ANALGESIC, ANTI-INFLAMMATORY, ACUTE ORAL TOXICITY AND PHYTOCHEMICAL STUDY OF *MAERUA TRIPHYLLA* A. RICH. (CAPPARIDACEAE)

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UNIVERSITY OF NAIROBI

DECLARATION

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

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DEDICATION

I dedicate this work to my father Samson Palia Wangusi for his immeasurable support, tremendous guidance and generous sacrifice towards my education.

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LIST OF ACRONYMS

ANOVA	Analysis of variance
COX	Cyclooxygenase
EDTA	Ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
GI	Gastrointestinal
IL	Interleukin
iNOS	Inducible nitric oxide synthase
LC_{50}	Lethal concentration of chemical in air responsible for the death of 50% of a population of animals
LD_{50}	Lethal dose responsible for the death of 50% of a population of animals
mm	Millimetres
M. triphylla	*Maerua triphylla*
NF-B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NSAIDS	Nonsteroidal anti-inflammatory drugs
OECD	Organization for Economic Co-operation and Development
STIs	Sexually Transmitted Infections
TNF	Tumor Necrosis Factor
V/V	Volume per volume
W/W	Weight by weight
WHO	World Health Organization

ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and opioids are used in the management of inflammation and pain. However, the use of these drugs is limited by cost, adverse effects, and the reappearance of symptoms after discontinuation. Given these limitations, the search for alternatives may be necessary. The roots of *Maerua triphylla* are used by Maasai and Kikuyu communities for the management of headaches, stomachaches, migraines, and rheumatism. However, data on the safety and efficacy of this plant is not available to support its use. The aim of this study was to investigate the safety (LD_{50}), phytochemical constituents, analgesic, and anti-inflammatory properties of root extracts of *M. triphylla*. Cold maceration was used to prepare methanol and aqueous root extracts of *M. triphylla.* The safety of these extracts was evaluated in *Wistar* rats using the Organization for Economic Co-operation and Development (OECD 425) guidelines. Phytochemical composition of the extracts was determined by standard qualitative methods. The acetic acid-induced writhing procedure was used to evaluate the analgesic activity of the extracts in *Swiss albino* mice. The anti-inflammatory activity of the extracts was determined in *Wistar* rats using the acetic acid-induced paw oedema method. The percentage yield from the aqueous extraction was 12.4% whereas the percentage yield from the methanol extraction was 6.2%. All the studied plant extracts had LD_{50} > 2000mg/kg bw and were classified as nontoxic according to the OECD 425 guidelines. Qualitative phytochemical screening revealed the presence of flavonoids, phenols, cardiac glycosides and alkaloids in both extracts. However, saponins were only present in the methanol extract. In the analgesic study, mice that received 100 mg/kg bw and 500 mg/kg bw of aqueous root extract of *M. triphylla* had significantly lower acetic acid-induced writhing in comparison to mice that received 75 mg/kg bw acetylsalicylic acid (reference drug) ($p< 0.05$). Additionally, mice that received 500 mg/kg bw of methanol root extract of *M. triphylla* had significantly lower acetic acid-induced writhing in comparison to mice that received 75 mg/kg bw acetylsalicylic acid ($p< 0.05$). In the anti-inflammatory study, there was no significant difference $(p>0.05)$ between the inhibitory activity of different doses of the aqueous root extract of *M. triphylla* and a 50 mg/kg dose of

diclofenac sodium (reference drug) on acetic acid-induced paw edema in rats. Moreover, there was no significant difference in the inhibitory activity of 100 mg/kg bw and 500 mg/kg bw doses of the methanol root extract of *M. triphylla* and a 50 mg/kg dose of diclofenac sodium ($p>0.05$). These findings suggest that the roots of *M. triphylla* may be useful in the mitigation of pain and inflammation and therefore support their ethnomedicinal use in the management of inflammation and pain. Further isolation, characterization and quantification of the specific phytochemical constituents in the root extracts of *M. triphylla* with anti-inflammatory and analgesic activity is recommended. Furthermore, the specific mode(s) through which these extracts exert their reported pharmacological activities should be established. Further toxicological studies on the plant extracts are recommended to fully determine their safety.

Keywords; *Maerua triphylla, analgesic, anti-inflammatory*

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Traditional medicine is described by the World Health Organization (WHO) as the expertise, understanding, as well as practises founded on models, experiences and beliefs indigenous of various traditions used in diagnosis, treatment and prevention of mental and physical ailments (WHO, 2022). Medicinal plants naturally contain numerous phytochemicals and hence are used worldwide in traditional medicine (Singh and Geetanjali, 2013). In poor rural areas, most Kenyan communities depend heavily on herbal treatments (Kigen et al., 2013). Some of these therapeutic plants apply in the treatment and management of inflammation and pain in diseases associated with these symptoms such as rheumatism. The International Association for the Study of Pain (IASP) describes pain as unwanted emotional or receptive sensation brought about by potential or actual damage to the tissue (Raja et al., 2020). Inflammation, on the other hand is the body's immune system's response to an irritant and this can be part of various pathological conditions like arthritis, rheumatism, and atherosclerosis (Vogl et al., 2013).

For these illnesses, treatment generally relies on a large number of commercial preparations such as Nonsteroidal Anti-inflammatory Drugs (NSAIDs) like acetylsalicylic acid and steroidal drugs such as prednisone. They do this by inhibiting cyclooxygenase enzymes (COX) that act on arachidonic acid and cause the synthesis of prostaglandins (Katzung, 2018). However, most of these drugs have adverse effects like peptic ulcer, dyspepsia, and gastrointestinal bleeding (McGettigan and Henry, 2011). Furthermore, conventional drugs have low efficacy and are costly (Amaral et al., 2007). Therefore, a large number of medicinal plants need to be investigated for their potential analgesic and anti-inflammatory activity to avoid these adverse effects. One such plant is *Maerua triphylla* that has been used traditionally for the management of rheumatism, headache, migraine and tooth aches (Alfred, 2020).

Maerua triphylla is an evergreen shrub belonging to the family Capparidaceae. In terms of ethnomedicinal use, *M. triphylla* has been used to suppress conditions like rheumatism, headache,

migraine, diarrhoea and stomach ache by the Maasai and Kikuyu communities (Alfred, 2020). *Maerua triphylla* has medicinal properties of the two conditions being investigated above but insufficient information is present on the safety, phytochemistry, and pharmacological properties of crude *M. triphylla* extracts. The study therefore aimed at assessing the acute oral toxicity (LD_{50}), phytochemical composition, analgesic, as well as anti-inflammatory properties of *M. triphylla* root extracts.

1.2 Problem statement

Many communities use *M. triphylla* to treat pain and inflammation because it is regarded as cheap, easily accessible and believed to be more effective than modern-day drugs. However, prior to this study there was no scientific evidence of the anti-inflammatory and analgesic properties of *M. triphylla* to confirm these ethnomedicinal uses.

1.3 Justification

Modern-day chemotherapy against autoimmune diseases like rheumatism has faced a huge blow due to the associated adverse effects of existing drugs and costly treatment (Maina et al., 2015). There was therefore a need for efficacious and cheaper alternatives such as medicinal plants to manage these conditions. Given the above facts, studies of the plants having potential analgesic and anti-inflammatory properties such as *M. triphylla* are important. Phytochemicals such as proline betaine isolated from *M. triphylla* have been shown to suppress inflammation (McLean et al., 1996). *Maerua triphylla* roots are also known to be toxic (Alfred, 2020). Scarce scientific data existed to support the safe ethnomedicinal use of *M. triphylla* to mitigate conditions associated with pain and inflammation hence making this study necessary.

1.4 Objectives

1.4.1 Overall objective

To assess the acute oral toxicity, phytochemical composition, analgesic, and anti-inflammatory properties of *M. triphylla* root extracts.

1.4.2 Specific objectives

i. To determine acute oral toxicity (LD_{50}) of *M. triphylla* root extracts in *Wistar* rats.

ii. To find out the phytochemical constituents of *M. triphylla* root extracts.

iii. To evaluate analgesic properties of *M. triphylla* root extracts.

iv. To examine the anti-inflammatory activities of *M. triphylla* root extracts.

1.5 Hypothesis

Maerua triphylla root extracts did not have analgesic or anti-inflammatory properties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pain and Inflammation

Pain is an unwanted emotional or receptive sensation brought about by potential or actual damage to the tissue (Raja et al., 2020). Bradykinin, prostaglandins and histamine are the major mediators of pain. Pain can be categorized in different ways (Saikat et al., 2010). First based on the duration of the pain, there is acute pain and chronic pain. Acute pain is transient. Usually, it manifests itself in ways that can be easily observed and described. It results from tissue injury or damage and normally disappears when the cause of pain is removed or as the injury heals. Then there is chronic pain which is pain persisting for more than three months. Secondly based on the tissue affected, there is somatic pain as a result of the activation of pain receptors in either musculoskeletal tissues or the body surface which may be caused by various factors such as excessive activity, trauma and inflammation. There is visceral pain which is pain that is brought about by internal organ damage. Finally, neuropathic pain that is associated with malfunction or injury to the peripheral nerves and spinal cord (Saikat et al., 2010).

Inflammation describes the body's normal defensive mechanism to tissue injury triggered by microbiologic agents, noxious compounds and physical trauma (Stankov, 2012). It usually subsides after the healing process but sometimes it becomes severe leading to diseases such as arthritis, rheumatism, cancer, allergy, gastritis, pancreatitis, endocarditis and meningitis and may be fatal (Richard et al., 2008, Burke et al., 2006). Inflammation normally occurs in three phases which include acute transitory inflammation, delayed subacute inflammation, and chronic proliferating phase. In phase one, enhanced vascular permeability causing local edema leads to the development of inflammatory discharge. In phase two, there is migration of phagocytes and leukocytes from blood to vascular tissues. Finally in phase three, there is degradation of the tissue and thereafter, fibrosis (Anilkumar, 2010).

2.2 Conventional management of pain and inflammation

NSAIDs such as acetylsalicylic acid, indomethacin and diclofenac are widely used in reducing fever, inflammation and pain. They suppress prostaglandin formation by suppressing cyclo-oxygenase enzymes. They are particularly effective when pain results from inflammation. However, for chronic or severe malignant pain, opioid analgesics like morphine are used (Richard et al., 2008). The greatest drawback of these drugs is reappearance of symptoms after discontinuation and toxicity. Table 2.1 shows the adverse effects of some of the common analgesic and anti-inflammatory drugs used.

Table 2.1: Adverse effects of some of the commonly used analgesic and anti-inflammatory drugs (Grover, 2018).

DRUGS	ADVERSE EFFECTS
	NSAIDS
Indomethacin	GIT bleeding, nausea, gastric ulcer formation, constipation
Aspirin	Hyperventilation, tinnitus, epigastric distress, vomiting, respiratory alkalosis, nausea, peptic ulcer, allergic and anaphylactic reactions
Ibuprofen	Headache, somnolence, nausea, GIT bleeding, dyspepsia, gastric ulcer
Diclofenac sodium	Vomiting, gastric or duodenal ulcer, nausea bleeding, duodenal or gastric ulcer, vomiting, dizziness
	OPIOID ANALGESICS
Methadone	Physical dependence, respiratory depression, light headedness, vomiting, dizziness, nausea, constipating, sedation
Morphine sulfate	Dysphoria, nausea, sedation, respiratory depression, hypotension, somnolence, increased sweating, dry mouth, constipation, vomiting
Codeine	Light-headedness, sedation, headache, sweating, lethargy, dizziness

There is therefore a need to develop potent anti-inflammatory and analgesic drugs with reduced adverse effects (Saha and Ahmed, 2009).

2.3 Herbal medicine used as analgesics and anti-inflammatory agents

The use of medicinal plants to treat various diseases is becoming popular due to the high cost and adverse effects of conventional medicines (Kumar et al., 2013). Different phytochemicals like flavonoids, alkaloids, xanthone, coumarin, sterols etc., have proved effective as pain-relieving and anti-inflammatory agents (Singh et al., 2008).

Throughout Europe, *Achillea millefolium* Linn is a herb used traditionally for its anti-inflammatory properties. Its pharmacological property has been shown to be due to phenolics and isoprenoids (David et al., 2010). *Desmodium podocarpum,* a shrub of the family Leguminosae present in China possesses anti-inflammatory and analgesic activities (Zhu et al., 2011). *Aconitum heterophyllum,* a plant belonging to the family Valeraneaceae is used for treatment of rheumatism and fever. Its ethanolic root extract inhibits subacute inflammation (Santosh et al., 2010).

Michrotrichi perotitii Dc of the family Asteracea*e* is a shrub widely dispersed in West Africa. Its dried or fresh leaves and flowers are used for the management of toothache. The n-butanol phase of its methanol leaf extract has anti-inflammatory and analgesic properties (Nuhu et al., 2010). In Nigeria, *Annona senegalensis* of the family Annonaceae is a perennial shrub widely grown there. Its roots are used to treat pains. Its ethanol leaf extract possesses therapeutic potential against pains and feverish conditions (Megwas et al., 2020). *Echinops kebericho* of the family Astereceae has been used in different parts of Ethiopia to treat and manage pain and inflammation (Abera, 2014; Getnet et al., 2016). Data obtained from studies elucidates that 80% methanol root extract of *Echinops kebericho* has significant analgesic and anti-inflammatory properties (Yimer et al., 2020).

Margaritaria discoidea is a tree commonly found in western Cameroon and Senegal. Its bark is used to treat inflammation, toothache and postpartum pains. Pain-relieving and anti-inflammatory properties related to the aqueous stem and bark extracts has been shown using murine models (Adedapo

et al., 2009). *Maerua triphylla* A. Rich is a shrub of the family Capparidaceae. It has been used traditionally in East Africa especially in Kenya by the Maasai and Kikuyu communities to treat conditions associated with pain and inflammation like rheumatism (Alfred, 2020).

2.4 Capparidaceae

Capparidaceae is a medium-sized family with members having considerable diversity in habitat, floral and fruit features. They are usually climbers, shrubs or trees. The leaves alternate and may be simple or digitately three to nine foliolate. Stipules may be present or not and rarely develop into spines (Hyde et al., 2020). Floral variation includes zygomorphy and actinomorphy with huge variation in number of stamens and pronounced basal intercalary elongation zones (Hall et al., 2002).

2.4.1 Classification and distribution

Capparidaceae belongs to the order Brassicales. It consists of about 39 genera and 650 species (Hyde et al., 2020). The largest genera are *Capparis* followed by *Maerua*, *Boscia* and *Cadaba* in that order. Their distribution is from almost sea level up to about 2600m above sea level. Africa harbors *Cadaba, Maerua, Thilachium, Boscia, Buchholzia, Bachmannia*, *Euadenia, Ritchiea* and *Cladostemon* (Kers, 2003).

2.4.2 Biological activities

Leaf, stem, bark, fruits, and root extracts of various plants belonging to the Capparidaceae family have been shown to have biological activity. The aerial part extract of *Cleome heratensis* is an excellent natural antioxidant (Nasseri et al., 2019). Fresh ground fruits and leaves of *Boscia senegalis* have pesticidal activity against *Prostephanus truncates* and *Callosobruchus maculants* adults (Seck et al., 1993). The seed extracts of *Buchholzia coriacae* have shown modest antimicrobial activity against *Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcis aerus, Cryptococcus neoformans,* and *Candida albicans* (Nweze, 2011). The aqueous alcoholic leaf extract of *Boscia angustifolia* has shown

remarkable inhibition against the H5N1 virus (Maha et al., 2016). Studies have shown that *Ritchiea longipedicelleta* exhibits antinociceptive activity (Margaret et al., 2018).

2.4.3 Phytochemistry

Proanthocyanidins, ferulic acids, sinapic, common flavonols and glucosinolates are widely present in Capparidaceae. Minimal quantities of tannins have been found in seed coats and ovules whereas myricetin rarely occurs. In Capparoideae, toxic protoalkaloids are important constituents. Glucocapparin is the predominant glucosinolate. Glucocleomin less frequently accompanies glucocapparin. Among fatty acids, n-eicos-11-enoic acid and erucic acid are absent (Kers, 2003). From branches and dried aerial parts of *M. triphylla*, quaternary ammonium compounds and betaines such as glycine betaine, 3-hydroxyprolinebetaine, 3-hydroxy-1,1-dimethyl pyrrolidinium and proline betaine have been identified (McLean et al., 1996).

2.4.4 Ethnomedicinal uses

Various plants belonging to Capparidaceae have been used by traditional healers to treat different illnesses. *Cleome gynandra* leaves are mixed in the form of paste and applied to the tooth to treat toothache (Sandhya et al., 2006). In South Africa, *Maerua cafra* root decoction is taken to cure female sterility and menorrhagia (Steenkamp, 2003). In Somalia, *Maerua denhardtiorum* fresh leaves decoction is used in treatment of migraines by washing the head using the extract. (Ichikawa, 1987).

In Tanzania, the peeled and crushed roots of *Maerua Kirkii* are applied to painful limbs to relieve the pain. Its roots have shown antibacterial activity against *Staphylococcus aureus* (Hedberg et al, 1982). In Kenya, a leaf maceration of *Maerua parvifolia* is gargled as a treatment for a pharyngitis and tonsilitis (Ichikawa, 1987). In the Dorobo and Chamus communities of Kenya, the roots of *Maerua Endlichii* soaked in warm water is taken as a purgative. (Ichikawa, 1987; Heine and Heine, 1988). *Maerua triphylla* is used for the treatment of rheumatism, migraine, headache, tooth ache, snake bites and respiratory problems by the Maasai and Kikuyu communities (Alfred, 2020).

2.4.5 The profile of *M. triphylla*

2.4.5.1 Description

Maerua triphylla is an evergreen shrub that grows up to 3 meters tall and occurs in dune bush, open woodland, or on forest fringes (Palgrave, 2002). Some of the vernacular names for this shrub include Mitumburu, Gathandika, Mukuriundu, Mukasi, Msingizi, Mukayi, Mkuturu and Mlala-mbuzi. The branches and trunk are often markedly flattened rather than round. The bark is grey to dark brown and liberally covered in many lenticels. It consists of leaves that alternate from simple to trifoliate having leaflets that are elliptical. It has notched, apex rounded terminal leaflets that are about twice in size compared to lateral leaflets. The leaves have ciliate margins and petiolules of only 10 mm or less in length while the petioles may be up to 60 mm long. The apex of the median leaflet may be apiculated and vary from broadly acute to rounded (Mollel, 2013).

The plant has bisexual flowers that are in short axillary heads. The green sepals are oblong-oblivate to elliptical and 5 to 9 mm long. The petals on the other hand are white, obovate to elliptical, and early falling. The flower has 12 to 16 stamens which are intricately folded in the unopened bud. The ovary is on a gynophore that is not more than 1.2 cm in length. The fruit is rod-shaped and elliptical and comparable to the globular capsule measuring between 5 and10 cm in length (Mollel, 2013). It is considerably restrained in between the seeds, the external wartlike, pubescent to glabrous varied seed count. The seeds are irregularly kidney shaped, brown and obscurely warted. Figure 2.1 depicts the various parts of *M. triphylla.*

A B

Figure 2.1: Parts of *M. triphylla*

A-Whole plant; B-Leaves and flowers

2.4.5.2 Classification and distribution

Maerua triphylla belongs to the domain Eukaryota, the Plantae kingdom, subkingdom Tracheophyte, and phylum Spermatophyte. Additionally, it belongs to subphylum Angiospermae, class Eudicots, subclass Rosidae, order Brassicales, family Capparidaceae, genus *Maerua* and species *triphylla*. *Maerua triphylla* occurs all the way from Somalia, Kenya and Ethiopia south to Mozambique and Zimbabwe. It is also found in the Seychelles, Mayotte, Madagascar and Peninsular Arabia (Palgrave, 2002).

2.4.5.3 Ethnomedicinal and economic uses

Maerua triphylla has various ethnomedicinal uses as shown in Table 2.2.

Table 2.2: Ethnomedicinal uses of *M. triphylla*.

Use	Used Part	References
Headache and toothache	Roots mixed with those of *Capparis cartilaginea* taken orally and applied topically for toothache	(Waswala-Olewe et al., 2014)
Rheumatism	Topical application of root and fruit decoction	
Respiratory problems	Oral intake of leaf and root decoction and infusion	
Breast cancer	Oral intake of roots mixed with *Uvaria acuminata* roots	
Wounds	Topical application of stem bark, leaf, and roots extract.	(Kimondo et al., 2015)
Aphrodisiac	Oral intake of stem bark, leaf and root concoction and infusion	
Tonic	Oral intake of leaf and stem bark decoction	
Boils	Topical application of leaf ash	(Hassan-Abdallah et al., 2013)
GIT Problems	Oral intake of root, bark and leaf decoction and infusion	
Burns	Topical application of leaf decoction	(Fratkin, 1996)
Eye ailments	Application of leaf maceration as lotion	(Mollel, 2013)
STIs	Root infusion and decoction taken orally	
Snake bite	Topical application of roots	(Dharani, 2019)

In Ethiopia and Tanzania during famine, cooked *M. triphylla* leaves are eaten (Luoga et al., 2000; Lulekal et al., 2011). During the famine period in Kenya, the roots are used to prepare porridge (Hamilton and Hamilton, 2006; Dharani, 2019). The Maasai community also considers the foliage of *M. triphylla* as

good fodder for goats and donkeys (Mbuvi et al, 2019). The flowers of *M. triphylla* provide nectar and pollen hence attract many bees and butterflies. In rocky sites, *M. triphylla* is planted for soil reclamation (Mollel, 2013). Acetone and hexane extracts of *M. triphylla* have acaricidal activities and good repellent properties against ticks (Zorloni, 2008).

2.4.5.4 Phytochemistry

Limited information is available on the phytochemistry of isolated *M. triphylla* crude extracts. From branches and dried aerial parts of *M. triphylla*, quaternary ammonium compounds and betaines such as glycine betaine, 3-hydroxyprolinebetaine, 3-hydroxy-1,1-dimethyl pyrrolidinium and proline betaine have been identified (McLean et al., 1996).

2.4.5.5 Toxicity

Maerua triphylla is recognized as toxic hence the need for thorough safety evaluations of its compounds and crude extracts (Alfred, 2020). Methanol:dichloromethane (1:1) extracts of *M. triphylla* leaves have cytotoxic activity corroborating its ethnomedicinal use in Tanzania against leukaemia and breast cancer (Augustino and Gillah, 2005; Matata et al., 2018). The uncooked roots of *M. tryphylla* are regarded as toxic and therefore boiling and reboiling them severally is suggested to make them non-poisonous and edible (Tairo, 2011).

2.5 Analgesic tests

There are several analgesic tests that can be used for investigating the analgesic activities of many agents. These include the formalin test (Dubuisson and Dennis, 1977), acetic acid-induced writhing procedure (Koster et al.,1959), Randall-Sellito assessment (Randall and Selitto, 1957), rat paw gradually increase-pressure test (Jensen et al., 1986) and acute thermal assays (Allen and Yaksh, 2004).

The acetic acid-induced writhing method is used widely to evaluate the pain-relieving activity of various agents against pain caused by inflammation. Here, pain is induced on the abdomen of the animal by a peritoneal injection of 0.6 % acetic acid causing spasms which are observed as writhing. The test substance is applied at known concentrations. After one hour, peritoneal injection of acetic acid is done

and the number of writhes observed and counted for 20 minutes (Moreno-Quirós et al., 2017). The mean count of writhes and the percentage inhibition of writhing is then determined as an index of analgesic activity.

The formalin test evaluates analgesic activity by producing a two-phase response: the early and the late phase (Spindola et al., 2012). Here, the experimental animals receive the treatment and controls 60 minutes before the beginning of the test. At first, intraplantar injection of 20µL of 2.5% formalin is done in either hind limb. The duration the animal bites, shakes or licks the injected paw is then recorded. The pain sensitivity in the early phase which is caused by activation of C-fibres is evaluated from 0 to 10 minutes. The neurogenic pain can be inhibited by drugs like opioids that act on the central nervous system (Hassani et al., 2015). Pain in the second phase involving release of local mediators like prostaglandins and spinal cord-reinforced synaptic transmission is determined from 15 to 30 minutes (Callegari et al., 2016). Peripherally acting drugs like NSAIDs can inhibit the inflammatory pain (Hassani et al., 2015).

The formalin-induced orofacial pain procedure is done to determine analgesic activity at the trigeminal nerve area related to several diseases. It involves subcutaneous application of 50µL of 2% formalin in the animal's vibrissae right cushion region. Observation is done by recording the time the animal scratches the area of formalin application in comparison to the controls (Cazanga et al., 2018, Magalhães et al., 2018)

Rat paw gradually increase-pressure test involves pressure application using an electronic equipment to the hind limbs of the rat. The application of linear pressure is repeated up to six times to get a measurement of triple close paw flick values. Quantification denoted as change in pressure is the difference between the mean of triple values after the administration of the stimuli and the mean of triple values observed before performing the experiment (Dutta et al., 2018).

Acute thermal assays which include tail-flick, Hargreaves and hot plate tests are the most common methods used to evaluate analgesia (Allen and Yaksh, 2004). In principle, the skin is innervated by high

threshold nerves whose thermoreceptors are activated by the heat. Behavioral response such as flicking the tail are observed when such stimuli is applied to the tail (Allen and Yaksh, 2004).

Randall-Sellito test measures pain by testing the effect of pressure applied on the gastrocnemius muscle. To reduce stress level in a dimly lit room, the animals are allowed to rest with a controlled temperature. After half an hour, the lower pelvic limb of the animal is placed on the analgesiometer and pressure is applied gradually until the animal senses pain by vocalizing or flicking the limb. The analgesiometer records this pressure. (Kiso et al., 2018, Valdes et al., 2018)

2.6 Anti-inflammatory test

Various methods have been employed in assessing the anti-inflammatory properties of various products and these include Carrageenan-induced intraplantar edema (Winter et al., 1962), Carrageenan-induced peritonitis (Ferrándiz and Alcaraz, 1991), Croton oil-induced ear edema in mice (Tubaro et al., 1986), formalin-induced paw edema (Ibironke and Ajiboye, 2007) and Pleurisy (Ammendola et al., 1975).

Acute and progressive swelling of the paw is induced by intraplantar injection of carrageenan. This swelling is a useful criterion in investigation of the anti-inflammatory activity that is proportional to the inflammatory response intensity. Inhibition of edema here is comparable to NSAIDs (Zitterl-Eglseer et al., 1997). The intact animal's hind paw volume is measured before testing. One hour after application of the test compound and controls, the volume should be measured by the paw injection in the plethysmometer at 30, 60, 120, and 180 minutes (Batista et al., 2016). Quantification of the inflammatory response is denoted as an increase in paw size.

Croton oil-induced ear edema is used to evaluate the inhibition of ear edema formation after applying croton oil topically. Here, the test substance is applied on the inner ear surface of one ear 60 minutes before testing. Similarly, acetone is applied on the other ear as the negative control. This is followed by measurement of the edema formation (Tubaro et al., 1986).

In carrageenan-induced peritonitis, carrageenan induces peritoneal cavity inflammation. The experimental animals are treated with the test substance and controls at known concentrations. Four hours after intraperitoneal injection of carrageenan, euthanization of the animal is done and heparinized PSB solution used to wash the peritoneum for polymorphonuclear cell counting. For analysis, comparison of the number of leukocytes is done with the test group (Sreeja et al.,2018).

In the formalin test, subacute inflammation is produced by application of 20µl of 1% formalin into the hind paw surface of the animal 60 minutes after the treatment administration (Ibironke and Ajiboye, 2007). Here, cell damage induces production of inflammation mediators such as serotonin.

In the pleurisy test, exudate volume is used to evaluate the systemic anti-inflammatory effect in the pleural region (Zanusso-Junior et al., 2010). Here, an oral administration of the controls and test substance is administered at known concentrations. Carrageenan induction of inflammation is done by injecting carrageenan into the pleural region and testing after one hour. The animals are euthanized six hours after induction of inflammation. Opening of the pleural cavity and rinsing with EDTA and a physiological solution is done. Cell count is done in a Neubauer chamber in comparison to a negative control (da Silva et al., 2018)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Ethical approval

The ethical authorization to perform the study was acquired from the Faculty of Veterinary Medicine Biosafety, Animal Use and Ethics Committee (BAUEC) of the University of Nairobi with reference number FVM BAUEC/2021/291(Appendix I). The research license for the study was acquired from the National Commission for Science, Technology and Innovation (NACOSTI) and a permit number assigned NACOSTI/P/21/9494(Appendix II).

3.2 Study area

Maerua triphylla roots were obtained from Kajiado County, Ilbisil area. With a total size of 21,900.9 square kilometers, Kajiado County is located in Kenya's southern region between 360 5’ and 370 5’ East Longitudes and between 10 0’ and 30 0’ South Latitudes. The highest altitude is 2500 metres above sea level whereas the lowest point is around 500 metres above sea level. The area’s temperature ranges from a mean of 12℃ to 28℃ whereas the mean rainfall received ranges from 450 to 1454mm per annum. The major economic activity undertaken by a majority of residents in the region is pastoralism (Kajiado County Integrated Development Plan 2018-2022).

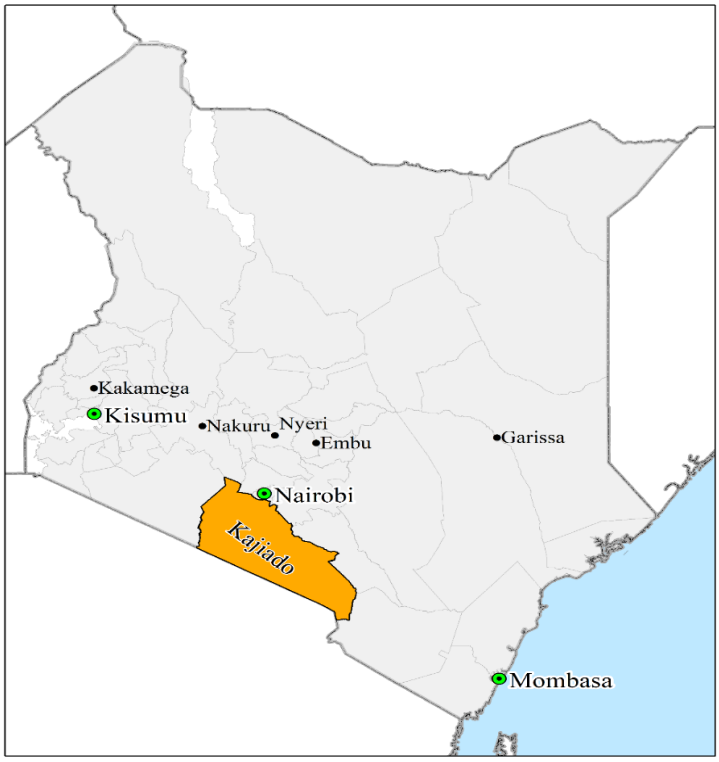

Figure 3.1: Kajiado County location in Kenya (Source: National Environmental Management Authority - Kajiado County)

3.3 Collection and authentication of plant specimen

Fresh roots of *M. triphylla* were obtained from Kajiado County, Ilbisil area with the assistance of a renowned local herbalist. Identification of the plant was performed by a taxonomist at the Land Resource Management and Agricultural Technology (LARMAT) Department, University of Nairobi and the voucher number LARMATCAP36 assigned to the specimen. A specimen was submitted to the herbarium for future reference.

3.4 Preparation of plant material

The obtained roots were cleaned with tap water, cut into small pieces, spread thinly on racks in a well-ventilated room. They were then air dried for 14 days before being crushed to powder with an electric grinder at the LARMAT Department, University of Nairobi. The resulting powder was put in a well labeled manila sack and stored in a cool and non-humid place awaiting extraction.

3.5 Preparation of plant extracts

Preparation of the methanol and aqueous extracts from the dried root powder of *M. triphylla* was done using the procedure illustrated by (Harborne, 1976) and later improved by (Bibi et al., 2012).

3.5.1 Aqueous extract

Two hundred and fifty (250) grams of the dried *M. triphylla* roots were precisely weighed on an analytical scale and placed into an extraction jar coated with aluminum foil. Gradual addition of 1.25 litres of distilled water to the powder was then done. The contents were shaken to form a mixture of homogeneous consistency. Extraction of phytochemicals present in the root powder was done using cold maceration with constant shaking for 24 hours. The same procedure was done again with another amount of 250 grams of the root powder.

The obtained mixture was then decanted and filtered through cotton gauze. The resultant filtrate was filtered using a Whatman No. 1 filter paper. The filtrate was kept at 4°C for storage in light-resistant bottles. The mouths of the bottles were covered with muslin cloth and affixed to a freeze drier after 24 hours. To create a freeze-dried product, this setup was left overnight. The obtained freeze-dried product was weighed and recorded before it was stored in tightly closed, light-resistant bottles at 4°C in a refrigerator pending bioassay. The freeze-dried product percentage yield was then determined as percentage weight by weight (%w/w).

Determination of percentage yield;

Percentage yield= (M1-M2/M3) ×100,

Where;

M1=mass of extract + container

M2=mass of empty container

M3=mass of the root powder sample

3.5.2 Methanol Extract

Two hundred and fifty (250) grams of the dried *M. triphylla* roots were precisely weighed on an analytical scale and placed into an extraction jar coated with aluminum foil. Gradual addition of 1 litre of analytical grade methanol to the *M. triphylla* root powder was done. Shaking of the contents was done until they produced a smooth, homogenous mixture. Cold maceration was used to extract the phytochemicals present in the root powder with constant shaking for two days. This procedure was done again with another amount of 250 grams of the root powder. The obtained mixture was decanted and filtered through cotton gauze. Filtration using a Whatman No. 1 filter paper of the resultant filtrate was done.

To concentrate the extract and get rid of the excess organic solvent, the obtained filtrates were mixed and reduced in vacuo at 60℃ using a rotary evaporator (Búchi-technik AG, Switzerland). Further concentration of the extract and solvent removal was done in a sand bath at 40℃ for 5 days. The dried product's percentage yield was then determined as percentage weight by weight (%w/w).

Determination of percentage yield;

Percentage yield= (M3-M2/M1) ×100,

Where;

M3=mass of extract + container

M2=mass of empty container

M1=mass of the initial root powder sample

The dried product was then kept at 4℃ in storage in well-closed, light-resistant bottles pending analysis.

3.6 Experimental animals

Sixty healthy, 8–10-week-old 25 male and 35 female *Wistar* rats weighing 110 ± 20 grams were used to evaluate the anti-inflammatory properties and acute oral toxicity of *M. triphylla* root extracts.

These animals were obtained from the Department of Public Health, Pharmacology and Toxicology (PHPT) of the University of Nairobi animal house.

Fifty healthy, 4–5-week-old 25 male and 25 female *Swiss albino* mice weighing 30 ± 5 grams were used to investigate the analgesic activities of *M. triphylla* root extracts. These mice were purchased from the animal breeding facility at Vet Farm Kabete and delivered to the Department of PHPT of the University of Nairobi animal house. The mice were given seven days to acclimatize before commencing the study.

All the animals were nulliparous and non-pregnant. They were housed at a 56-60% relative humidity and temperature of 25±3°C. The night and day cycle was kept at 12 hours each. The animals were nourished with commercial feeds with water being provided ad libitum.

3.7 Preparation of doses

In this study, the OECD (2008, Document No. 425) standards by Erhierhier et al., 2014 were adopted to prepare the doses for administration. Briefly to make a stock solution with a dosage level of 500mg/kg for administration to a 100g rat, the following formula described by Erhierhier et al., 2014 was followed:

$$\text{Animal dose (mg/kg bw)} = \frac{\text{bodyweight of the animal(g)}}{1000\ \text{g}} \times \text{selected dose}$$

Therefore, animal dose (mg/kg bw) = (100 g/ 1000g) × 500mg = 50 mg

In accordance to the OECD (2008, Document No. 425) guidelines, 50mg should be dissolved in 0.2 ml of the vehicle. Here, a 10 ml stock solution having a concentration of 500 mg/kg bw of either the methanolic or the aqueous root extracts of *M. triphylla* was made and serially diluted with normal saline to generate dosages of 100 mg/kg bw and 20 mg/kg bw. Similarly, this method was followed for the reference drug.

3.8 Acute oral toxicity study of aqueous and methanol root extracts of *M. triphylla*

The Up-and-Down procedure described by OECD (2008, Document No. 425) was used to evaluate the acute oral toxicity of the methanol and aqueous root extracts of *M. triphylla*. The *Wistar* rats were assigned randomly to two groups each with five (5) animals. The animals were each weighed and labeled with a permanent marker on their tails for identification. Prior to the commencement of the study, they were fasted for a night. Group I was administered orally with 2000mg/kg bw aqueous root extract of *M. triphylla* whereas Group II was given 2000mg/kg bw methanol root extract of *M. triphylla* orally using a gavage tube. Thereafter, wellness parameters such as lethargy, salivation, mucous membrane appearance, skin, hair, diarrhea, unconsciousness, changes in body weight, mortality as well as sleep were observed and recorded in 30 minutes, 60 minutes, 4 hours, 1 day, 2 days, 1 week, and 2 weeks correspondingly. The animals were weighed individually after 7 days and 14 days respectively and the weights documented.

3.9 Phytochemical screening of aqueous and methanol root extracts of *M. triphylla*

Identification of the phytoconstituents in the methanolic and aqueous root extracts of *M. triphylla* was carried out using analytical methods described by Harborne, (1998) and Kokate et al., 2007 with modification. The presence of different phytochemicals was detected using standard screening tests. The phytochemicals that were screened for included; tannins, saponins, flavonoids, terpenoids, sterols, phenolics, cardiac glycosides and alkaloids.

3.9.1 Saponins (Froth test)

Maerua triphylla root extract (0.1g) was dissolved in 5ml of distilled water and shaken for 5 minutes. In a water bath at 50°C, the mixture was then heated for 20 minutes. Frothing that lasted for at least 30 minutes indicated the presence of saponins.

3.9.2 Alkaloids (Dragendroff's test)

Maerua triphylla root extract (0.1g) of the extract was dissolved in 5 ml of distilled water. Acidification of the mixture was then done with 1M HCl and heated for 10 minutes in a water bath at

50°C. Treatment of the acidic medium with Dragendroff's reagent was done. The existence of alkaloids was detected by the appearance of a reddish-brown or orange precipitate.

3.9.3 Terpenoids (Salkowski test)

One millilitre of petroleum ether was mixed with 2 ml of chloroform in 0.1 g of the extract. After that, 2ml of 2M sulfuric acid was precisely put alongside to form a layer. The existence of terpenoids was indicated by the appearance of a reddish-brown hue at the interface.

3.9.4 Flavonoids (Alkaline reagent test)

Maerua triphylla root extract (0.1g) was dissolved in 2 ml of distilled water and then mixed with 2 ml of 5M sodium hydroxide. Positive results were indicated by the development of an intense/golden yellow precipitate.

3.9.5 Cardiac glycosides (Keller-Kiliani test)

Maerua triphylla root extract (0.1g) was dissolved in 2 ml glacial acetic acid with 2 drops of 5% ferric chloride solution. The underlying mixture was then carefully added 1 ml of concentrated sulfuric acid. At the interphase, the development of a greenish ring, brown, or violet was considered positive for the deoxysugar typical of cardiac glycosides.

3.9.6 Steroids (Salkowski test)

In 2 ml of chloroform, 0.1 g of the extract was dissolved. 3ml of 2M sulfuric acid was then gently added to the test tube's sidewalls forming a layer. The presence of steroids was depicted by the reddish-brown tint at the interphase.

3.9.7 Phenols (Ferric chloride test)

To determine whether the extract contained phenols, 0.1g of the extract was diluted in 2 ml of distilled water. One millilitre of 5% ferric chloride solution was added to the solution. The presence of phenolics was indicated by the appearance of green to blue tint.

3.9.8 Tannins (Ferric chloride test)

Maerua triphylla root extract (0.1g) was dissolved in 2ml of distilled water. Addition of two drops of 5% ferric chloride was done to the solution. The presence of tannins was indicated by the development of a blue-black precipitate.

3.10 Determination of the analgesic activity of the aqueous and methanol root extracts of *Maerua triphylla*

The acetic acid-induced writhing procedure developed by (Ur Rashid et al., 2015) was used to evaluate the analgesic activities of aqueous and methanol *M. triphylla* root extracts. For each extract, the *Swiss albino* mice were randomly assigned into five groups with five animals each and the treatments were as presented in Table 3.1.

Table 3.1: The treatment procedure used for the evaluation of the analgesic activities of methanol and aqueous root extracts of *M. triphylla* in *Swiss albino* mice

Group	Status	Treatment
V	Negative control	Normal saline (300 µl) + AA.
W	Positive control	75 mg/kg bw Acetylsalicylic acid + AA.
X	Experimental group I	20 mg/kg bw extract + AA.
Y	Experimental group II	100 mg/kg bw extract + AA.
Z	Experimental group III	500 mg/kg bw extract + AA.

Key: AA - 0.6% v/v acetic acid (200 µl).

All mice were fasted overnight before the commencement of the study. Each animal was weighed and marked using a permanent marker on its tail to enable identification. Groups V and W received 300µl normal saline and acetylsalicylic acid (75 mg/kg bw) orally as negative and positive controls respectively. Groups Z, Y and X received an oral treatment of 500 mg/kg bw, 100 mg/kg bw and 20 mg/kg bw respectively of the *M. triphylla* root extracts. After half an hour, writhing induction was done in each animal with a 0.6% v/v acetic acid injected intraperitoneally.

Five minutes after writhing induction, the animals were individually observed and counting of the number of writhes done for 30 minutes and documented. The mean count of writhes and the percentage inhibition of writhing was determined as an index of analgesic activity using the formula defined by Ur Rashid et al., 2015:

$$\% \text{ Writhing inhibition} = \frac{Wc - W}{Wc} \times 100$$

Where;

Wc = Average count of writhes in the negative control group.

W = Average count of writhes in the positive control or experimental group.

This experimental procedure was done for both the methanol and aqueous root extracts of *M. triphylla* and the results tabulated for data analysis.

3.11 Evaluation of the anti-inflammatory activity of the aqueous and methanol root extracts of *Maerua triphylla*

The anti-inflammatory activity of the methanol and aqueous root extracts of *M. triphylla* was assessed using the acetic acid-induced paw edema procedure with acetic acid (0.6% v/v) as the inflammation-inducing agent and diclofenac sodium as the standard drug. The *Wistar* rats were randomly assigned into five groups with five animals each and then treatments were performed as presented in Table 3.2.

Table 3.2. The treatment procedure for the evaluation of the anti-inflammatory activities of aqueous and methanol root extracts of *M. triphylla* in *Wistar* rats.

Group	Status	Treatment
V	Negative control	Normal saline (1.5ml) + AA.
W	Positive control	50 mg/kg bw Diclofenac sodium + AA.
X	Experimental group I	20 mg/kg bw extract + AA.

Y	Experimental group II	100 mg/kg bw extract + AA.
Z	Experimental group III	500 mg/kg bw extract + AA.

Key: AA - 0.6% v/v acetic acid (200 µl).

This anti-inflammatory testing procedure was performed as described by Winter et al. (1962) with modification. The rats were fasted overnight before the commencement of the study. Each animal was weighed and labeled with a permanent marker on its tails to enable identification. At first, the diameter of the intact rat's left hind paw was measured in mm in all groups using a digital vernier calliper (Ugo Basile, Italy) and recorded. Groups V and W received 1.5ml normal saline and 50 mg/kg bw diclofenac sodium orally as negative and positive controls respectively. Groups Z, Y and X received an oral treatment of 500 mg/kg bw, 100 mg/kg bw and 20 mg/kg bw respectively of the *M. triphylla* root extracts. Thirty minutes after administration of the treatments, 0.6% v/v acetic acid was injected into the sub-plantar tissue of the left hind paw. After inflammation induction, the diameter of the left hind paw was then measured hourly from hour one up to the fifth hour. Comparison between the paw diameter measured prior to the acetic acid injection and the same paw diameter after acetic injection was done by calculating the percentage inhibition of edema using the formula below:

$$\% \text{ Inhibition of edema} = \frac{T - T_0}{T} \times 100$$

Where;

T is difference in thickness of paw in negative control group

T_0 is difference in thickness of paw in the experimental or positive control group.

3.12 Data Analysis

The data obtained from analgesic and anti-inflammatory activities was tabulated on Microsoft Excel spreadsheet (2016), expressed as Mean ± Standard Error of the Mean (SEM) and analyzed using analysis of variance (ANOVA) and the two-sample t-test by GenStat statistical software 4th edition. This was followed by Tukey's *post hoc* test for pairwise comparison and separation of means at $\alpha = 0.05$.

Values with $p \leq 0.05$ were considered statistically significant. Acute oral toxicity data was quantitatively and qualitatively analyzed according to OECD guidelines (2008, Document No. 425) and LD_{50} value recorded.

CHAPTER FOUR

4.0 RESULTS

4.1 The appearance and percentage yield of *M. triphylla* root extracts

The appearance and percentage yield of the *M. triphylla* root extracts are as presented in Table 4.1 below.

Table 4.1: Appearance and percentage yield of *M. triphylla* root extracts

Solvent	Weight of root powder (grams)	Percentage yield (%w/w)	Appearance of the extract
Water	500	12.4%	Light brown powder
Methanol	500	6.2%	Dark -brown semi solid mass

4.2 Evaluation of the safety of the methanol and aqueous root extracts of *M. triphylla* on oral administration in *Wistar* rats

The oral administration of the methanol and aqueous root extracts of *M. triphylla* at the limit dosage level of 2000mg/ kg bw did not generate any clinical and physical changes in *Wistar* rats. Table 4.2 is a summary of observations in *Wistar* rats after administration of 2000 mg/kg bw dose of methanol and aqueous *M. triphylla* root extracts

Table 4.2: Observations in *Wistar* rats treated with 2000 mg/kg bw dose of methanol and aqueous *M. triphylla* root extracts.

Observations	Aqueous extract	Methanolic extract
Clinical signs	No abnormal effect	No abnormal effect
Physical changes	No abnormal effect	No abnormal effect
Number dead	0	0

No deaths occurred in both groups after administration of 2000mg/kg bw dose of *M. triphylla* extracts to the *Wistar* rats (Table 4.2). There were no substantial alterations in clinical signs of lacrimation, urinary incontinence, perspiration, salivation, defecation and respiration in the experimental animals (Table 4.2). Additionally, the oral administration of the methanol and aqueous root extracts of *M. triphylla* did not generate any physical alterations in the mucous membranes, eyes, skin and fur of the experimental animals (Table 4.2).

Table 4.3 is a summary of the mean weight gain in animals treated with the methanol and aqueous root extracts of *M. triphylla* over a period of 14 days.

Table 4.3: Effect of a 2000 mg/kg bw dose of methanol and aqueous root extracts of *M. triphylla* on the weight of *Wistar* rats.

Treatment (2000 mg/kg bw)	Mean Weight gain	
	Day 7	Day 14
Aqueous extract	25.46 ± 1.82^{a}	57.57 ± 3.96^{b}
Methanol extract	29.81 ± 2.15^{a}	67.57 ± 7.01^{c}

Values are presented as Mean ± standard error of the mean (SEM). Means with different superscript letters are significantly different (One-Way ANOVA and Tukey's test; $p < 0.05$).

There was no significant difference $(p>0.05)$ in weight gain between rats treated with a 2000 mg/kg bw aqueous root extract of *M. triphylla* and rats treated with a 2000mg/kg bw methanol root extract of *M. triphylla* on day 7 (Table 4.3). However, the weight gain in rats after 14 days of treatment with a 2000 mg/kg bw dosage level of the aqueous extract of *M. triphylla* was significantly greater $(p<0.05)$ than the weight gain after 7 days (Table 4.3). A similar pattern was noted in rats treated with the methanol extract of *M. triphylla* (Table 4.3). Furthermore, the weight gain in rats after 14 days of treatment with a 2000 mg/kg bw dosage level of the aqueous extract of *M. triphylla* was significantly lower $(p<0.05)$ than the weight gain in rats treated with a 2000 mg/kg bw dose of the methanol extract of *M. triphylla* (Table 4.3).

Owing to the absence of any toxicity and lethal effects in the rats, it was suggested that the LD_{50} of the methanol and aqueous root extracts of *M. triphylla* was above 2000mg/kg dose. Therefore, the extracts were classified as nontoxic according to the OECD 425 guidelines.

4.3 Phytochemical screening

Table 4.4 is a summary of the phytochemical composition of the methanol and aqueous root extracts of *M. triphylla*.

Table 4.4: Phytochemical composition of *M. triphylla* root extract

Phytochemical	Aqueous extract	Methanol extract
Saponins	-	+
Alkaloids	+	+
Terpenoids	-	-
Flavonoids	+	+
Cardiac glycosides	+	+
Steroids	-	-
Phenols	+	+
Tannins	-	-

(+): present, (-): absent

Cardiac glycosides, flavonoids, alkaloids and phenols were found to be present in both extracts. However, saponins were found to be present in the methanol extract only.

4.4 The effect of the methanol and aqueous root extracts of *Maerua triphylla* on acetic acid-induced writhing in *Swiss albino* mice

Table 4.5 is a summary of the effect of the methanol and aqueous root extracts of *M. triphylla* on acetic-acid induced writhing in mice. The effects are compared against 75mg/kg bw dose of acetylsalicylic acid (reference drug).

Table 4.5: Summary of the effect of methanol and aqueous root extracts of *M. triphylla* on acetic acid-induced writhing in *Swiss albino* mice

Treatment (mg/kg bw)	% Inhibition of writhing	
	Aqueous extract	**Methanol Extract**
Acetylsalicylic acid (75mg/kg bw)	81.08 ± 1.16^{a}	82.17 ± 2.11^{ab}
20	85.31 ± 1.13^{a}	81.84 ± 1.51^{a}
100	93.43 ± 1.26^{b}	88.43 ± 1.25^{bc}
500	95.97 ± 1.16^{b}	92.55 ± 1.20^{c}

Values are presented as Mean ± SEM. Means with different superscript letters along the column are significantly different (One-Way ANOVA and Tukey's test; $p < 0.05$).

The aqueous and methanol root extracts of *M. triphylla* inhibited acetic acid-induced writhing in a dose-dependent manner (Table 4.5). However, there was no significant difference in the effect of a 75 mg/kg bw dose of acetylsalicylic acid and a low dose of the aqueous *M. triphylla* root extract on acetic acid-induced writhing in mice ($p>0.05$; Table 4.5). Conversely, intermediate and high dosage levels of the aqueous root extract of *M. triphylla* produced a significantly higher inhibition of acetic acid-induced writhing when compared with a 75 mg/kg dose of acetylsalicylic acid ($p<0.05$; Table 4.5). Moreover, the inhibition of acetic acid-induced writhing by a 75 mg/kg dose of acetylsalicylic acid was not significantly different from the inhibition resulting from the use of low and intermediate doses of the methanol root extract of *M. triphylla* ($p>0.05$; Table 4.5). However, the 500mg/kg bw dose of the methanol root extract

of *M. triphylla* produced a significantly higher inhibition of acetic acid-induced writhing in comparison to the use of 75mg/kg bw dose of acetylsalicylic acid (*p<0.05;* Table 4.5).

This study also included a comparison of the effects of aqueous and methanol root extracts of *M. triphylla* on acetic acid-induced writhing in mice (Figure 4.1).

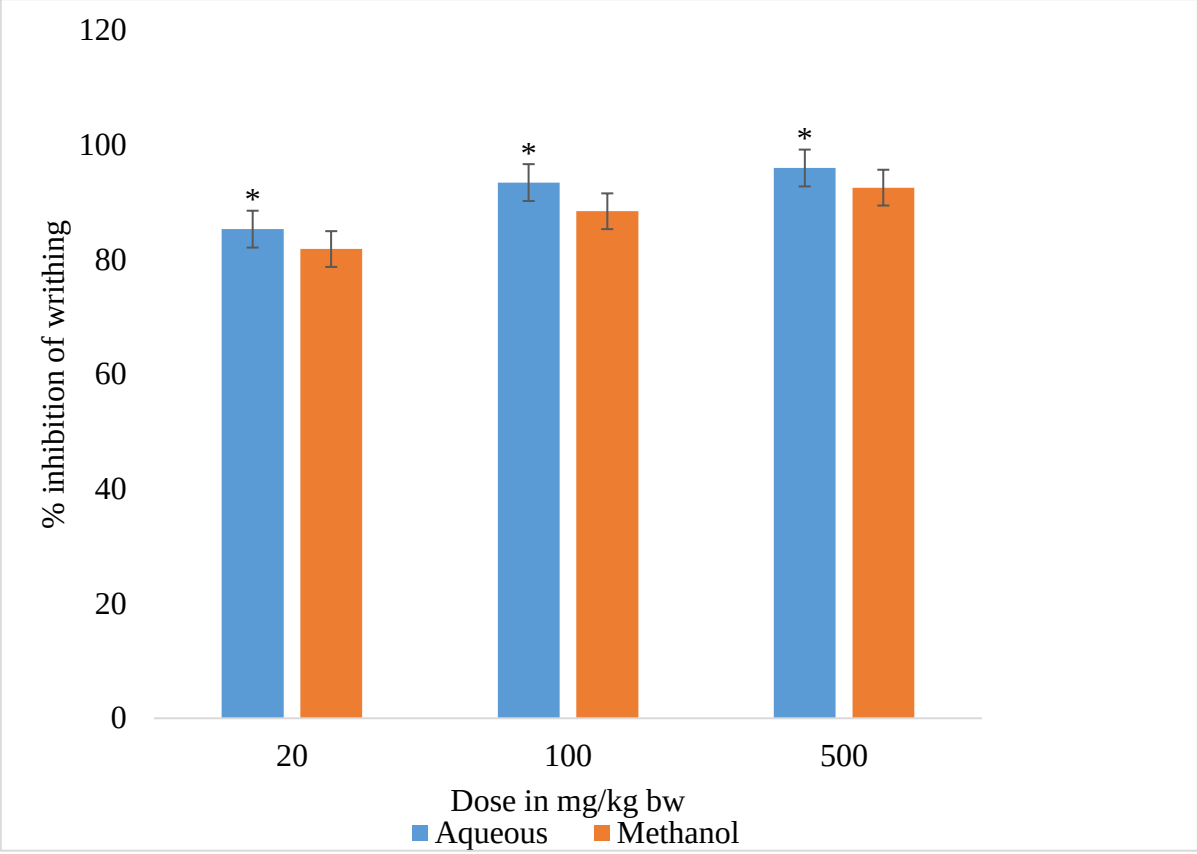

Figure 4.1: A comparison of the analgesic activities of the aqueous and methanol root extracts of *M. triphylla* in *Swiss albino* mice. Bars are plotted as Mean ± SEM. Bars with an asterisk at the same dose are significantly different (two-sample t-test; P<0.05).

At all dose levels, the animals given the aqueous root extract of *M. triphylla* showed significantly greater percentage inhibition of the acetic acid-induced writhing in comparison to that noted for mice given the methanol extract (p< 0.05; Figure 4.1).

4.5 The effect of the methanol and aqueous root extracts of *Maerua triphylla* on acetic acid-induced paw edema in *Wistar* rats

Table 4.6 is a summary of the effect of the methanol and aqueous root extracts of *M. triphylla* on acetic acid-induced paw edema in rats. The effects are compared against 50 mg/kg bw dose of diclofenac sodium (reference drug).

Table 4.6: Summary of the effect of the methanol and aqueous root extracts of *M. triphylla* on acetic acid-induced paw edema in *Wistar* rats

Treatment (mg/kg bw)	%Inhibition of edema	
	Aqueous extract	**Methanol extract**
Diclofenac sodium (50mg//kg bw)	24.23 ± 7.78^{a}	29.81 ± 5.76^{bc}
20	9.11 ± 5.79^{a}	7.57 ± 5.01^{a}
100	17.48 ± 5.20^{a}	19.77 ± 6.46^{ab}
500	28.63 ± 6.21^{a}	47.69 ± 6.57^{c}

Values are presented as Mean ± SEM. Means with different superscript letters along the column are significantly different from each other (Two-Way ANOVA and Tukey's test; $p< 0.05$).

There was no significant difference between the effect of different dosage levels of the aqueous extract of *M. triphylla* and a 50 mg/kg dose of diclofenac sodium on acetic acid-induced paw edema in rats ($p>0.05$; Table 4.6). A low dosage level of the methanol extract of *M. triphylla* produced a significantly lower ($p<0.05$) inhibition of acetic acid-induced paw edema than a 50 mg/kg dose of diclofenac (Table 4.6). There was no significant difference in the inhibitory activity of intermediate and high doses of the methanol extract of *M. triphylla* and a 50 mg/kg dose of diclofenac sodium on acetic acid-induced paw edema (Table 4.6).

4.6 The effect of duration of treatment on acetic acid-induced paw edema in *Wistar* rats

Table 4.7 is a summary of the effect of duration of treatment on acetic acid-induced paw edema in rats.

Table 4.7: Effects of the duration of treatment on acetic acid-induced paw edema in *Wistar* rats

Duration	% Inhibition of edema	
	Aqueous extract	Methanol extract
1 hour	16.63 ± 7.70^{a}	6.83 ± 10.32^{a}
2 hours	27.65 ± 3.12^{a}	12.89 ± 4.86^{ab}
3 hours	13.22 ± 6.52^{a}	39.61 ± 3.87^{c}
4 hours	11.92 ± 8.17^{a}	38.34 ± 6.27^{c}
5 hours	29.90± 8.57^{a}	33.37 ± 7.05^{bc}

Values are presented as Mean ± SEM. Means with different superscript letters along the column are significantly different (Two-Way ANOVA and Tukey's test; $p< 0.05$).

Figure 4.2 below further illustrates the onset and duration of action of *M. triphylla* root extracts in inhibiting acetic acid-induced paw edema in *Wistar* rats.

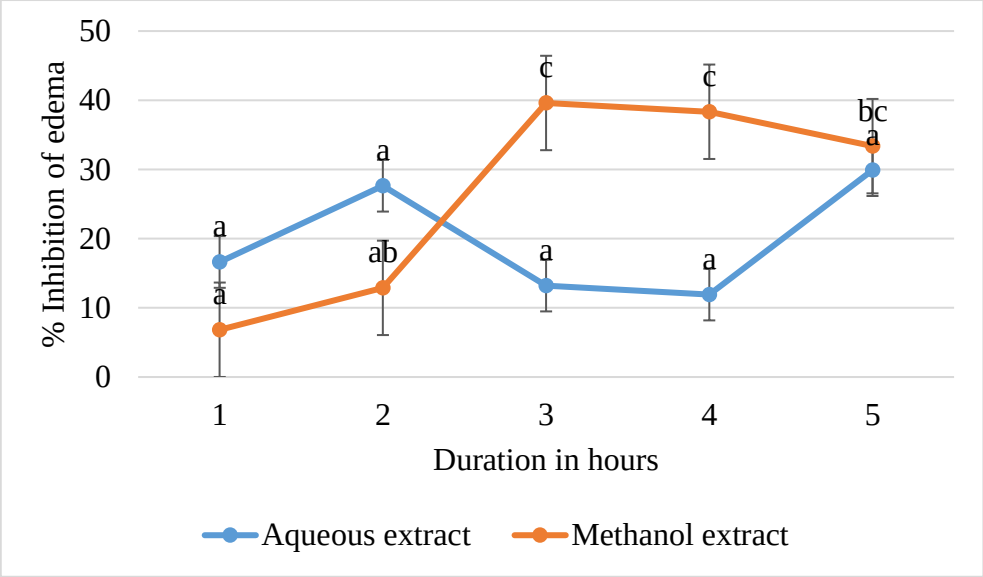

Figure 4.2: Effects of the duration of treatment on acetic acid-induced paw edema in *Wistar* rats. Values are presented as Mean ± SEM. Means with different superscript letters along the same line are significantly different (Two-Way ANOVA followed by Tukey's test; $p< 0.05$).

The duration of treatment did not significantly affect the efficacy of the aqueous extract of *M. triphylla* to inhibit acetic acid-induced paw edema in rats (p>0.5, Table 4.7, Figure 4.2). Conversely, the inhibition of acetic acid-induced paw edema in rats given the methanol extract was significantly higher *(p<0.05)* after 3, 4, and 5 hours relative to after 1 hour **(**Table 4.7, Figure 4.2). Moreover, there was no significant difference in the inhibition of acetic acid-induced paw edema in rats given the methanol extract after 2 hours relative to after 1 hour (*p>0.05;* Table 4.7, Figure 4.2).

4.7 The effect of treatment and duration on the acetic acid-induced paw edema in *Wistar* rats

There was no significant difference (*p>0.05*) between the effect of low, intermediate, and high dosage levels of the aqueous root extract of *M. triphylla* and the effect of 50mg/kg bw dose of diclofenac sodium on acetic acid-induced paw edema in rats after 1, 2, 3, 4 and 5 hours. However, the percentage inhibition of acetic acid-induced paw edema in rats treated with a low dose of the methanol root extract of *M. triphylla* after 1 hour was significantly lower *(p<0.05)* than in rats treated with a high dose after 3, 4 and 5 hours

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

The present study evaluated acute oral toxicity, phytochemical content, the analgesic and anti-inflammatory properties of the methanol and aqueous extracts of *M. triphylla* roots. The extensive ethnomedicinal use of *M. triphylla* throughout its distributional range suggests that it is taken below the toxic dosages. However, the WHO and FDA emphasize the scientific-based validation of efficacious and safe use of herbal medications (Setzer and Kimmel, 2003; WHO 1993). Therefore, rigorous clinical and toxicological studies of the compounds isolated from the species are necessary (Alfred, 2020) to determine their safer dose range (Saleem et al., 2016).

In this experiment, the acute oral toxicity effects of both the aqueous and methanol root extracts of *M. triphylla* were determined using the Up-and-Down procedure described by OECD (2008, Document No. 425). This procedure has as well been used in previous studies (Olela et al., 2020; Saleem et al., 2017; Otieno, 2016). There was no observable evidence of toxicity in the rats at the limit dosage level of 2000 mg/kg bw suggesting that the extracts are nontoxic at therapeutic doses. This study disagrees with previous findings of Hamilton and Hamilton, (2006) and Dharani, (2019) that found out that the roots of *M. triphylla* have to be boiled for a long time to render them nontoxic by either denaturing or exposing the toxic metabolites to degradation reactions. The toxicity here however could be due to repeated intake of the *M. triphylla* root porridge hence subacute or chronic adverse effects.

As stated by Husna et al., 2013, the nonappearance of notable signs of toxicity and death in the animals when given a specific test dose signifies that the LD_{50} is above this test dose. During the entire period of the study, there was no mortality or morbidity suggesting that the LD_{50} of the methanol and aqueous root extracts of *M. triphylla* was above 2000 mg/kg bw. Previous studies have indicated that the toxic effects reported after consumption of some plants or their products are as a result of the presence of toxic secondary metabolites like alkaloids (Molyneux et al., 2007). These results, therefore, suggest that

toxic metabolites in *M. triphylla* root extracts are either absent or present at very low amounts to induce any noticeable adverse effects.

Weight gain is an important toxicity index for rats (Wen et al., 2017; Saleem et al., 2017). The weight of the rats was measured before the beginning of the experiment at dosing, at 7 days and 14 days according to OECD (2008, Document No. 425). Significant weight gain was observed in rats that received both extracts after 7 days and 14 days. However, the weight gain in rats after 7 and 14 days of treatment with a 2000 mg/kg dosage level of the aqueous extract of *M. triphylla* was significantly lower than the weight gain in rats given a 2000 mg/kg dosage level of the methanol extract of *M. triphylla.* These results suggest that the aqueous extract of *M. triphylla* contained toxic metabolites or more toxic metabolites than the methanol extract. These toxic metabolites may have affected the normal metabolism of the animals hence the significantly lower weight gain (Gregus and Klaasen, 2001).

The phytochemical screening method of Harborne, (1998) and Kokate et al., 2007 used in this study to qualitatively screen for bioactive compounds in the *M. triphylla* root extracts showed the existence of flavonoids, cardiac glycosides, alkaloids and phenols in both extracts. This is in agreement with previous studies (Ker., 2003). Both extracts tested negative for terpenoids, steroids, and tannins. No saponins were present in the aqueous extract as opposed to the methanol extract that tested positive for saponins. This is in agreement with previous studies where the investigators found out that plant parts may not contain all phytochemicals (Otieno, 2016).

Phytochemical compounds such as saponins, flavonoids, cardiac glycosides, alkaloids and phenols found in the extracts have been found to have anti-inflammatory and analgesic properties in previous studies. Numerous studies have shown that alkaloids possess anti-inflammatory and analgesic properties (Chindo et al., 2010; Kaleem et al., 2013). Flavonoids also show analgesic and anti-inflammatory activity by inhibiting prostaglandin synthetase which in turn reduces prostaglandin synthesis and release (Hossinzadeh et al., 2002; Chatterjee et al., 2015; Tapas et al., 2008). Cardiac glycosides inhibit the

activation of NF-β signaling pathway by suppressing the hypersecretion of IL-8. Phenols reduce the expression and inhibit the function of iNOS. They also lower the level of prostaglandins and TNF-α (Nyamai et al., 2016).

The analgesic property evaluation of the extracts of M. *triphylla* was determined using the acetic acid-induced writhing technique on *Swiss albino* mice of either sex. Generally, the acetic acid-induced writhing procedure is normally selected as a standard procedure to evaluate the analgesic efficacy of drugs and natural products in the periphery. It acts by stimulating chemically induced stimulus (Zhen et al., 2015). The intraperitoneal injection of acetic acid causes the release of endogenous mediators like prostaglandin especially prostaglandin 2, histamine and serotonin in peritoneal fluids. This produces peritoneal inflammation which is associated with pain (Bose et al., 2007; Yasmen et al., 2018). This pain is characterized by abdominal muscle contractions, body elongation as well as an extension of forelimbs characterized as writhing whose frequency can be quantified (Olela et al., 2020). Agents which inhibit or reduce the acetic acid-induced writhing are regarded as possessing the analgesic effect.

Here, both root extracts of *M. triphylla* demonstrated significant inhibition of the acetic acid-induced writhing in mice. In this method, the release of free arachidonic acid via COX and prostaglandin production from tissue phospholipid in peritoneal fluids (Gupta and Singh, 2017) resulting from triggering of localized inflammation brings about the pain sensation (Bhattacharya et al., 2014). Furthermore, there is the release of other pain mediators such as histamine and bradykinins from the peritoneal cavity cell lining that further enhance stimulation of nociceptors. The increase in capillary permeability caused by an increase in prostaglandin concentration in the peritoneal cavity then intensifies inflammatory pain (Zakaria et al., 2008). These results suggest that the extracts were able to inhibit the prostaglandin synthesis which are inflammatory pain mediators. Therefore, it can be suggested that the flavonoids and alkaloids contained in the aqueous and methanolic root extracts of *M. triphylla* could be responsible for the analgesic effect of the extracts. This is similar to other results obtained in previous studies that have examined the analgesic activities of other medicinal plants (Safari et al., 2016).

The aqueous extract was more potent than the methanol extract throughout the dose levels. This suggests that the aqueous extract contains more bioactive compounds responsible for the analgesic effect compared to the methanol extract at similar dose levels. The three-dose levels of both the methanol and aqueous root extracts of *M. triphylla* generated a dose-dependent response to the acetic-induced pain. A similar response was also seen by Olela et al., 2020. Peak analgesic effect was noted at a dosage level of 500 mg/kg in both extracts with the analgesic effect of both extracts being greater than that of acetylsalicylic acid (75mg/kg bw) at all dose levels except for methanol extract at a dosage level of 20mg/kg bw. This could be attributed to the clearance and fast metabolism of the active compounds that were in an insufficient concentration in the lower dose level of the methanol extract (Maina et al., 2015).

Inflammation describes the body's normal defensive mechanism to tissue injury triggered by microbiologic agents, noxious compounds and physical trauma (Stankov, 2012). Some of the symptoms that characterize inflammation include the release of inflammatory mediators, vasodilation, increased blood flow, necrosis, tissue degeneration, and formation of exudates. Inflammation usually subsides after the healing process but sometimes it becomes severe which may be fatal leading to diseases such as arthritis and rheumatism (Richard et al., 2008).

In this experiment, the anti-inflammatory properties of *M. triphylla* root extracts in *Wistar* rats were investigated using the acetic-acid induced paw edema. The subplantar injection of acetic acid causes the discharge of inflammatory mediators like histamine, prostaglandins and serotonin (Yasmen et al., 2018). Therefore, there is increased vasculature permeability with greater vasodilation resulting in edema at the paw (Yasmen et al., 2018). An increase in paw size is used to quantify the inflammatory response seen post acetic acid injection.

The results obtained in this study suggest a remarkable ability of the root extracts of *M. triphylla* in inhibiting/reducing acetic acid-induced paw edema in rats. NSAIDs mitigate inflammation by inhibiting the activity of COX-2 and phospholipase A_2 (Necas and Bartosikova, 2013). Flavonoids,

cardiac glycosides, phenols, and saponins have been reported in other studies to also have potent anti-inflammatory attributes (Tapas et al., 2008; Nyamai et al., 2016). Therefore, the cardiac glycosides, flavonoids and phenols identified in both *M. triphylla* root extracts could be causing the anti-inflammatory property of the plant extracts by inhibiting prostaglandin synthesis in a similar manner to NSAIDs. The methanol extract had better percentage inhibition of inflammation compared to the aqueous extract at the intermediate and high dose levels. A similar kind of response was also observed by Uddin et al., 2020 and Hong et al., 2020. Apart from flavonoids, cardiac glycosides, and phenols, saponins were identified in the methanol extract but were absent in the aqueous extract. In previous studies, 3,4-seco-dammarane triterpenoid saponins isolated from *Cyclocarya paliurus* leaves have exhibited anti-inflammatory activity. This suggests that the saponins in the methanol root extract of *M. triphylla* could be responsible for the better potency of the methanol extract in inhibiting inflammation in comparison to the aqueous extract (Liu et al., 2020).

Peak anti-inflammatory effect was noted at a dosage level of 500 mg/kg in both the extracts with both extracts exhibiting a dose-dependent relationship. Similar results have also been observed by (Olela et al., 2020). The anti-inflammatory effect of the aqueous extracts was not significantly different from the standard drug diclofenac sodium at all doses. This could imply that the concentration of the phytochemicals causing the anti-inflammatory activity of the aqueous extract was similar at all the studied doses. In the methanol extract however, only the low methanol extract dose had a lower anti-inflammatory activity in comparison to the standard drug. This could be attributed the clearance and fast metabolism of the active compounds that were in an insufficient concentration in the lower dose level of the methanol extract (Maina et al., 2015).

The three doses of the aqueous extract and methanol extract together with their respective standard drugs (diclofenac sodium) achieved maximum anti-inflammatory activity in the second and third hours respectively. This indicates a gradual but constant movement of the phytochemicals that are bioactive across the cell membrane into the site of inflammation (Hossain et al., 2011). This would suggest that the

aqueous extract constituents diffused faster compared to the methanol extract constituents hence achieving the maximal anti-inflammatory effect at the second hour rather than the third hour in the methanol extract. From the results, the aqueous extract at the three dose levels had low percentage inhibition of the acetic acid-induced paw edema in rats during hour one. The methanol extract at the three dose levels had low percentage inhibition of the acetic acid-induced paw edema in rats during the first and second hour. This can be attributed to the absence of prostaglandins in this early phase of inflammation since the treatments were working similarly to NSAIDs by inhibiting the biosynthesis of prostaglandins (Necas and Bartosikova, 2013).

Previous studies have identified quaternary ammonium compounds and betaines such as glycine betaine, proline betaine, 3-hydroxyprolinebetaine and 3-hydroxy-1,1-dimethyl pyrrolidinium in the branches and dried aerial parts of *M. triphylla* (McLean et al., 1996). Proline betaine has been shown to significantly suppress IL-1β-induced inflammation with decreased levels of cytokines and inflammatory mediators including IL-6, iNOS, PGE2, TNF-α, COX-2 and NO (Haojie et al., 2020). This compound has also been shown to dock at the COX-2 receptor using GOLD docking fitness and therefore inhibiting the activity of COX-2 enzyme (Uddin et al., 2014). This suggests that proline betaine could be responsible for the anti-inflammatory and analgesic properties of the root extracts of *M. triphylla*.

5.2 CONCLUSION

Based on the results obtained in this study, the following conclusions were made:

1. The aqueous and methanol root extracts of *M. triphylla* have significant analgesic and anti-inflammatory activities which may be due to the presence of phytochemicals like flavonoids.
2. The aqueous and methanol root extracts of *M. triphylla* are non-toxic at therapeutic doses.
3. The aqueous and methanol root extracts of *M. triphylla* demonstrated a dose-dependent response to the acetic acid-induced pain and acetic acid-induced paw edema.
4. Validation of the ethnomedicinal use of the roots of *M. triphylla* in the management of pain and inflammation by communities.

5.3 RECOMMENDATIONS

Based on the study, the following recommendations may be drawn:

1. Isolation, characterization and quantification of the specific phytochemical constituents in the root extracts of *M. triphylla* with analgesic and anti-inflammatory activity is needed.
2. There is a need to determine the specific analgesic and anti-inflammatory mechanism(s) of action of the *M. triphylla* root extracts at cellular and molecular levels.
3. Evaluation of the subacute and chronic effects of the aqueous and methanol root extracts of *M. triphylla* on experimental animals is needed.

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APPENDICES

Appendix I: Ethical approval document

UNIVERSITY OF NAIROBI

FACULTY OF VETERINARY MEDICINE

DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
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REF: FVM BAUEC/2021/291

Brian Wangusi,

University of Nairobi

Dept. of PHP & Toxicology,

08/03/2021

Dear Wangusi,

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

Analgesic, Anti-inflammatory, Acute oral toxicity and Phytochemical studies of *Maerua triphylla*.

Brian Wangusi J56/33857/2019

We refer to your MSc. proposal submitted to our committee for review and your application letter dated 1st March 2021. We have reviewed your application for ethical clearance for the study.

The animal husbandry, analgesic, anti-inflammatory, acute oral toxicity protocols and number of mice to be used in the study meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.

We have also noted that KVB registered veterinary surgeons will supervise the study.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, Ph.D

Chairperson, Biosafety, Animal Use and Ethics Committee,

Faculty of Veterinary Medicine,

University of Nairobi

Appendix II: Research permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: **209882**

Date of Issue: **15/April/2021**

RESEARCH LICENSE

This is to Certify that Mr. BRIAN Muyukani WANGUSI of University of Nairobi, has been licensed to conduct research in Nairobi on the topic: ANALGESIC, ANTI-INFLAMMATORY, ACUTE ORAL TOXICITY AND PHYTOCHEMICAL STUDY OF MAERUA TRIPHYLLA A. RICH (CAPPARADACEAE) for the period ending: 15/April/2022.

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209882

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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Appendix III: Data output from the analysis of the effect of the aqueous root extract of *M. triphylla* on acetic acid-induced writhing in *Swiss albino* mice

GenStat Fifteenth Edition
GenStat Procedure Library Release PL23.1

Analysis of variance

Variate: %_Inhibition_of_number_of_writhes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	722.883	240.961	34.80	<.001
Residual	16	110.791	6.924		
Total	19	833.674			

Tukey's 95% confidence intervals
Treatment

Comparison	Significant
Aspirin vs 20mg/kg_AQ extract	no
Aspirin vs 100mg/kg_AQ extract	yes
Aspirin vs 500mg/kg_AQextract	yes
20mg/kg_AQ extract vs 100mg/kg_AQ extract	yes
20mg/kg_AQ extract vs 500mg/kg_AQextract	yes
100mg/kg_AQ extract vs 500mg/kg_AQextract	no

	Mean	
Aspirin	81.08	a
20mg/kg_AQ extract	85.31	a
100mg/kg_AQ extract	93.43	b
500mg/kg_AQextract	95.97	b

Summary statistics for %_Inhibition_of_number_of_writhes: Treatment 20mg/kg_AQ extract

Number of missing values =	0
Mean =	85.31
Median =	84.44
Minimum =	82.29
Maximum =	88.76
Standard error of mean =	1.133

Summary statistics for %_Inhibition_of_number_of_writhes: Treatment 100mg/kg_AQ extract

Number of missing values =	0
Mean =	93.43
Median =	93.9
Minimum =	90.45
Maximum =	96.67
Standard error of mean =	1.260

Summary statistics for %_Inhibition_of_number_of_writhes: Treatment 500mg/kg_AQextract

Number of missing values =	0
Mean =	95.97
Median =	96.63
Minimum =	92.71
Maximum =	98.81
Standard error of mean =	1.155

Summary statistics for %_Inhibition_of_number_of_writhes: Treatment Acetylsalicylic Acid

Number of missing values =	0
Mean =	81.08
Median =	80.49
Minimum =	78.57
Maximum =	85.42
Standard error of mean =	1.155

Appendix IV: Data output from the analysis of the effect of the methanol root extract of *M. triphylla* on acetic acid-induced writhing in *Swiss albino* mice

Analysis of variance

Variate: %_Inhibition_of_number_of_writhes

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	402.47	134.16	11.04	<.001
Residual	16	194.46	12.15		
Total	19	596.93			

Tukey's 95% confidence intervals

Treatment

Comparison	Significant
20mg/kg_MEOH extract vs Aspirin	no
20mg/kg_MEOH extract vs 100mg/kg_MEOH extract	yes
20mg/kg_MEOH extract vs 500mg/kg_MEOHextract	yes
Aspirin vs 100mg/kg_MEOH extract	no
Aspirin vs 500mg/kg_MEOHextract	yes
100mg/kg_MEOH extract vs 500mg/kg_MEOHextract	no

	Mean	
20mg/kg_MEOH extract	81.84	a
Aspirin	82.17	ab
100mg/kg_MEOH extract	88.43	bc
500mg/kg_MEOHextract	92.55	c

Summary statistics for %_Inhibition_of_number_of_writhes: Treatment 20mg/kg_MEOH extract

Number of values =	5
Mean =	81.84
Median =	81.25
Minimum =	77.33
Maximum =	86.02
Standard deviation =	3.385
Standard error of mean =	1.514

Summary statistics for %_Inhibition_of_number_of_writhes: Treatment 100mg/kg_MEOH extract

Number of values =	5
Mean =	88.43

Median =	88.75
Minimum =	84
Maximum =	91.56
Standard deviation =	2.792
Standard error of mean =	1.249

Summary statistics for %_Inhibition_of_number_of_writhes: Treatment 500mg/kg_MEOHextract

Number of values =	5
Mean =	92.55
Median =	92.47
Minimum =	88.42
Maximum =	95
Standard deviation =	2.677
Standard error of mean =	1.197

Summary statistics for %_Inhibition_of_number_of_writhes: Treatment Acetylsalicylic Acid

Number of values =	5
Mean =	82.17
Median =	82.5
Minimum =	76.29
Maximum =	87.37
Standard deviation =	4.712
Standard error of mean =	2.107

Appendix V: Data output from the comparison of the inhibition of acetic acid-induced writhing by aqueous and methanol root extracts of *M. triphylla*

Two-sample t-test

Variate: %_Inhibition_of_number_of_writhes
Group factor: Treatment
Test for equality of sample variances

Test statistic F = 1.79 on 4 and 4 d.f.
Probability (under null hypothesis of equal variances) = 0.59

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
20mg/kg_AQ extract	5	85.31	6.413	2.532	1.133
20mg/kg_MEOH extract	5	81.84	11.456	3.385	1.514

Difference of means: 3.464
Standard error of difference: 1.890

95% confidence interval for difference in means: (-0.8954, 7.823)

Test of null hypothesis that mean of %_Inhibition_of_number_of_writhe with Treatment = 20mg/kg_AQ extract is equal to mean with Treatment = 20mg/kg_MEOH extract

Test statistic t = 1.83 on 8 d.f.
Probability = 0.104

Two-sample t-test

Variate: %_Inhibition_of_number_of_writhe
Group factor: Treatment
Test for equality of sample variances

Test statistic F = 1.02 on 4 and 4 d.f.
Probability (under null hypothesis of equal variances) = 0.99

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
100mg/kg_AQ extract	5	93.43	7.941	2.818	1.260

100mg/kg_MEOH extract	5	88.43	7.795	2.792 1.249

Difference of means: 4.998
Standard error of difference: 1.774

95% confidence interval for difference in means: (0.9070, 9.089)

Test of null hypothesis that mean of %_Inhibition_of_number_of_writhe with Treatment = 100mg/kg_AQ extract is equal to mean with Treatment = 100mg/kg_MEOH extract

Test statistic t = 2.82 on 8 d.f.
Probability = 0.023

Two-sample t-test

Variate: %_Inhibition_of_number_of_writhe
Group factor: Treatment
Test for equality of sample variances

Test statistic F = 1.07 on 4 and 4 d.f.
Probability (under null hypothesis of equal variances) = 0.95

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
500mg/kg_AQextract	5	95.97	6.672	2.583	1.155
500mg/kg_MEOHextract	5	92.55	7.165	2.677	1.197

Difference of means: 3.418
Standard error of difference: 1.664

95% confidence interval for difference in means: (-0.4182, 7.254)

Test of null hypothesis that mean of %_Inhibition_of_number_of_writhe with Treatment = 500mg/kg_AQextract is equal to mean with Treatment = 500mg/kg_MEOHextract

Test statistic t = 2.05 on 8 d.f.
Probability = 0.074

Appendix VI: Data output from the analysis of the effect of duration on the anti-inflammatory activity of the aqueous root extract of *M. triphylla* on acetic acid-induced paw edema in *Wistar* rats

Analysis of variance

Variate: %_inhibition_of_edema

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Duration	4	5581.	1395.	1.27	0.290
Treatment	3	5431.	1810.	1.64	0.186
Duration.Treatment	12	2106.	175.	0.16	0.999
Residual	80	88130.	1102.		
Total	99	101247.			

Tukey's 95% confidence intervals

Duration

Comparison	Difference	Lower 95%	Upper 95%	Significant
4-hour vs 3-hour	-1.30	-30.59	28.00	no
4-hour vs 1-hour	-4.71	-34.00	24.59	no
4-hour vs 2-hour	-15.73	-45.02	13.56	no
4-hour vs 5-hour	-17.98	-47.27	11.32	no
3-hour vs 1-hour	-3.41	-32.70	25.88	no
3-hour vs 2-hour	-14.43	-43.73	14.86	no
3-hour vs 5-hour	-16.68	-45.97	12.62	no
1-hour vs 2-hour	-11.02	-40.32	18.27	no
1-hour vs 5-hour	-13.27	-42.56	16.03	no
2-hour vs 5-hour	-2.25	-31.54	27.05	no

	Mean	
4-hour	11.92	a
3-hour	13.22	a
1-hour	16.63	a
2-hour	27.65	a
5-hour	29.90	a

Appendix VII: Data output from the analysis of the effect of treatment on the anti-inflammatory activity of the aqueous root extract of *M. triphylla* on acetic acid-induced paw edema in *Wistar* rats

Analysis of variance

Variate: %_inhibition_of_edema

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Duration	4	5581.	1395.	1.27	0.290
Treatment	3	5431.	1810.	1.64	0.186
Duration. Treatment	12	2106.	175.	0.16	0.999
Residual	80	88130.	1102.		
Total	99	101247.			

Tukey's 95% confidence intervals

Treatment

Comparison	Significant
20mg/kg_AQ vs 100mg/kg_AQ	no
20mg/kg_AQ vs Diclofenac sodium only	no
20mg/kg_AQ vs 500mg/kg_AQ	no
100mg/kg_AQ vs Diclofenac sodium only	no
100mg/kg_AQ vs 500mg/kg_AQ	no
Diclofenac sodium only vs 500mg/kg_AQ	no

	Mean	
20mg/kg_AQ	9.11	a
100mg/kg_AQ	17.48	a
Diclofenac sodium only	24.23	a
500mg/kg_AQ	28.63	a

Appendix VIII: Data output of the analysis of the effect of treatment and duration on the anti-inflammatory activity of the aqueous root extract of *M. triphylla* on acetic acid-induced paw edema in *Wistar* rats

Analysis of variance

Variate: %_inhibition_of_edema

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Duration	4	5581.	1395.	1.27	0.290
Treatment	3	5431.	1810.	1.64	0.186
Duration.Treatment	12	2106.	175.	0.16	0.999
Residual	80	88130.	1102.		
Total	99	101247.			

Tukey's 95% confidence intervals
Duration. Treatment

Comparison	Significant
4-hour 20mg/kg_AQ vs 3-hour 20mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 4-hour 100mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 1-hour 20mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 3-hour 100mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 5-hour 20mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 1-hour Diclofenac sodium only	no
4-hour 20mg/kg_AQ vs 3-hour Diclofenac sodium only	no
4-hour 20mg/kg_AQ vs 1-hour 100mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 5-hour 100mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 4-hour Diclofenac sodium only	no
4-hour 20mg/kg_AQ vs 1-hour 500mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 2-hour 20mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 4-hour 500mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 3-hour 500mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 2-hour Diclofenac sodium only	no
4-hour 20mg/kg_AQ vs 2-hour 500mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
4-hour 20mg/kg_AQ vs 5-hour Diclofenac sodium only	no
4-hour 20mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
3-hour 20mg/kg_AQ vs 4-hour 100mg/kg_AQ	no
3-hour 20mg/kg_AQ vs 1-hour 20mg/kg_AQ	no
3-hour 20mg/kg_AQ vs 3-hour 100mg/kg_AQ	no
3-hour 20mg/kg_AQ vs 5-hour 20mg/kg_AQ	no

3-hour 20mg/kg_AQ vs 1-hour Diclofenac sodium only	no
3-hour 20mg/kg_AQ vs 3-hour Diclofenac sodium only	no
3-hour 20mg/kg_AQ vs 1-hour 100mg/kg_AQ	no
3-hour 20mg/kg_AQ vs 5-hour 100mg/kg_AQ	no
3-hour 20mg/kg_AQ vs 4-hour Diclofenac sodium only	no
3-hour 20mg/kg_AQ vs 1-hour 500mg/kg_AQ	no
3-hour 20mg/kg_AQ vs 2-hour 20mg/kg_AQ	no
3-hour 20mg/kg_AQ vs 4-hour 500mg/kg_AQ	no
3-hour 20mg/kg_AQ vs 3-hour 500mg/kg_AQ	no
3-hour 20mg/kg_AQ vs 2-hour Diclofenac sodium only	no
3-hour 20mg/kg_AQ vs 2-hour 500mg/kg_AQ	no
3-hour 20mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
3-hour 20mg/kg_AQ vs 5-hour Diclofenac sodium only	no
3-hour 20mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
4-hour 100mg/kg_AQ vs 1-hour 20mg/kg_AQ	no
4-hour 100mg/kg_AQ vs 3-hour 100mg/kg_AQ	no
4-hour 100mg/kg_AQ vs 5-hour 20mg/kg_AQ	no
4-hour 100mg/kg_AQ vs 1-hour Diclofenac sodium only	no
4-hour 100mg/kg_AQ vs 3-hour Diclofenac sodium only	no
4-hour 100mg/kg_AQ vs 1-hour 100mg/kg_AQ	no
4-hour 100mg/kg_AQ vs 5-hour 100mg/kg_AQ	no
4-hour 100mg/kg_AQ vs 4-hour Diclofenac sodium only	no
4-hour 100mg/kg_AQ vs 1-hour 500mg/kg_AQ	no
4-hour 100mg/kg_AQ vs 2-hour 20mg/kg_AQ	no
4-hour 100mg/kg_AQ vs 4-hour 500mg/kg_AQ	no
4-hour 100mg/kg_AQ vs 3-hour 500mg/kg_AQ	no
4-hour 100mg/kg_AQ vs 2-hour Diclofenac sodium only	no
4-hour 100mg/kg_AQ vs 2-hour 500mg/kg_AQ	no
4-hour 100mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
4-hour 100mg/kg_AQ vs 5-hour Diclofenac sodium only	no
4-hour 100mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
1-hour 20mg/kg_AQ vs 3-hour 100mg/kg_AQ	no
1-hour 20mg/kg_AQ vs 5-hour 20mg/kg_AQ	no
1-hour 20mg/kg_AQ vs 1-hour Diclofenac sodium only	no
1-hour 20mg/kg_AQ vs 3-hour Diclofenac sodium only	no
1-hour 20mg/kg_AQ vs 1-hour 100mg/kg_AQ	no
1-hour 20mg/kg_AQ vs 5-hour 100mg/kg_AQ	no
1-hour 20mg/kg_AQ vs 4-hour Diclofenac sodium only	no
1-hour 20mg/kg_AQ vs 1-hour 500mg/kg_AQ	no
1-hour 20mg/kg_AQ vs 2-hour 20mg/kg_AQ	no
1-hour 20mg/kg_AQ vs 4-hour 500mg/kg_AQ	no
1-hour 20mg/kg_AQ vs 3-hour 500mg/kg_AQ	no
1-hour 20mg/kg_AQ vs 2-hour Diclofenac sodium only	no
1-hour 20mg/kg_AQ vs 2-hour 500mg/kg_AQ	no
1-hour 20mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
1-hour 20mg/kg_AQ vs 5-hour Diclofenac sodium only	no

1-hour 20mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
3-hour 100mg/kg_AQ vs 5-hour 20mg/kg_AQ	no
3-hour 100mg/kg_AQ vs 1-hour Diclofenac sodium only	no
3-hour 100mg/kg_AQ vs 3-hour Diclofenac sodium only	no
3-hour 100mg/kg_AQ vs 1-hour 100mg/kg_AQ	no
3-hour 100mg/kg_AQ vs 5-hour 100mg/kg_AQ	no
3-hour 100mg/kg_AQ vs 4-hour Diclofenac sodium only	no
3-hour 100mg/kg_AQ vs 1-hour 500mg/kg_AQ	no
3-hour 100mg/kg_AQ vs 2-hour 20mg/kg_AQ	no
3-hour 100mg/kg_AQ vs 4-hour 500mg/kg_AQ	no
3-hour 100mg/kg_AQ vs 3-hour 500mg/kg_AQ	no
3-hour 100mg/kg_AQ vs 2-hour Diclofenac sodium only	no
3-hour 100mg/kg_AQ vs 2-hour 500mg/kg_AQ	no
3-hour 100mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
3-hour 100mg/kg_AQ vs 5-hour Diclofenac sodium only	no
3-hour 100mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
5-hour 20mg/kg_AQ vs 1-hour Diclofenac sodium only	no
5-hour 20mg/kg_AQ vs 3-hour Diclofenac sodium only	no
5-hour 20mg/kg_AQ vs 1-hour 100mg/kg_AQ	no
5-hour 20mg/kg_AQ vs 5-hour 100mg/kg_AQ	no
5-hour 20mg/kg_AQ vs 4-hour Diclofenac sodium only	no
5-hour 20mg/kg_AQ vs 1-hour 500mg/kg_AQ	no
5-hour 20mg/kg_AQ vs 2-hour 20mg/kg_AQ	no
5-hour 20mg/kg_AQ vs 4-hour 500mg/kg_AQ	no
5-hour 20mg/kg_AQ vs 3-hour 500mg/kg_AQ	no
5-hour 20mg/kg_AQ vs 2-hour Diclofenac sodium only	no
5-hour 20mg/kg_AQ vs 2-hour 500mg/kg_AQ	no
5-hour 20mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
5-hour 20mg/kg_AQ vs 5-hour Diclofenac sodium only	no
5-hour 20mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
1-hour Diclofenac sodium only vs 3-hour Diclofenac sodium only	no
1-hour Diclofenac sodium only vs 1-hour 100mg/kg_AQ	no
1-hour Diclofenac sodium only vs 5-hour 100mg/kg_AQ	no
1-hour Diclofenac sodium only vs 4-hour Diclofenac sodium only	no
1-hour Diclofenac sodium only vs 1-hour 500mg/kg_AQ	no
1-hour Diclofenac sodium only vs 2-hour 20mg/kg_AQ	no
1-hour Diclofenac sodium only vs 4-hour 500mg/kg_AQ	no
1-hour Diclofenac sodium only vs 3-hour 500mg/kg_AQ	no
1-hour Diclofenac sodium only vs 2-hour Diclofenac sodium only	no
1-hour Diclofenac sodium only vs 2-hour 500mg/kg_AQ	no
1-hour Diclofenac sodium only vs 2-hour 100mg/kg_AQ	no
1-hour Diclofenac sodium only vs 5-hour Diclofenac sodium only	no
1-hour Diclofenac sodium only vs 5-hour 500mg/kg_AQ	no
3-hour Diclofenac sodium only vs 1-hour 100mg/kg_AQ	no
3-hour Diclofenac sodium only vs 5-hour 100mg/kg_AQ	no
3-hour Diclofenac sodium only vs 4-hour Diclofenac sodium only	no

3-hour Diclofenac sodium only vs 1-hour 500mg/kg_AQ	no
3-hour Diclofenac sodium only vs 2-hour 20mg/kg_AQ	no
3-hour Diclofenac sodium only vs 4-hour 500mg/kg_AQ	no
3-hour Diclofenac sodium only vs 3-hour 500mg/kg_AQ	no
3-hour Diclofenac sodium only vs 2-hour Diclofenac sodium only	no
3-hour Diclofenac sodium only vs 2-hour 500mg/kg_AQ	no
3-hour Diclofenac sodium only vs 2-hour 100mg/kg_AQ	no
3-hour Diclofenac sodium only vs 5-hour Diclofenac sodium only	no
3-hour Diclofenac sodium only vs 5-hour 500mg/kg_AQ	no
1-hour 100mg/kg_AQ vs 5-hour 100mg/kg_AQ	no
1-hour 100mg/kg_AQ vs 4-hour Diclofenac sodium only	no
1-hour 100mg/kg_AQ vs 1-hour 500mg/kg_AQ	no
1-hour 100mg/kg_AQ vs 2-hour 20mg/kg_AQ	no
1-hour 100mg/kg_AQ vs 4-hour 500mg/kg_AQ	no
1-hour 100mg/kg_AQ vs 3-hour 500mg/kg_AQ	no
1-hour 100mg/kg_AQ vs 2-hour Diclofenac sodium only	no
1-hour 100mg/kg_AQ vs 2-hour 500mg/kg_AQ	no
1-hour 100mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
1-hour 100mg/kg_AQ vs 5-hour Diclofenac sodium only	no
1-hour 100mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
5-hour 100mg/kg_AQ vs 4-hour Diclofenac sodium only	no
5-hour 100mg/kg_AQ vs 1-hour 500mg/kg_AQ	no
5-hour 100mg/kg_AQ vs 2-hour 20mg/kg_AQ	no
5-hour 100mg/kg_AQ vs 4-hour 500mg/kg_AQ	no
5-hour 100mg/kg_AQ vs 3-hour 500mg/kg_AQ	no
5-hour 100mg/kg_AQ vs 2-hour Diclofenac sodium only	no
5-hour 100mg/kg_AQ vs 2-hour 500mg/kg_AQ	no
5-hour 100mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
5-hour 100mg/kg_AQ vs 5-hour Diclofenac sodium only	no
5-hour 100mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
4-hour Diclofenac sodium only vs 1-hour 500mg/kg_AQ	no
4-hour Diclofenac sodium only vs 2-hour 20mg/kg_AQ	no
4-hour Diclofenac sodium only vs 4-hour 500mg/kg_AQ	no
4-hour Diclofenac sodium only vs 3-hour 500mg/kg_AQ	no
4-hour Diclofenac sodium only vs 2-hour Diclofenac sodium only	no
4-hour Diclofenac sodium only vs 2-hour 500mg/kg_AQ	no
4-hour Diclofenac sodium only vs 2-hour 100mg/kg_AQ	no
4-hour Diclofenac sodium only vs 5-hour Diclofenac sodium only	no
4-hour Diclofenac sodium only vs 5-hour 500mg/kg_AQ	no
1-hour 500mg/kg_AQ vs 2-hour 20mg/kg_AQ	no
1-hour 500mg/kg_AQ vs 4-hour 500mg/kg_AQ	no
1-hour 500mg/kg_AQ vs 3-hour 500mg/kg_AQ	no
1-hour 500mg/kg_AQ vs 2-hour Diclofenac sodium only	no
1-hour 500mg/kg_AQ vs 2-hour 500mg/kg_AQ	no
1-hour 500mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
1-hour 500mg/kg_AQ vs 5-hour Diclofenac sodium only	no

1-hour 500mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
2-hour 20mg/kg_AQ vs 4-hour 500mg/kg_AQ	no
2-hour 20mg/kg_AQ vs 3-hour 500mg/kg_AQ	no
2-hour 20mg/kg_AQ vs 2-hour Diclofenac sodium only	no
2-hour 20mg/kg_AQ vs 2-hour 500mg/kg_AQ	no
2-hour 20mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
2-hour 20mg/kg_AQ vs 5-hour Diclofenac sodium only	no
2-hour 20mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
4-hour 500mg/kg_AQ vs 3-hour 500mg/kg_AQ	no
4-hour 500mg/kg_AQ vs 2-hour Diclofenac sodium only	no
4-hour 500mg/kg_AQ vs 2-hour 500mg/kg_AQ	no
4-hour 500mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
4-hour 500mg/kg_AQ vs 5-hour Diclofenac sodium only	no
4-hour 500mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
3-hour 500mg/kg_AQ vs 2-hour Diclofenac sodium only	no
3-hour 500mg/kg_AQ vs 2-hour 500mg/kg_AQ	no
3-hour 500mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
3-hour 500mg/kg_AQ vs 5-hour Diclofenac sodium only	no
3-hour 500mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
2-hour Diclofenac sodium only vs 2-hour 500mg/kg_AQ	no
2-hour Diclofenac sodium only vs 2-hour 100mg/kg_AQ	no
2-hour Diclofenac sodium only vs 5-hour Diclofenac sodium only	no
2-hour Diclofenac sodium only vs 5-hour 500mg/kg_AQ	no
2-hour 500mg/kg_AQ vs 2-hour 100mg/kg_AQ	no
2-hour 500mg/kg_AQ vs 5-hour Diclofenac sodium only	no
2-hour 500mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
2-hour 100mg/kg_AQ vs 5-hour Diclofenac sodium only	no
2-hour 100mg/kg_AQ vs 5-hour 500mg/kg_AQ	no
5-hour Diclofenac sodium only vs 5-hour 500mg/kg_AQ	no

	Mean	
4-hour 20mg/kg_AQ	-1.60	a
3-hour 20mg/kg_AQ	1.25	a
4-hour 100mg/kg_AQ	5.96	a
1-hour 20mg/kg_AQ	9.15	a
3-hour 100mg/kg_AQ	9.82	a
5-hour 20mg/kg_AQ	14.38	a
1-hour Diclofenac sodium only	15.89	a
3-hour Diclofenac sodium only	17.95	a
1-hour 100mg/kg_AQ	19.42	a
5-hour 100mg/kg_AQ	20.26	a
4-hour Diclofenac sodium only	20.49	a
1-hour 500mg/kg_AQ	22.05	a
2-hour 20mg/kg_AQ	22.36	a
4-hour 500mg/kg_AQ	22.82	a

3-hour 500mg/kg_AQ	23.84	a
2-hour Diclofenac sodium only	27.03	a
2-hour 500mg/kg_AQ	29.29	a
2-hour 100mg/kg_AQ	31.93	a
5-hour Diclofenac sodium only	39.80	a
5-hour 500mg/kg_AQ	45.14	a

Summary statistics for %_inhibition_of_edema: Treatment 20mg/kg_AQ

Number of values =	25
Mean =	9.110
Median =	9.09
Minimum =	-50
Maximum =	68.75
Standard deviation =	28.97
Standard error of mean =	5.794

Summary statistics for %_inhibition_of_edema: Treatment 100mg/kg_AQ

Number of values =	25
Mean =	17.48
Median =	26.67
Minimum =	-60
Maximum =	56.25
Standard deviation =	26.02
Standard error of mean =	5.204

Summary statistics for %_inhibition_of_edema: Treatment 500mg/kg_AQ

Number of values =	25
Mean =	28.63
Median =	30.77
Minimum =	-44.44
Maximum =	85.71
Standard deviation =	31.06
Standard error of mean =	6.211

Summary statistics for %_inhibition_of_edema: Treatment Diclofenac sodium only

Number of values =	25
Mean =	24.23
Median =	36
Minimum =	-66.67
Maximum =	88.89
Standard deviation =	38.88
Standard error of mean =	7.776

Summary statistics for %_inhibition_of_edema: Duration 1-hour (AQ extract and diclofenac sodium)

Number of values =	20
Mean =	16.63
Median =	30.20
Minimum =	-60
Maximum =	48
Standard deviation =	34.44
Standard error of mean =	7.702

Summary statistics for %_inhibition_of_edema: Duration 2-hour (AQ extract and diclofenac sodium)

Number of values =	20
Mean =	27.65
Median =	30.2
Minimum =	0
Maximum =	51.85
Standard deviation =	13.95
Standard error of mean =	3.120

Summary statistics for %_inhibition_of_edema: Duration 3-hour (AQ extract and diclofenac sodium)

Number of values =	20
Mean =	13.22
Median =	15.48
Minimum =	-54.54
Maximum =	58.33
Standard deviation =	29.15
Standard error of mean =	6.519

Summary statistics for %_inhibition_of_edema: Duration 4-hour (AQ extract and diclofenac sodium)

Number of values =	20
Mean =	11.92
Median =	13.89
Minimum =	-66.67
Maximum =	55.55
Standard deviation =	36.54
Standard error of mean =	8.171

Summary statistics for %_inhibition_of_edema: Duration 5-hour (AQ extract and diclofenac sodium)

Number of values =	20
Mean =	29.89
Median =	36.81
Minimum =	-42.86
Maximum =	88.89
Standard deviation =	38.32
Standard error of mean =	8.570

Appendix IX: Data output from the analysis of the effect of duration on the anti-inflammatory activity of the methanol root extract of *M. triphylla* on acetic acid-induced paw edema in *Wistar* rats

Analysis of variance

Variate: %_inhibition_of_edema

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Duration	4	18618.1	4654.5	6.01	<.001
Treatment	3	21587.4	7195.8	9.29	<.001
Duration.Treatment	12	5354.2	446.2	0.58	0.855
Residual	80	61976.8	774.7		
Total	99	107536.5			

Tukey's 95% confidence intervals

Duration

Comparison	Difference	Lower 95%	Upper 95%	Significant
1-hour vs 2-hour	-6.06	-30.62	18.51	no
1-hour vs 5-hour	-26.54	-51.11	-1.98	yes
1-hour vs 4-hour	-31.51	-56.07	-6.94	yes
1-hour vs 3-hour	-32.78	-57.34	-8.21	yes
2-hour vs 5-hour	-20.48	-45.05	4.08	no
2-hour vs 4-hour	-25.45	-50.01	-0.88	yes
2-hour vs 3-hour	-26.72	-51.29	-2.16	yes
5-hour vs 4-hour	-4.96	-29.53	19.60	no
5-hour vs 3-hour	-6.24	-30.80	18.33	no
4-hour vs 3-hour	-1.27	-25.84	23.29	no

	Mean	
1-hour	6.83	a
2-hour	12.89	ab
5-hour	33.37	bc
4-hour	38.34	c
3-hour	39.61	c

Appendix X: Data output from the analysis of the effect of treatment on the anti-inflammatory activity of the methanol root extract of *M. triphylla* on acetic acid-induced paw edema in *Wistar* rats

Analysis of variance

Variate: %_inhibition_of_edema

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Duration	4	18618.1	4654.5	6.01	<.001
Treatment	3	21587.4	7195.8	9.29	<.001
Duration.Treatment	12	5354.2	446.2	0.58	0.855
Residual	80	61976.8	774.7		
Total	99	107536.5			

Tukey's 95% confidence intervals

Treatment

Comparison	Significant
20mg/kg_MEOH vs 100mg/kg_MEOH	no
20mg/kg_MEOH vs Diclofenac sodium only	yes
20mg/kg_MEOH vs 500mg/kg_MEOH	yes
100mg/kg_MEOH vs Diclofenac sodium only	no
100mg/kg_MEOH vs 500mg/kg_MEOH	yes
Diclofenac sodium only vs 500mg/kg_MEOH	no

	Mean	
20mg/kg_MEOH	7.57	a
100mg/kg_MEOH	19.77	ab
Diclofenac sodium only	29.81	bc
500mg/kg_MEOH	47.69	c

Appendix XI: Data output from the analysis of the effect of treatment and duration on the anti-inflammatory activity of the methanol root extract of *M. triphylla* on acetic acid-induced paw edema in *Wistar* rats

Analysis of variance

Variate: %_inhibition_of_edema

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Duration	4	18618.1	4654.5	6.01	<.001
Treatment	3	21587.4	7195.8	9.29	<.001
Duration. Treatment	12	5354.2	446.2	0.58	0.855
Residual	80	61976.8	774.7		
Total	99	107536.5			

Tukey's 95% confidence intervals

Duration.Treatment

Comparison	Significant
1-hour 20mg/kg_MEOH vs 1-hour 100mg/kg_MEOH	no
1-hour 20mg/kg_MEOH vs 2-hour 20mg/kg_MEOH	no
1-hour 20mg/kg_MEOH vs 2-hour 100mg/kg_MEOH	no
1-hour 20mg/kg_MEOH vs 5-hour 20mg/kg_MEOH	no
1-hour 20mg/kg_MEOH vs 1-hour 500mg/kg_MEOH	no
1-hour 20mg/kg_MEOH vs 4-hour 20mg/kg_MEOH	no
1-hour 20mg/kg_MEOH vs 2-hour Diclofenac sodium only	no
1-hour 20mg/kg_MEOH vs 1-hour Diclofenac sodium only	no
1-hour 20mg/kg_MEOH vs 3-hour 20mg/kg_MEOH	no
1-hour 20mg/kg_MEOH vs 5-hour 100mg/kg_MEOH	no
1-hour 20mg/kg_MEOH vs 5-hour Diclofenac sodium only	no
1-hour 20mg/kg_MEOH vs 4-hour 100mg/kg_MEOH	no
1-hour 20mg/kg_MEOH vs 3-hour 100mg/kg_MEOH	no
1-hour 20mg/kg_MEOH vs 2-hour 500mg/kg_MEOH	no
1-hour 20mg/kg_MEOH vs 3-hour Diclofenac sodium only	no
1-hour 20mg/kg_MEOH vs 4-hour Diclofenac sodium only	no
1-hour 20mg/kg_MEOH vs 3-hour 500mg/kg_MEOH	yes
1-hour 20mg/kg_MEOH vs 4-hour 500mg/kg_MEOH	yes
1-hour 20mg/kg_MEOH vs 5-hour 500mg/kg_MEOH	yes
1-hour 100mg/kg_MEOH vs 2-hour 20mg/kg_MEOH	no
1-hour 100mg/kg_MEOH vs 2-hour 100mg/kg_MEOH	no
1-hour 100mg/kg_MEOH vs 5-hour 20mg/kg_MEOH	no
1-hour 100mg/kg_MEOH vs 1-hour 500mg/kg_MEOH	no
1-hour 100mg/kg_MEOH vs 4-hour 20mg/kg_MEOH	no
1-hour 100mg/kg_MEOH vs 2-hour Diclofenac sodium only	no

1-hour 100mg/kg_MEOH vs 1-hour Diclofenac sodium only	no
1-hour 100mg/kg_MEOH vs 3-hour 20mg/kg_MEOH	no
1-hour 100mg/kg_MEOH vs 5-hour 100mg/kg_MEOH	no
1-hour 100mg/kg_MEOH vs 5-hour Diclofenac sodium only	no
1-hour 100mg/kg_MEOH vs 4-hour 100mg/kg_MEOH	no
1-hour 100mg/kg_MEOH vs 3-hour 100mg/kg_MEOH	no
1-hour 100mg/kg_MEOH vs 2-hour 500mg/kg_MEOH	no
1-hour 100mg/kg_MEOH vs 3-hour Diclofenac sodium only	no
1-hour 100mg/kg_MEOH vs 4-hour Diclofenac sodium only	no
1-hour 100mg/kg_MEOH vs 3-hour 500mg/kg_MEOH	no
1-hour 100mg/kg_MEOH vs 4-hour 500mg/kg_MEOH	no
1-hour 100mg/kg_MEOH vs 5-hour 500mg/kg_MEOH	yes
2-hour 20mg/kg_MEOH vs 2-hour 100mg/kg_MEOH	no
2-hour 20mg/kg_MEOH vs 5-hour 20mg/kg_MEOH	no
2-hour 20mg/kg_MEOH vs 1-hour 500mg/kg_MEOH	no
2-hour 20mg/kg_MEOH vs 4-hour 20mg/kg_MEOH	no
2-hour 20mg/kg_MEOH vs 2-hour Diclofenac sodium only	no
2-hour 20mg/kg_MEOH vs 1-hour Diclofenac sodium only	no
2-hour 20mg/kg_MEOH vs 3-hour 20mg/kg_MEOH	no
2-hour 20mg/kg_MEOH vs 5-hour 100mg/kg_MEOH	no
2-hour 20mg/kg_MEOH vs 5-hour Diclofenac sodium only	no
2-hour 20mg/kg_MEOH vs 4-hour 100mg/kg_MEOH	no
2-hour 20mg/kg_MEOH vs 3-hour 100mg/kg_MEOH	no
2-hour 20mg/kg_MEOH vs 2-hour 500mg/kg_MEOH	no
2-hour 20mg/kg_MEOH vs 3-hour Diclofenac sodium only	no
2-hour 20mg/kg_MEOH vs 4-hour Diclofenac sodium only	no
2-hour 20mg/kg_MEOH vs 3-hour 500mg/kg_MEOH	no
2-hour 20mg/kg_MEOH vs 4-hour 500mg/kg_MEOH	no
2-hour 20mg/kg_MEOH vs 5-hour 500mg/kg_MEOH	yes
2-hour 100mg/kg_MEOH vs 5-hour 20mg/kg_MEOH	no
2-hour 100mg/kg_MEOH vs 1-hour 500mg/kg_MEOH	no
2-hour 100mg/kg_MEOH vs 4-hour 20mg/kg_MEOH	no
2-hour 100mg/kg_MEOH vs 2-hour Diclofenac sodium only	no
2-hour 100mg/kg_MEOH vs 1-hour Diclofenac sodium only	no
2-hour 100mg/kg_MEOH vs 3-hour 20mg/kg_MEOH	no
2-hour 100mg/kg_MEOH vs 5-hour 100mg/kg_MEOH	no
2-hour 100mg/kg_MEOH vs 5-hour Diclofenac sodium only	no
2-hour 100mg/kg_MEOH vs 4-hour 100mg/kg_MEOH	no
2-hour 100mg/kg_MEOH vs 3-hour 100mg/kg_MEOH	no
2-hour 100mg/kg_MEOH vs 2-hour 500mg/kg_MEOH	no
2-hour 100mg/kg_MEOH vs 3-hour Diclofenac sodium only	no
2-hour 100mg/kg_MEOH vs 4-hour Diclofenac sodium only	no
2-hour 100mg/kg_MEOH vs 3-hour 500mg/kg_MEOH	no
2-hour 100mg/kg_MEOH vs 4-hour 500mg/kg_MEOH	no
2-hour 100mg/kg_MEOH vs 5-hour 500mg/kg_MEOH	yes
5-hour 20mg/kg_MEOH vs 1-hour 500mg/kg_MEOH	no

5-hour 20mg/kg_MEOH vs 4-hour 20mg/kg_MEOH	no
5-hour 20mg/kg_MEOH vs 2-hour Diclofenac sodium only	no
5-hour 20mg/kg_MEOH vs 1-hour Diclofenac sodium only	no
5-hour 20mg/kg_MEOH vs 3-hour 20mg/kg_MEOH	no
5-hour 20mg/kg_MEOH vs 5-hour 100mg/kg_MEOH	no
5-hour 20mg/kg_MEOH vs 5-hour Diclofenac sodium only	no
5-hour 20mg/kg_MEOH vs 4-hour 100mg/kg_MEOH	no
5-hour 20mg/kg_MEOH vs 3-hour 100mg/kg_MEOH	no
5-hour 20mg/kg_MEOH vs 2-hour 500mg/kg_MEOH	no
5-hour 20mg/kg_MEOH vs 3-hour Diclofenac sodium only	no
5-hour 20mg/kg_MEOH vs 4-hour Diclofenac sodium only	no
5-hour 20mg/kg_MEOH vs 3-hour 500mg/kg_MEOH	no
5-hour 20mg/kg_MEOH vs 4-hour 500mg/kg_MEOH	no
5-hour 20mg/kg_MEOH vs 5-hour 500mg/kg_MEOH	no
1-hour 500mg/kg_MEOH vs 4-hour 20mg/kg_MEOH	no
1-hour 500mg/kg_MEOH vs 2-hour Diclofenac sodium only	no
1-hour 500mg/kg_MEOH vs 1-hour Diclofenac sodium only	no
1-hour 500mg/kg_MEOH vs 3-hour 20mg/kg_MEOH	no
1-hour 500mg/kg_MEOH vs 5-hour 100mg/kg_MEOH	no
1-hour 500mg/kg_MEOH vs 5-hour Diclofenac sodium only	no
1-hour 500mg/kg_MEOH vs 4-hour 100mg/kg_MEOH	no
1-hour 500mg/kg_MEOH vs 3-hour 100mg/kg_MEOH	no
1-hour 500mg/kg_MEOH vs 2-hour 500mg/kg_MEOH	no
1-hour 500mg/kg_MEOH vs 3-hour Diclofenac sodium only	no
1-hour 500mg/kg_MEOH vs 4-hour Diclofenac sodium only	no
1-hour 500mg/kg_MEOH vs 3-hour 500mg/kg_MEOH	no
1-hour 500mg/kg_MEOH vs 4-hour 500mg/kg_MEOH	no
1-hour 500mg/kg_MEOH vs 5-hour 500mg/kg_MEOH	no
4-hour 20mg/kg_MEOH vs 2-hour Diclofenac sodium only	no
4-hour 20mg/kg_MEOH vs 1-hour Diclofenac sodium only	no
4-hour 20mg/kg_MEOH vs 3-hour 20mg/kg_MEOH	no
4-hour 20mg/kg_MEOH vs 5-hour 100mg/kg_MEOH	no
4-hour 20mg/kg_MEOH vs 5-hour Diclofenac sodium only	no
4-hour 20mg/kg_MEOH vs 4-hour 100mg/kg_MEOH	no
4-hour 20mg/kg_MEOH vs 3-hour 100mg/kg_MEOH	no
4-hour 20mg/kg_MEOH vs 2-hour 500mg/kg_MEOH	no
4-hour 20mg/kg_MEOH vs 3-hour Diclofenac sodium only	no
4-hour 20mg/kg_MEOH vs 4-hour Diclofenac sodium only	no
4-hour 20mg/kg_MEOH vs 3-hour 500mg/kg_MEOH	no
4-hour 20mg/kg_MEOH vs 4-hour 500mg/kg_MEOH	no
4-hour 20mg/kg_MEOH vs 5-hour 500mg/kg_MEOH	no
2-hour Diclofenac sodium only vs 1-hour Diclofenac sodium only	no
2-hour Diclofenac sodium only vs 3-hour 20mg/kg_MEOH	no
2-hour Diclofenac sodium only vs 5-hour 100mg/kg_MEOH	no
2-hour Diclofenac sodium only vs 5-hour Diclofenac sodium only	no
2-hour Diclofenac sodium only vs 4-hour 100mg/kg_MEOH	no

2-hour Diclofenac sodium only vs 3-hour 100mg/kg_MEOH	no
2-hour Diclofenac sodium only vs 2-hour 500mg/kg_MEOH	no
2-hour Diclofenac sodium only vs 3-hour Diclofenac sodium only	no
2-hour Diclofenac sodium only vs 4-hour Diclofenac sodium only	no
2-hour Diclofenac sodium only vs 3-hour 500mg/kg_MEOH	no
2-hour Diclofenac sodium only vs 4-hour 500mg/kg_MEOH	no
2-hour Diclofenac sodium only vs 5-hour 500mg/kg_MEOH	no
1-hour Diclofenac sodium only vs 3-hour 20mg/kg_MEOH	no
1-hour Diclofenac sodium only vs 5-hour 100mg/kg_MEOH	no
1-hour Diclofenac sodium only vs 5-hour Diclofenac sodium only	no
1-hour Diclofenac sodium only vs 4-hour 100mg/kg_ MEOH	no
1-hour Diclofenac sodium only vs 3-hour 100mg/kg_MEOH	no
1-hour Diclofenac sodium only vs 2-hour 500mg/kg_MEOH	no
1-hour Diclofenac sodium only vs 3-hour Diclofenac sodium only	no
1-hour Diclofenac sodium only vs 4-hour Diclofenac sodium only	no
1-hour Diclofenac sodium only vs 3-hour 500mg/kg_MEOH	no
1-hour Diclofenac sodium only vs 4-hour 500mg/kg_MEOH	no
1-hour Diclofenac sodium only vs 5-hour 500mg/kg_MEOH	no
3-hour 20mg/kg_MEOH vs 5-hour 100mg/kg_MEOH	no
3-hour 20mg/kg_MEOH vs 5-hour Diclofenac sodium only	no
3-hour 20mg/kg_MEOH vs 4-hour 100mg/kg_MEOH	no
3-hour 20mg/kg_MEOH vs 3-hour 100mg/kg_MEOH	no
3-hour 20mg/kg_MEOH vs 2-hour 500mg/kg_MEOH	no
3-hour 20mg/kg_MEOH vs 3-hour Diclofenac sodium only	no
3-hour 20mg/kg_MEOH vs 4-hour Diclofenac sodium only	no
3-hour 20mg/kg_MEOH vs 3-hour 500mg/kg_MEOH	no
3-hour 20mg/kg_MEOH vs 4-hour 500mg/kg_MEOH	no
3-hour 20mg/kg_MEOH vs 5-hour 500mg/kg_MEOH	no
5-hour 100mg/kg_MEOH vs 5-hour Diclofenac sodium only	no
5-hour 100mg/kg_MEOH vs 4-hour 100mg/kg_MEOH	no
5-hour 100mg/kg_MEOH vs 3-hour 100mg/kg_MEOH	no
5-hour 100mg/kg_MEOH vs 2-hour 500mg/kg_MEOH	no
5-hour 100mg/kg_MEOH vs 3-hour Diclofenac sodium only	no
5-hour 100mg/kg_MEOH vs 4-hour Diclofenac sodium only	no
5-hour 100mg/kg_MEOH vs 3-hour 500mg/kg_MEOH	no
5-hour 100mg/kg_MEOH vs 4-hour 500mg/kg_MEOH	no
5-hour 100mg/kg_MEOH vs 5-hour 500mg/kg_MEOH	no
5-hour Diclofenac sodium only vs 4-hour 100mg/kg_MEOH	no
5-hour Diclofenac sodium only vs 3-hour 100mg/kg_MEOH	no
5-hour Diclofenac sodium only vs 2-hour 500mg/kg_MEOH	no
5-hour Diclofenac sodium only vs 3-hour Diclofenac sodium only	no
5-hour Diclofenac sodium only vs 4-hour Diclofenac sodium only	no
5-hour Diclofenac sodium only vs 3-hour 500mg/kg_MEOH	no
5-hour Diclofenac sodium only vs 4-hour 500mg/kg_MEOH	no
5-hour Diclofenac sodium only vs 5-hour 500mg/kg_MEOH	no
4-hour 100mg/kg_MEOH vs 3-hour 100mg/kg_MEOH	no

4-hour 100mg/kg_MEOH vs 2-hour 500mg/kg_MEOH	no
4-hour 100mg/kg_MEOH vs 3-hour Diclofenac sodium only	no
4-hour 100mg/kg_MEOH vs 4-hour Diclofenac sodium only	no
4-hour 100mg/kg_MEOH vs 3-hour 500mg/kg_MEOH	no
4-hour 100mg/kg_MEOH vs 4-hour 500mg/kg_MEOH	no
4-hour 100mg/kg_MEOH vs 5-hour 500mg/kg_MEOH	no
3-hour 100mg/kg_MEOH vs 2-hour 500mg/kg_MEOH	no
3-hour 100mg/kg_MEOH vs 3-hour Diclofenac sodium only	no
3-hour 100mg/kg_MEOH vs 4-hour Diclofenac sodium only	no
3-hour 100mg/kg_MEOH vs 3-hour 500mg/kg_MEOH	no
3-hour 100mg/kg_MEOH vs 4-hour 500mg/kg_MEOH	no
3-hour 100mg/kg_MEOH vs 5-hour 500mg/kg_MEOH	no
2-hour 500mg/kg_MEOH vs 3-hour Diclofenac sodium only	no
2-hour 500mg/kg_MEOH vs 4-hour Diclofenac sodium only	no
2-hour 500mg/kg_MEOH vs 3-hour 500mg/kg_MEOH	no
2-hour 500mg/kg_MEOH vs 4-hour 500mg/kg_MEOH	no
2-hour 500mg/kg_MEOH vs 5-hour 500mg/kg_MEOH	no
3-hour Diclofenac sodium only vs 4-hour Diclofenac sodium only	no
3-hour Diclofenac sodium only vs 3-hour 500mg/kg_MEOH	no
3-hour Diclofenac sodium only vs 4-hour 500mg/kg_ MEOH	no
3-hour Diclofenac sodium only vs 5-hour 500mg/kg_ MEOH	no
4-hour Diclofenac sodium only vs 3-hour 500mg/kg_ MEOH	no
4-hour Diclofenac sodium only vs 4-hour 500mg/kg_ MEOH	no
4-hour Diclofenac sodium only vs 5-hour 500mg/kg_ MEOH	no
3-hour 500mg/kg_ MEOH vs 4-hour 500mg/kg_ MEOH	no
3-hour 500mg/kg_ MEOH vs 5-hour 500mg/kg_ MEOH	no
4-hour 500mg/kg_ MEOH vs 5-hour 500mg/kg_ MEOH	no

	Mean	
1-hour 20mg/kg_ MEOH	-5.65	a
1-hour 100mg/kg_ MEOH	0.05	ab
2-hour 20mg/kg_ MEOH	0.83	ab
2-hour 100mg/kg_ MEOH	2.57	ab
5-hour 20mg/kg_ MEOH	6.54	abc
1-hour 500mg/kg_ MEOH	10.18	abc
4-hour 20mg/kg_ MEOH	10.60	abc
2-hour Diclofenac sodium only	10.76	abc
1-hour Diclofenac sodium only	22.76	abc
3-hour 20mg/kg_ MEOH	25.53	abc
5-hour 100mg/kg_ MEOH	25.84	abc
5-hour Diclofenac sodium only	32.71	abc
4-hour 100mg/kg_ MEOH	33.87	abc
3-hour 100mg/kg_ MEOH	36.50	abc
2-hour 500mg/kg_ MEOH	37.40	abc
3-hour Diclofenac sodium only	37.47	abc

4-hour Diclofenac sodium only	45.34	abc
3-hour 500mg/kg_ MEOH	58.95	bc
4-hour 500mg/kg_ MEOH	63.54	bc
5-hour 500mg/kg_ MEOH	68.40	c

Summary statistics for %_inhibition_of_edema: Treatment 20mg/kg_MEOH

Number of values =	25
Mean =	7.569
Median =	0
Minimum =	-62.5
Maximum =	41.18
Standard deviation =	25.05
Standard error of mean =	5.009

Summary statistics for %_inhibition_of_edema: Treatment 100mg/kg_MEOH

Number of values =	25
Mean =	19.77
Median =	30.77
Minimum =	-100
Maximum =	56.67
Standard deviation =	32.32
Standard error of mean =	6.464

Summary statistics for %_inhibition_of_edema: Treatment 500mg/kg_MEOH

Number of values =	25
Mean =	47.69
Median =	53.33
Minimum =	-87.5
Maximum =	76.92
Standard deviation =	32.87
Standard error of mean =	6.573

Summary statistics for %_inhibition_of_edema: Treatment Diclofenac sodium only

Number of values =	25
Mean =	29.81
Median =	27.78
Minimum =	-25
Maximum =	88.24
Standard deviation =	28.80
Standard error of mean =	5.759

Summary statistics for %_inhibition_of_edema: Duration 1-hour (MEOH extract and diclofenac sodium)

Number of values =	20
Mean =	6.832
Median =	24.45

Minimum =	-100
Maximum =	80
Standard deviation =	46.15
Standard error of mean =	10.32

Summary statistics for %_inhibition_of_edema: Duration 2-hour (MEOH extract and diclofenac sodium)

Number of values =	20
Mean =	12.89
Median =	11.80
Minimum =	-22.22
Maximum =	52.94
Standard deviation =	21.73
Standard error of mean =	4.859

Summary statistics for %_inhibition_of_edema: Duration 3-hour (MEOH extract and diclofenac sodium)

Number of values =	20
Mean =	39.61
Median =	39.05
Minimum =	4.76
Maximum =	65.38
Standard deviation =	17.32
Standard error of mean =	3.872

Summary statistics for %_inhibition_of_edema: Duration 4-hour (MEOH extract and diclofenac sodium)

Number of values =	20
Mean =	38.34
Median =	45.23
Minimum =	-17.65
Maximum =	73.68
Standard deviation =	28.02
Standard error of mean =	6.265

Summary statistics for %_inhibition_of_edema: Duration 5-hour (MEOH extract and diclofenac sodium)

Number of values =	20
Mean =	33.37
Median =	35.29
Minimum =	-13.33
Maximum =	88.24
Standard deviation =	31.51
Standard error of mean =	7.046

Appendix XII: Data output from the analysis of the acute oral toxicity of the aqueous and methanol root extracts of *M. triphylla* in *Wistar* rats

Analysis of variance

Variate: Weight_of_treated_animals

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	6399.66	2133.22	93.60	<.001
Residual	16	364.65	22.79		
Total	19	6764.32			

Tukey's 95% confidence intervals

Treatment

Comparison	Significant
2000 mg/kg_AQ_day7 vs 2000 mg/kg_MEOH_day7	no
2000 mg/kg_AQ_day7 vs 2000 mg/kg_AQ_day14	yes
2000 mg/kg_AQ_day7 vs 2000 mg/kg_MEOH_day14	yes
2000 mg/kg_MEOH_day7 vs 2000 mg/kg_AQ_day14	yes
2000 mg/kg_MEOH_day7 vs 2000 mg/kg_MEOH_day14	yes
2000 mg/kg_MEOH_day14 vs 2000 mg/kg_AQ_day14	yes

	Mean	
2000 mg/kg_AQ _day7	25.46	a
2000 mg/kg_MEOH_day7	29.81	a
2000 mg/kg_AQ_day14	57.57	b
2000 mg/kg_MEOH_day14	67.57	c

Summary statistics for Weight_of_treated_animals: Treatment 2000 mg/kg_AQ_day14

Number of values =	5
Mean =	57.57
Median =	57.38
Minimum =	52.42
Maximum =	63.44
Standard deviation =	3.958
Standard error of mean =	1.770

Summary statistics for Weight_of_treated_animals: Treatment 2000 mg/kg_AQ_day7

Number of values =	5
Mean =	25.46
Median =	25.14
Minimum =	23.3
Maximum =	27.32

Standard deviation =	1.822
Standard error of mean =	0.815

Summary statistics for Weight_of_treated_animals: Treatment 2000 mg/kg_MEOH_day14

Number of values =	5
Mean =	67.57
Median =	69.33
Minimum =	55.67
Maximum =	73.84
Standard deviation =	7.012
Standard error of mean =	3.136

Summary statistics for Weight_of_treated_animals: Treatment 2000 mg/kg_MEOH_day7

Number of values =	5
Mean =	29.81
Median =	32.14
Minimum =	22.56
Maximum =	34.24
Standard deviation =	4.797
Standard error of mean =	2.145