1996; Jung *et al*., 1997). NSAIDS is one of the most widely used class of analgesic drugs (Raama *et al.*, 2010), however, although most

clinically important NSAIDS possess good analgesic activity, there should be a long-term administration, especially to treat chronic painful diseases (Bordgers *et al*., 2013).

These conventional drugs may have various severe side effects. The major adverse reactions of ibuprofen include the effects on the gastrointestinal tract (GIT), the kidney and the coagulation system (Rocca *et al.,* 2005). Diclofenac is known hepatotoxic drug in certain individuals and it also causes deposition of urates crystals in kidneys, liver, heart and spleen (Modi *et al.,* 2012). Sulindac causes serious gastrointestinal effects including inflammation, ulceration, bleeding, stomach perforations, large and small intestines perforations, which can be fatal (Modi *et al.,* 2012).

2.3 Conventional Management of Pyrexia

Antipyretic drugs achieve their antipyretic activity by preventing or inhibiting COX_2 expression to reduce the elevated body temperature by inhibiting PGE_2 biosynthesis (Shukla and Mehta, 2015). By inhibition of the activity of the enzyme cyclooxygenase and reduction of the PGE_2 levels in the hypothalamus, fever is reduced (Mwangi *et al.,* 2015). There are two types of COX enzymes; COX-1 enzyme that produces prostaglandins that support platelets and protect the stomach and COX-2 enzyme that produces prostaglandins responsible for inflammation (Mitchell *et al.,* 1993).

According to Subedi *et al.* (2016) the most commonly prescribed antipyretic medications worldwide are non-steroidal anti-inflammatory drugs. They inhibit thrombolytic aggregation and are used primarily to manage pain, fever and inflammation (Miller, 2016). NSAIDs block the activity of the COX enzymes therefore reducing prostaglandins throughout the body leading to a reduction of the ongoing pain, fever and inflammation (Yorio *et al.,* 2011). Since the prostaglandins that protect the stomach also are reduced, NSAIDs can result in ulceration of the stomach walls leading to bleeding (Thomas *et al.,* 2008).

2.4 Conventional Management of Inflammation

Non-steroidal anti-inflammatory drugs are able to relieve inflammation and the associated pain by inhibiting cyclooxygenase enzymes involved in the production of prostaglandins (Lima *et al.,* 2011). Diclofenac, according to Todd and Sorkin, (1988); Skoutakis *et al*. (1986) can be used to suppress inflammation by inhibiting prostaglandins synthesis and/or production. Aspirin is the most widely used drug in the world today, because of its ability to act as anti-inflammatory medicine (Serhan *et al*., 2004; Schwab *et al*., 2007). However, patients with a history of peptic ulcer or other gastrointestinal disorders are prone to gastro duodenal lesions on prolonged use of aspirin.

2.5 Nociception assays

There are a number of nociception models that can be used for screening of the antinociceptive properties of various agents. They include; the formalin assay (Spindola *et al.,* 2012) and acute thermal assays like the hot plate test, tail flick test and Hargreaves tests (Allen and Yaksh, 2004). These assays use behaviors such as withdrawal, licking, flinching (shaking of the injured paw) and lifting to measure the existence of pain (Abbort *et al*. 1996).

Formalin produces a biphasic pain response; phase I also known as neurogenic pain and phase II also known as inflammatory pain (Spindola *et al.,* 2012). The first phase starts from 0-10 minutes after formalin injection (Hassani *et al.,* 2015) and mediators such as amino acids and kinins (Spindola *et al.,* 2012) are released. This phase results from the direct activation of primary afferent sensory neurons (Mc Namara *et al.,* 2007) and therefore, drugs that act primarily on the central nervous system, like opoids, can inhibit neurogenic pain (Hassani *et al.,* 2015). The second phase starts from 20-30 minutes after formalin injection leading to the release of inflammatory mediators like bradykinin, histamine, prostaglandins, ILs and TNF-α (Hassani *et al.,* 2015). This phase reflects a combination of peripheral input and spinal cord sensitization (Spindola *et al.,* 2012) and it's sensitive to peripherally-acting drugs like NSAIDs and corticosteroids (Hassani *et al.,* 2015).

Acute thermal assays; the hot plate, tail flick and Hargreaves tests are among the most commonly used nociception models (Allen and Yaksh, 2004). The manipulation, in principle, serves to activate high threshold sensory fibres that innervate the skin. These fibres transducer temperatures in the range of those that produce escape behavior when applied to the skin. The frequency of discharge is usually proportional to the intensity of the stimulus that's exposed to the skin. Such stimuli evoke a behavioral response like foot withdrawal when it is applied to the foot. The advantage of these assays is that they allow repeated and multiple testing using a single animal because the stimulus is transitory and produces no tissue damage (Allen and Yaksh, 2004).

2.5. Pyrexia Assays

Brewer's yeast is commonly used to induce pyrexia in rat models. It is an exogenous pyrogen and it contains a lipopolysaccharide that binds to the lipopolysaccharides binding protein (LBP) (Ukwuani *et al.* 2012). This binding leads to the production of IL-1, IL-6 and TNFα, (endogenous cytokines), that penetrates the blood-brain barrier and act on the thermoregulatory center in the hypothalamus. This in turn, activates the arachidonic acid pathway resulting in the synthesis and release of PGE2, ultimate mediators of pyrexia (Gege-Adebayo, 2013).

Lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, is an exogenous pyrogens and it's also commonly used to induce pyrexia in rat models (Anochie *et al.,* 2013). LPB, an immunological protein, binds to LPS and this complex in turn binds to the CD14 receptor of the macrophage nearby. This results in the synthesis and release of IL-1, IL-6 and TNFα (endogenous cytokines). These cytokines in turn activates the arachidonic acid pathway leading to the production of PGE2 (Cannon *et al*., 1990; Klir *et al*., 1994; Anochie *et al*., 2013).

Turpentine oil also serves as a pyrogenic stimulus (Leon, 2002). Turpentine-induced pyrexia is associated with the production of endogenous cytokines that do enhance the production of prostaglandins leading to body temperatures elevation (Zhu *et al*., 2011). Polyinosinic: polycytidylic acid (poly I: C), a double-stranded RNA that is used to model viral infections *in vivo*, is a potent pyrogen (Fortier *et al.*, 2004).

2.6 Inflammation assays

Carrageenan, derived from the Irish word "carraigin" meaning Irish moss, refers to a species of red alga *Chondrus crispus* found along the rocky areas of the Atlantic coast of British Isles, Europe, and North America (Morris, 2003). It also refers to its mucopolysaccharide extract that was discovered in 1862 by the British pharmacist Stanford (Morris, 2003). Carrageenan, 1-3% in normal saline,

is commonly used as an intra-plantar injection in doses of 50–150 µl. For the modeling of specific pathophysiological conditions higher concentrations have been used (Necas and Bartosikova, 2013).

Carrageenan-induced edema is a biphasic, age-weight dependent event that involves various mediators that do produce the inflammatory response. In the early phase, the detectable mediators include bradykinin, histamine and serotonin while prostaglandins and COX-2 are the mediators involved in the late phase. The early phase cannot be blocked by NSAIDS but the late phase can because, NSAIDS alleviate inflammation by reducing the production of prostaglandins and COX-2 (Necas and Bartosikova, 2013). Increase in paw size is used to quantify the inflammatory response that's usually maximal at around the fifth hour post Carageenan injection.

Administration of 20µl of 1% formalin into the dorsal surface of the left hind paw of the rats one hour after the treatments have been administered produces sub-acute inflammation (Ibironke and Ajiboye, 2007). This is caused by cell damage and provokes the production of inflammation mediators like bradykinin, serotonin and histamine. Dextran, a polysaccharide of high molecular weight, when injected in the sub-plantar tissue of hind paw in animal models, produces an anaphylactic reaction. This reaction, due to the production of histamine and serotonin from

mast cells, is characterized by edema formation as well as extravasation (Van Wauve and Goosens, 1989).

Intra-plantar injection of the complete Freud's adjuvants leads to the induction of chronic inflammation. The pro-inflammatory cytokines produced include; IL-6, TNFα, and IL-1β (Raghavendra *et al*., 2004). The cotton pellet granuloma test also produces sub-acute inflammation (ChaitanyaB, 2012). A sterilized cotton pellet, 30mg, is introduced subcutaneously in the groin region of the rats. After the treatments, the animals are killed, using ether or chloroform, the pellets removed, dried overnight at 60°C and weighed (Ibironke and Ajiboye, 2007).

2.7 Role of medicinal plants in disease management

Traditional medicine refers to the therapeutic practices that have been in existence before the modern medicine development and spread and are still in use today (Pal and Shukla, 2003). About 75-80% of the world population still relies on herbal medicine for primary health care (Kambo, 2000). According to Pal and Shukla, 2003, the use of herbal medicine exceeds that of conventional drugs by 2-3 times. However, there is little information regarding the literature on medicinal plants use in Kenya (Kigen *et al.,* 2013). Most communities in Kenya, especially from the poor rural areas still rely on herbal remedies (Kigen *et al.,* 2013). For many years, medicine had depended exclusively on leaves, flowers and barks of plants; only recently has synthetic drugs come into use.

Nature provides numerous remedies to cure most ailments of mankind from its plants, animals and other sources and therefore, it acts as a good source of salvation for man's health (Saha *et al*., 2012). The search for new drugs to combat diseases and infections has aroused the interest of scientists all over the world in herbal remedies. Medicinal plants are very important because they replace or assist conventional drugs in the management of various diseases (Bordgers *et al.,* 2013). Natural compounds, mostly from plants, have been the manifestation of traditional medicine for thousands of years (Ginsburg and Deharo, 2011). Since time immemorial man has been using plants as traditional medicines for health care (Uddin *et al.,* 2011; Rauf *et al*., 2014).

2.6 Herbal Management of Pain, Pyrexia and Inflammation

Currently, there are many studies in literature that have been conducted on various medicinal plants and revealed their potential in managing pain, pyrexia and inflammation. The study on the assessment of the antinociceptive activity of *Piper cubeba* L. essential oil in animal models by Mothana *et al.* (2016) demonstrated a significant antinociceptive activity. Maina *et al.* (2015) also demonstrated the antinociceptive activity of dichloromethane: methanolic leaf and root bark extracts of *Carissa edulis* in rats. Studies by Silva *et al.* (2015) revealed that the crude ethanolic extract of *Annona vepretorum* has significant antinociceptive properties. Similarly, Safari *et al.* (2016a) demonstrated a significant antinociceptive activity of the aqueous bark extracts of *Acacia nilotica*.

According to the research conducted by Mwonjoria *et al*. (2011), the herbal extract of *Solanum incanum* in possesses antipyretic properties. Studies by Antonisamy *et al.* (2011) have revealed that fliedelin isolated from *Azima tetracantha* Lam. posses antipyretic activity. The study by Alam *et al.* (2016) demonstrated the antipyretic activities of five medicinal plants; *Alhagi Maurorum, Echinops echinatus, Panicum turgidum, Fagonia cretica* and *Cymbopogon jwarancusa.* Intahphuak *et al.* (2010) also demonstrated the antipyretic activity of virgin coconut oil.

Research by Mradu *et al.* (2013) demonstrated the anti-inflammatory activity of the methanolic stem bark extracts of *Tinospora cordifolia* Wild fruits of *Emblica officinalis* and rhizomes of *Cyperus rotundus* Linn in rodents. Significant anti-inflammatory activities of the ethanolic and aqueous extracts of *Kalanchoe pinnatta* (Lam.) Pers ware also demonstrated by Matthew *et al.* (2013) on carrageenan-induced edema in rats. The study by Shukla and Mehta, (2015) demonstrated the *in vivo* anti-inflammatory properties of *Caesalpinia bonducella* F. Similarly, Arul *et al.* (2005) demonstrated the anti-inflammatory activity of the leaves of *Aegle Marmelos* Corr.

According to Bent and Ko (2004), herbs are normally considered to be safe because they are ‘natural’. However, a number of dangerous and lethal side effects of these herbs have been reported. These include; allergic reactions,

interactions with drugs and other herbs, direct toxic effects and effects from contaminants (Bent and Ko, 2004).

2.8 *Terminalia brownii*

Terminalia brownii (Plate 2.1) belongs to the family Combretaceae (Machumi *et al.,* 2013). According to Mbwambo *et al.* (2007), *T. brownii* has various vernacular names in different regions; Koloswa (Northern region, Kenya), Kiukuu or Muuku (Kamba, Kenya), lbukoi (Samburu, Kenya), Weba (Ethiopia), Orbukoi (Maasai, Tanzania) and Mwalambe or Mbarao in Kiswahili. *T. brownii* occurs in many parts of Africa; Kenya, Ethiopia, Tanzania, Uganda, Eritrea and the Democratic Republic of Congo (Mbwambo *et al.,* 2007).

This tree grows near rivers and wadies in dry areas, it also grows in moist savannas of semi-arid regions, and it thrives best on sandy loam soils (Schmidt, 2010). *T. brownii* is a small deciduous tree that is less than 20m tall. The tree has a flat or round crown with its branches appearing from whorls giving the tree a layered appearance. Its leaves are alternate and spirally arranged. Flowers are small, white in color and have an unpleasant smell (Schmidt, 2010).

Plate 2.1. A mature *T. brownii* tree

Terminalia brownii has a wide range of medicinal uses in African traditional medicine (Machumi *et al.*, 2013). The barks of *T. brownii* are boiled or chewed to treat abdominal pains (Kipkore *et al*., 2016). Hot water extract of *T. brownii* is taken orally, one glass 2-3 times daily for two weeks to treat chronic joint pains (Wambugu *et al*., 2011). A concoction made from the barks of *T. brownii* and the roots of *Toddalia asiatica* is used to treat arthritis (Kigen *et al*., 2014). The bark is also chewed to treat coughs and joint stiffness (Kaigongi and Musila, 2015). Dried and ground barks are used as a clotting agent. Tying up the cut with a strip

of a fresh bark can also speed up the clotting (Kaigongi and Musila, 2015). For the treatment of liver disease and jaundice, the barks of *T. brownii* are usually concocted with the barks of *Croton macrostachyus* and then the patient takes a cup of the infusion (Belayneh and Bussa, 2014).

According to Mbwambo *et al.* (2007), the leaves are used to treat heartburn, diarrhea, gastric ulcers, stomach ache and colic. The leaves and bark infusions are mixed with meat to treat hepatitis. The barks from stem, trunk and branches are used to treat gonorrhea, syphilis, urethral pain, urogenital infections and leucorrhoea. Stem bark decoctions are taken against body swellings, malaria, epilepsy, urino-genital problems, and as anthelminthic.

Terminalia brownii has been revealed to possess antimicrobial (Kareru *et al*., (2008a); Machumi *et al.*, (2013); Mbwambo *et al.*, (2007) and anti-plasmodial properties (Machumi *et al.,* 2013). Phytochemical composition of *T. brownii* has also been evaluated using different solvents. Ethyl acetate and n-hexane extracts of *T. brownii* showed the presence of five compounds; arjungenin, β- sitosterol, betulinic acid, monogynol A and stigmasterol (Opiyo *et al.,* 2011). Methanolic leaf extracts of *T. brownii* in Ethiopia have also been reported to possess phytosterols, coumarins, flavonoids, tannins, polyphenols, and saponins (Periasamy *et al.,* 2015). Phytochemical screening of ethyl acetate stem bark extract of *T. brownii* revealed the presence of 3-*O*-β-D-glucopyranosyl-β-

sitosterol, an oleanane-type triterpenoid, seven ellagic acid derivatives and seven known triterpenoids (Machumi *et al.,* 2013). Fourier Transform Infrared (FTIR) spectroscopy of crude dry powder of *T. brownii* revealed the presence of saponins (Kareru *et al.,* 2008b). The safety dose range of the methanolic bark extracts of *T. brownii* has been demonstrated to be up to 2000 mg/kg bw (Periasamy *et al*., 2015)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and preparation of plant materials

The plant barks were collected from Kitui County, Kenya. *Terminalia brownii* was locally identified with the help of a local herbalist. Bark samples were collected, sorted, cleaned and transported in polythene bags to the Biochemistry and Biotechnology laboratories at Kenyatta University (KU) for further processing. Plant specimens were then taken to the East Africa Herbarium, National Museums of Kenya for botanical authentication and a specimen deposited at Kenyatta University Herbarium. The barks of *T. brownii* were cut into small pieces and dried at room temperature for two weeks. They were then ground into fine homogenous powder via an electric mill and sieved through a mesh sieve.

3.2 Extraction of the plant material

To obtain the extract, 200g of the sample's powder was soaked in methanol and stirred for six hours. The extract was filtered using Whatman's filter paper No.1 and the filtrate concentrated under reduced pressure and vacuum using a rotary evaporator. The concentrate was then put in an airtight container and stored at 4 ^{o}C before use in bioassay studies.

3.3 Experimental animals

Adult male Wister rats, *Rattus norvegicus,* 2-3 months old and weighing between 140-180g were used to assess the anti-nociceptive, antipyretic and anti-inflammatory activities of *T. brownii* using (Khan *et al.,* 2013). Animal breeding was carried out in the Animal Breeding and Experimentation Facility of the Department of Biochemistry and Biotechnology, KU. The rats were then allowed to acclimatize for 48 hours before beginning the experiment. The rats were caged and maintained well under standard laboratory conditions. They were fed on a rodent pellets and supplied with water *ad libitum* (Vogel, 2002). All the experiments conducted in the present study followed the guidelines for the care and use of experimental animals in laboratories.

3.4 Bio screening

3.4.1 Evaluation of anti-nociceptive activities

The formalin assay was used to evaluate the anti-nociceptive activities of the *T. brownii's* bark extracts. The rats were divided into six groups with five rats each and the treatments were as shown in Table 3.1.

Table 3.1. Treatment procedure used for the evaluation of the anti-nociceptive activities of methanolic bark extracts of *T. brownii* in *R. norvegicus*

Group	Status	Treatment
I	Normal control	None
II	Negative control	Formalin + DMSO
III	Positive control	Formalin + 15 mg/kg Diclofenac +DMSO
IV	Experimental group A	Formalin + 50 mg/kg extract + DMSO
V	Experimental group B	Formalin + 100 mg/kg extract + DMSO
VI	Experimental group C	Formalin + 150 mg/kg extract + DMSO

Subcutaneous administration of 0.1 ml of 2.5% formalin into the sub-plantar region of the left hind paw led to the induction of nociceptive behaviors; biting, licking and lifting (Tjolsen *et al*., 1992). Formalin was administered thirty minutes after the various treatments were given. One rat at a time was placed in a transparent glass cage to allow proper observation of the nociceptive behaviors. The time that the rats spent licking and biting the injected paw was scored in two distinct phases. The early phase was scored for the first five minutes after formalin injection and the late phase was scored 15-30 minutes after formalin injection. The following formula was then used to calculate percentage inhibition of paw licking;

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

Where;

C- The vehicle treated control group value for each phase

T - The treated group value for each phase

3.4.2 Evaluation of antipyretic activities

The antipyretic activity of *T. brownii's* bark extracts was evaluated by using 100% turpentine oil as the fever inducing agent and Aspirin, dissolved in 10% DMSO, as the standard drug. The rats were divided into six groups, each group having 5 rats, and the treatments were as shown in Table 3.2.

Table 3.2. Treatment procedure for the evaluation of antipyretic activities of methanolic bark extracts T. brownii in *R. norvegicus*

Group	Status	Treatment
I	Normal control	None
II	Negative control	Turpentine + DMSO
III	Positive control	Turpentine + 100 mg/kg Aspirin + DMSO
IV	Experimental group A	Turpentine +50 mg/kg extract + DMSO
V	Experimental group B	Turpentine +100 mg/kg extract + DMSO
VI	Experimental group C	Turpentine +150 mg/kg extract + DMSO

Before fever induction, the rats were weighed and their rectal temperature taken by inserting a well lubricated thermistor probe of a digital thermometer about 3 cm (Grover, 1990) in to the rectum. The digital thermometer (Mode YB-009, Shenzhen Osykyoo Technology Co., Ltd, Guadong, China) was calibrated against a mercury thermometer. After obtaining the initial temperatures, fever was induced using 25ml/kg bw turpentine oil. Rats that experienced a temperature rise by 0.8°C after an hour were considered pyretic and therefore given the respective treatments. Temperatures were recorded at hourly intervals up to the fourth hour after administration of the treatments. Rectal temperatures before and after

treatments were compared by calculating the percentage change and percentage inhibition using the following formula (Hukkeri *et al.*, 2006; Ray, 2006);

$$\frac{B - Cn}{B} \times 100$$

Where,

B - Rectal temperature at 1 hour after turpentine administration

C_n - Rectal temperature after drug administration

3.4.3 Evaluation of anti-inflammatory activity

The anti-inflammatory activity of *T. brownii's* extract was evaluated using Carrageenan (0.l ml, 1% w/v in normal saline) as the inflammation inducing agent and diclofenac as the reference drug. The experimental units were divided into six groups (n=5) and then treatments were done as shown in Table 3.3.

Table 3.3. Treatment procedure used for the evaluation of anti-inflammatory activities of Methanolic bark extracts of T. brownii in *R. novegicus*

Group	Status	Treatment
I	Normal control	None
II	Negative control	Carrageenan + DMSO
III	Positive control	Carrageenan + DMSO + 15 mg/kg diclofenac
IV	Experimental group A	Carrageenan + DMSO + 50 mg/kg extract
V	Experimental group B	Carrageenan + DMSO + 100 mg/kg extract
VI	Experimental group C	Carrageenan + DMSO + 150 mg/kg extract

Evaluation of the anti-inflammatory activity was carried out as described by Winter *et al*. (1962). The paw diameter of the rats was measured in mm using a

SDC041 digital vanier calipers (Xuzhou Smile Trading Company Ltd., Jiangsu, China) and recorded before inflammation induction. The inflammation-inducing agent was then injected into the sub-plantar tissue of left hind paw one hour after administration of the treatments. Paw diameter was then measured one hour after induction of inflammation up to the forth hour (Bamgbose and Noamesi, 1981). Paw diameter measured prior to the Carrageenan injection was then compared with the diameter of the same paw after Carrageenan injection by calculating the percentage inhibition and percentage change using the formulae below;

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

Where,

Ct = Paw diameter at 1 hour after Carrageenan administration

Tt = Paw diameter after Treatment

3.5 Qualitative phytochemical screening

Presence or absence of the selected phytochemicals in the extract was done using methods of analysis as described by Harbone, (1998) and Kotake, (1994). Standard screening tests for detecting the presence of different phytochemicals were employed. The secondary metabolites that were tested for include; saponins, cardiac glycosides, flavonoids, terpenoids, phenolics, alkaloids, and sterols.

3.5.1 Saponins (Froth test)

Two milliliters of the plant extract was mixed with 2 ml of sodium bicarbonate solution and shaken vigorously to test for saponins. The extract was then allowed to stand for 15-20 minutes and classified for saponin content as follows:

a) Negative - No froth
b) Weakly positive - Froth less than 1 cm
c) Positive - Froth 1.2 cm high
d) Strongly positive - Froth greater than 2 cm high

3.5.2 Alkaloids

To test for alkaloids, 5 ml of the extract was first acidified with 1 M HCl. This acidic medium was heated and then treated with Dragendroff‘s reagent. The formation of an orange or reddish brown precipitate indicated the presence of alkaloids.

3.5.3 Terpenoids (Salkowski test)

To test for terpenoids, 1 ml of ethyl acetate/petroleum ether was added to 0.5 g of the extract and then mixed into 2 ml of chloroform. Three milliliters of 2 M sulphulic acid (H_2SO_4) was then carefully added alongside to form a layer. The formation of a reddish brown coloration at the interface was regarded as positive results for the presence of terpenoids.

3.5.4 Flavonoids (Sodium hydroxide test)

The presence of flavonoids in the extract was tested by mixing 2 ml of the extract with 2 ml of 5 M sodium hydroxide. An intense/golden yellow precipitate indicated positive results.

3.5.5 Cardiac glycosides (Keller-Kilian test)

To test for cardiac glycosides presence, 0.5 g of the extract was dissolved in 2 ml glacial acetic acid containing 2 drops of 10% ferric chloride solution. One milliliter of concentrated H_2SO_4 was then carefully added underlying this mixture. Formation of either a violet, brown or greenish ring at the interphase was regarded as positive for the deoxysugar characteristic of cardenolides.

3.5.6 Steroids

The presence of steroids in the extract was tested by dissolving 0.5 g of the extract in 2 ml of chloroform. Three milliliters of 2M H_2SO_4 was then carefully added to the sides of the test tube forming a layer. The reddish brown color at the interphase depicted the presence of steroids.

3.5.7 Phenols

To screen for the presence of phenols in the extract, 1 ml of ferric chloride solution was added to 2 ml of the extract. Formation of blue to green colour indicated the presence of phenolics.

3.6 Statistical analysis

All the data was recorded and tabulated on Excel spreadsheet and the minitab statistical software version 17.1.0 (Penn State University, 1972) was used for analysis. The data was subjected to descriptive statistics and the results were expressed as mean ± standard error of mean. One-way analysis of variance (ANOVA) was used to determine the statistical significant differences among groups. Values of $p \leq 0.05$ were considered to be significant. This was followed by Tukey's tests to separate the means and obtain the specific significant differences among the different groups. Values with the same superscripts were considered to have no statistical significant differences.

CHAPTER FOUR

4.0 RESULTS

4.1 Antinociceptive activity of methanolic bark extracts of *T. brownii* on formalin-induced pain in *R. novegicus*

The administration of the methanolic bark extract of *T. brownii* reduced the formalin-induced pain in both early and late phases and this was indicated by the reduction in paw licking time (Table 4.1).

In the early phase, treatment with the extract at the dose levels; 50, 100 and 150 mg/kg bw exhibited a dose dependent trend and reduced paw licking time by 4.62%, 23.59% and 44.96%, respectively (Table 4.1). On the other hand, the reference drug (diclofenac) reduced the paw licking time by 44.79%. The antinociceptive effects extract at the dose levels of 50, 100 and 150 mg/kg bw were significantly different amongst each other ($p < 0.05$, Table 4.1). At the dose level of 150 mg/kg bw, the extract was comparable to the standard control drug ($p > 0.05$, Table 4.1). The extract at the dose levels of 100 and 150 mg/kg bw was significantly different from the negative and normal control groups ($p < 0.05$, Table 4.1). In this phase, the extract at the dose level of 150 mg/kg bw demonstrated the highest antinociceptive activity.

Table 4.1. Anti-nociceptive activity of methanolic bark extracts of *T. brownii* in *R. novegicus*

Group	Treatment	Paw Licking Time After Treatment (Sec)	
		Early Phase	**Late Phase**
Normal control	DMSO	0.00±0.00[d] (100.00%)	0.00±0.00[d] (100.00%)
Negative control	Formalin + DMSO	117.00 ±4.11[a] (00.00%)	191.20±4.80[a] (00.00%)
Positive control	Formalin + Diclofenac + DMSO	64.60±2.29[c] (44.79%)	85.40±3.78[c] (55.33%)
Experimental Group A	Formalin + 50 mg/kg bw+ DMSO	111.60±4.34[a] (4.62%)	122.80±4.89[b] (35.77%)
Experimental Group B	Formalin + 100 mg/kg bw+ DMSO	89.40±4.43[b] (23.59%)	96.00±5.44[c] (49.79%)
Experimental Group C	Formalin + 150 mg/kg bw+ DMSO	64.40±5.95[c] (44.96%)	78.60±7.11[c] (58.89%)

Values were expressed as Mean ± SEM for the five rats per group. Statistical comparisons were made within a column and values with the same superscript were not significantly different by ANOVA followed by Tukey's post hoc test ($p > 0.05$). Values in brackets indicate percentage paw licking inhibition. Formalin = 2.5%; DMSO = 10%; Diclofenac = 15 mg/kg.

In the late phase, the methanolic bark extracts of *T. brownii* exhibited a dose dependent response on the formalin-induced pain. The standard drug and the extract at the dose levels of 50, 100 and 150 mg/kg bw reduced the paw licking time by 55.33%, 35.77%, 49.79% and 58.89 respectively (Table 4.1). The antinociceptive effect of the extract at the dose levels of 100 and 150 mg/kg was not significantly different ($p > 0.05$, Table 4.1). However, the antinociceptive activity of the extract at the dose levels of 100 and 150 mg/kg bw was significantly different from the antinociceptive activity of the extract at the dose level of 50 mg/kg ($p < 0.05$, Table 4.1). The antinociceptive activity of the extract at dose levels 100 and 150 mg/kg bw was comparable to that of the reference drug, diclofenac ($p > 0.05$, Table 4.1). The extract at the three dose levels was significantly different from the negative and normal control groups ($p < 0.05$, Table 4.1). In this phase, the extract at the dose level of 150 mg/kg bw demonstrated the highest antinociceptive activity.

4.2 Antipyretic activity of methanolic bark extracts of *T. brownii* on turpentine oil-induced pyrexia in *R. novegicus*

The methanolic bark extracts of *T. brownii* demonstrated antipyretic activity on the turpentine-induced pyrexia in rats and this was indicated by reduction in the rectal temperatures after extract administration (Figure 4.1, Table 4.2). In the first one hour, the methanolic bark extracts of *T. brownii* at the dose levels of 50, 100 and 150 mg/kg bw and aspirin, the reference drug, lowered the elevated rectal

temperatures by 1.15%, 2.76%, 4.28% and 4.85% respectively (Table 4.2) . The extract at the three dose levels showed a dose dependent response (Figure 4.1, Table 4.2). The antipyretic activity of the extract among the three dose levels was significantly different ($p < 0.05$, Table 4.2). The antipyretic activity of the extract at the dose level of 150 mg/kg bw was comparable to that of the reference drug ($p > 0.05$, Table 4.2). The extract at the three dose levels was significantly different from the negative control group ($p < 0.05$, Table 4.2)

Table 4.2. Antipyretic activity of methanolic bark extracts of *T. brownii* in *R. novegicus*

Group	Treatment	% Change in Rectal Temperatures after Treatment				
		Ohrs	**1h**	**2h**	**3h**	**4h**
Normal Control	DMSO	100.00±0.00 (0.00%)	100.00±0.09^{ab} (0.00%)	99.95±0.13^{a} (0.05%)	99.95±0.05^{b} (0.05%)	99.95±0.10^{b} (0.05%)
Negative Control	Turpentine +DMSO	100.00±0.00 (0.00%)	100.47±0.23^{a} (-0.47%)	101.04±0.36^{a} (-1.05%)	101.57±0.30^{a} (-1.57%)	102.35±0.24^{a} (-2.35%)
Positive Control	Turpentine +Asprin+DMSO	100.00±0.00 (0.00%)	95.42±0.23^{d} (4.85%)	95.78±0.32^{cd} (4.22%)	95.99±0.34^{d} (4.01%)	95.99±0.46^{d} (4.01%)
Experimental Group A	Turpentine +50 mg/kg+DMSO	100.00±0.00 (0.00%)	98.90±0.29^{b} (1.15%)	97.81±0.50^{b} (2.19%)	98.02±0.37^{c} (1.98%)	97.76±0.40^{c} (2.25%)
Experimental Group B	Turpentine +100 mg/kg+DMSO	100.00±0.00 (0.00%)	97.24±0.35^{c} (2.76%)	97.34±0.29^{bc} (2.66%)	97.45±0.27^{c} (2.55%)	97.35±0.35^{cd} (2.65%)
Experimental Group C	Turpentine +150 mg/kg+DMSO	100.00±0.00 (0.00%)	95.72±0.55^{d} (4.28%)	95.62±0.48^{d} (4.38%)	95.99±0.23^{d} (4.02%)	96.24±0.26^{d} (3.76%)

The values were expressed as Mean± SEM for five rats per group. Statistical comparisons were made within a column and values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p > 0.05$). Percentage reduction in rectal temperature is given within the brackets. Turpentine oil =25 ml/kg bw; DMSO = 10%; Asprin = 15 mg/kg.

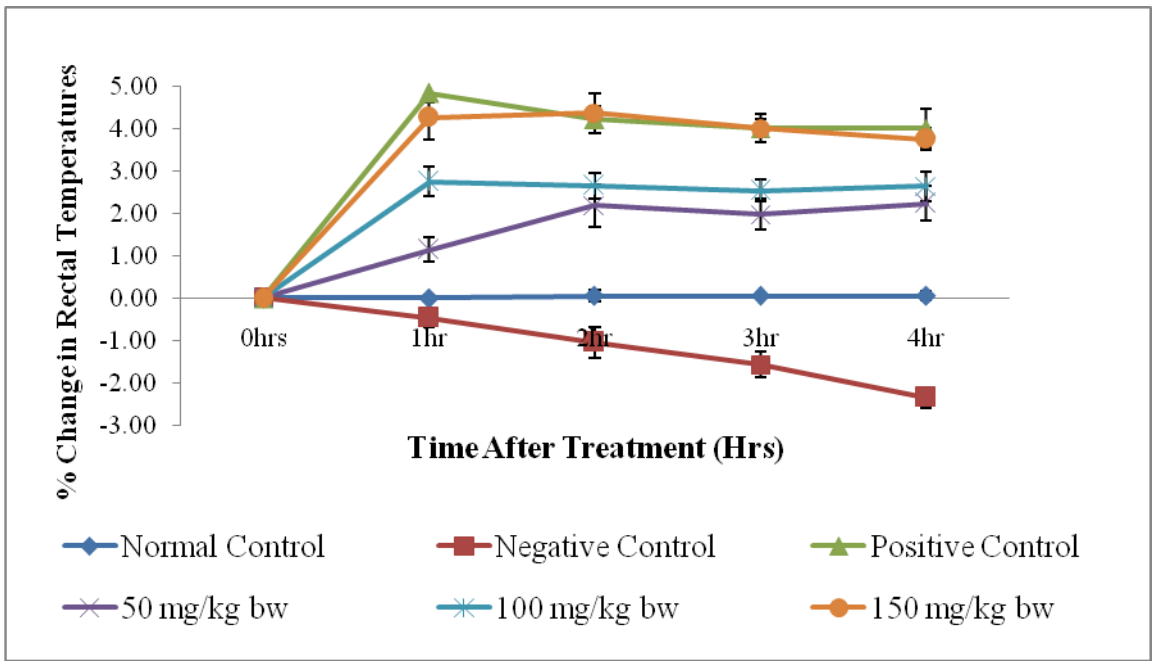

Figure 4.1. Antipyretic activity of the methanolic bark extracts of *T. brownii* in *R. novegicus*

In the second hour after administration of the treatments, the *T. brownii* extract at the dose levels of 50, 100 and 150 mg/kg bw and the reference drug lowered the elevated rectal temperatures by 2.19%, 2.66%, 4.38% and 4.22%, respectively (Figure 4.1, Table 4.2). The extract at the three dose levels demonstrated a dose dependent response (Figure 4.1, Table 4.2). The antipyretic activity of the extract at the dose level of 150 mg/kg bw was significantly different from the antipyretic activity of the extract at the dose levels of 50 and 100 mg/kg bw ($p < 0.05$, Table 4.2). In this hour, the antipyretic activity of the extract at the dose levels of 100 and 150 mg/kg bw was comparable to that of the reference drug ($p > 0.05$, Table 4.2). The extract at the three dose levels was significantly different from the negative and normal control groups ($p < 0.05$, Table 4.2)

In the third hour, the extract at the dose levels of 50, 100 and 150 mg/kg bw and the reference drug reduced the turpentine-induced pyrexia by 1.98%, 2.55%, 4.02% and 4.01%, respectively (Figure 4.1, Table 4.2). The extract at the three dose levels showed a dose dependent response (Figure 4.1). The antipyretic activity of the extract at the dose level of 150 mg/kg bw was significantly different from the antipyretic activity of the extract at the dose levels of 50 and 100 mg/kg bw ($p < 0.05$, Table 4.2). The antipyretic activity of the extract at the dose level of 150 mg/kg bw was comparable to that of the standard drug ($p >$

0.05). The extract at the three dose levels was significantly different from the negative and normal control groups ($p < 0.05$, Table 4.2).

Four hours after the administration of the treatments, the extract at the dose levels of 50, 100 and 150 mg/kg bw and the reference drug reduced the elevated rectal temperatures by 2.55%, 2.65%, 3.76% and 4.01%, respectively (Figure 4.1, Table 4.2). The extracts of *T. brownii* at the three dose levels demonstrated a dose dependent response on the turpentine oil-induced pyrexia (Figure 4.1). The antipyretic activity of the extract at the dose level of 150 mg/kg bw was significantly different from the antipyretic activity of the extract at the dose level of 50 mg/kg bw ($p < 0.05$). Compared to the reference drug and the extract at the dose level of 100 mg/kg bw, the extract at the dose level of 150 mg/kg bw did not show a significant difference ($p > 0.05$, Table 4.2). The extract at the three dose levels was significantly different from the negative and normal control groups ($p < 0.05$, Table 4.2). The extract at the dose level of 150 mg/kg bw exhibited the highest antipyretic activity in all the four hours.

4.3 Anti-inflammatory activity of methanolic bark extracts of *T. brownii* on carrageenan-induced edema in *R. novegicus.*

The methanolic bark extract of *T. brownii* demonstrated anti-inflammatory activity on the carrageenan-induced paw edema in the experimental rats and this was indicated by the reduction of the paw diameter after the administration of the

treatments (Figure 4.2, Table 4.3). In the first hour after administration of the treatments, the extract at the dose levels of 50, 100 and 150 mg/kg bw and the reference drug, diclofenac, did not demonstrate anti-inflammatory activity, -4.11%, -5.39%, -3.08% and -0.28%, respectively (Figure 4.2, Table 4.3). The extract at the three dose levels showed no significant difference ($p > 0.05$, Table 4.3). At the dose levels of 50 and 150 mg/kg bw, the extract was comparable to the reference drug ($p > 0.05$, Table 4.3). The extract at the three dose levels was not significantly different from the negative control group ($p > 0.05$, Table 4.3).

Table 4.3. Anti-inflammatory activity of methanolic bark extracts of *T. brownii* in *R. novegicus*

Groups	Treatment	% Change in paw diameter after treatment				
		0hr	**1h**	**2h**	**3h**	**4h**
Normal Control	DMSO	100.00±0.00 (0.00%)	100.00±0.00^{b} (0.00%)	100.00±0.00^{b} (0.00%)	100.00±0.00^{b} (0.00%)	100.00±0.00^{b} (0.00%)
Negative Control	Carrageenan +DMSO	100.00±0.00 (0.00%)	105.40±1.43^{a} (-5.45%)	109.34±2.82^{a} (-9.14%)	112.57±2.98^{a} (-12.63%)	111.08±2.50^{a} (-11.13%)
Positive Control	Carrageenan+Diclofenac+DMSO	100.00±0.00 (0.00%)	100.28±0.21^{b} (-0.28%)	88.88±1.92^{c} (11.12%)	79.31±0.62^{c} (20.69%)	74.67±1.44^{e} (25.33)
Experimental Group A	Carrageenan +50 mg/kg+DMSO	100.00±0.00 (0.00%)	104.10±0.91^{ab} (-4.11%)	100.73±0.54^{b} (-0.73%)	97.12±0.84^{b} (2.88%)	94.44±1.53^{bc} (5.56%)
Experimental Group B	Carrageenan +100mg/kg+DMSO	100.00±0.00 (0.00%)	105.39±1.25^{a} (-5.39%)	98.43±0.83^{b} (1.575)	94.18±1.32^{b} (5.82%)	88.79±1.14^{cd} (11.21%)
Experimental Group C	Carrageenan +150mg/kg+DMSO	100.00±0.00 (0.00%)	103.08±1.27^{ab} (-3.08%)	89.95±1.77^{c} (10.05%)	85.35±2.13^{c} (14.65%)	79.59±4.59^{de} (20.41%)

The values were expressed as Mean± SEM for five rats per group. Statistical comparisons were made within a column and values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test ($p > 0.05$). Percentage reduction in paw diameter is given within the brackets. Carrageenan = 1%; DMSO = 10%; Asprin = 15 mg/kg.

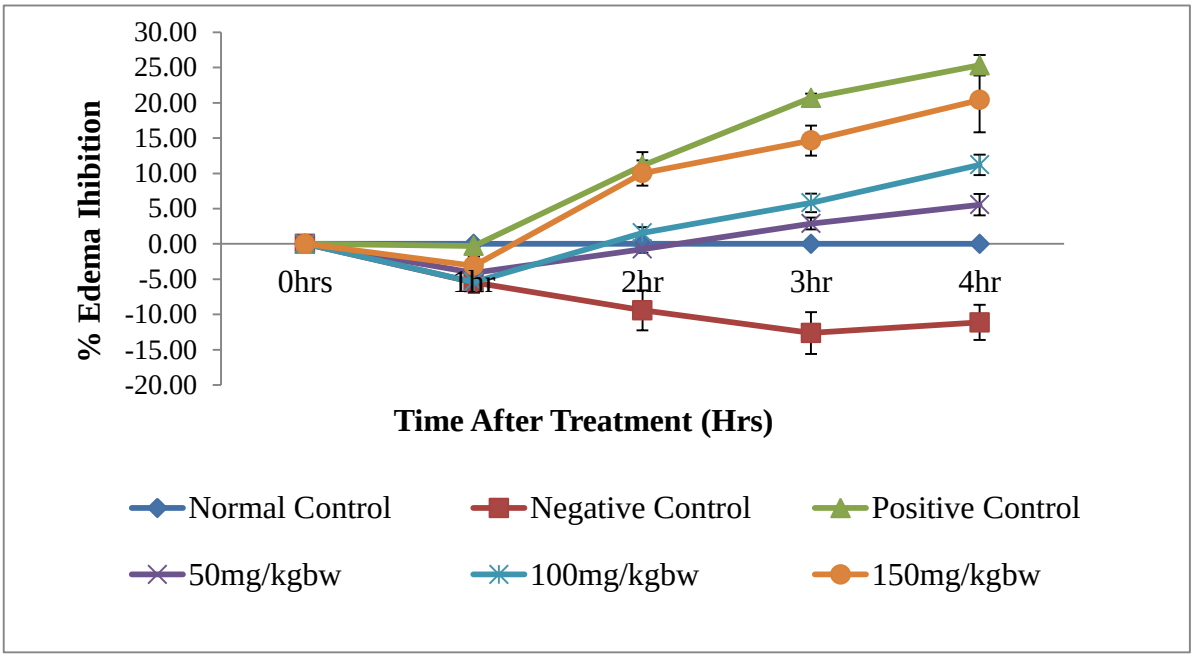

Figure 4.2. Anti-inflammatory activity of methanolic bark extracts of *T. brownii* in *R. novegicus*

In the second hour after the administration of the treatments, the extract at the dose levels of 50, 100 and 150 mg/kg bw and the reference drug reduced the Carrageenan-induced paw edema in the left hind paw of the experimental rats by -0.73%, 1.57%, 10.05% and 11.12%, respectively (Figure 4.2, Table 4.3). The three dose levels of the extract exhibited a dose-dependent response (Figure 4.2, Table 4.3). The anti-inflammatory activity of the extract at the dose level of 150 mg/kg bw was significantly different from the anti-inflammatory activity of the extract at the dose levels of 50 and 100 mg/kg bw ($p < 0.05$, Table 4.3). Compared to the reference drug, the extract at the dose level of 150 mg/kg bw did not show a significant difference ($p > 0.05$). The extract at the three dose levels was significantly different from the negative control group ($p < 0.05$, Table 4.3).

In the third hour after the administration of the treatments, the extract at the dose levels of 50, 100 and 150 mg/kg bw and the reference drug reduced the Carrageenan-induced paw edema by 2.88%, 5.82%, 14.65% and 20.69%, respectively (Figure 4.2, Table 4.3). The extract at the three dose levels demonstrated a dose dependent response (Figure 4.2, Table 4.3). The anti-inflammatory activity of the extract at the dose level of 150 mg/kg bw was significantly different from the anti-inflammatory activity of the extract at the dose levels of 50 and 100 mg/kg bw ($p < 0.05$, Table 4.3). However, compared to the reference drug, the extract at the dose level of 150 mg/kg bw did not show

significant difference ($p > 0.05$). The extract at the three dose levels was significantly different from the negative control group ($p < 0.05$, Table 4.3).

In the fourth hour after the administration of the treatments, the methanolic bark extracts of *T. brownii* at the dose levels of 50, 100 and 150 mg/kg bw and the reference drug reduced the Carrageenan-induced paw edema by 5.56%, 11.21%, 20.14% and 25.33%, respectively (Figure 4.2, Table 4.3). The three dose levels of the extract demonstrated a dose dependent response (Figure 4.2, Table 4.3). The anti-inflammatory activity of the extract at the dose level of 150 mg/kg bw was significantly different the anti-inflammatory activity of the extract at the dose level of 50 mg/kg bw ($p < 0.05$, Table 4.3). However, the anti-inflammatory activity of the extract at the dose level of 150 mg/kg bw was comparable to that of the reference drug and the extract at the dose level of 100 mg/kg bw ($p > 0.05$, Table 4.3). The extract at the three dose levels was significantly different from the negative control group ($p < 0.05$, Table 4.3). The extract at the dose level of 150 mg/kg bw exhibited the highest anti-inflammatory activity in all the four hours.

4.4 Phytochemical screening

Qualitative phytochemical screening of the methanolic bark extracts of *T. brownii* showed the presence of cardiac glycosidases, steroids, flavonoids, alkaloids, phenols, saponins and terpenoids (Table 4.4).

Table 4.4. The phytochemical composition of *T. brownii*

Phytochemical	Presence/absence
Alkaloids	+
Cardiac glycosides	+
Flavonoids	+
Phenols	+
Saponins	+
Steroids	+
Terpenoids	+

The presence of the phytochemicals was denoted by the (+) sign

CHAPTER 5

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

The present study evaluated for the antinociceptive, antipyretic and anti-inflammatory properties of the methanolic bark extracts of *T. brownii* in *R. novegicus.* The antinociceptive properties of the extract were evaluated on formalin-induced pain in the left hind paw of male Wistar rats. Acute thermal assays like the hot plate, tail flick and Hargreave's tests are other models that have been used to screen for the antinociceptive activities of various agents (Allen and Yaksh, 2004). Acetic acid can also be used to induce pain (Spindola *et al.,* 2012). However, the formalin assay was chosen over other models because; it has the potential to mimic human clinical pain conditions (Ibironke and Ajiboye, 2007), the freely moving unrestrained animal allows for the observation of spontaneous pain-related responses (Mc Namara *et al.,* 2007) and it produces a response in two distinct phases and this allows researchers to model both acute and chronic pain using a single noxious chemical.

Formalin evoked a biphasic pain response; phase I also known as neurogenic pain and phase II also known as inflammatory pain (Spindola *et al.,* 2012). The first phase starts from 0-10 minutes after formalin injection (Hassani *et al.,* 2015) and mediators such as amino acids and kinins (Spindola *et al.,* 2012) are released. This phase results when the primary afferent sensory neurons are directly

activated (Mc Namara *et al.,* 2007) and therefore, drugs that act primarily on the CNS, opoids for example, can inhibit neurogenic pain (Hassani *et al.,* 2015). The second phase starts from 20-30 minutes after formalin injection leading to the release of inflammatory mediators like bradykinin, histamine, prostaglandins, ILs and TNF-α (Hassani *et al.,* 2015). This phase reflects a combination of peripheral input and spinal cord sensitization (Spindola *et al.,* 2012) and it's sensitive to peripherally-acting drugs like NSAIDs and corticosteroids (Hassani *et al.,* 2015).

In the present study, 2.5% formalin was injected into the sub plantar region of the left hind paw of the experimental animals. This concentration of formalin was chosen because it evokes a maximum response and according to Clavelou *et al*. (1995), higher concentrations of formalin may lead to other behavioral responses that may interfere with the primary antinociceptive behavior. Under normal physiological conditions, rats tend to lick their forepaws (Mwangi *et al.,* 2015) and so as to show that the paw licking was entirely due to formalin, the hind paw was chosen.

The methanolic bark extracts of *T. brownii* demonstrated a significant antinociceptive activity by reducing the paw licking time in both phases. These results showed that the extract was able to inhibit the activation of the primary afferent sensory neurons and the release of inflammatory pain mediators. It can therefore be suggested that the methanolic bark extracts of *T. brownii* contain

centrally and peripherally acting analgesic phytochemicals. These results are similar with the results of previous studies that have evaluated and revealed the antinociceptive activities of other medicinal plants (Silva *et al.,* (2015); Mothana *et al.,* (2016); Safari *et al.,* (2016a)).

The dose levels of the extract used in this study were 50, 100 and 150mg/kg bw and were in a similar dose range used by Safari *et al.* (2016b), Maina *et al.*(2015) and Ishola *et al.* (2014). The three dose levels of the methanolic bark extracts of *T. brownii* produced a dose-dependent response to the formalin-induced pain and this kind of response was also observed by Chatterjee *et al.* (2015). In the present study it was observed that the extract at the lower dose levels of 50 and 100mg/kg bw was not as effective as the higher dose level of the extract, 150mg/kg bw. This could be explained by the fast metabolism and clearance of the active principle(s) that was/were in an inadequate concentration in these lower dose levels of the extract (Maina *et al.,* 2015). It can also be suggested that at the dose level of 150mg/kg bw, the extract demonstrated the highest antinociceptive activity due to the presence of a sufficient concentration of the active principle(s) in the extract.

The antinociceptive effect of the methanolic bark extracts of *T. brownii* can be attributed to one or more groups of the phytochemicals observed in the extract. Several studies on medicinal plants revealed the presence of different phytochemicals such as steroids, saponins, alkaloids, flavonoids among others.

Various medicinal properties of these chemical constituents, like the antinociceptive, antipytretic, anti-inflammatory and other properties have been revealed (Subedi *et al.,* 2016).

Flavonoids exhibit antinociceptive activity (Hossinzadeh *et al.,* 2002). Flavonoids inhibit the activity of the enzyme endo-peroxidase (prostaglandin synthetase) leading to a reduction of the synthesis and release of prostaglandins, a pain mediator (Chatterjee *et al.,* 2015). Several studies have also revealed that alkaloids and saponins do posses antinociceptive properties (Kaleem *et al*. (2013); Hassan *et al.* (2010); Chindo *et al.* (2010)). Therefore, the flavonoids, saponins and alkaloids observed in the methanolic bark extract of *T. brownii* could be also responsible for the antinociceptive activity of the extract.

The methanolic bark extracts of *T. brownii* demonstrated a significant antipyretic activity in all the four hours after the administration of the treatments. These results relate to the findings of Alam *et al.* (2016), Intahphuak *et al.* (2010) and Antonisamy *et al.* (2011). Turpentine oil is an exogenous pyrogen that is widely used to induce fever in experimental rats and mice (Maina *et al.,* 2015). Other exogenous pyrogens that have been used to induce fever include; brewer's yeast, Lipopolysaccharides (Anochie *et al.,* 2013) and polyinosinic: polycytidylic acid (Frontier *et al.*, 2004).

Steam-distilled turpentine induces pyrexia faster (within an hour or two) and experimental animals acquire a higher tolerance to it as compared to the other exogenous pyrogens (Soszynski and Krajewska, 2002). It is against this background that turpentine oil was chosen as a model for screening the antipyretic activity of the methanolic bark extracts of *T. brownii* in this study. In the present study, crude turpentine was distilled to eliminate impurities and obtain pure turpentine oil. These impurities could have interfered with the tests or even killed the experimental rats as it was observed in the pilot study.

The most commonly prescribed drugs for the management of pyrexia are the NSAIDs such as diclofenac, aspirin and ibuprofen (Maina *et al.,* 2015). NSAIDs inhibit the activity of cyclooxygenase (COX-2), the enzyme that converts arachidonic acid to prostaglandins. The findings of this study suggest that the methanolic bark extract of *T. brownii* was able to inhibit the activity of COX-2 enzyme hence its antipyretic activity.

The dose levels of the extract used in the present study to evaluate the antipyretic activity of *T. brownii* were 50, 100 and 150mg/kg bw and were similar to the dose levels used by Maina *et al.* (2015), Afsar *et al*. (2015) and Akuodorl *et al*. (2013). These dose levels were chosen after carrying out a pilot study on a number of dosages. The methanolic bark extracts of *T. brownii* exhibited a dose-dependent response on the turpentine oil-induced pyrexia in the experimental rats. These

findings were consistent with the findings of other studies on medicinal plants from Cholistan desert Pakistan (Alam *et al.,* 2016), *Piper cubeba* L. essential oil (Mothana *et al.,* 2016) and ethyl acetate roots extracts of *Ocimum sanctum* (Kumar *et al*., 2015).

The extract at the dose level of 150mg/kg bw demonstrated the highest antipyretic activity compared to the lower dose levels of 50 and 100mg/kg bw in all the four hours after the administration of the treatments. This can be explained by the possible existence of an adequate concentration of the active principle(s) in the dose level of 150mg/kg bw compared to the lower dose levels of the extract. It can also be explained by the fast metabolism and clearance of the active principle(s) present in inadequate concentrations in the lower dose levels of the extract. The reference drug, Aspirin, demonstrated the highest antipyretic activity compared to the three dose levels of the extract. This can be suggested to be as a result of the reference drug exhibiting a better blockage of the biosynthesis of prostaglandins than the extract (Maina *et al.,* 2015).

The extract at the dose levels of 100 and 150mg/kg bw and the standard drug achieved their maximum antipyretic activity in the first, second and first hours respectively and then their activity decreased subsequently. This could probably be due to the metabolism and excretion of the respective treatments. However, the extract at the dose level of 50mg/kg bw achieved its maximum activity at the

fourth hour. This could be due to the slow diffusion of the active principle(s) that were present in very low concentrations in the extract across the cell membrane into the peritoneal cavity (Hossain *et al*., 2011).

The antipyretic activity of the methanolic bark extracts of *T. brownii* can be attributed to the presence of one or more groups of the phytochemical secondary metabolites observed in the extract. Steroids and terpenoids have been reported to inhibit the activity of prostaglandin synthetase, the enzyme that stimulates the production and release of prostaglandins, the primary mediator in fever induction (Niazi *et al*., 2010). Flavonoids can inhibit fever in two ways; by decreasing the release of arachidonic acid and by interfering with the eicosanoids biosynthesis pathways involved in fever production (Subedi *et al.,* 2016). These two actions in turn suppress mediators like prostaglandins responsible for fever (Subedi *et al.,* 2016). Saponins also posses antipyretic activity (Zakaria *et al.,* 2007). It can therefore be suggested that the terpenoids, steroids, flavonoids and saponins observed in the extract could be responsible for its antipyretic activity.

The methanolic bark extracts of *T. brownii* demonstrated a significant anti-inflammatory activity on the carrageenan-induced hind paw edema in *Rattus. novegicus.* These findings were consistent with the findings of previous similar studies such as the studies by Mradu *et al.* (2013), Shukla and Mehta, (2015), Arul *et al.* (2005) and Matthew *et al.* (2013).

Carrageenan-induced paw edema is one of the most feasible methods used to screen anti-inflammatory agents (Chatterjee *et al.,* 2015). Freund's adjuvant (Raghavendra *et al.,* 2004), dextran (Van Wauve and Goosens, 1989), cotton pellet granuloma (Maina *et al.,* 2015) and formalin (Ibronke and Ajiboye, 2007) are other inflammatory models. Carrageenan-induced paw edema is a simple and routine model for the evaluation of inflammation (Necas and Bartosikora, 2013) and produces an inflammatory response in two phases (Erdemoglu *et al.,* 2009; Thomazzi *et al.,* 2010) and hence, the phlogistic agent of choice in this study.

Carrageenan is a natural carbohydrate derived from a number of seaweeds of the class Rhodophyceae (Necas and Bartosikora, 2013). Sub plantar injection of carrageenan in the rat hind paw induces a biphasic edema; the early and late phases (Kapewangolo *et al.,* 2015; Nivsarkar *et al.,* 2009). The key inflammatory mediators detectable during the early phase (1 hour) of the carrageenan-induced edema include; serotonin, histamine and kinins (Chatterjee *et al.,* 2015). The late phase occurs after the first one hour of the carrageenan-induced edema and the key mediators detectable in this phase include prostaglandins and inducible cox-2 (Necas and Bartosikora, 2013).

Non-steroidal anti-inflammatory drugs such as indomethacin, aspirin and diclofenac are the conventional drugs used to manage inflammation (Mwangi *et*

al., 2015). The anti-inflammatory effect of NSAIDs is attributed to their inhibitory effect on the activity of COX-2 enzyme that converts arachidonic acid to the inflammatory mediator prostaglandins (Shukla and Mehta, 2015). Two types of COX enzymes exists; COX-1 enzyme that produces prostaglandins that are responsible for supporting platelets and protecting the stomach and COX-2 enzyme that produces prostaglandins that are responsible for inflammation (Mitchell *et al.,* 1993). Therefore, NSAIDS inhibit only the late phase of carrageenan-induced inflammation where prostaglandins and COX-2 enzymes are the detectable mediators (Necas and Bartosikora, 2013). It can therefore be suggested that the methanolic bark extracts of *T. brownii* reduced the carrageenan-induced paw edema by inhibiting the activity of COX-2 enzyme.

The dose levels used in this study for the evaluation the anti-inflammatory activities of the methanolic bark extracts of *T. brownii* were 50, 100 and 150mg/kg bw. These dose levels were similar to the dose ranges used by Safari *et al.* (2016c) and Falodun *et al.* (2006). The methanolic bark extracts of *T. brownii* demonstrated a dose-dependent response on the carrageenan-induced paw edema. A similar trend was also observed by Mwangi *et al.* (2015), Chatterjee *et al.* (2015) and Shukla and Mehta, (2015).

The methanolic bark extract of *T. brownii* demonstrated a minimal anti-inflammatory activity at the lower dose levels of 50 and 100 mg/kg body weight

as compared to the highest dose level of 150 mg/kg body weight and the reference drug in all the four hours after treatment. These findings indicate that the extract at the dose level of 150mg/kg was able to inhibit the activity of COX-2 better than the lower dose levels probably due to the presence of a sufficient concentration of the active principle. However, the anti-inflammatory activity of the extract at the dose level of 150mg/kg bw was comparable to the anti-inflammatory activity of the standard drug indicating that the two treatments inhibited the activity of COX-2 with a similar magnitude.

The extract at the three dose levels achieved maximum anti-inflammatory activity in the fourth hour indicating a slow but steady passive diffusion of the bioactive chemical constituents across the cell membrane into the peritoneal cavity (Hossain *et al*., 2010). However, the reference drug and the extract at the three dose levels did not inhibit the carrageenan-induced edema during the first hour. This can be explained by the absence of prostaglandins in this early phase of inflammation since the treatments were working by inhibiting the biosynthesis of prostaglandins just like the NSAIDS (Necas and Bartosikora, 2013).

The phytochemical screening of the methanolic bark extracts of *T. brownii* revealed the presence of various phytochemicals in the extract some of which could have been responsible for its anti-inflammatory activity. Flavonoids inhibit the activity of the enzyme prostaglandin synthetase (Chatterjee *et al.,* 2015).

Flavonoids have also been reported as potent anti-inflammatory agents in another study (Tapas *et al.,* 2008). Steroids reduce inflammation by inhibiting phospholipase A2 which is responsible for the hydrolyization of arachidonic acid from the membrane phospholipids leading to the formation of prostanoids and leukotrienes (Mencarelli, 2009).

Triterpenoids inhibit the production of prostaglandins and also suppresses the function of macrophages and neutrophils, hence their anti-inflammatory activity (Salminen *et al.,* 2008). Phenols reduce the level of inflammatory mediators like TNF-α and prostaglandins (Nyamai *et al*., 2016). They also lower the expression and inhibit the function of iNOS (Nyamai *et al*., 2016). Cardiac glycosides suppress hyper secretion of IL-8, a protein implicated in lungs inflammation thus inhibiting the activation of NF-β signaling pathway (Nyamai *et al*., 2016). This study therefore suggested that the flavonoids, steroids and terpenoids observed in the extract, acting either individually or synergistically could have been responsible for its anti-inflammatory activity.

5.2 Conclusion

The data obtained in this study revealed significant antinociceptive, antipyretic and anti-inflammatory properties of methanolic bark extracts of *T. brownii* which may be due to the presence of bioactive ingredients with a pharmacological potential. The extract demonstrated a dose dependent response to the formalin-

induced pain, turpentine oil-induced pyrexia and carrageenan-induced inflammation. At the dose level of 150mg/kg bw, the extract exhibited the highest antinociceptive, antipyretic and anti-inflammatory activities. The anti-nociceptive, anti-pyretic and anti-inflammatory activities of the extract at the dose level of 150mg/kg bw was comparable to the anti-nociceptive, anti-pyretic and anti-inflammatory activities of the respective reference drugs.

The results obtained in the present study supports the use of the plant species by traditional medicine practitioners in Kenya for the management and treatment of the three conditions studied; pain, fever and inflammation.

5.3 Recommendations

i. The leaves of *T. brownii* should be used instead of the barks to enhance the conservation of this medicinal plant

ii. Phytochemical screening of *T. brownii* to be done during the dry season since the samples of the present study were collected during the wet season

iii. Standardization of the crude plant extracts

5.4 Suggestions for further studies

i. Elucidation of the full mechanisms of antinociceptive, anti-inflammatory and antipyretic activities of *T. brownii*.

ii. Bio-screening of aqueous extracts of *T. brownii* to establish how they would compare with the organic solvent extracts in terms of the antinociceptive, antipyretic and anti-inflammatory activities.

iii. Combination therapy study of different parts of *T. brownii* like roots, bark and leaves to create a rationale for a combination therapy for pain, fever and inflammation.

iv. Bioassay-guided isolation of active phytochemical ingredients which could further be developed into antinociceptive, antipyretic and anti-inflammatory drugs.

v. Detailed and quantitative antinociceptive, antipyretic and anti-inflammatory studies at cellular level.

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APPENDICES

Appendix I. Analysis of the anti-nociceptive activity of *T. brownii* on formalin-induced pain *in R. novegicus*- Raw data

One-way ANOVA: Phase 1 versus TREATMENT

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENT	5	45775	9154.94	114.27	0.000
Error	24	1923	80.12		
Total	29	47698			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
8.95079	95.97%	95.13%	93.70%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENT	N	Mean	Grouping
Negative Control	5	117.00	A
50mg/kgbw	5	111.60	A
100mg/kgbw	5	89.40	B
Postive Control	5	64.60	C
150mg/kgbw	5	64.40	C
Normal Control	5	0.000000	D

Means that do not share a letter are significantly different.

One-way ANOVA: Phase 2 versus TREATMENT

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENT	5	97059	19411.7	164.74	0.000
Error	24	2828	117.8		
Total	29	99887			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
10.8551	97.17%	96.58%	95.58%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENT	N	Mean	Grouping
Negative Control	5	191.20	A
50mg/kgbw	5	122.80	B
100mg/kgbw	5	96.00	C
Postive Control	5	85.40	C

```
150mg/kgbw         5     78.60      C
Normal Control     5  0.000000        D

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

Appendix II. Analysis of the anti-nociceptive activity of *T. brownii* on formalin-induced pain in *R. novegicus* – Percentage inhibition

One-way ANOVA: PHASE 1 versus TREATMENTS

```
Source      DF  Adj SS   Adj MS  F-Value  P-Value
TREATMENTS   5   33439  6687.81   138.64    0.000
Error       24    1158    48.24
Total       29   34597


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
6.94538  96.65%     95.96%      94.77%
Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS        N      Mean  Grouping
NORMAL CONTROL    5     100.0  A
150mg/kgbw        5     44.96    B
POSITIVE CONTROL  5     44.79    B
100mg/kgbw        5     23.59      C
50mg/kgbw         5      4.62        D
NEGATIVE CONTROL  5  0.000000        D

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

One-way ANOVA: PHASE 2 versus TREATMENTS

```
Source      DF   Adj SS   Adj MS  F-Value  P-Value
TREATMENTS   5  26549.6  5309.93   196.81    0.000
Error       24    647.5    26.98
Total       29  27197.2


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
5.19426  97.62%     97.12%      96.28%


Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS        N      Mean  Grouping
NORMAL CONTROL    5     100.0  A
150mg/kgbw        5     58.89    B
POSITIVE CONTROL  5     55.33    B
100mg/kgbw        5     49.79    B
50mg/kgbw         5     35.77      C
NEGATIVE CONTROL  5  0.000000        D
```

Means that do not share a letter are significantly different

Appendix III. Analysis of the anti-pyretic activity of *T. brownii* on turpentine oil-induced pyrexia in *R. novegicus* - Raw data

One-way ANOVA: 0hrs(°C) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	11.431	2.28613	30.84	0.000
Error	24	1.779	0.07412		
Total	29	13.210			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.272259	86.53%	83.73%	78.96%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
POSITIVE CONTROL	5	38.8400	A
150MG/KG	5	38.840	A
100MG/KG	5	38.400	A B
NEGATIVE CONTROL	5	38.3600	A B
50MG/KG	5	38.2800	B
NORMAL CONTROL	5	37.000	C

Means that do not share a letter are significantly different.

One-way ANOVA: 1hr(°C) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	8.843	1.7685	11.48	0.000
Error	24	3.696	0.1540		
Total	29	12.539			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.392428	70.52%	64.38%	53.94%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
NEGATIVE CONTROL	5	38.5400	A
50MG/KG	5	37.840	A B
100MG/KG	5	37.340	B C
150MG/KG	5	37.180	B C
POSITIVE CONTROL	5	37.060	C
NORMAL CONTROL	5	37.000	C

Means that do not share a letter are significantly different.

One-way ANOVA: 2hr(°C) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	10.470	2.0939	17.87	0.000
Error	24	2.812	0.1172		
Total	29	13.282			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.342296	78.83%	74.42%	66.92%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
NEGATIVE CONTROL	5	38.760	A
50MG/KG	5	37.440	B
100MG/KG	5	37.380	B
POSITIVE CONTROL	5	37.200	B
150MG/KG	5	37.140	B
NORMAL CONTROL	5	36.9800	B

Means that do not share a letter are significantly different.

One-way ANOVA: 3hr(°C) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	12.367	2.47333	43.91	0.000
Error	24	1.352	0.05633		
Total	29	13.719			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.237346	90.14%	88.09%	84.60%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
NEGATIVE CONTROL	5	38.9600	A
50MG/KG	5	37.520	B
100MG/KG	5	37.420	B C
POSITIVE CONTROL	5	37.280	B C
150MG/KG	5	37.2800	B C
NORMAL CONTROL	5	36.980	C

Means that do not share a letter are significantly different.

One-way ANOVA: 4hr(°C) versus TREATMENTS

```
Source      DF  Adj SS   Adj MS  F-Value  P-Value
TREATMENTS   5  16.850  3.36993    75.45    0.000
Error       24   1.072  0.04467
Total       29  17.922


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.211345  94.02%     92.77%      90.65%


Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS        N     Mean  Grouping
NEGATIVE CONTROL  5  39.2600  A
50MG/KG           5   37.420    B
150MG/KG          5  37.3800    B C
100MG/KG          5  37.3800    B C
POSITIVE CONTROL  5   37.280    B C
NORMAL CONTROL    5  36.9800      C

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

Appendix IV. Analysis of the anti-pyretic properties of *T. brownii* on turpentine oil-induced pyrexia in *R. novegicus* – Percentage change

One-way ANOVA: 1hr(°C) versus TREATMENTS

```
Source      DF  Adj SS   Adj MS  F-Value  P-Value
TREATMENTS   5  116.23  23.2456    47.33    0.000
Error       24   11.79   0.4912
Total       29  128.02


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.700825  90.79%     88.87%      85.61%


Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS        N     Mean  Grouping
NEGATIVE CONTROL  5  100.470  A
NORMAL CONTROL    5  100.000  A B
50MG/KG           5   98.850    B
100MG/KG          5   97.239      C
150MG/KG          5   95.724        D
POSITIVE CONTROL  5   95.416        D

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

One-way ANOVA: 2hr(°C) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	120.36	24.0711	35.21	0.000
Error	24	16.41	0.6836		
Total	29	136.76			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.826819	88.00%	85.50%	81.26%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
NEGATIVE CONTROL	5	101.045	A
NORMAL CONTROL	5	99.947	A
50MG/KG	5	97.808	B
100MG/KG	5	97.344	B C
POSITIVE CONTROL	5	95.779	C D
150MG/KG	5	95.624	D

Means that do not share a letter are significantly different.

One-way ANOVA: 3hr(°C) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	123.851	24.7701	63.48	0.000
Error	24	9.364	0.3902		
Total	29	133.215			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.624646	92.97%	91.51%	89.02%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
NEGATIVE CONTROL	5	101.566	A
NORMAL CONTROL	5	99.9464	B
50MG/KG	5	98.016	C
100MG/KG	5	97.453	C
POSITIVE CONTROL	5	95.986	D
150MG/KG	5	95.985	D

Means that do not share a letter are significantly different.

One-way ANOVA: 4hr(°C) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	149.36	29.8722	57.18	0.000
Error	24	12.54	0.5224		

```
Total       29  161.90


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.722781  92.26%     90.64%      87.90%


Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS        N     Mean  Grouping
NEGATIVE CONTROL  5  102.348  A
NORMAL CONTROL    5   99.947    B
50MG/KG           5   97.755      C
100MG/KG          5   97.351      C D
150MG/KG          5   96.243        D
POSITIVE CONTROL  5   95.987        D

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

Appendix V. Analysis of the anti-pyretic properties of *T. brownii* on turpentine oil-induced pyrexia in *R. novegicus* – Percentage inhibition

One-way ANOVA: 1hr(°C) versus TREATMENTS

```
Source      DF  Adj SS   Adj MS  F-Value  P-Value
TREATMENTS   5  116.22  23.2447    46.97    0.000
Error       24   11.88   0.4948
Total       29  128.10


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.703443  90.73%     88.80%      85.51%



Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS        N     Mean  Grouping
POSITIVE CONTROL  5    4.584  A
150MG/KG          5    4.276  A
100MG/KG          5    2.761    B
50MG/KG           5    1.150      C
NORMAL CONTROL    5  -0.0001      C D
NEGATIVE CONTROL  5   -0.470        D

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

One-way ANOVA: 2hr(°C) versus TREATMENTS

```
Source      DF  Adj SS   Adj MS  F-Value  P-Value
TREATMENTS   5  120.36  24.0718    35.70    0.000
Error       24   16.18   0.6742
Total       29  136.54


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.821126  88.15%     85.68%      81.48%


Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS        N    Mean  Grouping
150MG/KG          5   4.376  A
POSITIVE CONTROL  5   4.221  A B
100MG/KG          5   2.656    B C
50MG/KG           5   2.192      C
NORMAL CONTROL    5   0.053        D
NEGATIVE CONTROL  5  -1.045        D

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

One-way ANOVA: 3hr(°C) versus TREATMENTS

```
Source      DF   Adj SS   Adj MS  F-Value  P-Value
TREATMENTS   5  123.851  24.7702    64.16    0.000
Error       24    9.265   0.3860
Total       29  133.116


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.621327  93.04%     91.59%      89.12%


Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS        N    Mean  Grouping
150MG/KG          5   4.015  A
POSITIVE CONTROL  5   4.014  A
100MG/KG          5   2.547    B
50MG/KG           5   1.984    B
NORMAL CONTROL    5  0.0536      C
NEGATIVE CONTROL  5  -1.566        D

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

One-way ANOVA: 4hr(°C) versus TREATMENTS

```
Source      DF  Adj SS   Adj MS  F-Value  P-Value
```

```
TREATMENTS   5  149.36  29.8720    57.34    0.000
Error       24   12.50   0.5209
Total       29  161.86
Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.721767  92.28%     90.67%      87.93%


Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS        N    Mean  Grouping
POSITIVE CONTROL  5   4.013  A
150MG/KG          5   3.757  A
100MG/KG          5   2.649  A B
50MG/KG           5   2.245    B
NORMAL CONTROL    5   0.053      C
NEGATIVE CONTROL  5  -2.348        D

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

Appendix VI. Analysis of the anti-inflammatory activity of *T. brownii* on carrageenan-induced inflammation in *R. novegicus* – Raw data

One-way ANOVA: 0hrs(mm) versus TREATMENTS

```
Source      DF  Adj SS   Adj MS  F-Value  P-Value
TREATMENTS   5  10.729  2.14570    23.98    0.000
Error       24   2.147  0.08947
Total       29  12.876


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.299113  83.32%     79.85%      73.94%



Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS        N    Mean  Grouping
NEGATIVE CONTROL  5   5.894  A
POSITIVE CONTROL  5  5.5600  A B
100MG/KG          5   5.216    B
50MG/KG           5  5.1480    B
150MG/KG          5   5.030    B
NORMAL CONTROL    5  3.9640      C

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

One-way ANOVA: 1hr(mm) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	13.663	2.73256	44.79	0.000
Error	24	1.464	0.06101		
Total	29	15.127			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.247012	90.32%	88.30%	84.87%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
NEGATIVE CONTROL	5	6.2040	A
POSITIVE CONTROL	5	5.5760	B
100MG/KG	5	5.492	B
50MG/KG	5	5.3560	B
150MG/KG	5	5.180	B
NORMAL CONTROL	5	3.9640	C

Means that do not share a letter are significantly different.

One-way ANOVA: 2hr(mm) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	17.003	3.40066	51.42	0.000
Error	24	1.587	0.06614		
Total	29	18.591			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.257177	91.46%	89.68%	86.66%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
NEGATIVE CONTROL	5	6.4280	A
50MG/KG	5	5.186	B
100MG/KG	5	5.136	B
POSITIVE CONTROL	5	4.940	B C
150MG/KG	5	4.5140	C
NORMAL CONTROL	5	3.9640	D

Means that do not share a letter are significantly different.

One-way ANOVA: 3hr(mm) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	22.531	4.5062	43.13	0.000
Error	24	2.508	0.1045		
Total	29	25.039			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.323249	89.98%	87.90%	84.35%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
NEGATIVE CONTROL	5	6.632	A
50MG/KG	5	5.000	B
100MG/KG	5	4.918	B
POSITIVE CONTROL	5	4.4080	B C
150MG/KG	5	4.2800	C
NORMAL CONTROL	5	3.9640	C

Means that do not share a letter are significantly different.

One-way ANOVA: 4hr(mm) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	23.962	4.79245	52.93	0.000
Error	24	2.173	0.09054		
Total	29	26.135			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.300896	91.69%	89.95%	87.01%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
NEGATIVE CONTROL	5	6.542	A
50MG/KG	5	4.864	B
100MG/KG	5	4.628	B C
POSITIVE CONTROL	5	4.1500	C D
150MG/KG	5	3.976	D
NORMAL CONTROL	5	3.9640	D

Means that do not share a letter are significantly different.

Appendix VII. Analysis of the anti-inflammatory activity of *T. brownii* on carrageenan-induced inflammation in *R. novegicus* – Percentage change

One-way ANOVA: 1hr(mm) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	145.4	29.077	5.73	0.001
Error	24	121.7	5.071		
Total	29	267.1			

Model Summary

```
      S    R-sq  R-sq(adj)  R-sq(pred)
2.25195  54.43%     44.94%      28.80%


Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS        N     Mean  Grouping
NEGATIVE CONTROL  5   105.40  A
100MG/KG          5   105.39  A
50MG/KG           5  104.105  A B
150MG/KG          5   103.08  A B
POSITIVE CONTROL  5  100.280    B
NORMAL CONTROL    5    100.0    B

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

One-way ANOVA: 2hr(mm) versus TREATMENTS

```
Source      DF  Adj SS  Adj MS  F-Value  P-Value
TREATMENTS   5  1440.4  288.07    21.97    0.000
Error       24   314.7   13.11
Total       29  1755.0


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
3.62095  82.07%     78.33%      71.98%


Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS        N     Mean  Grouping
NEGATIVE CONTROL  5   109.34  A
50MG/KG           5  100.728    B
NORMAL CONTROL    5    100.0    B
100MG/KG          5   98.431    B
150MG/KG          5    89.95      C
POSITIVE CONTROL  5    88.88      C

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

One-way ANOVA: 3hr(mm) versus TREATMENTS

```
Source      DF  Adj SS  Adj MS  F-Value  P-Value
TREATMENTS   5  3389.0  677.81    50.07    0.000
Error       24   324.9   13.54
Total       29  3713.9


Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)
3.67938  91.25%     89.43%      86.33%
```

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
NEGATIVE CONTROL	5	112.57	A
NORMAL CONTROL	5	100.0	B
50MG/KG	5	97.122	B
100MG/KG	5	94.18	B
150MG/KG	5	85.35	C
POSITIVE CONTROL	5	79.307	C

Means that do not share a letter are significantly different.

One-way ANOVA: 4hr(mm) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	4482.7	896.54	31.82	0.000
Error	24	676.2	28.18		
Total	29	5158.9			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
5.30816	86.89%	84.16%	79.52%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
NEGATIVE CONTROL	5	111.08	A
NORMAL CONTROL	5	100.0	B
50MG/KG	5	94.44	B C
100MG/KG	5	88.79	C D
150MG/KG	5	79.59	D E
POSITIVE CONTROL	5	74.67	E

Means that do not share a letter are significantly different.

Appendix VIII. Analysis of the anti-inflammatory activity of *T. brownii* on carrageenan-induced inflammation in *R. novegicus* – Percentage inhibition

One-way ANOVA: 1hr(mm) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	146.6	29.329	5.71	0.001
Error	24	123.2	5.135		
Total	29	269.9			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.26597	54.34%	44.82%	28.65%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
NORMAL CONTROL	5	0.000000	A
POSITIVE CONTROL	5	-0.280	A
150MG/KG	5	-3.08	A B
50MG/KG	5	-4.105	A B
100MG/KG	5	-5.39	B
NEGATIVE CONTROL	5	-5.45	B

Means that do not share a letter are significantly different.

One-way ANOVA: 2hr(mm) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	1448.5	289.70	22.01	0.000
Error	24	315.9	13.16		
Total	29	1764.4			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.62811	82.09%	78.36%	72.02%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
POSITIVE CONTROL	5	11.12	A
150MG/KG	5	10.05	A
100MG/KG	5	1.569	B
NORMAL CONTROL	5	0.000000	B
50MG/KG	5	-0.728	B
NEGATIVE CONTROL	5	-9.41	C

Means that do not share a letter are significantly different.

One-way ANOVA: 3hr(mm) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	3399.7	679.93	50.52	0.000
Error	24	323.0	13.46		
Total	29	3722.6			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.66846	91.32%	89.52%	86.44%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
POSITIVE CONTROL	5	20.693	A

150MG/KG	5	14.65	A
100MG/KG	5	5.82	B
50MG/KG	5	2.878	B
NORMAL CONTROL	5	0.000000	B
NEGATIVE CONTROL	5	-12.63	C

Means that do not share a letter are significantly different.

One-way ANOVA: 4hr(mm) versus TREATMENTS

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TREATMENTS	5	4493.7	898.75	31.96	0.000
Error	24	675.0	28.12		
Total	29	5168.7			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
5.30316	86.94%	84.22%	79.60%

Grouping Information Using the Tukey Method and 95% Confidence

TREATMENTS	N	Mean	Grouping
POSITIVE CONTROL	5	25.33	A
150MG/KG	5	20.41	A B
100MG/KG	5	11.21	B C
50MG/KG	5	5.56	C D
NORMAL CONTROL	5	0.000000	D
NEGATIVE CONTROL	5	-11.13	E

Means that do not share a letter are significantly different.

RESEARCH PERMIT

THIS IS TO CERTIFY THAT:
MISS. JANE WANJA MBIRI
of SOUTHEASTERN KENYA UNIVERSITY, 170-90200 KITUI,has been permitted to conduct research in *Nairobi County*
on the topic: *ANTINOCICEPITIVE, ANTIPYRETIC AND ANTI-INFLAMMATORY PROPERTIES OF TERMINALIA BROWNII IN RATTUS NOVEGICUS*
for the period ending:
4th July,2017

Permit No : NACOSTI/P/16/48885/11446
Date Of Issue : 5th July,2016
Fee Recieved :Ksh 1000

Applicant's Signature

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 Officers 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

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National Commission for Science, Technology and Innovation

RESEARCH CLEARANCE PERMIT

Serial No. A 9954

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