PHYTOCHEMICAL, ANTI-INFLAMMATORY, ANTINOCICEPTIVE, ANTIPYRETIC AND TOXICITY ANALYSIS OF DICHLOROMETHANE AND METHANOL EXTRACTS OF FIVE SELECTED PLANTS USING ANIMAL MODEL

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REG. NO. I84/31990/2015

A Thesis Submitted in Fulfillment of the Requirements for the Award of the Degree of Doctor of Philosophy in (Medical Biochemistry) in the School of Pure andApplied Sciences, Kenyatta University

MARCH 2022

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DECLARATION

I declare that the work presented in this thesis is my original work and has not been presented for a degree or any other award in any other University or institution.

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DEDICATION

This work is whole heartedly dedicated to the soul of my late cousin Mr Sebastian Kimathi Muguongo. Eternal rest grant unto him Oh dear Lord, and May your Perpetual Light Shine on him. Your contribution enabled me achieve my dream. In my heart you will live forever.

ACKNOWLEDGEMENTS

I highly express my sincere gratitude to my University supervisors, Prof. Joseph J. N. Ngeranwa for your guidance in animal handling techniques, Prof. Alex K. Machocho for your guidance in plant extraction and phytochemical analysis and Prof. Silas Kiruki for your guidance in biochemical analysis of the blood samples. Thanks for your excellence, immeasurable and unwavering professional guidance. I also appreciate Prof. E. N. Njagi for the support offered when identifying the area of study. I would also like to thank Kenyatta University and Department of Biochemistry, Microbiology and Biotechnology for providinga serene learning environment.

I acknowledge the support offered by Mr. Christopher Gituma, Mr. Anderson Kaumbuthu and Mr. Collins Mwirigi from Imenti North, Meru County for invaluable contributions madewhen selecting the herbal plants.

I acknowledge the support offered by HELB through partial scholarship granted. Thank you very much.

I wish to acknowledge the outstanding support offered my dear wife Beth Karambu and my sons Vince Rayan Karani and Ivan Azariah Mutethia for the moral support offered throughout this journey. To my parents Angelo Gitonga Kireria and Mary Kamathi Gitonga,thank you for showing me that I have the potential to pursue higher education and the supportyou have offered me all through. To my brothers Wilson Kiriinya, Isaiah N. Gitonga, Anderson Kaumbuthu and sisters Lucy N. Kiunga, Lilian Karamuta, Glory Nthangi and Norah Karimi, your moral and any financial support are highly appreciated and acknowledged.

To almighty God I reckon my utmost thanks for His wonderful provision.

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ABBREVIATIONS AND ACRONYMS

ALP	Alanine phosphatase
ALT	Alanine amino transferase
ANOVA	Analysis of variance
API	American Proficiency Institute
AST	Aspartate amino transferase
Camp	Cyclic adenine monophosphate
CAMs	Cell adhesion molecules
CCK	Cholecystokinin
CNS	Central nervous system
COX	Cyclooxygenase
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
FAO	Food and Agricultural organization
GC – MS	Gas chromatography coupled to mass spectrophotometer
GGT	Gamma glutamyl transferase
GLP-1	Glucagon like peptide – 1
GPS	Global Positioning System
HIV/AIDs	Human immunodeficiency virus / Acquired immunodeficiency Syndrome
ICAM	Intracellular adhesion molecule
IDF	International diabetes federation
IL	Interleukin
LPS	Lipopolysaccharide
MeOH	Methanol
MUFAs	Monounsaturated fatty acids
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIST	National institute of standards and technology
NIST – MS	National Institute of Standards and Technology – Mass Spectral
Nm	Nanometer
PGE_2	Prostaglandin E_2
PLA	Phospholipase A
PUFAs	Polyunsaturated fatty acids
SEM	Standard error mean
SPSS	Statistical package for social scientists
SOPs	Standard operating procedures
TLR_4	Toll like receptor 4
TNF	Tumor necrotic factor
UNEP	United nations environmental programme

UV	Ultraviolet
UV-VIS	Ultraviolet – visible
WHO	World health organization

ABSTRACT

Inflammation results from irritants causing body injury. Pain is discomfort associated with illness or injury while pyrexia can be defined as elevated body temperature. Common conventional anti-inflammatory, analgesic and antipyretic agents are expensive and have severe adverse effects. Traditional medicines are regarded by various communities as safe, efficacious with little or no adverse side effects. The study aim was to evaluate phytochemical profile, *in vivo* anti-inflammatory, antinociceptive, antipyretic activities as well as safety associated with *Senna didymobotyra, Eucalyptus saligna, Bidens pilosa, Mangifera indica* and *Prunus africana*. Plant samples were collected from Kanjagi sub - location, Kirima – Itune location, Giaki division, Meru County in Kenya. Extractions of phytochemicals were carried out using dichloromethane and methanol. Edema, pain and fever were induced in test animals using 1% carrageenan (0.5 ml), 2.5% v/v formalin (0.05 ml) and 20% v/v turpentine respectively. Wistar rats and albino mice were used in this study. The animal models were grouped into normal, negative, positive and experimental test groups I - III. Experimental test groups I, II and III were treated with 50, 100 and 150 mg/kg of the plant extract respectively. Anti-inflammatory and antinociceptive activities associated with extracts were evaluated against the standard anti-inflammatory and antinociceptive drug (Diclofenac), while antipyretic activities were established against aspirin. The herbal extract of *Senna didymobotyra, Eucalyptus saligna, Bidens pilosa, Mangifera indica* and *Prunus africana* and the reference drugs indicated some *in vivo* anti- inflammatory, antinociceptive and antipyretic effects. For anti-inflammatory activity, the extracts were associated with reduced hind paw diameter in relation to what was observed in control groups. The inhibitory rates observed in paw edema ranged from 1.59 - 11.05%, Diclofenac edema inhibition was ranging between 0.1 and 8.78%. For the analgesic study, the *Senna didymobotyra, Eucalyptus saligna, Bidens pilosa, Mangifera indica* and *Prunus africana* extract reduced paw licking time by 1.38-48.26% during early phase and between 28.45-83.90% during late phase while diclofenac pain inhibitory rates ranged between 12.20-80.20% in both phases. The herbal extract of *Senna didymobotyra, Eucalyptus saligna, Bidens pilosa, Mangifera indica* and *Prunus africana* were associated with 0.16 and 3.95% antipyretic activity while aspirin was associated with 1.52 and 3.60% antipyretic activities. The qualitative phytochemical evaluation indicated positive results for alkaloids, cardiac glycosides, flavonoid, saponins, steroids, tannins terpenoids and phenolics compound which are associated with anti-inflammatory, antinociceptive and antipyretic activities. The herbal extract of *Senna didymobotyra, Eucalyptus saligna, Bidens pilosa, Mangifera indica* and *Prunus africana* were not associated with any significant repeated dose toxicity at $p<0.05$. The study, confirms the role of *Senna didymobotyra, Eucalyptus saligna, Bidens pilosa, Mangifera indica* and *Prunus africana* extract by Meru community in disease management associated with inflammation, pain and fever. The results support the need to preserve or protect the biodiversity of the *Senna didymobotyra, Eucalyptus saligna, Bidens pilosa, Mangifera indica* and *Prunus africana*

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Inflammation is an immunological response that is not specific and occurs as a result of pathogenic invasion, physical injury, chemical or allergic reactions (Stankov, 2012). Inflammation reaction process facilitates recognition of pathogenic molecules or injuries which facilitates activations and enzymatic release of inflammatory mediators. This process also facilitates migration of immunological cells to the affected body site which leads to tissue breakdown and this is followed by healing process (Maskerey *et al*., 2011). Persistence of the inflammation process is determined by the inability of the body to eliminate the irritant or as a result of deregulation mechanisms common in the resolution phase. Persistence inflammation process is common feature in chronic inflammation which is very common in diseases like asthma, atherosclerosis and rheumatoid arthritis (Punchard, 2004; Maskerey *et al*., 2011).

The drugs of choices in management of inflammation and inflammatory diseases are the corticosteroids and the non-steroid anti-inflammatory drugs (NSAIDs). These drugs lead to adverse side effects like gastrointestinal ulcers and bleeding (Punchard, 2004). The inflammatory mediators produced by the inflamed cells or tissues lead to pain, redness, edema of tissue or cell function loss (Nijkamp and Parnham, 2005).

Pain is an unpleasant personal sensation leading to actual or potential damage of the

tissues transmitted to the brain for interpretation (Cole, 2002). Pain cannot be described just as a symptom used in diagnoses of various diseases and conditions but as a physiological protective function. The ability of the organism to detect and respond to noxious stimuli facilitates the organism to engage appropriate protective mechanism for wellbeing and survival (Ezeja *et al*., 20011; Prystupa *et al*., 2013). If a patients is not relieved from pain he/she may suffer or lead to inability to carry out daily activities leading to increased host costs as well as loss of economic performance to both victim and the society (Prystupa *et al*., 2013). Non-steroidal anti-inflammatory drugs (NSAIDs) and the opioids can be used in management of mild and severe pain respectively. The common available analgesics leads to serious side effects like; addiction, respiratory depression, nausea and vomiting, ulcerationa and gastrointestinal bleeding (Sani *et al*., 2013).

Pyrexia or fever is an elevated body temperature above 37.5°C (Visundra and Divya, 2013). Pyreixia is associated with trauma, microbial infections, chemicals or drugs triggering the production and release of pro-inflammatory cytokines such as interferons, tumor necrosis factor-alpha (TNF-α) and interleukin (IL). The released cytokines in circulatory system migrate and in the hypothalamus they facilitate the synthesis of prostaglandin E_2 (PGE_2) and their release to the pre-optic hypothalamus region leading to elevated body temperature because they promote generation of heat while decreasing heat loss mechanism (Kumar *et al*., 2012; Anochie and Ifesinachi, 2013).

Fever is associated with symptoms like chills, cold sensation and sweating. It is a

common symptom exhibited in cases of diseases like typhoid, malaria and arthritis (Kumar *et al*., 2012; Anochie and Ifesinachi, 2013). Antipyretic drugs are used in management and treatment of fever. Antipyretic drugs inhibit the biosynthesis of PGE_2 hence leading to body temperature reduction. These drugs are toxic to hepatocytes, glomeruli, cardiac muscles and brain cortex (Paschapur *et al*., 2009).

Use of herbal medicine by human in management of diseases dates back to the prehistoric times (Dery *et al*., 1999; Fabricant and Farnsworth, 2001; Shi *et al*., 2010). Previously, they were regarded as the option for the poor the poor people who cannot afford conventional drugs but nowadays most of the people are opting to herbal medications because they believe that herbal concortions are less toxic (Cunningham, 1993; Black and Cox, 1996; Mworia 2000). Herb concoctions can be prepared either from the exudates, seeds, tuber or roots, stems and leaves (Mukherjee, 2002). Some of the diseases affecting the developing countries are malaria, hypertension, cancer, tuberculosis and human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDs) (WHO, 2013). Communicable and non-communicable diseases are all associated with inflammation, pain and fever. By 2004, the World Health Organisation (WHO) estimated that over 80% of world population was using traditional herbal medicine for primary healthcare (Choudhry *et al*., 2004).

Phytochemical compounds present in medicinal plants are associated with disease prevention, healing and some great effects on the metabolic processes (Obianjunwa *et al*., 2004). Since the plants contains more than phytochemical compounds, they can be used to manage a variety of diseases either in human or livestocks (Doss and Anand, 2012).

Secondary metabolites of the plants have no nutritive value but they help the plants and also the animals fight some diseases affecting them (Doss and Anand, 2012). Some secondary metabolites biosynthesized by different plants are flavonoids, saponins, alkaloids, glycosides, tannins, steroids and terpenoids (Venkatesh *et al*., 2011). Different phytochemical compounds are known to elicit some pharmacological activities in human and livestock bodies like some are associated with anticancer, antimicrobial, hormonal regulation, antioxidant, antidiabetic, hepatoprotective, antihelmithic and enzyme stimulation activies (Venkataswamy *et al*., 2010).

International Diabetes Federation (IDF) estimates that around 194 million people are diabetic and the figure is expected to increase by 2025 to almost 333 million all over the world (NIDDK, 2005). Developing countries have rare cases of diabetes based on the survey carried out on the 75 communities living in 32 countries because they observe their traditional lifestyle (Wild *et al*., 2000). Currently, cancer cases are on increases in developing and developed countries with more than 7 million death cases associated with cancer reported yearly worldwide (WHO, 2013). According to World Health Organization, new cases of diabetes will be more than 16 million per year by 2020 (WHO, 2002). American National Cancer Institute associates more than a third of cancer cases with the diet. This means that right diet choice is one of the best ways to prevent cancer (UNEP, 1995). Copper and vitamin C are known to kill cancer cells (Bakhru, 2006). The major parasitic associated disease worldwide is malaria. In Africa, more than 500 million malaria cases are recorded every year with approximately 3 million people succumbing due to malaria infection. Malaria mostly affects children within the age limit of 0 – 5 years and

the expectant mothers (WHO, 2008).

Approximately, 3.5 billion people use herbal medicines in management of livestock and human diseases worldwide (FAO, 1997). Currently, the use of plant associated medicines is increasing because of the belief that plant associated medicines have less or no unwanted toxicological effects (Jacobsson *et al*., 2009). In developing counries, traditional medicinemen or women provide health care services to approximately 80% of the people living in rural regions (WHO, 2001). Medicinal plants are used to manage different diseases by the African communities (Environmental Centre International, 2003). In Ethiopia, it is approximated that almost 80% of the population use plants products to manage different health associated problems (Dawit and Ahadu, 1993). Approximately 90% of the Kenyan populations have used herbal products to manage man or livestock diseases (Chirchir *et al*., 2006).

Complememtary or alternative medicines are used globally to alleviate inflammation, fever and pain. Herbal medicine involves the use of plants extracts or products in treatment or management of diseases or conditons. Herbs variety form one region to the other depending with the regional flora and the use of various herbs in management of various disease vary throughout the various communities in the world (Pant *et al*., 2012). Most of the conventional drugs used in management of various diseases affecting human were originally isolated from the plants though nowadays most of the pharmaceutical companies are synthetically manufacturing them (Saleheen *et al*., 2010).

Researches on drug discovery and design are now targeting natural products like the herbal products in management and treatment of various diseases. This is accomplished

through documentation and scientifically authenticating the traditional knowledge in medicinal plants through isolation and analysis of the active pharmacological compounds (Chowdury *et al*., 2015). Some of the herbal plants that have caught the attention of the researchers include *M. indica, S. didymobotyra, E. saligna, P. africana* and *B. pilosa* because they are associated with various phytocompounds with some pharmacological activities like antileshmanial (Wambui *et al*., 2007) antimicrobial (Mutai *et al*., 2009), antiviral and hepatoprotective activities (Parvez *et al*., 2015). Herbal medicinal plants desirable characteristics include affordability, accessibility and *in vivo* safety

M. indica, S. didymobotyra, E. saligna, P. africana and *B. pilosa* are distributed in various parts of Africa and Kenya. Studies indicate that these plants are used traditionally in various communities in management of health problems such as stomachache, indigestion and also as digestion enhancer (Kiringi *et al*., 2006), joint pain, back pain and pneumonia (Kareru *et al*., 2007), malaria, sterility and coughs (Kokwaro, 2009), sexually transmitted infections (Kipkore *et al*., 2014) and chronic joint-ache (Wambugu *et al*., 2011).

M. indica, S. didymobotyra, E. saligna, P. africana and *B. pilosa* were traditionally used in management and treatment of pain, pyrexia and inflammation in the Meru community of Kenya. However, there is no scientific data on the supporting the identification and isolation of the pharmacologically active phytocompounds against fever, pain and inflammation. This background triggers the need for this study to bio-screen both methanolic and dichloromethane plant part extracts of *M. indica, S. didymobotyra, E.*

saligna, P. africana and *B. pilosa* against pain, pyrexia and inflammation. This study is necessary as a preliminary step in development of herb pharmacologically active product against fever, pain and inflammation. The study aims tries to scientifically justify and document the use *M. indica, S. didymobotyra, E. saligna, P. africana* and *B. pilosa* extract or products as potential agents for management and treatment of pyrexia, pain and inflammation. The study also revealed knowledge gaps that can lead to further studies.

1.2 Statement of the problem

Inflammation, fever and pain are the common pathological signs and sypmtoms associated with various diseases in human which are affecting millions of the population globally. The prevalence of diseases associated with inflammation, fever and pain are on increase like Covid – 19, cancer, Diabetes Mellitus type 1 and type 2, hepatitis, gastritis and meningitis. There is an increased prevalence rate of cancer, diabetes and cardiovascular associated diseases. The reports according to medical experts indicate that the prevalence rates of non – communicable diseases are estimated to overtake that of communicable diseases (FAO, 1995). Most of the infectious and non- infectious diseases are associated with inflammation, fever and pain and leads to increased mortality rates especially in children, expectant mothers and those with other underlying conditions (Kabi, 2004). Most of the Kenyan are poor and can not afford basic medical services (Meme, 1998). Drugs commonly used in management of inflammation, fever and pains are non-steroidal antiinflammatory drugs (NSAIDs) and corticosteroids.

There are several anti-inflammatory, antipyretic and analgesic drugs drugs in the market.

These drugs are associated with resistance development and adverse side effects which include gastritis, gastrointestinal bleeding, ulcers, cardiovascular complications and hyperglycemia. The drugs are also ineffective in management of all these conditions separately; hence they are managed by combining various drugs (Hoque *et al*., 2011; Alamgeer *et al*., 2015). Successful increased use of the herbal products in management of diseases leaves a roomto explore alternative molecules from them that can be used in management of fever, pain and inflammation. Most communities living in Kenya, uses traditional herbal medicines in management of various diseases. Numerous studies have established the phytochemical components of various medicinal plants commonly used by the Kenyan communities to manage various diseases (Roja and Rao, 2000; Rukunga and Simons, 2006). Anti-inflammatory, antinociceptive and antipyretic activities of various medicinal plants used in Meru community of Kenya is yet to be established. Plants are potential candidates sources potential pharmacological compounds for management of pyrexia, pain and inflammation.

1.3 Justification

Use of herb medicine is a common practice in both prevention and management of human and livestock diseases in developing countries. Some of the diseases signs and symptoms managed by herbal medicines include fever, pain and inflammation. Some plants extracts may have better or almost equal analgesic, antipyretic and inflammatory activities when compared with standard painkillers, antipyretics and antiinflammatory drugs. The study tried to quantify *in vivo* analgesic, antinociceptive and anti – inflammatory activities *Prunus Africana*, *Eucalyptus saligna*, *Bidens pilosa*, *Mangifera indica* and *Senna didymobotyra* plant samples collected from Meru County because they

are commonly used in management of pain, fever and inflammation. Quantification of the phytochemicals indicates the effectiveness of the plants in this study. The plants commonly used by local herbalist were grouped into five families and the most common plant used in management of pain, fever and pyrexia was selected for this study.

The positive results on the anti-inflammatory, analgesic and antipyretic activities supported the use of these plants in management of diseases associated with fever, pain and inflammation and supports that these plant phytochemicals are strong candidates for development of antipyretic, analgesic and antiinflammatory drugs. The information generated from the study supports the use of above mentioned plants in management of fever, pain and inflammation. It can also be used to search for alternative molecules that can be used to manage fever, pain and inflammation. The study aim was also to confirm and document scientifically the use of *Prunus Africana, Eucalyptus saligna, Bidens pilosa, Mangifera indica* and *Senna didymobotyra* as an alternative remedy in management and treatment of fever, pain and inflammation in Meru community of Kenya.

1.4 Hypothesis

Ho_1: *Prunus africana, Eucalyptus saligna, Senna didymobotyra, Bidens pilosa* and *Mangifera indica* dichloromethane and methanol extracts contain phytochemical associated with pharmacological activities.

Ho_2: *Prunus africana, Eucalyptus saligna, Senna didymobotyra, Bidens pilosa* and *Mangifera indica* dichloromethane and methanol extracts are associated with anti-

inflammatory activity.

Ho_3: *Prunus africana, Eucalyptus saligna, Senna didymobotyra, Bidens pilosa* and *Mangifera indica* dichloromethane and methanol extracts are associated with antinociceptive activity.

Ho_4: *Prunus africana, Eucalyptus saligna, Senna didymobotyra, Bidens pilosa* and *Mangifera indica* dichloromethane and methanol extracts are associated with antipyretic activity.

Ho_5: *Prunus africana, Eucalyptus saligna, Senna didymobotyra, Bidens pilosa* and *Mangifera indica* dichloromethane and methanol extracts are not associated with any toxic effects.

1.5 Objectives of the study

1.5.1 General objective

To investigate *in vivo* antiinflammation, antinociceptive and antipyretic activities associated with dichloromethane and methanol extract of *Prunus africana, Eucalyptus saligna, Sennadidymobotyra, Bidens pilosa* and *Mangifera indica*.

1.5.2 Specific objectives

i. To determine qualitative and quantitative phytochemical components present in dichloromethane and methanol extract of *Prunus africana, Eucalyptus saligna, Senna didymobotyra, Bidens pilosa* and *Mangifera indica* collected from Meru County.

ii. To determine anti-inflammatory activities associated with dichloromethane and methanol extracts of *Prunus africana, Eucalyptus saligna, Senna didymobotyra,*

Bidens pilosa and *Mangifera indica* using mice.

iii. To determine the antinociceptive activities of *Prunus africana, Eucalyptus saligna, Senna didymobotyra, Bidens pilosa* and *Mangifera indica* using mice.

iv. To determine the antipyretic activities of *Prunus africana, Eucalyptus saligna, Senna didymobotyra, Bidens pilosa* and *Mangifera indica* using rat.

v. To evaluate sub-acute (repeated dose) toxic effects associated with dichloromethane and methanol extract of *Prunus africana, Eucalyptus saligna, Senna didymobotyra, Bidens pilosa* and *Mangifera indica* using mice.

1.6 Significance of the study

The data generated by this research helps to inform the local herbalists of Meru community on the effectiveness and role of *Prunus africana, Eucalyptus saligna, Senna didymobotyra, Bidens pilosa* and *Mangifera indica* plants traditionally used in management of pain, fever and inflammation especially the patients who cannot afford the conventional medicines. It will also help in conservation of such plant and encourage further research on the same plants. The study also forms a base for further research on the five selected herbal plants in the study.

1.7 Limitation of the study

The limitation of the study was that only one route of drug admistration was employed and this could not give a clear picture on the effectiveness of the plant products on management of fever, pain and inflammation because drug effectiveness vary with the route of administration. .

1.8 Scope of the study

The purpose of the study was to establish the phytochemical presence in *Prunus africana* and *Mangifera indica* stem bark extract, *Bidens pilosa* stalks, *Eucalyptus saligna* and *Senna didymobotyra* leaves extract since these plant parts are used traditionally in Meru community in management of fever, inflammation and pain. The study also established the antipyretic, analgesic and antiinflammatory activities of the methanol and dichloromethane plant extracts using animal models. The Swiss albino mice were used to test for inflammation and pain while Wistar rats were used for testing the antipyretic activity. The Wistar rats were selected for antipyretic activity because their size allows one to take the rectal temperatures unlike the mice. The inflammation was induced by use of carrageenan, while fever and pain were induced by use of turpentine and formalin respectively. The acute toxicity studies of the plant extract were established after administering the plant extract for 28 days in animal models. The macroscopic and biochemical assays were used to establish the organ damage at the end of toxicity tests.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pain

The unpleasant emotional and sensory feeling associated with either existing or imminent tissue damage is referred to as pain. Patients suffering from either acute or chronic diseases experience pain. Pain results to extensive suffering to the patients. There are different forms of pain and they occur to warn the organism on the prevailing injuries or diseases (Aage, 2006). Pain affects negatively the quality of the patient life and impact negatively also the patient's family. Pain quality and intensity varies in relation to different external and internal factors, leading to different pain experiences in different circumstances. Individual way of pain perception varies with time (Helms, 2008). Pain intensity cannot be measured but personal pain perception can be determined by the circumstances and emotional state of pain acquisition and the life threating signal perceived (Apkarian *et al.,* 2005).

Pain perception depends on various factors such as attention, expectation (Serham *et al*., 2015);arousal (Isailoyic *et al*., 2015) and distraction and can affect various physiological functioning of the body (Todd *et al*., 2015). Pain stimuli are passed very quickly in fractions of seconds. Acute pain sensitizes against an impending and ensuing danger. Sometimes pain stimulus may disappear leaving no trail (Apkarian *et al.,* 2005). Central nervous system can develop some adaptive changes in cases of repeated pain stimuli leading to pain inhibition (Apkarian *et al.,* 2005). The glial cells are responsible for the synthesis of the compounds involved in modifying pain sensation (Uttara *et al*., 2009).

The central and peripheral nervous system can also generate pain without receptors (pathological pain). The pathological pain (non – receptor pain) results from nervous system damage and has different manifestation and clinical presentation from the physiological pain (Sela *et al*., 2015).

Nociceptive pain warns against dangers associated with tissue damage. The pain receptors in this case may be as a result of external tissues or internal tissues damage without any nervous system disorder or damage (Allan and Siegel, 2002). This type of pain may result from either physical or chemical factors acting on the pain receptors. The stimulation from these factors can be converted to electrical impulses which are then conducted to be recognized as pain by the undamaged nervous system (WHO, 2013). This pain stimulus first of all is detected by the reticular system which leads to generation of non – specific impulses. The non- specific impulses leads to cerebral cortex activity stimulation and also the limbic system stimulation which determines the pain emotional response. The brain centres forms the defense reflex (Allan and Siegel, 2002).

Nociceptors are nerve ending which response to irritation leading to pain. They are found in dental pulp, cornea of the eye, skin, muscles, joint capsule, periosteum, meninges, peritoneum, pleura and ligaments (Matre *et al*., 2006). They facilitate the transmission of information to brain through chemical, thermal, electrical, biological and mechanical stimulation. Pain perception occurs when the pain stimuli is transmitted to spinal cord and finally to the central parts of the brains (Allan *et al*., 2009).

The impulses are transmitted from the spinal cord dorsal horn to neurons dorsal horn

located in substantia gelatinosa and finally they are transmitted into the brain. The basic pain sensation takes place in the thalamus (Prystupa *et al*., 2013). The pain impulse continues up to the limbic system which is also referred to as the emotional centre and also to the cerebral cortex for perception and interpretation (Dubin and Patapoutian, 2010). Nociceptors are found at nerve fibre endings and they are of two types involved in pain transmission designated as Aδ and C. The Aδ fibres are involved in production of well-defined sharp pain commonly stimulated by physical blow, electrical shock and cut. The Aδ fibres are myelinated hence facilitates the action potential to travel towards the central nervous system at a rate of 20 meters/ second. The Aδ fibers transmission is so fast hence the body responds very fast to pain stimulus (Duenas *et al*., 2016). This leads to affected body part retraction before pain perception. This results into quick response. The Aδ fibres practically lack the opioid receptors. The pharmacological modification of Aδ fibre receptors is limited hence it is difficult to block the "sharp", "fast" pain (Redii *et al*., 2013).

The small C fibres are involved in the transmission of aching and dull burning sensation also referred to as "second pain" (Burger, 2008; Patel and Kopf, 2010). The C fibres are susceptible to damage because they are very thin. They lack myelin sheath hence conduction of stimuli is slow (Wolf, 2004; D'Mello *et al*., 2008). The C fibres cover a broad area hence the patient is not able to locate the actual pain site. The C fibres are responsible for responding to chemical, mechanical and thermal stimuli (Lamont *et al*., 2000; Ohara *et al*., 2005; Schaible, 2006; Marchand, 2008). Pain conduction through the C fibres can be described as rapid, pulsing and hitching. Different receptors are located at the end of the C fibres and the major receptor is the opioid receptors. Ganglion is involved in the synthesis of the protein forming the part of the opioid receptors. Inactive receptors

are found in the nerve endings in the cell membrane and they are triggered by inflammation. Different cytokines originating from the inflammatory cells penetrate the perineurium and are able to activate receptors. Activated opioid receptors are capable of reacting to both exogenous and endogenous opioids (Goldstein, 2007; Lu *et al*., 2008).

The non-steroidal anti – inflammatory drugs are associated with inhibition of prostaglandin synthesis while corticosteroids reduce the sensitivity of the nerve fibres leading to an increased pain threshold. This is achieved due to proper co-ordination between the nervous and immune system (Scholz and Woolf, 2002; Gereau *et al*., 2014). Pain can be categorized into two phases based on the nerve fibres involved (Deng *et al*., 2016). The first phase is associated with the Aδ nerve fibres which are fast conductors and the second phase is associated with the C nerve fibres (Gatchel *et al*., 2014). The physiological pain is very important for human safety because it serves as a warning sign (Fishman *et al*., 2018).

The chemical substances are released when tissues are damaged into extracellular tissues to modulate pain transmission. Nerve ending irritation activates the pain receptors. The chemical mediators involved in pain activation are: bradykinin, histamine, acetylcholine, prostaglandins, substance P and leukotrienes (Gatchel *et al*., 2007; Woof, 2008; Redii *et al*., 2013). Other reactions associated with tissue injury mediators include:- altered permeability of the capillary, vasodilatation and vasoconstriction. Prostaglandins lead to tissue inflammation and it stimulates the release of other mediators associated with inflammation. Drugs such as aspirin blocks the enzyme cyclooxygenase 2 which is involved in the process of prostaglandin synthesis hence it is involved in pain reduction

(Matoc *et al*., 2010; Patel and Kopf, 2010; Redii *et al*., 2013; Artemiadis and Zis, 2018).

Aspirin is commonly used in management of pain resulting from tissue inflammation (Goldstein, 2007; Kapur *et al.*, 2014). The body in build chemical systems are used in pain management. The fibres in brain stem, peripheral tissues and dorsal horn releases the endogenous opioids and the neuromodulators that block the neurons from transmitting pain impulses (Sang, 2000; Rojagopal, 2006). The natural substances resembling the opioids that are responsible for pain relief are known as the endorphins. The levels of endorphins varies from one individual to the other and this explains why different people experience pain in different intensities (Sang, 2000; Sinatra, 2002; Zhou *et al*., 2008).

The nervous system is capable of detecting and interpreting various range of mechanical, thermal, environmental and endogenous chemical pain irritants. Acute pain is self – limiting and is associated with useful biologic purposes (Zhu *et al.,* 2005; Basbaum *et al*., 2009; Putapontian, 2010). It is associated with spasm in the skeletal muscles and sympathetic nervous system stimulation resulting associated with specific disease or tissue injury. In case of persistent injury, the component of central and peripheral nervous system are involved in pain transmission pathway which is associated with exhibition of huge plasticity leading to enhanced pain signals hence leading to hypersensitivity (Cole, 2002; Kilic *et al*., 2012; Zwakhalen *et al.,* 2016). Plasticity can favour the protective reflexes and this can be associated with beneficial effects but if the changes persist, this may result into chronic pain condition state. Chronic pain is not associated with any distinguishable end point but it may may arise from psychological states which is not associated with any

biologic purpose (Zwakhalen *et al*., 2006; Woof, 2008; Redii *et al*., 2013). Persistent pain is associated with peripheral nerves properties changes and it results from diseases such as cancer, arthritis and diabetes. Chronic pain is associated with nerve fibre damage which leads to increased alteration and firing of the neurotransmitter and conduction properties (Rojagopal, 2006; Wilson, 2011).

The clinical characteristics associated with pain and can all be evaluated subjectively include: duration, location, quality and intensity (Wolf, 2004; Goldstein, 2007). Pain location facilitates determination of the causes pain and does not correspond always to injury site or disease process. It is particularly difficult to locate pain in deep organs leading to hinderance in the location of the disease. Internal organs lack pain receptors, it is the overlying peritoneum that have sensory innervations. Pain intensity experience varies with individual patients and it is very difficult pain characteristic to assess (Lamont *et al*., 2000; WHO, 2009).

Pain can be classified on the basis of its presumed underlying pathophysiology such as nociceptive or neuropathic pain (Portenoy, 1989). Nociceptive pain is caused by the ongoing activation of A-δ and C-nociceptors in response to a noxious stimulus like injury, disease, inflammation (Coda and Bonila, 2001). Pain arising from visceral organs is called visceral pain, whereas that arising from tissues such as skin, muscle, joint capsules, and bone is called somatic pain. Somatic pain may be further categorized as superficial (cutaneous) or deep somatic pain (Coda and Bonila, 2001). In contrast to neuropathic pain, the nervous system associated with nociceptive pain is functioning

properly.

Generally, there is a close correspondence between pain perception and stimulus intensity, and the pain is indicative of real or potential tissue damage. Differences in how stimuli are processed across tissue types contribute to the pain's varying characteristics (Chapman and Nakamura, 1999). For example, cutaneous pain is often described as a well-localized sharp, pricking, or burning sensation; deep somatic pain, as a diffuse dull or aching sensation; and visceral pain, as a deep cramping sensation that may be referred to other sites. This is known as referred pain (Coda and Bonila, 2001). Associated clinical pain states like hyperalgesia and allodynia which reflect sensitization (Meyer *et al*., 1985; Coda and Bonila, 2001).

Neuropathic pain is caused by aberrant signal processing in the peripheral or central nervous system (Portenoy, 1996). In other words, neuropathic pain reflects nervous system injury or impairment. Common causes of neuropathic pain include trauma, inflammation, metabolic diseases (like diabetes), infections (like, herpes zoster), tumors, toxins, and primary neurological diseases (Woolf, 2001). Neuropathic pain can be broadly categorized as peripheral or central in origin (Portenoy, 1996). Painful peripheral mononeuropathy and polyneuropathy, deafferentation pain, sympathetically maintained pain, and central pain are subdivisions of these categories.

Neuropathic pain is sometimes called "pathologic" pain because it serves no purpose (Woolf, 2000). A chronic pain state may occur when pathophysiologic changes become independent of the inciting event (Sensitization plays an important role in this process.

Although central sensitization is relatively short lived in the absence of continuing noxious input, nerve injury triggers changes in the CNS that can persist indefinitely (Galer *et al*., 2001). Thus, central sensitization explains why neuropathic pain is often disproportionate to the stimulus (like hyperalgesia, and allodynia) or occurs when no identifiable stimulus exists (like persistent pain and pain spread). Neuropathic pain may be continuous or episodic and is perceived in many ways (like burning, tingling, prickling, shooting, electric shock-like, jabbing, squeezing, deep aching, spasm, or cold) (Galer *et al*., 2001).

Acute pain was once defined simply in terms of duration is now viewed as a "complex, unpleasant experience with emotional and cognitive, as well as sensory, features that occur in response to tissue trauma. In contrast to chronic pain, relatively high levels of pathology usually accompany acute pain and the pain resolves with healing of the underlying injury (Chapman and Nakamura, 1999). Acute pain is usually nociceptive, but may be neuropathic. Common sources of acute pain include trauma, surgery, labor, medical procedures, and acute disease states. Acute pain serves an important biological function, as it warns of the potential for or extent of injury. A host of protective reflexes (such as withdrawal of a damaged limb, muscle spasm, autonomic responses) often accompany it. However, the "stress hormone response" prompted by acute injury also can have adverse physiologic and emotional effects. Even brief intervals of painful stimulation can induce suffering, neuronal remodeling, and chronic pain; (Carr and Goudas, 1999). associated behaviors (like: bracing, abnormal postures and excessive reclining) may further contribute to the development of chronic pain. Therefore,

increasing attention is being focused on the aggressive prevention and treatment of acute pain to reduce complications, including progression to chronic pain states (Coda and Bonila, 2001).

Chronic pain was once defined as pain that extends 3 or 6 months beyond onset or beyond the expected period of healing (Turk, 2001). However, new definitions differentiate chronic pain from acute pain based on more than just time. Chronic pain is now recognized as pain that extends beyond the period of healing, with levels of identified pathology that often is low and insufficient to explain the presence and/or extent of the pain (Jacobsen and Mariano, 2001). Chronic pain is also defined as a persistent pain that "disrupts sleep and normal living, ceases to serve a protective function, and instead degrades health and functional capability (Chapman and Stillman, 1996). Thus, unlike acute pain, chronic pain serves no adaptive purpose. Chronic pain may be nociceptive, neuropathic, or both and caused by injury (such as, trauma and surgery), malignant conditions, or a variety of chronic non-life-threatening conditions (like: arthritis, fibromyalgia and neuropathy). In some cases, chronic pain exists *de novo* with no apparent cause. Although injury often initiates chronic pain, factors pathogenetically and physically remote from its cause may perpetuate it (Turk, 2001). Environmental and affective factors also can exacerbate and perpetuate chronic pain, leading to disability and maladaptive behavior.

Pain associated with potentially life-threatening conditions such as cancer is often called "malignant pain" or "cancer pain." However, there is movement toward the use of new

terms such as “pain associated with human immunodeficiency virus (HIV) infection” or “pain associated with cancer.” (The term “cancer pain” is used in this monograph for the sake of brevity.) Cancer pain includes pain caused by the disease itself (such as tumor invasion of tissue, compression or infiltration of nerves or blood vessels, organ obstruction, infection, inflammation) and/or painful diagnostic procedures or treatments such as biopsy, postoperative pain, toxicities from chemotherapy or radiation treatment (Chepman and Foley, 1993). There are several reasons why some experts feel that cancer pain merits a discrete category. First, its acute and chronic components and multiple etiologies make it difficult to classify based on duration or pathology alone. Second, cancer pain differs from chronic noncancer pain (CNCP) in some significant ways such as time frame, levels of pathology, treatment strategies (Jacobsen and Mariano, 2001). However, there is little evidence to support a distinction between these pain types based on underlying neural processes. Therefore, many pain experts categorize cancer pain as acute or chronic pain (Turk, 2001).

A subtype of chronic pain is CNCP, which refers to persistent pain not associated with cancer. In contrast to patients with chronic cancer pain, patients with CNCP often report pain levlevels that only weakly correspond to identifiable levels of tissue pathology and/or respond poorly to standard treatments (Dunajcik, 1999; Jacobsen and Mariano, 2001). As CNCP may last for many years, some consider use of the traditional term for such pain, “chronic nonmalignant pain,” inappropriate. Thus, there is movement toward use of alternate terms such as “chronic noncancer pain” and “chronic noncancer- related pain.” Causes of CNCP include acute injury that has proceeded to chronic pain like

whiplash and various chronic conditions (Turk, 2001). In some cases, there is no discernable cause, and the pain is considered the disease. CNCP can affect virtually any body system or region, and pain severity ranges from mild to excruciating. Some types of CNCP have well-defined characteristics and patterns, whereas others do not. Neuropathic and myofascial CNCP can be particularly hard to diagnose, as they may occur in the absence of a known injury or disease process (Dunajcik, 1999). Because of its chronicity and impact on daily activities, patients with CNCP may experience vocational, interpersonal, and/or psychological problems (Becker *et al*., 1997). If the symptoms of CNCP consume the attention of and incapacitate the patient, he or she may suffer from a psychosocial disorder known as "chronic pain syndrome" (CPS) (Dunajcik, 1999). The pain experienced by these patients is real, and not all patients with CNCP develop this syndrome. Appropriate management of both CNCP and CPS requires aninterdisciplinary approach that addresses the complex interaction of physical, psychological, and social factors that contribute to the ongoing pain (Jacobsen and Mariano, 2001).

2.2 Pyrexia

Pyrexia is commonly known as fever (Axelrod and Diringer, 2008). It is the elevation of body temperature above 37.5°C due to physiological changes affecting the temperature set point regulator in the hypothalamus (Karakitsos and Karabinis, 2008). Increased change in set points is associated with shivering and increased muscle tone. Numbers of illnesses are associated with fever as a natural reaction. General symptoms associated with fevers include: cold sensation, chills and sweating though they may miss in serious illness. Fever may occur in cases of infections associated with parasites, bacteria, viruses and immunological reactions such as: immunological abnormalities, defects in collagen and

in cases of acquired immunodeficiency. Fever may also be associated with tissue destruction especially in cases of: trauma, infarction (local necrosis) and in cases of both tissues and vessels inflammatory reactions. Inflammation of specific tissues or organs like in cases of hepatitis, sarcoidisis, inflammation of intraabdominal and intestine, granulomatous, and carcinoma also lead to fever. Fever may also result from administration of specific drugs, salts, foreign proteins and also in cases of dehydration (Karakitsos and Karabinis, 2008).

2.3 Inflammation

Inflammation originates from the word "inflammare" meaning to burn. Inflammation is an important central process involved in defense mechanisms in animal cells in cases of injuries and infections (Isailovic *et al*., 2015; Todd *et al*., 2015). Inflammation can progress regularly to acute or chronic (Isailovic *et al*., 2015; Serham *et al*., 2015). Chronic inflammation commonly results from diseases like cancer, cardiovascular diseases and neurodegenerative disorders (Uttara *et al*., 2009).

Inflammation mechanism represents a well-organized chain of dynamic changes in both vascular and cellular events associated with certain specific changes in humoral secretions. At the inflamed site there is physical changes like accumulation of leucocytes like neutrophils and eosinophils), fluids and plasma (Huether and McCance, 2015). Immune defense cells contribute to increase event of inflammation through a release of mediators and other signaling molecules such as serotonin, leukotreines, histamines, nitrogen and oxygen derived free radicals and prostaglandins (Anwikar and Bhitre, 2010). Inflammation occurs in phases either as an acute and chronic inflammation. Each inflammatory phase is

associated with a specific mediatory mechanism (Serham *et al*., 2015). Immune responses involved in the acute inflammation category are classified into either cellular or vascular (Nguyen, 2012). Microvasculature responses appear normally after few minutes of tissue injury or as a result of microbial infection associated with named vascular events resulting from certain inflammatory stimuli (Nguyen, 2012). These occur rapidly and lead eventually to vasodilation which makes up the vessels to be more permeable. This results into inflammatory mediators entry into the inflamed site leading to interstitial edema (Porter, 2013).

In cases of inflammatory responses, white blood cells infiltration from the circulatory system plays a very essential role (Kumar *et al*., 2013; Goljan, 2014). Specific chemotactic agents like the amino terminal N –formyl methionyl groups of the microbial endotoxins, histamine, interleukins, leukotriene B and the C5a complement fragment stimulate an intense infiltration of the leukocyte in an inflamed site within a period of few minutes (Kumar *et al*., 2012; Bitencourt *et al*., 2013). In cases of acute inflammation, neutrophils are recruited at the inflamed site (Curcic *et al*., 2015). Inflammation cellular events are associated with capturing, trundling and formation of an adhesion to the endothelium of the microvasculature (Nourshargh *et al*., 2010). The mobilization pathway events are facilitated by the cell adhesion molecules (CAMs) like the ICAM -1, ICAM -2, selectin and integrins. Three families of the selectin group of CAM are the L-selectin which are produced by the white blood cells and the E-selectin and P – selectin which are produced by the endothelial cells (Springer *et al*., 2012). The high affinity adhesion of the white blood cells presented in the endothelium in cases of acute inflammation is associated by

interaction between the adhesion molecules (CAM-1 and CAM -2) and the integrins (Ogra *et al.,* 2012). After the stationary adhesion in acute inflammation, the white leucocytes can leave post capillary venules through pseudopodia in endothelial cells to sub - endothelial space. This process is known as leucocytes extravasations or transendothelial migration (Siers, 2013).

Chronic inflammation is associated with mononuclear cell infiltration such as the monocytes and lymphocytes. It is also associated with proliferation of the fibroblasts, connective tissue formation, collagen fibers and this may result in formation of granuloma (Gleension *et al*., 2011). Chronic inflammation tissue degeneration results from reactive nitrogen/oxygen species and proteases generated from the infiltrated inflammatory cells (Murakumi, 2012). A genetic alteration in p53 is associated with chronic inflammation associated with arthritis, cancer and inflammatory bowel diseases (Kong, 2013; Niederhuber, 2014; Ogrunc *et al*., 2014).

Chronic inflammation results from recurrent acute inflammatory processes lasting for weeks, months or years (Paramita *et al.,* 2017). Inflammation is a normal physiological process but it is associated with adverse symptoms like severe pain. Conventional anti-inflammatory agents such like the steroids and the non-steroidal drugs used to alleviate adverse symptoms associated with inflammation (Oyekachukwu *et al*., 2017). Corticosteroids inhibit the phospholipase A2 (PLA2) while the non-steroidal drugs inhibit the cyclooxygenase activity (Oyekachukwu *et al*., 2017). Though these drugs are able to alleviate the negative effects of the inflammatory processes, they have severe adverse effects like gastric bleeding, peptic ulcers, nausea and vomiting (Paramita *et al*., 2017).

The non-selective non- steroidal drugs inhibit both the COX-2 and COX-1 leading to inhibition of both prostaglandin E2 (PGE-2) and prostacyclin. Both prostaglandin E2 and prostacyclin aprotects gastric mucosa from stomach acid (Paramita *et al*., 2017). Selective COX inhibitors are associated with increased cases of stroke and risks of heart attack (Oyekachukwu *et al.,* 2017). There is an increased cases of resistance with the use of corticosteroids (Vazquez-Tello *et al*., 2013). Due to the side effects associated with both corticosteroids and the NSAIDs there is a great need for alternative drugs to manage inflammation without or with minimal side effects, especially the ones derived from plants (Ismail *et al*., 2012).

The inflammatory response must be well ordered and controlled and a variety of interconnected cellular and soluble mechanisms are activated when tissue damage and infection occur (Isailovic *et al*., 2015). Examples include cytokines, by-products of the plasma enzyme systems (complement, the coagulation, kinin and fibrinolytic pathways), lipids (prostaglandins, leukotrienes, platelet activating factor), and vasoactive mediators. Once leucocytes have arrived at a site of inflammation, they release mediators which control the later accumulation and activation of other cells (Todd *et al*., 2015).

Cytokines are soluble proteins which regulate host-cell function through interaction with specific receptors. They are produced by neutrophils, lymphocytes, monocytes/ macrophagesand endothelial cells. A wide range of cytokines has been identified and many have overlapping and complementary activities (Serham *et al*., 2015). Increasing numbers of cytokines are being discovered. Broad groupings of cytokine families are

now known including interleukins (ILs), tumour necrosis factors (TNFs), interferons (IFNs) and colony stimulating factors (CSFs). Another way of grouping cytokines is by their action – either pro-inflammatory or antiinflammatory. The importance of the balance between these two opposing actions in many ways similar to the coagulation system with procoagulants and profibrinolytics and anti-coagulants is now becoming better appreciated (Uttara *et al*., 2009).

Cytokines make up a major class of soluble intercellular signalling molecules and possess typical hormonal activities in that: (i) they are secreted by a single cell type, react specifically with other cell types (target cells) and regulate specific vital functions controlled by feedback mechanisms; (ii) they act at short range in a paracrine or autocrine manner; and (iii) they interact first with high-affinity cell surface receptors (distinct for each type or even subtype) and regulate gene transcription (Huether and McCance, 2015). This altered transcription (which can be enhanced or inhibited) results in changes in cell behaviour. Target cells may be in any body compartment (sometimes a long distance from the site of secretion). Other types of cytokine act mostly on neighbouring cells in the micro-environment where released (Anwikar and Bhitre, 2010).

During paracrine secretion, some cytokines may escape cell binding and spill over into the general circulation (Nguyen, 2012). Cytokines are synthesised, stored and transported by various cell types, not only those of the immune system (Porter, 2013). They control the direction, amplitude, and duration of immune responses and the remodelling of tissues. Individual cytokines have multiple, overlapping and sometimes contradictory,

functions depending on local concentration, cell type they are acting on, and the presence of other mediators. Thus, the information which an individual cytokine conveys depends on the pattern of regulators to which a cell is exposed, and not on just one cytokine (Kumar *et al.*, 2013). Because of the potent and profound biological effects of cytokines, it is not surprising that their activities are tightly regulated, mainly at the levels of secretion and receptor expression (Goljan, 2014).

Pro-inflammatory cytokines up-regulate inflammatory reactions. Anti-inflammatory cytokines are generally T-cellderived cytokines and down-regulate inflammatory reactions (Biten-Court *et al*., 2013). TNF-α and IL-1 have a central role in inflammatory response since administration of their antagonists, such as IL- 1 receptor antagonist (IL-1ra), soluble fragment of IL-1 receptor, or monoclonal antibodies to TNF-α and soluble TNF receptor, block various responses in models of inflammation (Curcic *et al.,* 2015). Some have also been used in the treatment of human chronic inflammatory states such as rheumatoid arthritis (Nourhargh *et al*., 2010). On the other hand, anti-inflammatory cytokines (IL-4, IL-10, IL-13) are responsible for the down-regulation of inflammation (Ogra *et al*., 2012).

Anti-inflammatory cytokines suppress the production of pro-inflammatory cytokines and their strong anti-inflammatory activity may suggest a role in the management of many inflammatory conditions. IL-10 has been extensively investigated in both healthy volunteers and in patients with both chronic and acute inflammatory diseases (Siers, 2013). Patients with rheumatoid arthritis, inflammatory bowel disease, and hepatitis C

infections have received IL-10 for extended periods (up to several months). The safety profile in patients receiving IL-10 has been very good to date, with no evidence of an increased susceptibility to either bacterial or viral infections (Gleension *et al*., 2011). Future clinical studies in acute inflammatory diseases will need to address the unresolved issues of the quantitative importance of IL-10's anti-inflammatory and immunosuppressive properties in critically ill patients. It is possible that treatment targeted to increasing tissue levels rather than plasma concentrations will be more useful (Murakumi, 2012).

2.4 Methods of inflammation induction

2.4.1 Carrageenan inflammation induction method

This method is considered as the standard inflammation induction model specifically for inducing both nonimmune and acute inflammatory response and it is commonly employed when testing for the anti-inflammatory activities of both synthetic and natural products chemical compounds (Muhammad *et al*., 2012; Mansouri *et al*., 2015). Carrageenan is a polysaccharide obtained from seaweeds (Rhodophyceae) and it is made up of galactose – related monomers (Hafeez *et al*., 2013). The lambda, kappa and iota are the three forms of carragenaan. Lambda types does not form a strong gel at room temperature. Carrageenan is known to be antigenic and has no systemic effects after administration (Hussain *et al*., 2015).

Carrageenan induce acute inflammation when administered to the animal model with a peak effect achieved after 3-5 hours (Pareek *et al*., 2011; Dhalendra *et al*., 2013). Carrageenan induces inflammation by stimulating the production of various mediators

hence inflammation induced through carrageenan administration is bi-phasic. The first phase (first hour) is associated with production of: kinins, histamine, serotonin and small amounts of prostaglandins and the late phase which is experienced in the course of the second to the fourth hour following the administration of carragenaan led to production offree radicals (Asfar *et al*., 2015); prostaglandins, proinflammatory cytokines like IL-1β, TNF-α (Samriti *et al*., 2016); and neutrophils infiltration (Hafeez *et al*., 2013). Anti-inflammatory drugs work well in the second phase of inflammation (Mansouri *et al*., 2015; Mondal *et al*., 2016;).

2.4.2 Egg albumin inflammation induction method

Egg albumin when injected to experimental animal models like mice or rat can be used to induce inflammation. To determine the paw circumference change after administration of the egg albumin, vernier caliper is used (Salawu *et al*., 2008; Kolawole and Dapper, 2016). Egg albumin induces inflammation because it leads to a release of 5 – hydroxytryptamine in the intercellular space (Perianayagam *et al*., 2012). Egg albumin edema is biphasic. The egg albumin first phase edema occurs in the first and second hours after administration and the late phase edema start from the third hour up to the fifth hour after the administration (Anosike *et al*., 2012).

2.4.3 Croton oil edema induction method

This is one of the easiest methods of investigating the anti-inflammatory activities of substances. Croton oil is commonly used to investigate topical anti-inflammatory activities of substances. Croton oil leads to cutaneous inflammation because it is a highly inflammatory irritant agent. Inflammation resulting from croton oil administration is

associated with increased migration of leukocytes, vasodilation and liberation of mediators of inflammatoion locally like: prostaglandins, 5-hydroxytryptamine and histamine (Araujo *et al.*, 2004). To demonstrate edema inhibition potential of a substance using croton oil test method, 5% of the croton oil is administerd topically to the inner left ear surface of the rat or mouse. The right ear should not be treated with anything. The experimental drug under investigation is administered topically after 30 minutes on the left ear inner surface. Euthanize mice after 6 hours. Edematous response is measured by taking the weight of two plugs measuring 6mm in diameter collected from right and left ears using dermatologic punch (Oliveira *et al*., 2013).

2.5 Pain induction experimental methods

2.5.1 Tail-flick experimental model

This involves immersion of small portion of the experimental animal tail in hot water or exposure to radiant heat at 55^0C or very cold water to evoke the nociceptive stable spine mediated response. Response takes place in jerk and flick forms of the tail movement away from heat source or vigorous body movement. The tail or the body movement is often recorded as a measure of pain experienced and this is commonly known as "tail-flick latency" (Keefe *et al*., 1991; Milind *et al*., 2013).

2.5.2 Hot plate pain induction method

This method is commonly employed to investigate thermal antinociception in animal models such as mice and rats. Animal paws are heat sensitive. The method is well suited for investigating analgesic effect of central origin because in peripheral analgesic this method is associated with little or no analgesic activity. During hot plate method of pain

induction, the rat or mice is placed in a cylindrical open ended container with metallic floor. Boiling liquid or thermode is used to heat the metallic floor to a constant temperature of about 55^0C. The animals respond either by licking the paw or jumping when their footpads come into contact heated metallic floor. The response reaction time occurs in matter of seconds (Le Bar *et al*., 2001; Paschapur *et al*., 2009).

2.5.3 Acetic acid pain induction writhing test

This method is commonly employed in the processes of investigating peripheral anti-inflammatory activities of the non-steroidal drugs. Acetic acid is administerd to the test animals peritoneally. This results into animal writhing or stretching which is characterized with abdominal contraction which is recorded within the first thirty minutes (Mishra *et al*., 2011). Acetic acid introduction to the peritoneal cavity leads to peripheral receptor system sensitization especially on the peritoneal cavity cell lining by increased release of both prostaglandins $PGF_{2\alpha}$ and PGE_2. Bradykinins, histamine and serotonin are also released to stimulate the endings of the nervous system (Akindele *et al*., 2012; Bhattacharya *et al*., 2014).

2.5.4 Formalin pain induction method

Formalin pain induction method is best suited to establish the drug analgesic activity and the nociceptive processes (Cheng *et al*., 2005; Arzi *et al*., 2013). This method establishes a continuous and not transient pain induction process with two well established phases. The two phases are categorized as early and late nociceptive response phases. The phases are distinguished on the basis of the underlying mechanisms and the duration. The early

phase last for only 5-10 minutes and it is associated with intense pain. Early phase starts immediately following the administration of formalin due to direct nerve end stimulation (Sufka *et al*., 1998; Jeong and Lee, 2002).

The late phases occur following the little or lack of nociceptive behavior which follow the early phase. The late phase is a prolonged continuous process lasting for 15-60 minutes following the administration of formalin peritoneally. It is associated with moderate pain due to inflammation associated with release of inflammatory mediators affecting the spine dorsal horn. The inflammatory mediators associated with late phase include: serotonin, prostaglandin, bradykinin and histamine. Analgesic acts differently in distinct inflammatory phases (Sharma *et al*., 2013; Gong *et al*., 2014). Nociception is induced by administering 2.5% formalin subcutaneously in the peritoneal region. High formalin concentration exceeding 5% should not be used to induce pain because it leads to unquantifiable pain leading to hindrances of the primary behaviors associated with pain. To increase the test sensitivity, low formalin concentration is recommended (Tjolsen *et al*., 1992).

Formalin pain induction model is best compared with the other pain induction techniques because it is associated with continuous and not a transient pain induction. This resembles the clinical pain. This method is good for discriminating the pain components associated with central and peripheral pain. In this method, the animal models used are not or slightlyrestrained during the investigation (Svendsen and Hau 1994; Gong *et al*., 2014).

2.6 Pyrexia induction experimental methods

2.6.1 Brewer's yeast pyrexia induction method

Brewer's yeast consists of lipopolysaccharides in the cell wall and it is an exogenous pyrogenic substance that leads to pyrexia through increasing the synthesis of the prostaglandins (Akpan *et al.,* 2012). The basal body temperature is recorded before pyrexia induction. To induce fever by use of brewer's yeast, it is injected subcutaneously at a concentration of 15-20%. After 17–20 hours upon the brewer's yeast administration, the rectal body temperature is expected to increase by a range of 0.5-1^{0}C. Animals in which rectal temperature fails to change by 0.5-1^{0}C are excluded in pyrexia testing. After administration of the antipyretic substance under investigation the rectal temperature change is monitored after every hour (Kumar *et al.,* 2015).

2.6.2 D-amphetamine pyrexia induction method

Amphetamine acts on the brain leading to release of biogenic amines in the nerve ends. This is followed by increased release of cAMP in the body leading also to increased synthesis of prostaglandins from arachidonic acid through hydrolysis of phospholipids by receptor mediation (Akpan *et al*., 2012). The changes in body temperature are monitored using digital clinical thermometer. To induce fever, D-amphetamine is administered intraperitoneally. The rectal temperature is taken and recorded after one hour upon the administration of D-amphetamine. The expected temperature change is supposed to be achieved within the first five hours following the administration of D-amphetamine (Agbaje and Ajidahum, 2011).

2.6.3 Turpentine oil fever induction method

Turpentine oil is extracted from *Pinus palustril*. Turpentine oil tissue irritation leads to inflammation, fever, body weight loss, formation of abscesses, production of cytokine and changes in levels of acute phase protein (Renckens *et al.,* 2005). Turpentine induces endogenous pyrogen synthesis and release of cytokines. The endogenous pyrogen act on the hypothalamus at the thermoregulatory center leading to pyrexia due to increased concentration of prostaglandin E_2 (Vasundra and Divya, 2013). To induce pyrexia, turpentine is administered intraperitoneally at a concentration of 20% and the quantity administered depends with the animals body weight. Fever induction occurs after 1 hour after the administration of the turpentine oil. The body temperature of the animal model issupposed to change by approximately 0.7-1.0^{0}C otherwise the animal are excluded for the study (Gitahi *et al*., 2015).

2.7 Conventional drugs used in management of pyrexia, inflammation and pain

The non-steroidal and steroidal (corticosteroids) anti-inflammatory agents are the drugs of choice in inflammation management. Glucocorticoids and the mineralocorticoids which occur naturally as hormones which are secreted by the adrenal glands are classical examples of steroidal drugs. They have various side effects though they have immediate, powerful and short-lived inflammation relief (Dewick, 2009; Becker, 2013).

Glucocorticoids have anti-rheumatic and anti-inflammatory effect because they affect the metabolism of fat, protein and carbohydrate. Their mode of action involves phospholipase inhibition hence they prevent phospholipid release needed for prostaglandin synthesis from their storage. Malfunctioning of adrenal gland leads to health problems such as acne,

overweight, decrease in infection resistance and Addison's disease. Mineralocorticoids like aldosterone and fluorocortisone have minor analgesic and are involved in regulation of electrolyte hence promoting sodium, hydrogen carbonate and chloride reabsorption in the kidney (Dewick, 2009; Becker, 2013).

The anti-inflammatory non – steroidal drugs used in management of fever, inflammation and mild pain are diclofenac, aspirin, celecoxib, naproxen, piroxicam, diflunisal, ibuprofen and ketorolac (Burger, 2008; Deghrigue *et al*., 2015). The non-steroidal anti-inflammatory drugs inhibit pro-inflammatory prostaglandins synthesis by blocking COX-1 and 2 (Mworia *et al*., 2015). This reduces peripheral nervous tissue sensitization hence less pain due to decreased stimulation of the nerves (Mworia *et al*., 2015). The COX-1 and 2 catalyzes oxygenation of arachidonic acid resulting to generation of prostanoids (Francischi *et al*., 2002; Malkowski *et al*., 2016).

The cyclooxygenase are bifunctional homodimeric enzymes which contribute to a wide range of both pathophysiological and physiological functions. Most tissues constitutively express COX-1 while COX-2 in the inflammatory cells is induced rapidly. Renal functions maintenance and platelet aggregation are facilitated by prostanoids produced through the action of COX-1. These prostanoids also help in preservation of the gastrointestinal tract integrity through mucus formation stimulation in the small intestine and the stomach. They also facilitate blood flow and the protection of the mucosa lining of the gastric by decreasing the levels of acid secreted and facilitating the secretion of bicarbonate (Reanmongkol and Songkram, 2013).

Ibuprofen and indomethacin inhibit the COX enzymes reversibly by competing for the active sites with the arachidonic acid. Paracetamol which is also known as acetaminophen is classified as a NSAIDs and is the commonly used analgesic with minimal anti- inflammatory activity. Paracetamol is capable of inhibiting the enzyme COX-1 and this leads to its antipyretic and analgesic activity. It is very safe in management of fever associated with viral infection in children (Dewick, 2009). Metabolism of paracetamol involves the cytochrome P_{450} leading to electron transfer in a number of reactions resulting to hydrogen peroxide and superoxide formation and this is associated with liver cells injury(Clayman, 2016).

Salicylate used to treat fever and pain includes acetylsalicylic acid (aspirin), methyl salicylate, choline magnesium salicylate and salsalate. Aspirin was developed in 1899 in search of a salicylate derivative with less irritation on the stomach in relation to salicylic acid. Aspirin is used in management of fever, inflammation and pyrexia in adults but should be avoided in children since it leads to Reye's syndrome (Greenstein *et al*., 2007).

2.8 Herbal medicines

Herbal medicines use in management and prevention of diseases affecting human can be traced back to the early civilizations (Azaizeh *et al*., 2003). This can be traced way back between 5000 – 4000 B.C especially in India, China, Greece and Egypt (Farnsworth, 1994; Dery *et al*., 1999; Ermias *et al*., 2008). The practice of using plants products as medicine vary from one community to the other. Due to lack of standard way on their use, the

practice is commonly regarded as folk, alternative, unorthodox and indigenous medicine. Most of the time the knowledge regarding the use of herbal medicine is transferred from one generation to the other orally (Cotton, 1996).

Tradition medicine incorporates both the use of plants and animal products. Sometimes it is also associated with some exercises or manual techniques and spiritual beliefs to maintain or restore human health (WHO, 2003). Almost 1 – 10 percentage of 250,000 – 500,000 plant species found worldwide are used to produce some herbal medical products or as food by different communities (Borris, 1996; Tilahun and Mirutse, 2007). Approximately, more than 80% of the world populations have used herbal medicines. Previously herbal medicines were regarded as an option for the poor but nowadays even the rich people are opting for the herbal medicines because they are associated with low adverse effects (*Ermias et al*., 2008). This as led to more than 40% of the people even in developed Countries choosing herbal medicine in management of different illnesses (Lanfranco, 1999; Demma *et al*., 2009). More than 80% of the people in different parts of the world are using traditional medicines for the provision of the basic care therapy (Azaizeh *et al*., 2003). Medical plant products are used in management of both infectious and non-infectious diseases especially in India and other countries in Asia (Jamir *et al*., 1999).

Most people in Africa are using traditional medicines in management of diseases because they are more accessible and affordable (Steenkamp, 2003). Malaria associated fever in Africa affects approximately 60% of the population especially the children. The bigger

percentages of the population are known to have successfully managed this condition using alternative medicines at their homes (Kassaye *et al*., 2006).

In Ethiopia and Kenya, approximately 80 and 90% of the population are poor respectively. The poor population can only afford to use herbal medicines to manage some of the health problem affecting them (Abebe, 1996; Abebe, 2001). In Kenya, around 90% of the people have an experience of using herbal products in management of either human or livestock related diseases (Chirchir *et al*., 2006). Herbal derived products are usually used as pesticides, antivenom, painkiller, antimicrobial and in management of wounds (Tadeg *et al*., 2005).

2.9 Medicinal plants used in management of pain, pyrexia and inflammation

Most of the plants used in either management of human or livestock diseases contain organic compounds associated with pharmacological activities. The organic compounds are well metabolized in the body hence have less or no toxicological effects in relation to the conventional drugs (Anselem, 2004). Some communities in the have realized the importance of the medicinal plants and they have started the practice of domesticating them (Cowley, 2002). In Meru community, various plants are used either in management of fever, pain or inflammation or all. The plants selected in this study were:- *Prunus africana, Mangifera indica*, *Senna didymobotyra, Eucalyptus saligna* and *Bidens pilosa* (Adongo, 2012).

2.9.1 *Prunus africana*

Prunus africana (Hook.f) also referred to as red stinkwood or African cherry is classified in the family Rosaceae. It is commonly found in the Sub-Saharan Africa along the equator

especially in central and eastern part of Africa. It is a common plant species in highlands areas of Kenya especially the in Mt Kenya, Tugen hills, Aberdares and Mt Elgon (Van Wyk*et al*., 1997; Gachie *et al*., 2012; Kadu *et al*., 2012). The plant is evergreen and can grow to a height exceeding 24 M and trunk diameter of more than 1 M with black to brown roughstem bark which is reddish brown on the inner side. The local name for this plant varies from one community to the other. The local names for the plant are 'Mwiria' in Meru community of Kenya (Adongo, 2012), "garge" and "gurayu" in Gedeo and Oromo communities of Ethiopia, respectively (Mesfin *et al*., 2009). The plant is used commonly for timber production, though the roots, leaves and the stem bark are traditionally used for production of herbal medicines in Africa especially for management of benign prostatic cancer (Ferreti *et al*., 2010).

In Europen countries, *Prunus africana* is widely used in management of health related problems such as: benign prostate hypertrophy, fever, loss of appetite, pain, inflammation, kidney diseases and infectious diseases like urinary tracts infections and gonorrhea (Van Wyk *et al*., 1997; Neuwinger, 2000). Locally in Kenya, it is used in management of both infectious and non infectious diseases. Some of the health related issues managed by different products extracted fron the *Prunus africana* plant especially by the Meru community of Kenya are: amoeba infestation, body weakness, chest pain (Kareru *et al*., 2007); malaria, babesiosis and cancer affecting breast, prostate and colon (Gachie *et al*., 2012). Highly purified products are derived by different pharmaceutical companies from the plant bark, patented, and commonly used in the management of both malignant and benign prostatic gland hypertrophy and hyperplasias (Breza *et al*., 1998). This has led to both local and international attention for the plants products as excellence potential

candidates for the management of prostate cancer in men (Schipmarn, 2001; Gathumbi *et al*., 2002).

Figure 2.1: *Prunus africana* captured from Meru County, Kenya

2.9.2 *Eucalyptus saligna*

Eucalyptus saligna is an evergreen flowering plant. It is commonly referred to as bluegum and belongs to Myrtaceae family (Ritter, 2014). It is a fast growing plant species and a mature plant can be more than 125 Metres in height. *Eucalyptus saligna* has persistent rough bark (Saulle *et al*., 2018). The leaves average length is 14 cm with average width of 2.65 cm. It flowers after attaining an age of 3 – 4 years. The plant bears fruits which are bell shaped and dark brown in colour. The flowers are yellow to white in colour. It does well in a vast of environmental conditions especially in cool climatic regions (Saulle *et al*., 2018). The local name for the plant changes from one community to the other. "Mubau" is the local name for *Eucaliptus saligna* in Meru community of Kenya (Adongo, 2012). The plant is widely cultivated to produce timber, paper, volatile oils and

for both perfume and pharmaceutical industries (Barbosa *et al*., 2016). Several phytochemicals that are active pharmacologically have been identified from *Eucalyptus saligna* extract. Because of the presence of bioactive phytochemical compounds like the tannin, saponin, alkaloids, terpenoids, flavonoids and glycosides *Eucalyptus saligna* is associated with antifungal, antidiabetic, antimicrobial and antioxidant activities. Different communities have used the plant in management of different health related issues. The plants have been used traditionally in various communities in management of burns, lung diseases, rheumatism and infections affecting the upper respiratory tract (Ghisalberti, 1996). The local communities of Meru in Kenya are traditionally using *Eucalyptus saligna* herbal products in management of pain, fever, measles and chicken pox (Adongo, 2012).

Figure 2.2: *Eucalyptus saligna* captured in Meru County, Kenya

2.9.3 *Senna didymobotyra*

Senna didymobotyra is locally known as “Murao” in Meru community of Kenya and

belongs to the Caesalpinoideae family (Tabuti, 2007). The plant can grow to attain height averaging to 60 cm (Hutchings *et al.,* 1996). It grows to form bushes in wet areas especially on the banks of the streams or rivers and shores of the lakes (Nyamwamu *et al*., 2015). Several phytochemical compounds associated with various pharmacological activities have been isolated and identified from the *Senna didymobotyra* extracts (Nagappan, 2012). These include tannins, saponins, alkaloids, flavonoids, anthraquinones, terpenoids and steroids (Nyamwamu *et al*., 2015). The plant have been used to treat malaria and fever (Nagappan,2012); hypertension and inflammation (Tabuti, 2007); venereal diseases and skeletal muscle abscesis (Kamatenesi-Mugisha, 2004); bacterial infections (Reddy *et al*., 2010; Korir *et al*., 2012); abdominal pain (Singh *et al*., 2003); diarrhea (Sunarno, 1997); East coast fever in livestocks (Njoroge and Bussmann, 2007); swelling and fever in Meru community of Kenya (Tabuti, 2007).

Figure 2.3: *Senna didymobotyra* picture captured in Meru County, Kenya

2.9.4 *Bidens pilosa*

Bidens pilosa Linn. Var. *Radiata* (Asteraceae) is a botanical name for the Spanish needle and it is distributed in both subtropical and tropical countries. It is a weed plant commonly found in Japan, Africa, China and America. It is believed that it originated from South America and is widely by various communities as food and for medicinal sources (Grubben and Denton, 2004). It is commonly known as ghost needle weed, cobbler's pegs, pitchforks, broomstick or grab a leg weed (Bartolome *et al*., 2013). The plant can grow to attain an average height of 1.5 meter but in very good conditions it can attain a height of 2.0 metres. The plant is slender, erect branching herb with a life span of less than one year. The plant mostly grows as weed in tea, cassava, coffee, vegetables, maize, pasture, cotton, rubber, papaya, rice, citrus, sorghum and tobacco fields (Connelly, 2009). The biochemical activities of *Bidens pilosa* are directly associated with phytochemical compounds present (Adebayo *et al*., 2017).

Bidens pilosa are associated with anti-hypertensive, hepatoprotective, anti-hyperglycemic, antiulcerogenic, anti-leukemic, antipyretic, anticancer, anti-virus, antibiotic, anti-rheumatic and diuretic activities (Chianga *et al*., 2004; Chianga *et al*., 2007). It is traditionally used by various communities as a remedy for inflammatory diseases like hepatitis, ulceration, laryngitis and also in management of pain especially in cases of headache (Abajo *et al*., 2004). Bidens pilosa juice is also used traditionally to manage snakebites, lung diseases, fever, accident shock, wounds, insect sting and eye infections (Khan *et al*., 2001).

The *B.pilosa* leaves are also used locally in management of threatened abortion,

toothache, jaundice, cough, fever and intestinal helminthiasis. The leaves and flowers concortion can also be used to in management of flank pain and the whole plant is very useful in management of febrile convulsion and treatment of bone fractures. The flowers part only of the plant can be used to treat diarrhoea, stomach upset resulting from food poisoning and also dysentery. In some communities in Peru, the plant is widely accepted in management of diabetes, angina, dysmenorrhea, dysentery, dropsy, body water retention, hepatitis and edema. Various communities in Cameroon use the plant in management of high blood pressure and also as smooth muscle relaxant (Dimo *et al*., 2001). The plant juice is also used by communities found in the islands of the Middle America to treat ulcers, body water retention and choleritis. In various communities of Africa, the plant is widely used in treatment of angina, edema, conjunctivitis, diabetes, influenza and diarrhea (Dimo *et al*., 2002). Various communities found in tropical Africa use the plant in treatment of influenza, otitis, dysentery and ophthalmia (Dimo *et al*., 1998).

“Mucege / Munyugunyugu” is the local name for *Bidens pilosa* in Meru community of Kenya (Benhura and Chitsiku, 1997). The plants is commonly used in Meru community for management of wounds infections, fever, common cold/flu, jaundice (Nguelefack *et al.,* 2005); diarrhoea, conjunctivitis, snake bite, hepatitis (Sundararajan *et al.,* 2006); small pox and anaemia (Bartolome *et al*., 2013).

Figure 2.4: *Bidens pilosa* picture captured in Meru County, Kenya

2.9.5 *Mangifera indica*

Mangifera indica is evergreen, with a crown which is dome shaped, grows to average height of 22.5 metres and is classified in Anacardiaceae family. "Muembe" is the local name for *Mangifera indica* in Meru community. The plants species is found both in tropical and subtropical countries but the origin can be traced in Asia (Ross, 1999). The plant is cultivated mainly for food productions but some communities are using different plant parts such as the roots, stem barks or the leaves for management of various diseases affecting both human and livestock (Stoiloval *et al*., 2005). Its fruit is very rich in vitaminsespecially vitamin A, B and C. It also contains carbohydrates, proteins, minerals and fats (Wauters *et al.,* 1995). The pharmacologically active compounds contained in the fruits are maniferol,

mangiferic acid, mangiferene and resinol (Dweck, 2001); the plants leaves are rich in glucoside mangiferine (Wauters *et al.,* 1995); while the stem bark contains approximately 18% tannin (Dweck, 2001). The plants is used in management of hypertension, rheumatism, pain, bronchitis, asthma, anaemia, wound infections (Shah *et al*., 2010); pneumonia and common cold in Meru community of Kenya (Stoilova *et al.,* 2005).

Figure 2.5: *Mangifera indica* picture captured in Meru County, Kenya

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant sample collection

Plant samples involved were all collected from Kanjagi sub-location, Kirima-Itune location, Giaki division, Imenti North sub-county, Meru County, Kenya. The plants were collected from Meru County because they are locally used by the community in management of fever, pyrexia and inflammation. All plant samples were growing at an altitude ranging from 1500 to 1520 M above the sea level. Random sampling method was used to collect various plants samples in the study. The samples were well labeled and given a special identification number after collection. The study area map was generated using ArcMap GIS 10.5 (ESRI Company) software (Figure 3.1). The location co-ordinates where the plants samples were collected from were established by a use of a hand-held GPS machine (Garmin etrex 20X), (Table 3.1). The coordinates obtained were used to identify the specific location of plant sample collection in Google Earth through digitization and exportation of the coordinates to KMZ (keyhole markup language zipped) and ArcMap GIS 10.5 (ESRI Company) software (Figure 3.1).

The plants sampled were *Prunus africana, Eucalyptus saligna, Senna didymobotyra, Bidens pilosa* and *Mangifera indica*. The plants botanical identity was confirmed at the National Museums of Kenya herbarium by a plant taxonomist and assigned a voucher numbers: GMG - V1 for *Senna didymobotyra,* GMG – V2 for *Mangifera indica,* GMG – V3 for *Eucalyptus saligna,* GMG - V4 for *Bidens pilosa* and GMG – V5 for *Prunus africana*. The plant parts collected were: stalks for *Bidens pilosa,* stem bark for

Mangifera indica and *Prunus africana*, leaves for *Eucalyptus saligna* and *Senna didymobotyra*. The plant samples were collected based on plant parts used by local herbalists in Meru community in management of fever, pain and inflammation. Ten local herbalists from Meru County were involved in selecting the plants used traditionally by Meru community in management of pain, fever and inflammation.

The collected plants sample materials were well packed in a khaki paper pockets and immediately transported for processing at Kenyatta University. At Kenyatta University, the specimens were cleaned in running water before drying them under a shade in the Biochemistry laboratories. Plant sample extraction, qualitative plants sample screening, anti-inflammatory, analgesic, antipyretic and toxicity tests were excuted at animal experimental laboratory, Department of Biochemistry, Microbiology and Biotechnology at Kenyatta University. The quantititave plant sample screening was carried out at the Kenya Government chemist. The plant samples were collected from *Prunus africana, Eucalyptus saligna, Senna didymobotyra, Bidens pilosa* and *Mangifera indica* plants species commonly used herbalists in Meru community to manage pain, hyperthemia and inflammation associated with headache, stomachache, chest pain, arthritis, toothache, typhoid fever and malaria.

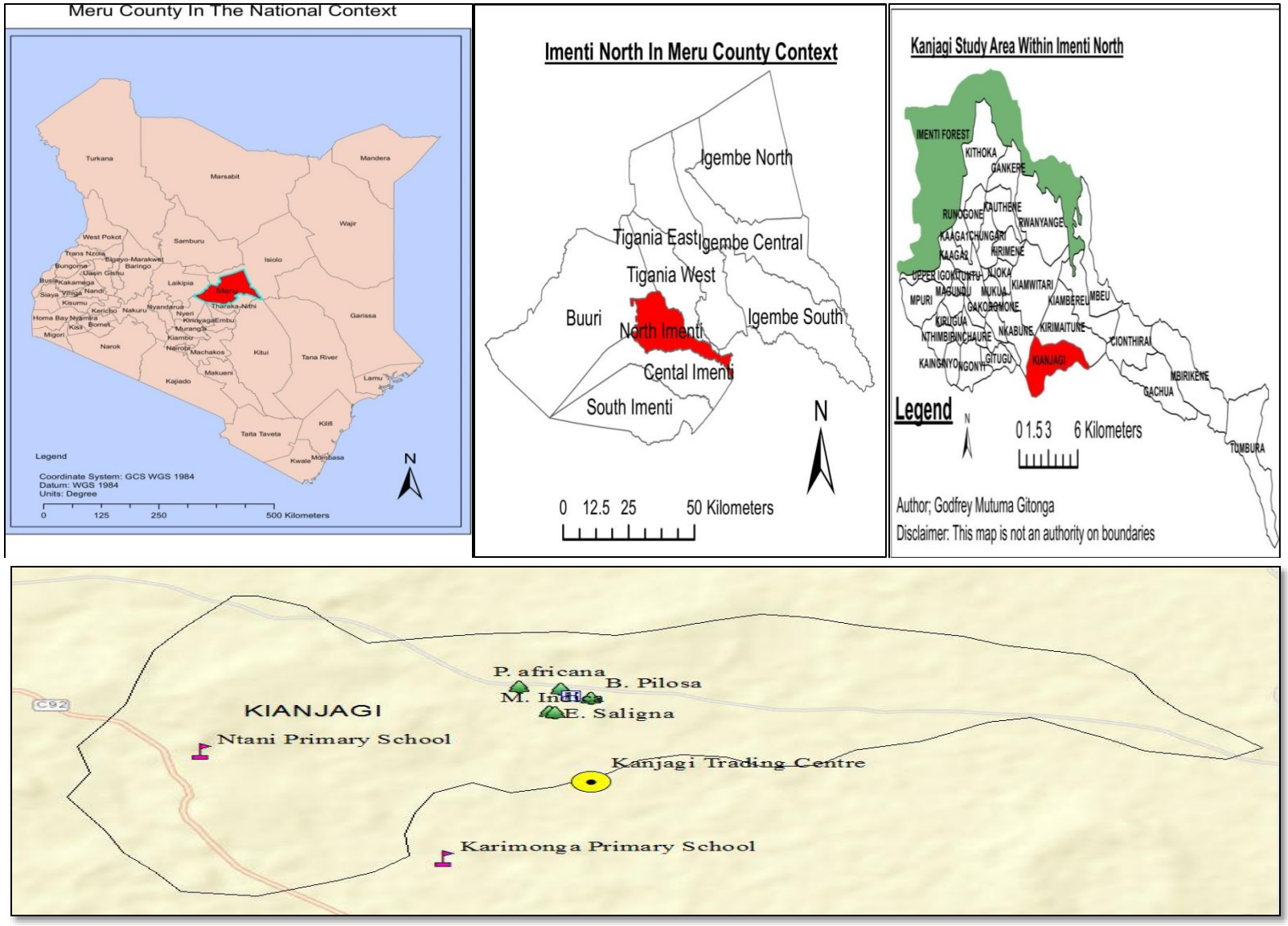

Figure 3.1: Map showing Kanjagi, Nyaki East, Imenti North Sub – County, Meru County, Kenya

Table 3.1: Coordinates of sites of plant sample collection

Plant species	Latitude	Longitude
Prunus africana	0.017421^0	37.728795^0
Eucalyptus saligna	0.015006^0	37.730277^0
Senna. Didymobotyra	0.017195^0	37.730843^0
Bidens pilosa	0.016355^0	37.732398^0
Mangifera. Indica	0.014933^0	37.730601^0

3.2 Plant sample preparation and extraction

The plants samples in the Biochemistry laboratories were chopped and allowed to dry under a well-airated shelter for three weeks (21 days) until they were completely dry. The dry samples were then crushed using an electric mill (Zhengzhou Yize Machinery Co.,Ltd. Henan, China). The powdered plant samples were sieved to remove large particles. The powdered plant samples were extracted using dichloromethane and methanol in a ratio of 1 gram of plant sample to 20 mL of the solvent. The plant sample was extracted with constant stirring for 48 hours at a room temperature. This was done by Filtration of plant extract was carried out using Whatman's paper #1. Filtrate was concentrated using rotor evaporator (Buchi Rotavapor R – 200, Labequip Ltd, Ontario, Canada) in a regulated temperature of 40^0C. The collected plant concentrate was stored in stoppered containers for further use at a temperatures averaging to 4^0C.

3.3 Qualitative phytochemical screening of plant extracts

Standard qualitative tests established presence or absence of various phytochemicals in dichloromethane and methanolic plant extracts. The phytochemical screening procedures for the plant sample extracts were carried out following the modified procedures described by Yadav and Agaewala, 2011; Sofowara, 1993; Trease and Evans, 1989 and Harborne, 1973.

3.3.1 Tannins test

To test for tannins, 1 gram of the plant sample extract was placed in a test tube and recostituented by use of respective extraction solvent to make 1ml. 2ml of 5% ferric chloride was then added to the test tube. Formation of greenish black colour was an indicator of the presence of tannins (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.2 Saponins test

To test for saponins, 1 gram of the plant sample extract was placed in a graduated cylinder and recostitued by use of respective extraction solvent to make 1 ml. 2 ml of distilled water was added to the graduated cylinder. This was shaken for 15 minutes; formation of 1 cm layer of foam was an indicator of the presence of Saponins (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.3 Flavonoids test

To test for flavonoids, 5 ml of dilute ammonia solution was placed in a test tube. 1ml of aqueous plant extract filtrate was added. This was followed by addition of concentrated Sulphuric acid. Yellow color change was an indicator for the presence of flavonoids (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.4 Alkaloids test

To test for alkaloids, 1 g of plant sample extract was reconstituted by respective solvent to make 2 ml in a test tube. Approximately 2 ml of concentrated hydrochloric acid was

added. Few drops of Mayer's reagent were also added. Formation ofgreen colour was an indicator of alkaloids presence (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.5 Betacyanin and anthocyanin test

Plant sample extract (1 g) was placed in a test tube and reconstituted with respective solvent to make 1ml. Approximately, 1ml of 2M sodium hydroxide was then added. This was heated at 100^0C for 5 minutes. Formation of yellow colour was an indicator of betacyanin and anthocyanin presence in the plant sample (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.6 Quinones test

To test for quinones, 1 g of the plant sample extract was reconstituted by use of respective extraction solvent to make 1ml. The plant extract was placed in a test tube and 1ml of concentrated Sulphuric acid was added. Formation of a red colour was an indicator for the presence of quinones (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.7 Glycosides test

To test for glycosides, 1 g of plant extract sample was recostitued by use of respective extraction solvent to make up 2 ml. The plant extract sample (2 ml) was placed in a test tube and 10% of the ammonia solution and 3 ml of chloroform was added. Formation of pink colour was an indicator for glycosides present (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.8 Cardiac glycosides test

To test for cardiac glycosides, 0.5ml of the plant sample extract reconstituted from 0.5 g of the plant sample extract by respective solvent was added in a test tube and few drops of 5% ferric chloride and 2 ml of glacial acetic acid was added. Brown ring at interface is a sign for cardiac glycoside the presence (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.9 Terpenoids test

To test for the presence of terpenoids, 0.5 ml of the plant sample extract reconstituted from 0.5 g of the plant sample by respective solvent was placed in a test tube. 2 ml of chloroform was added followed by careful addition of concentrated Sulphuric acid. Red brown colour formation at interface was an indicator of terpenoids presence (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.10 Triterpenoids test

To test for the presence of triterpenoids, 1.5 ml of the plant extract reconstituted from 0.5g of the plant sample extract by respective extraction solvent was placed in a test tube. 1 ml of Libermann-Buchard reagent was then added to the plant extract sample. Formation of blue green colour was an indicator of triterpenoids presence (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.11 Phenols testing

To test for the presence of phenols, 1 ml of the plant extract reconstituted from 0.5 g of

the plant sample extract by respective extraction solvent was placed in a test tube. 2ml of distilled water was added. This was followed by addition of few drops of 10% ferric chloride. Formation of blue black colour was an indicator for the presence of phenols (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.12 Coumarins test

To test for the presence of coumarins, 1ml of the plant extract reconstituted from 0.5g of the plant sample by respective extraction solvent was placed in a test tube. 1ml of 10% sodium hydroxide solution was then added. Formation of yellow colour indicated the coumarins presence (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.13 Steroids test

To test for the presence of steroids, 1ml of the plant sample extract reconstituted from 0.5g of the plant sample by respective extraction solvent was placed in a test tube. 5 ml of chloroform was added to the plant sample and the filtrate was collected. 2 ml of the filtrate was placed in a test tube and 2 ml of Sulphuric acid was added. This was followed by addition of 2 ml of acetic anhydrite. Colour change from violet to green or blue was an indicator for the presence ofsteroids (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.3.14 Acids test

To test for the presence of acids, 1 ml of the plant sample extract reconstituted from 0.5g

of the plant sample by respective extraction solvent was placed in a test tube and a solution of sodium bicarbonate was added. Effervescence formation was an indicator for the presence of acids (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993; Yadav and Agaewala, 2011).

3.4 Quantitative evaluation of the phytochemicals

The standard quantitative methods were used in order to evaluate the amount of secondary metabolites like alkaloid, flavonoids, saponins, steroids, tannins and phenolic compounds in the plant extracts.

3.4.1 Quantitative evaluation of phenolic compounds

Gallic acid and Folin-Ciocalteau reagents were used to determine the total phenolic compounds in the plant sample extract according to the standards by Rasineni *et al*. (2008). Plant sample extracts approximately 500 mg were homogenized using 10 ml of n – hexane. Centrifuged for 20 minutes at 10,000 revolutions per minute. Supernatant was processed to determine total quantity of phenolic compound in the plant samples. To determine the total phenolic compounds, approximately 2.5 ml supernatant added to a test tube and before an addition of 0.5 ml of the 2N Folin-Ciocalteau reagent. This was followed by addition 2 ml 10% sodium bicarbonate in ethanol. This was incubated at 20^0C for 5 minute. Absorbance was established at wavelength of 650 nm in triplicates. Standard gallic acid stock solution was prepared by adding 100 mg gallic acid in 100 ml distilled water. The stock solution were diluted to 0.25, 0.5, 1, 2, 4, 8, 16, 32 mg/100 ml. 0.25 ml of 2N Folin-Ciocalteau and 10% w/v sodium carbonate solution was added to

each of each of the 2.5 ml of the serially diluted standard and incubated for 5 minutes. The blank solution used inthis test was a mixture of 10% sodium carbonate, distilled water and 2N Folin-Ciocalteau. UV-VIS spectro-photometer (UV-1700 Pharmaspec, UV-VIS Spectrophotometer, Shimadzu Japan), was used to read the absorbance. The quantitative total phenolic contentwas expressed as mg/g dry weight gallic acid equivalent.

3.4.2 Quantitative evaluation of flavonoids

Kumaran and Karunakaran (2007) method with slight modification was employed to quantify total flavonoids contents. To quantify total flavonoid content, 5 ml of the 2% aluminium trichloride mixed with alcohol (methanol) was mixed with the equal amount of the extract. The mixture was incubated for one hour and the absorbance was determined at 420 nm. The blank sample did not contain aluminium trichloride. Total flavonoid in the samples was determined using standard curve of pyrocatechol with a range of 0.25 to 16 mg/ml. The results were expressed as mg/g pyrocatechol equivalent.

3.4.3 Quantitative evaluation of alkaloids

The alkaloid quantity in the plant samples were determined by simple modification of a method by Edeoga *et al*. (2005). 100 ml acetic acid (20% in ethanol) was used to extract 2.5 g of the plant samples. The plant sample solutions in acetic acid were allowed to stand for 4 hours. Filtrate was obtained and concentrated to attain 25 ml. Addition of drop by drop of concentrated ammonium hydroxide to concentrated filtrate resulted to formation precipitate. The solution was left to stand for sometime to allow settlement of the precipitates which were collected and then washed using dilute ammonium hydroxide after which the precipitates were collected through filtration. The pellets were collected

throughfiltration, dried and then weighed (Edeoga *et al.*, 2005).

3.4.4 Quantitative evaluation of Saponins

The Saponins quantity in the plant samples were determined by simple modification of a method by Edeoga *et al*. (2005). 100 ml (20% aqueous ethanol) was added to a test tube containing 10 g of the plant samples. The mixture was placed in a water bath shaker and the temperature adjusted for 4 hours at 55^0C. Whatmann filter paper No 540 was used to filter the mixture to obtain the filtrate. Water bath was used to concentrate the filtrate by adjusting the temperature to 90^0C to obtain 40 ml of the filtrated content. The concentrate obtained was placed in separating funnel to which 10 ml of diethyl ether was added. The mixture was vigorously shaken before aqueous layer formed was recovered. Ether layer was not recovered. The n–butanol was added to the aqueous layer and the mixture was washed twice in the separating funnel using 10 ml of 5% of aqueous sodium chloride. The upper part was retained after which it was heated to evaporation using a water bath and then dried to obtain a constant weight in an oven (Edeoga *et al*., 2005).

3.4.5 Quantitative evaluation of steroid

1 ml of plant extract sample was placed in a test tube and this was followed by addition of (4N, 2ml) Sulphuric acid and (0.5% w/v, 2ml) iron (III) chloride. Potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml) was added to this mixture. The mixture was incubated in a water bath with constant shaking at temperature of 70^0C for 30 minutes. The absorbance was taken at 780 nm to determine the quantity of steroid in the plant sample.

3.4.6 Quantitative evaluation of tannins

To obtain the quantitative amount of tannin in the plant sample, 70% acetone was used to extract 2g of the plant samples three times. The extracted samples were centrifuged and the supernatant was removed. Aliquots were obtained for each plant extracts and the volume was adjusted to 3ml using distilled water. The solution was mixed with 1ml, 0.016M of $K_3Fe(CN)_6$ Potassium ferricyanide) after which 1ml, 0.02 M of $FeCl_3$ mixed with 0.10 M HCl was added and mixed thoroughly through vortexing. Vortexing was repeated and the tubes remained undisturbed for 15 minutes to allow the content to settle down. Water,1% gum Arabic and H_3PO_4 in the ratio of 3:1:1 was used to prepare the stabilizer. 5 ml of the stabilizer was added to the plant sample mixtures followed by vortexing. Absorbance against the blank was obtained at 700 nm. The standard graphical plot for the samples was obtained using different concentrations of gallic acid (Gurib–Fakim, 2006).

3.5 GC-MS analysis of plant sample extract

The crude plant extracts of methanol and DCM were re-extracted using DCM and ethyl acetate in the ratio of 1:1. After re-extraction, the crude was cleaned by soaking the sample in carbonated charcoal for one hour. The dried plant sample extract was filtered to obtained a clear colourless sample which was then dried by passing the sample through anhydrous Na_2SO_4 before it was loaded to the GC-MS. Combined gas chromatography and mass spectrometry analysis for both dichlomethane and methanolic extracted plant samples was carried out by a use of Agilent equipment 7890A installed with a software by Mass Hunter to facilitate identification of the volatile compounds from the plant sample extract. The equipment comprised an inert capillary tube with a diameter of 30 x 0.25

mm ID x 0.2 μm film; Helium flowing at 1.0 ml/min was used as a carrier gas. The injector was operated at 250^{0}C and the oven temperature was increased from 50-300^{0}C gradually for 10 minutes. To identify the phytochemical components of the plant samples from the gas chromatography peak areas, NIST library data was used.

3.6 Antipyretic activity analysis

Antipyretic pharmacological activity of dichloromethane and methanol extracts in turpentine induced pyrexia on rats was evaluated based on the protocol given by Brito (1994) and Metowogo (2008). The rats were preferred in this activity because their size can allow collection of rectal temperature by inserting the clinical thermometer into their rectum. Experimental rats were divided into 6 groups. Each group was made up of 5 rats. The normal control group (Group I) was intraperitoneally administered with 0.05 ml of 10% dimethyl sulfoxide (DMSO). Pyrexia was induced through intraperitoneal administration of 0.05ml, 20% turpentine at a dose level of 20ml/Kg body weight.

The animals were observed for one hour and the body temperature change was noted. Animals with rectal temperature change greater than 0.8°C were included in this study. The animals in the group labeled Group II (negative control) were orally administerd with normal saline 2ml/Kg body weight after pyrexia induction. The positive control animals (Group III) were intraperitoneally administered with aspirin at a dose level of 100mg/Kg body weight after fever induction using 20% turpentine. The animals in the experimental test groups A, B and C were intraperitoneally administered with 0.25 ml either of methanol or DCM plant extracts at a dose level range of 50, 100 or 150 mg/Kg

body weight after fever induction (Table 3.2). All plant sample extracts were prepared for administration by dissolving them in DMSO.

Table 3.2: Antipyretic activities and treatment evaluation protocol

Animal groups	**Treatment**
Normal test	10% DMSO (0.05 ml)
Negative test	Distilled turpentine + 10% DMSO
Positive test	Distilled turpentine + 100 mg/kg Aspirin
Experiment test A	Distilled turpentine + 50 mg/kg Plant extracts
Experiment test B	Distilled turpentine + 100 mg/kg Plant extract
Experiment test C	20% distilled turpentine + 150 mg/kg Plant extract

Key: 20% turpentine

The rectal temperature of the rats was collected after insertion (3 cm) of lubricated digital clinical thermometer into the rectum of the animal model. The Wistar albino rat body temperature was recorded over the first hour prior to fever induction and recorded at an interval of 20 minutes. The animals with a rectal temperature increase of at least 0.8^0C after fever induction were included in the antipyretic study. The body temperature of each animal in group was recorded one hour after pyrexia induction. The rectal body temperature of the rat before and after treatment with both conventional antipyretic drug (100 mg/Kg body weight) and the plant extracts (50, 100, 150 mg/Kg body weight) were recorded at an interval of one hour for four hours. This was compared and the rectal temperature percentage change using the formula described by Hukkeri *et al*. (2006) and Ray (2006).

$$\%\ \text{change in rectal temperature} = \frac{B - C^n}{B}\ X\ 100$$

Where;

B = Rectal temperature after one hour of fever induction using turpentine.

C^n = Rectal temperature after drug administration or plant extracts.

3.7 Anti – inflammatory activity analysis

Methanol and dichloromethane anti-inflammatory activity of selected plant species extract was evaluated according to experimental by Akah and Nwambie (1994). Normal control experimental group was administered with 0.05 ml of 10% DMSO intraperitoneally. The negative control group mice were intraperitoneally injected with 0.05 ml of 10% DMSO and 0.05 ml of 1% fresh carrageenan. The positive control test group mice received 0.05ml of 1% fresh carrageenan and Diclofenac. The test groups labeled A, B and C were intraperitoneally injected with 50, 100 and 150 mg/kg body weight doses respectively of methanolic and dichlomethanolic extract of the selected plants and 0.05ml suspension of 1% fresh carrageenan. The design summary is indicated Table 3.3.

Table 3.3: Protocol for evaluating the anti-inflammatory activity of the selected plant extracts

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

Key: bw- body weight; 1% carrageenan (0.05ml)

Inflammation was induced 1 hour after the administration of the conventional antiinflammatory drugs or after the administration of either DCM or methanol plant extract of the selected plants. This was to allow the pharmacologically active compounds

in either the conventional drug or the plants extracts action to take place. The injected paw linear circumference was determined in millimeters (mm) after a periodical interval of one hour after carrageenan administration by use of digital vernier caliper (IP67, Qingdao Tide Machine Tool Supply Co Ltd., Shadong, China). Paw circumference change after carrageenan administration of carrageenan indicated the level of inflammation. Extracts anti-inflammatory activity was expressed in terms of percentage paw edema inhibition calculated using an equation described by Jia *et al*. (2003).

$$\%\ \text{Paw edema inhibition} = \frac{C^t - T^t}{C^t}\ X\ 100$$

Where:-

Ct = Paw diameter after 1st hour of carrageenan injection (control)

Tt = Paw diameter post treatment

3.8 Analgesic activity analysis

Experimental test animals were grouped and treated as demostrated on table below (Table 3.4). The experimental animals were grouped into six groups each with 5 animals. Normal control experimental group was administered with 0.05 ml of 10% DMSO intraperitoneally. The negative control group mice were intraperitoneally injected with 0.05 ml of 10% DMSO and 0.5 ml of 20% formalin. The positive control test group mice received 0.05ml of 20% formalin and Diclofenac. The test groups labeled A, B and C were intraperitoneally injected with 50, 100 and 150 mg/kg body weight doses respectively of methanolic and dichlomethanolic extract of the selected plants and 0.05ml suspension of 20% formalin. One hour after treatment, nociception in mice in all groups

apart from normal control group was induced through sub-cutaneous administration of formalin (0.05 ml) to left hind dorsal paw of the mice at concentration level of 2.5% v/v.

Table 3.4: Protocol for evaluating the antinociceptive activities of dichloromethane and methanolic extract of the selected plants in mice model

Animal groups	Treatment
Normal test	10% DMSO
Negative test	0.05 ml Formalin 2.5% v/v
Positive test	Diclofenac (100 mg/kg bw) + Formalin
Test group A	Plant extract (50 mg/kg bw) + Formalin
Test group B	Plant extract (100 mg/kg bw) + Formalin
Test group C	Plant extract (150 mg/kg bw)+ Formalin

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

Before the start of experiment, individual mouse was placed for 30 minutes in a transparent glass chamber. Mice were held gently before injecting them with formalin and they were returned in the glass chamber for observation after pain induction. The paw licking time on injected paw was observed and recorded and was used as an indicator parameter of pain as described by Tjolsen *et al*. (1992). The two distinguishing (early and late phases) periods of intensive biting/licking of the hind paw were identified and the time spent recorded separately. The first five minutes after administration of formalin marked the early phase while the 15^{th} -30^{th} minutes after pain induction using formalin marked the late phase. The percentage change in paw licking inhibition was calculated as per the formula described by Dubuisson and Dennis (1977).

$$\%\ \text{licking time inhibition} = \frac{C-T}{T}\ X\ 100$$

Where:-

C = Normal control values

T = The experimental test value

3.9 Toxicity testing

Adult healthy male and female Wistar rats aged 5-6 weeks old and weighing 120-150 grams were used to carry out the toxicity test. Toxicity test was established by adopting the protocol described by Ngari *et al.* (2013) with minimal modifications. Wistar rats between 5-6 weeks old and weighing approximately 120 – 150 g were divided into eleven groups each with five animals. The experimental groups were orally administered with 200 mg/Kg doses of either dichlomethane or methanol extract of *S. didymobotyra, E. saligna, P. africana, M. indica* and *B. pilosa*. The eleventh group animals acted as normal control test group were administered with normal saline. All the groups were treated and observed for four weeks (28 days). This is summarized in the table below (Table 3.5).

The body weight of all the animals involved in the study was determined and recorded before and after every 7 days after the commencement of the study for the entire experimental period. On the 28^{th} day, blood for biochemical parameter testing was collected by bleeding the animals from the tail and from the cardiacpuncture and placed in coagulant lacking vials and blood sample for hematological studies was placed in a heparinized vacuatainers. The rats were euthanized by placing them in a desiccator jar containing chloroform soaked cotton wool. The liver, testes, kidneys, spleen,lungs, heart and the brain were harvested from the euthanized rats and weighed before fixing them in 10% formalin.

Table 3. 5: Protocol for evaluating the toxicity of extract of the selected plants using rats

Animal groups	Treatment (200 mg/kg bw)
I (Normal control)	Normal saline
II (Experimental A)	MeOH *M. indica* stem bark extract
III (Experimental B)	DCM *M. indica* stem bark extract
IV (Experimental C)	MeOH *S. didymobotyra* leaves extract
V (Experimental D)	DCM *S. didymobotyra* leaves extract
VI (Experimental E)	MeOH *E. saligna* leaves extract
VII (Experimental F)	DCM *E. saligna* leaves extract
VII (Experimental G)	MeOH *B. pilosa* stalks extract
IX (Experimental H)	DCM *B. pilosa* stalks extract
X (Experimental I)	MeOH *P. africana* stem bark extract
XI (Experimental J)	DCM *P. africana* stem bark extract

Key: bw-body weight; DCM – dichloromethane; MeOH - Methanol

3.9.1 Hematological analysis

Hematological parameters assays were carried out by use of an automated hematological analyzer. Hematological parameters determined included the red blood cells (RBC) count, red cell distribution width (RDW), White blood cells (WBC) count, hemoglobin level (Hb), packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), plateletcrit (PCT) and platelet count.

3.9.2 Biochemical analysis

The blood samples were collected in biochemical vacuatainers (red tubes) and allowed to coagulate. The blood samples were centrifuged to obtain serum at 3000RPM (revolutions per minute) for 10 minutes. The obtained serum was stored at -20^{0}C in a refrigerator for further analysisof both renal and liver functioning tests using fully automated chemistry analyzer (Hycel Lisa 300). The renal functioning parameters determined in this study

were the levels of blood urea nitrogen (BUN) and serum creatinine. The livers function test parameters determined in this study were the levels of gamma glutamate aminotransferase (γGT), direct bilirubin, total bilirubin, total protein, albumin levels, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

3.10 Data analysis

The data collected was recorded in an excel sheet. The descriptive statistics were generated by use Minitab version 17 to obtain the means and standard errors. The data was subjected to analysis of variance (ANOVA) test to compare the levels of significance between different test groups at a $P < 0.01$. This was followed by Tukey's post hoc test to indicate the significance different between the means of different study groups. The data on antipretic and antiinflammation activity of different test groups was subjected to repeated measure analysis of variance (ANOVA) test to compare the levels of significance at different time intervals. The phytocompounds present in the plants were generally proposed in relation to their fragmentation behavior in reference to their spectra as published in the National Institute of Standards and Technology (NIST) library-MS database. The hydrocarbon range of C5 - C32 were used to determine the retention time of the proposed phytocompounds. To identify the phytocompounds of the spectra, above 60% match with the library-MS database was required. The phytocompounds name, molecular formula and molecular mass of the plant extracts were determined. The peak area normalization percentage was used to determine the relative concentration of the phytocompounds. All quantitative and qualitative data was

analyzed and results were presented well by use of either graphs or tables.

CHAPTER FOUR

RESULTS

4.1 Methanolic and DCM crude plants extracts yields

Prunus africana stem bark methanolic extraction yielded a dark brownish paste (65 mg/g dry weight). DCM stem bark extraction of *Prunus africana* yielded a dark brownish paste (59 mg/g dry weight). *Eucalyptus saligna* methanolic leaves extraction yielded a brownish paste (164 mg/g dry weight). DCM leaves of *Eucalyptus saligna* extraction yielded a brownish paste (141 mg/g dry weight). *Senna didymobotyra* methanolic leaves extraction yielded a dark green gum (86 mg/g dry weight). DCM leaves extraction of *Senna didymobotyra* yielded a dark green gum (74 mg/g dry weight). *Bidens pilosa* methanolic stalks extraction yielded a blackish paste (75 mg/g dry weight). DCM stalks extraction of *Bidens pilosa* yielded a blackish paste (64 mg/g dry weight). *Mangifera indica* methanolic stem bark extraction yielded a dark brownish paste (95 mg/g dry weight) and the DCM stalks extraction yielded a dark brown paste (86 mg/g dry weight). The crude plants extract in both solvents is as indicated in table 4.1.

Table 4.1: Methanolic and DCM crude plants extract yields (mg/g dry weight)

	Solvent		
Plant parts	**DCM**	**MeOH**	**Extract state**
Prunus africana stem bark	59	65	Dark brownish paste
Eucalyptus saligna leaves	164	141	Brownish paste
Senna didymobotyra Leaves	74	86	Dark green gum
Bidens pilosa stalks	64	75	Blackish paste
Mangifera indica stembark	86	95	Dark brown paste

DCM= dichloromethane and MeOH = methanol

4.2 Qualitative phytochemical analysis

The phytochemical analysis of *P. Africana, E. saligna, S. didymobotyra, B. pilosa* and *M. indica* crude extracts in different solvents is summarized in table 4.2. The result indicates the presence of various medically important compounds from different parts of the plants involved. The phytochemical compound analysis of both DCM and Methanolic extracts of the plants parts of *P. Africana, E. saligna S. didymobotyra, B. pilosa* and *M. indica* indicated the presence of various phytochemicals such as flavonoids, terpenoids, Saponins, alkaloids, phenolics, steroids, tannins, anthocyanin and betacyanin, quinones, coumarins, glycosides and cardiac glycosides as indicated in table 4.2. Tannins, terpenoids, phenol, alkaloids, steroid and acids tested positive in both methanolic and dichlomethane extracts of all the plants. It is only anthocyanin and betacyanin phytochemical compound which tested negative in all the plant extracts (Table 4.2).

4.3 Quantitative phytochemical compounds in plants extracts

The quantitative analysis of phytochemical compounds of both DCM and Methanolic extracts of the plants parts of *P. Africana, E. saligna, S. didymobotyra, B. pilosa* and *M. indica* indicated variance in phytochemical compound quantities from one plant to the other. The concentration of the phytochemical compound also varied in relation to the solvent used in the process phytochemical extraction as indicated in table 4.3.

Table 4.2: Qualitative screening of the phytochemicals in plants

	Plant samples									
	Prunus africana		***Eucalyptus saligna***		***Senna didymobotyra***		***Bidens pilosa***		***Mangifera indica***	
Plant part	**Barks**		**Leaves**		**Leaves**		**Stalks**		**Barks**	
Phytochemicals	**DCM**	**MeOH**	**DCM**	**MeOH**	**DCM**	**MeOH**	**DCM**	**MeOH**	**DCM**	**MeOH**
Carbohydrates	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+
Anthocyanin and Betacyanin	-	-	-	-	-	-	-	-	-	-
Quinones	+	+	-	-	+	+	-	+	+	+
Glycosides	-	+	+	+	-	+	-	+	-	-
Cardiac glycoside	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	-	+	+	+	+	+	+
Triterpenoids	+	+	+	+	-	-	+	-	-	_
Phenols	+	+	+	+	+	+	+	+	+	+
Coumarins	+	-	-	-	-	-	+	-	-	+
Steroids	+	+	+	+	+	+	+	+	+	+
Acids	+	+	+	+	+	+	+	+	+	+

KEY: + = present, - = not present.

Table 4.3: Quantitative analysis of phytochemical in plants

Plant parts	Class of phytochemicals and amounts in medicinal plants in mg/Kg						
	Solvent	**Total phenols**	**Flavonoids**	**Alkaloids**	**Saponins**	**Steroids**	**Tannins**
Prunus africana barks	DCM	3.98 ± 0.54^{e}	2.98 ± 0.54^{e}	10.98 ± 0.54^{e}	4.98 ± 0.54^{bc}	3.98 ± 0.54^{ab}	2.98 ± 0.54^{d}
	MeOH	4.11 ± 0.32^{de}	3.11 ± 0.32^{de}	13.11 ± 0.32^{de}	5.11 ± 0.32^{b}	5.01 ± 0.32^{a}	3.11 ± 0.32^{cd}
Eucalyptus saligna leaves	DCM	4.76 ± 0.65^{de}	3.76 ± 0.65^{de}	13.76 ± 0.65^{cde}	3.76 ± 0.65^{cd}	1.76 ± 0.65^{de}	2.76 ± 0.65^{d}
	MeOH	5.02 ± 0.22^{cde}	4.02 ± 0.22^{bcde}	16.02 ± 0.22^{cd}	3.02 ± 0.22^{d}	3.02 ± 0.22^{bcd}	3.02 ± 0.22^{cd}
Senna didymobotyra leaves	DCM	2.58 ± 0.67^{f}	3.58±0.67de	16.58 ± 0.67^{cd}	3.78 ± 0.67^{cd}	1.58 ± 0.67^{e}	5.18 ± 0.67^{b}
	MeOH	3.87 ± 0.53^{ef}	3.87 ± 0.53^{cde}	17.87 ± 0.53^{c}	4.07 ± 0.53^{bcd}	1.87 ± 0.53^{de}	6.97 ± 0.53^{a}
Bidens pilosa stalks	DCM	6.34 ± 0.52^{bc}	5.34 ± 0.52^{ab}	23.21 ± 2.3^{b}	5.34 ± 0.52^{b}	2.34 ± 0.52^{cde}	5.34 ± 0.52^{b}
	MeOH	8.56 ± 0.45^{a}	5.56 ± 0.45^{a}	30.45 ± 3.7^{a}	7.56 ± 0.45^{a}	3.56 ± 0.45^{bc}	5.56 ± 0.45^{b}
Mangifera indica bark	DCM	5.32 ± 0.02^{cd}	4.32 ± 0.02^{abcd}	15.32 ± 0.02^{cd}	3.32 ± 0.02^{d}	1.32 ± 0.02^{e}	4.32 ± 0.02^{bc}
	MeOH	6.91 ± 0.21^{b}	4.91 ± 0.21^{abc}	17.91 ± 0.21^{c}	4.91 ± 0.21^{bc}	1.91 ± 0.21^{de}	4.91 ± 0.21^{b}
	F Value	42.66	11.10	45.94	24.62	20.93	28.38
	P Value	0.000	0.000	0.000	0.000	0.000	0.000

Quantities of phytochemicals are expressed as Mean ± SEM of the three replicas of each plant sample powder. Column statistical comparison are made and different superscripts indicates significant different. Analysis was done using one – way ANOVA and Turkey'stestt ($p < 0.05$).

4.3.1 Gas chromatography mass spectrometry analyses of plant extracts

The GC-MS analysis of plants sample extracts established the percentage peak area abundance of the identified phytocompounds of every sample. This relates to the quantitative amount of the phytocompounds presence in the plant sample. Retention time,molecular weight and molecular peak area abundance were established. Percentage peak area abundance indicates relative proportion of the phytocompounds present in different plants sample extracts (Tables 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 4.10, 4.11, 4.12 and 4.13; Figures 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9 and 4.10).

4.3.1.1 Compounds from DCM stem bark extract of *Prunus africana*

As indicated in table 4.4 and figure 4.1, the DCM stem bark extract of the *Prunus africana* contained 52 chemical compounds identified by NIST2011 database. The results indicate that this plant has diverse constituents of the chemical compounds which contribute to the observed bioassay activities. The chemical structure, molecular weight and their percentage abundance is as indicated in table 4.4. The peak area and retention time of each of the compound identified in the plant sample is as indicated in figure 4.1.

Table 4.4: Compounds present in DCM stalk extract of *Prunus africana*

Sr No	RT (Min)	Chemical Name	Chemical formula	Molecular weight	% Abundance
1	4.2300	2 – Butanone (KD)*	C_4H_8O	72.1057	0.374
2	4.7181	Styrene	C_8H_8	104.1491	1.043
3	4.8580	1, 3 – Cyclohexadiene, 1 – methyl – 4 – (1 – methylethyl) – (MT)	$C_{10}H_{16}$	136.2340	0.661
4	5.1523	3 – Carene (MT)	$C_{10}H_{16}$	136.23	1.249
5	5.3109	.alpha. – Pinene(MT)	$C_{10}H_{16}$	136.2340	1.078
6	5.5876	Camphene(MT)	$C_{10}H_{16}$	136.2340	
7	5.7420	Benzene, 1 – ethyl – 2 – methyl –(AH)*	C_9H_{12}	120.1916	0.382
8	5.8187	Benzaldehyde	C_7H_6O	106.1219	0.303
9	5.8788	Benzene – 1, 3, 5 – trimethyl –(AH)*	C_9H_{12}	120.1916	0.291
10	6.0176	Bicyclo[3. 1. 1] heptane, 6, 6 – dimethyl – 2 methylene-(1S)- (MT)	$C_{10}H_{16}$	136.2340	0.990
11	6.1586	Benzonitrile (AH)	C_7H_5N	103.1213	0.182
12	6.2166	Benzene, tert – butyl – (AH)*	$C_{10}H_{14}$	134.2182	0.457
13	6.2736	Decane*	$C_{10}H_{22}$	142.2817	0.365
14	6.3399	Benzofuran (F)	C_8H_6O	118.1326	0.146
15	6.4923	1H – Indene, 2, 3 – dihydro – 5 – methyl –(AH)	$C_{10}H_{12}$	132.2023	0.797
16	6.7431	Benzene, 1 – methyl – 3 – (1 – methylethyl) – (MT)	$C_{10}H_{14}$	134.2182	0.875
17	6.8073	Limonene (MT)	$C_{10}H_{16}$	136.2340	1.058
18	6.8726	Eucalyptol (MT)*	$C_{10}H_{18}O$	154.2493	1.006
19	7.0871	Benzene, 1, 2 – diethyl -*	$C_{10}H_{14}$	134.2182	0.458
20	7.1431	Benzene, 1 – methyl – 2 – propyl -*	$C_{10}H_{14}$	134.2182	0.395
21	7.2084	Decane, 4 – methyl – (AH)*	$C_{11}H_{24}$	156.3083	0.519
22	7.2364	Benzene, 1, 3 – diethyl -*	C_8H_{10}	106.1650	0.321
23	7.2436	Benzene, 1, 2, 3, 5 – tetramethyl – (AH)*	$C_{10}H_{14}$	134.2182	0.512
24	7.2436	Benzene, 1 – ethyl – 2, 4 – dimethyl -*	$C_{10}H_{14}$	134.2182	1.039
25	7.8353	Undecane*	$C_{11}H_{24}$	156.3083	0.386
26	8.3110	Bicyclo[2. 2. 1] heptan – 2 – ol, 1, 3, 3- trimethyl - *	$C_{10}H_{18}O$	154.2493	0.729
27	8.9328	Maleic hydrazide*	$C_4H_4N_2O_2$	112.0868	0.048

Sr No	RT (Min)	Chemical Name	Chemical formula	Molecular weight	% Abundance
28	9.2147	3 – Cyclohexen – 1 – ol, 4 – methyl – 1 (1 – methylethyl) – (MT)*	$C_{10}H_{18}O$	154.2493	0.967
29	9.3504	Dodecane (AH)	$C_{12}H_{26}$	170.3348	0.796
30	9.4489	3 – Cyclohexene – 1 – methanol, .alpha., .alpha. 4 – trimethyl – (MT)	$C_{10}H_{18}O$	154.2493	0.987
31	10.7940	Tridecane (HC)	$C_{13}H_{28}$	184.3614	0.728
32	10.8034	1, 3 – Benzodioxole, 5 – (2 – propenyl) -	$C_{10}H_{10}O_2$	162.1852	0.693
33	11.0821	Phenol, 2 – methyl – 5 – (1 – methylethyl) – (PC)*	$C_{10}H_{14}O$	150.2176	0.973
34	12.0563	1 – Tetradecanol	$C_{14}H_{30}O$	214.3874	0.646
35	12.1568	Tetradecane	$C_{14}H_{30}$	198.3880	0.816
36	12.8294	1,4–Methanoazulene, decahydro– 4, 8, 8 – trimethyl – 9 – methylene -, [1S – (1. Alpha., 3a. be (TTP)	$C_{15}H_{24}$	204.3511	0.975
37	13.7040	Phenol, 2, 4 – bis (1, 1 – dimethylethyl)- (PC)	$C_{14}H_{22}O$	206.32	0.879
38	14.5807	1 – Hexadecene	$C_{16}H_{32}$	224.4253	0.925
39	16.0938	Methyl tetradecanoate*	$C_{15}H_{30}O_2$	242.3975	0.473
40	16.8482	1 – Octadecanol	$C_{18}H_{38}O$	270.4937	0.719
41	17.6140	1, 2 – Benzenedicarboxylic acid, bis(2 – methylpropyl) ester (FAD)	$C_{16}H_{22}O_4$	278.3435	0.879
42	18.2255	Hexadecanoic acid, methyl ester (FAD)*	$C_{17}H_{34}O_2$	270.4507	0.729
43	18.5861	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.3435	0.142
44	18.8949	Hexadecanoic acid, ethyl ester (FAD)*	$C_{18}H_{36}O_2$	284.4772	0.891
45	18.9032	1 – hexadecanol	$C_{16}H_{34}O$	242.4406	0.563
46	19.8722	9, 12 – Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	294.4721	0.387
47	19.9323	9 – Octadecenoic acid (Z) -, methyl ester (FAD)	$C_{19}H_{36}O_2$	296.4879	0.564
48	20.1675	Octadecanoic acid, methyl ester (FAD)*	$C_{19}H_{38}O_2$	298.5038	0.486
49	22.4619	Hexanedioic acid, bis(2 – ethylhexyl) ester (FAD)*	$C_{22}H_{42}O_4$	370.5665	0.719
50	23.6982	Bis(2 – ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.5561	0.492
51	26.9233	Squalene (TTP)*	$C_{30}H_{50}$	410.7180	1.598
52	11.0821	Thymol (MT)*	$C_{10}H_{14}O$	150.2176	1.768

Legend: AH stands for Aromatic hydrocarbon, FAD for Fatty acid derivatives, PC for phenolic compounds, TTP for triterpenoids, MT for monoterpenoids, KD for ketone derivatives, PS for phytosterols and SD for sugar derivatives. * indicates that the phytochemical is absent in MeOH extract of *Prunus africana*.

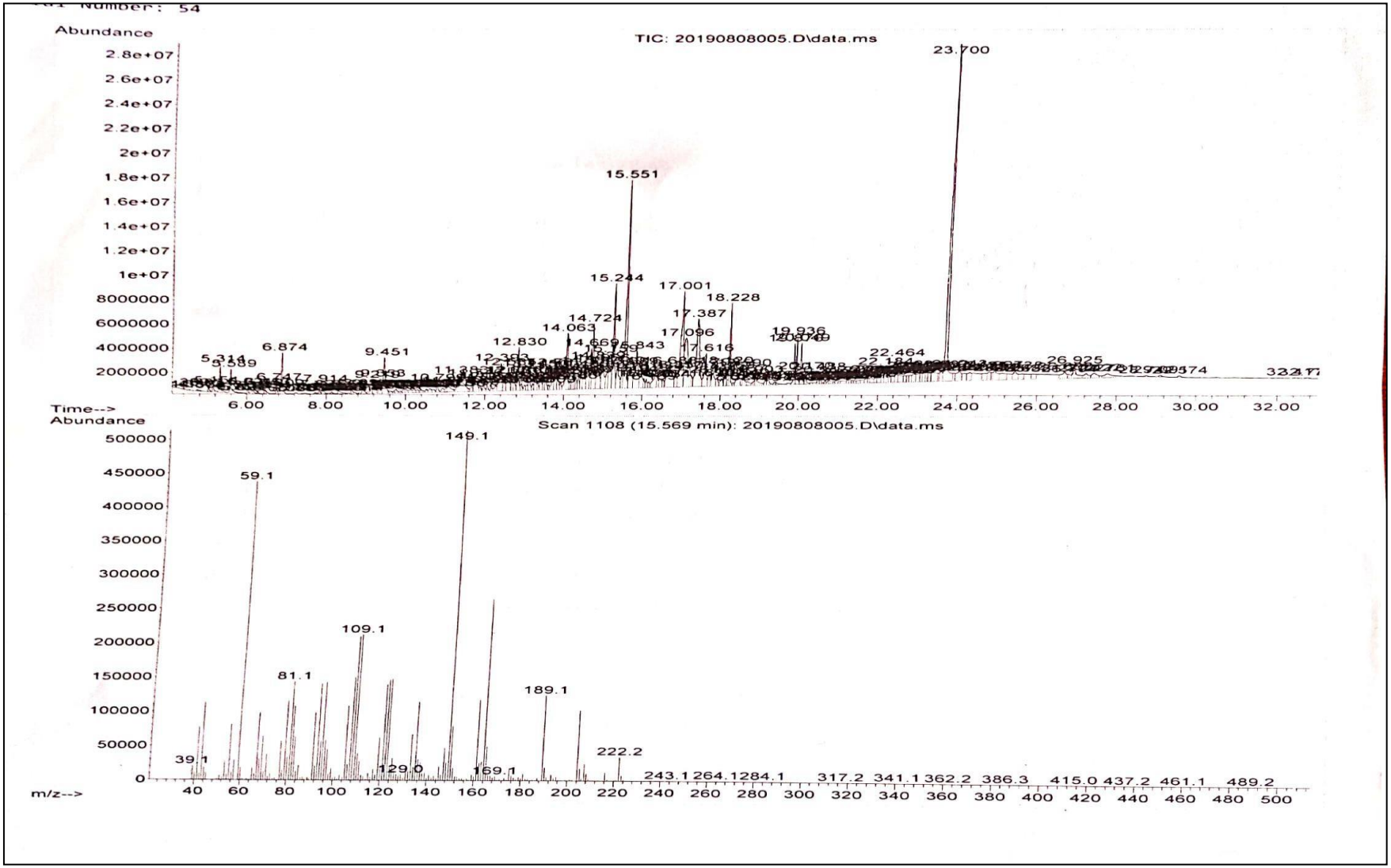

Figure 4.1: GC – MS chromatogram of DCM stalk extract of *Prunus africana*

4.3.1.2 Compounds from MeOH stem bark extract of *Prunus africana*

As indicated in table 4.5 and figure 4.2, the MeOH stem bark extract of the *Prunus africana* contained 46 chemical compounds identified by NIST2011 database. The result indicates that this plant has diverse constituents of the chemical compounds which contribute to the observed bioassay activities. The chemical structure, molecular weight and their percentage abundance is as indicated in table 4.5. The peak area and retention time of each of the compound identified in the plant sample is as indicated in the figure 4.2.

Table 4.5: Compounds present in MeOH stem bark extract of *Prunus africana*

Sr no	RT (Min)	Chemical name	Chemical formula	Molecular weight	% Abundance
1	12.5795	Acetophenone, 2 – chloro -*	C_8H_7ClO	154.594	0.368
2	12.6489	Pentobarbital*	$C_{11}H_{18}N_2O_3$	226.2722	0.271
3	13.7039	Phenol, 2, 4 – bis (1, 1 – dimethylethyl (PC)	$C_{14}H_{22}O$	206.3239	0.731
4	7.0497	Benzyl alcohol*	C_7H_8O	108.1378	0.289
5	7.8601	Benzoic acid, methyl ester*	$C_8H_8O_2$	136.1479	0.320
6	4.7087	2-Propenoic acid, butyl ester*	$C_7H_{12}O_2$	128.1690	0.713
7	4.7180	Styrene	C_8H_8	104.1491	0.991
8	4.8869	Ethanol,2– butoxy-*	$C_6H_{14}O_2$	118.1742	0.320
9	5.3087	3 – Carene (MT)	$C_{10}H_{16}$	136.2340	1.152
10	5.3087	.alpha. – Pinene(MT)	$C_{10}H_{16}$	136.2340	1.407
11	5.5864	Camphene(MT)	$C_{10}H_{16}$	136.2340	0.984
12	5.8175	Benzaldehyde	C_7H_6O	106.1219	0.175
13	6.0165	Bicyclo[3 . 1. 1] heptane, 6, 6 – dimethyl – 2 methylene-(1S)-,(MT)	$C_{10}H_{16}$	136.2340	0.758
14	6.1564	Benzonitrile(AH)	C_7H_5N	103.1213	0.376
15	6.3347	Benzofuran(F)	C_8H_6O	118.1326	0.211
16	6.4911	1H-Indene, 2, 3 – dihydro – 5 – methyl – (AH)	$C_{10}H_{12}$	132.2023	0.558
17	6.7399	Benzene, 1– methyl–3–(1– methylethyl) –(MT)	$C_{10}H_{14}$	134.2182	0.693
18	6.8062	Limonene (MT)	$C_{10}H_{16}$	136.2340	1.002
19	7.0497	Phenol, 3 – methyl –(PC)*	C_7H_8O	108.1378	0.739
20	7.0850	Benzene, propyl -*	C_9H_{12}	120.1916	0.247
21	7.8332	Undecane	$C_{11}H_{24}$	156.3083	0.521
22	8.4177	Phenylethyl alcohol*	$C_8H_{10}O$	122.1644	0.535
23	9.2291	1 – Dodecanol*	$C_{12}H_{26}O$	186.3342	0.358
24	9.3493	Dodecane	$C_{12}H_{26}$	170.3348	0.758
25	9.4509	3-Cyclohexene – 1 – methanol, .alpha.,.alpha. 4 – trimethyl –(MT)	$C_{10}H_{18}O$	154.2493	0.853

Sr No.	RT (Min)	Chemical name	Chemical formula	Molecular Weight	% Peak area
26	10.7908	Tridecane	$C_{13}H_{28}$	184.3614	0.346
27	10.8084	1, 3 – Benzodioxole, 5 – (2 – propenyl) -	$C_{10}H_{10}O_2$	162.1852	0.116
28	10.9774	Naphthalene, 2 – methyl -*	$C_{11}H_{10}$	142.1971	0.056
29	11.8593	Phenol, 4 – methyl – (PC)*	C_7H_8O	108.1378	0.765
30	12.0572	1 – Tetradecanol	$C_{14}H_{30}O$	214.3874	0.725
31	12.1577	Tetradecane	$C_{14}H_{30}$	198.3880	0.523
32	12.6489	Naphthalene, 1, 2 – dimethyl -*	$C_{12}H_{12}$	156.2237	0.383
33	12.7505	Diphenylmethane*	$C_{13}H_{12}$	168.2344	0.235
34	12.8272	1, 4 – Methanoazulene, decahydro – 4, 8, 8 – trimethyl – 9 – methylene -, (1S – (1. Alpha., 3a.be (TTP)	$C_{15}H_{24}$	204.3511	1.068
35	13.9412	Benzene, 1, 1’ – ethylidenebis -	$C_{14}H_{14}$	182.2610	0.461
36	14.5786	1 – Hexadecene	$C_{16}H_{32}$	224.4253	0.431
37	14.6604	Hexadecane*	$C_{16}H_{34}$	226.4412	0.273
38	16.8460	1 – Octadecanol	$C_{18}H_{38}O$	270.4937	0.591
39	16.9155	Octadecane*	$C_{18}H_{38}$	254.4943	0.490
40	18.0989	1, 2 – Benzenedicarboxylic acid, bis(2 – methylpropyl) ester (FAD)	$C_{16}H_{22}O_4$	278.3435	0.552
41	18.5839	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.3435	0.277
42	19.8700	9,12– Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.4721	0.612
43	19.9311	9 – Octadecanoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.4879	0.446
44	20.1643	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298.5038	0.497
45	20.7819	1 – Hexadecanol	$C_{16}H_{34}O$	242.4406	0.575
46	23.6847	Bis(2 – ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.5561	0.176

Legend: AH stands for Aromatic hydrocarbon, FAD for Fatty acid derivatives, PC for phenolic compounds, TTP for triterpenoids, MT for monoterpenoids, KD for ketone derivatives, PS for phytosterols and SD for sugar derivatives. * indicates that the phytochemical is absent in DCM extract of *Prunus africana*.

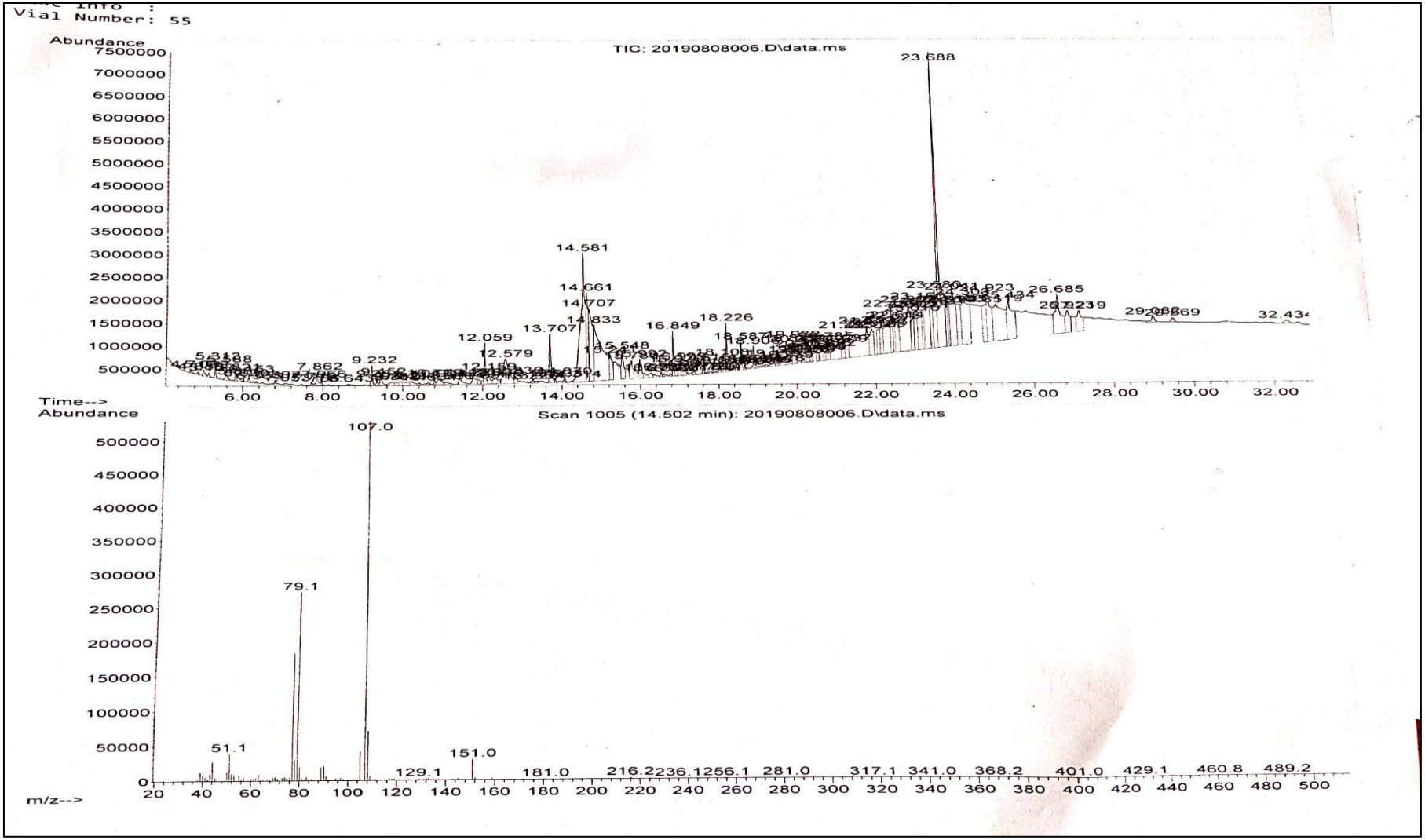

Figure 4.2: GC – MS chromatogram of DCM stalk extract of *Prunus africana*

4.3.1.3 Compounds from DCM leave extracts of *Eucalyptus saligna*

As indicated in table 4.6 and figure 4.3, the DCM leaves extract of the *Eucalyptus saligna* contained 31 chemical compounds identified by NIST2011 database. The result indicates that this plant has diverse constituents of the chemical compounds which contribute to the observed bioassay activities. The chemical structure, molecular weight and their percentage abundance is as indicated in table 4.6. The peakarea and retention time of each of the compound identified in the plant sample is as indicated in figure 4.3.

Table 4.6: Compounds present in DCM leave extract of *Eucalyptus saligna*

No.	RT (Min)	Chemical name	Chemical formula	Molecular weight	%Abundance
1	11.0798	Thymol (MT)	$C_{10}H_{14}O$	150.22	0.768
2	13.6986	Phenol, 2, 4 – bis (1, 1 – dimethylethyl) – (PC)*	$C_{14}H_{22}O$	206.32	1.296
3	4.7065	Styrene*	C_8H_8	104.15	1.089
4	5.1397	3 – Carene (MT)	$C_{10}H_{16}$	136.24	0.984
5	5.2982	.alpha, - Pinene (terpene)	$C_{10}H_{16}$	136.23	1.282
6	5.5770	Camphene (MT)	$C_{10}H_{16}$	136.24	0.963
7	5.7335	Benzene, 1 – ethyl – 2 – methyl –(AH)	C_9H_{12}	120.1916	0.697
8	5.8703	Benzene, 1, 3, 5 – trimethyl – (AH)	C_9H_{12}	120.19	0.324
9	6.0091	Bicyclo[3. 1. 1] heptane, 6, 6 – dimethyl – 2 – methylene – (1S) – (B-pinene) (MT)	$C_{10}H_{16}$	136.2340	0.968
10	6.1501	Benzonitrile (AH)	C_7H_5N	103.04	0.375
11	6.3273	Benzofuran (F)	C_8H_6O	118.1	0.358
12	6.4859	1H – Indene, 2, 3 – dihydro – 5 – methyl – (AH)	$C_{10}H_{12}$	132.2023	0.837
13	6.7377	Benzene, 1 – methyl – 3 – (1 – methylethyl) – (AH)	$C_{10}H_{14}$	134.2182	0.039
14	6.8019	Limonene (MT)	$C_{10}H_{16}$	136.23	1.287
15	6.8672	Eucalyptol (MT)	$C_{10}H_{18}O$	154.249	1.657
16	7.2475	Cyclohexene, 1 – methyl – 4 – (1 – methylethylidene) – (EO)	$C_{10}H_{16}$	136.2340	0.987
17	7.8310	Undecane	$C_{11}H_{24}$	156.31	0.638
18	8.3015	Bicyclo[2. 2. 1] heptan – 2 – ol, 1, 3, 3 – trimethyl – (MT)	$C_{10}H_{18}O$	154.2493	0.754
19	9.1316	Borneol (Terpene)	$C_{10}H_{18}O$	154.25	0.987
20	9.2083	3 – Cyclohexen – 1 – ol, 4 – methyl – 1 (1 – methylethyl) – (MT)	$C_{10}H_{18}O$	154.2493	0.826
21	9.4373	3 – Cyclohexene – 1 – methanol, .alpha., .alpha. 4 – trimethyl - (MT)	$C_{12}H_{20}O_2$	196.29	
22	10.7907	Tridecane	$C_{13}H_{28}$	184.37	0.258
23	10.8011	1, 3 – Benzodioxole, 5 – (2 – propenyl) -	$C_{10}H_{10}O_2$	162.1852	0.758
24	11.0798	Phenol, 2 – methyl – 5 – (1 – methylethyl) – (PC)	$C_{10}H_{14}O$	150.2176	0.917
25	12.0540	1- Tetradecanol	$C_{14}H_{30}O$	214.393	1.036
26	12.1545	Tetradecane	$C_{14}H_{30}$	198.39	1.116

Sr No.	RT (Min)	Chemical name	Chemical formula	Molecular weight	% Peak area abundance
27	12.8250	1, 4 – Methanoazulene, decahydro – 4, 8, 8 – trimethyl – 9 – methylene -, [1S – (1 .alpha., 3a. be((TTP)	$C_{15}H_{24}$	204.3511	0.947
28	13.6986	Phenol, 2, 4 – bis (1, 1 – dimethylethyl) – (PC)	$C_{14}H_{22}O$	206.32	
29	18.5807	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	0.169
30	18.5807	1,2 – Benzenedicarboxylic acid, bis(2 – methylpropyl) ester (FAD)	$C_{16}H_{22}O_4$	278.3435	0.837
31	23.6804	Bis(2 – ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.564	0.279

Legend: AH stands for Aromatic hydrocarbon, FAD for Fatty acid derivatives, P for phenolics, PS for phytosterols and SD for sugar derivatives and * for phytocompounds absent in MeOH leaves extract of *E saligna.*

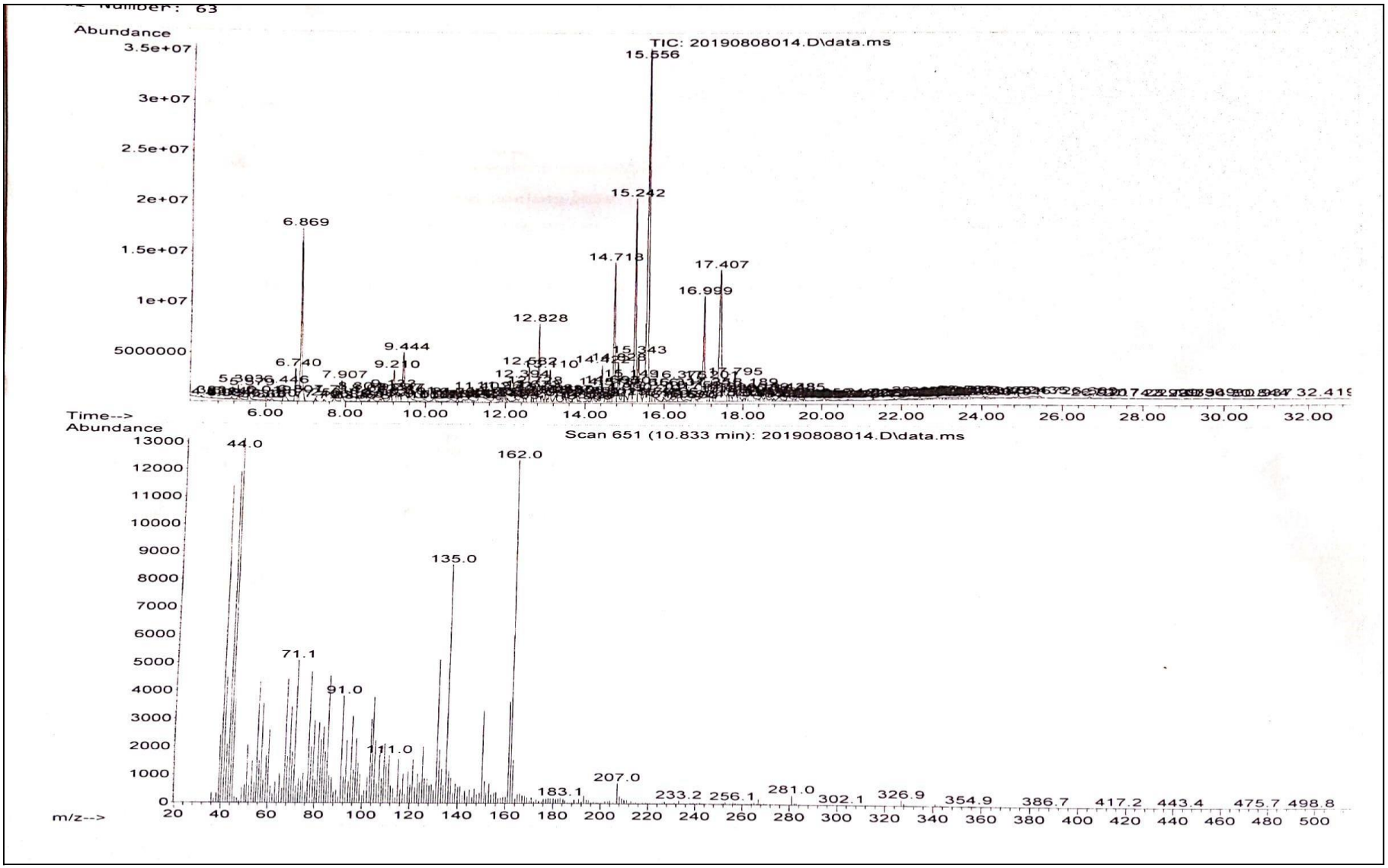

Figure 4.3: GC – MS chromatogram of DCM leave extract of *Prunus africana*

4.3.1.4 Compounds from MeOH leaves extract of *Eucalyptus saligna*

As indicated in table 4.7 and figure 4.4, the MeOH leaves extract of the *Eucalyptus saligna* contained 42 chemical compounds identified by NIST2011 database. The result indicates that this plant has diverse constituents of the chemical compounds which contribute to the observed bioassay activities. The chemical structure, molecular weight and their percentage abundance is as indicated in table 4.7. The peak area and retention time of each of the compound identified in the plant sample is as indicated in figure 4.4.

Table 4.7: Compounds present in MeOH stalk extract of *Eucalyptus saligna*

Sr No.	RT (Min)	Chemical Name	Chemical formula	Molecular weight	% Peak area
1	4.7575	Cyclohexanone*	$C_6H_{10}O$	98.15	0.623
2	5.2880	.alpha. – Pinene (Terpene)	$C_{10}H_{16}$	136.23	1.168
3	5.2880	3 – Carene (MT)	$C_{10}H_{16}$	136.24	1.325
4	5.5668	Camphene (MT)	$C_{10}H_{16}$	136.24	0.261
5	5.7243	Benzene, 1 – ethyl – 2 – methyl – (AH)	C_9H_{12}	120.1916	0.731
6	6.0031	Bicyclo[3. 1. 1] heptane, 6, 6 – dimethyl – 2 methylene -, (1S) – (MT)	$C_{10}H_{16}$	136.2340	1.369
7	6.1461	Benzonitrile (AH)	C_7H_5N	103.04	0.752
8	6.2591	Benzene, 1, 3, 5 – trimethyl -	C_9H_{12}	120.1916	0.049
9	6.3233	Benzofuran (F)	C_8H_6O	118.1	0.016
10	6.7368	Benzene, 1 – methyl – 3 – (1 – methylethyl) – (MT)	$C_{10}H_{14}$	134.2182	0.603
11	6.7980	Limonene (MT)	$C_{10}H_{16}$	136.23	0.691
12	6.8643	Eucalyptol (MT)	$C_{10}H_{18}O$	154.249	2.152
13	7.0519	Benzyl Alcohol*	C_7H_8O	108.14	0.218
14	7.2457	Cyclohexene, 1 – methyl – 4 – (1 – methylethylidene) -	$C_{10}H_{16}$	136.2340	1.197
15	7.5265	Benzene, 1 – ethyl – 3, 5 – dimethyl – *	$C_{10}H_{14}$	134.2182	0.975
16	7.7804	1H – Indene, 2, 3 – dihydro – 5 – methyl – (AH)	$C_{10}H_{12}$	132.2023	1.476
17	7.8291	Undecane (AH)	$C_{11}H_{24}$	156.31	0.711
18	7.9814	Phenol, 4 – methyl – (PC)*	C_7H_8O	108.14	0.693
19	8.2395	Benzene, 1, 2, 3, 5 – tetramethyl – (AH)*	$C_{10}H_{14}$	134.2182	0.045
20	8.3027	Bicyclo[2. 2. 1] heptan – 2 – ol, 1, 3, 3 – trimethyl – (MT)	$C_{10}H_{18}O$	154.2493	0.745
21	9.1317	Borneol (Terpene)	$C_{10}H_{18}O$	154.25	0.971
22	9.2084	3 – Cyclohexen – 1 – ol, 4 – methyl – 1 – (1 – methylethyl) – (MT)	$C_{10}H_{18}O$	154.2493	1.039
23	9.4426	3 – Cyclohexene – 1 – methanol, .alpha., .alpha. 4 – trimethyl – (MT)	$C_{10}H_{18}O_2$	170.2487	0.838
24	9.9805	1, 4 – Benzenediamine, N, N – diethyl -*	$C_{10}H_{16}N_2$	164.2474	0.056
25	10.7909	Tridecane	$C_{13}H_{28}$	184.37	0.987

Sr No.	RT (Min)	Chemical name	Chemical Formula	Molecular weight	% Peak area
26	10.7982	1,3 – Benzodioxole, 5 – (2 – propenyl)-	$C_{10}H_{10}O_2$	162.18500	0.853
27	11.0655	Phenol, 2 – methyl – 5 - (1- methylethyl)- (PC)	$C_{10}H_{14}O$	150.2176	0.616
28	12.0531	1 – Tetradecanol	$C_{14}H_{30}O$	214.393	0.258
29	12.1526	Tetradecane	$C_{14}H_{30}$	198.39	0.758
30	12.8324	1, 4 – Methanoazulene, decahydro – 4, 8, 8 – trimethyl – 9 – methylene -, [1S – (1 .alpha., 3a. be (TTP)	$C_{15}H_{24}$	204.3511	0.972
31	13.6978	Phenol, 2, 4 – bis (1, 1 – dimethylethyl) – PC)	$C_{12}H_{22}O$	206.32	0.838
32	14.5766	1 – Hexadecene*	$C_{16}H_3$	224.42	1.036
33	16.0896	Methyl tetradecanoate*	$C_{15}H_{30}O_2$	242.40	0.946
34	17.6099	1,2 – Benzenedicarboxylic acid, bis (2 – methylprophyl) ester (FAD)	$C_{16}H_{22}O_4$	278.3435	1.145
35	18.2192	Hexadecanoic acid, methyl ester (FAD)*	$C_{17}H_{34}O_2$	270.4507	1.643
36	18.5809	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	0.925
37	19.8670	9, 12 – Octadecadienoic acid (Z, Z) - , methyl ester*	$C_{18}H_{32}O_2$	280.4455	0.836
38	19.9260	9 – Octadecanoic acid (Z) -, methyl ester*	$C_{19}H_{36}O_2$	296.4879	0.854
39	20.1613	Octadecanoic acid, methyl ester*	$C_{19}H_{38}O_2$	298.50	0.912
40	23.6796	Bis (2 – ethylexyl) phthalate*	$C_{24}H_{38}O_4$	390.564	0.135
41	26.9118	Squalene (TTP)*	$C_{30}H_{50}$	410.73	0.958
42	11.0655	Thymol (MT)	$C_{10}H_{14}O$	150.22	1.084

Legend: AH stands for Aromatic hydrocarbon, FAD for Fatty acid derivatives, MT for Monoterpenoid, TTP for triterpenoid, PC for phenolic compound and * for the phytocompounds absent in DCM *E. saligna* leaves extract.

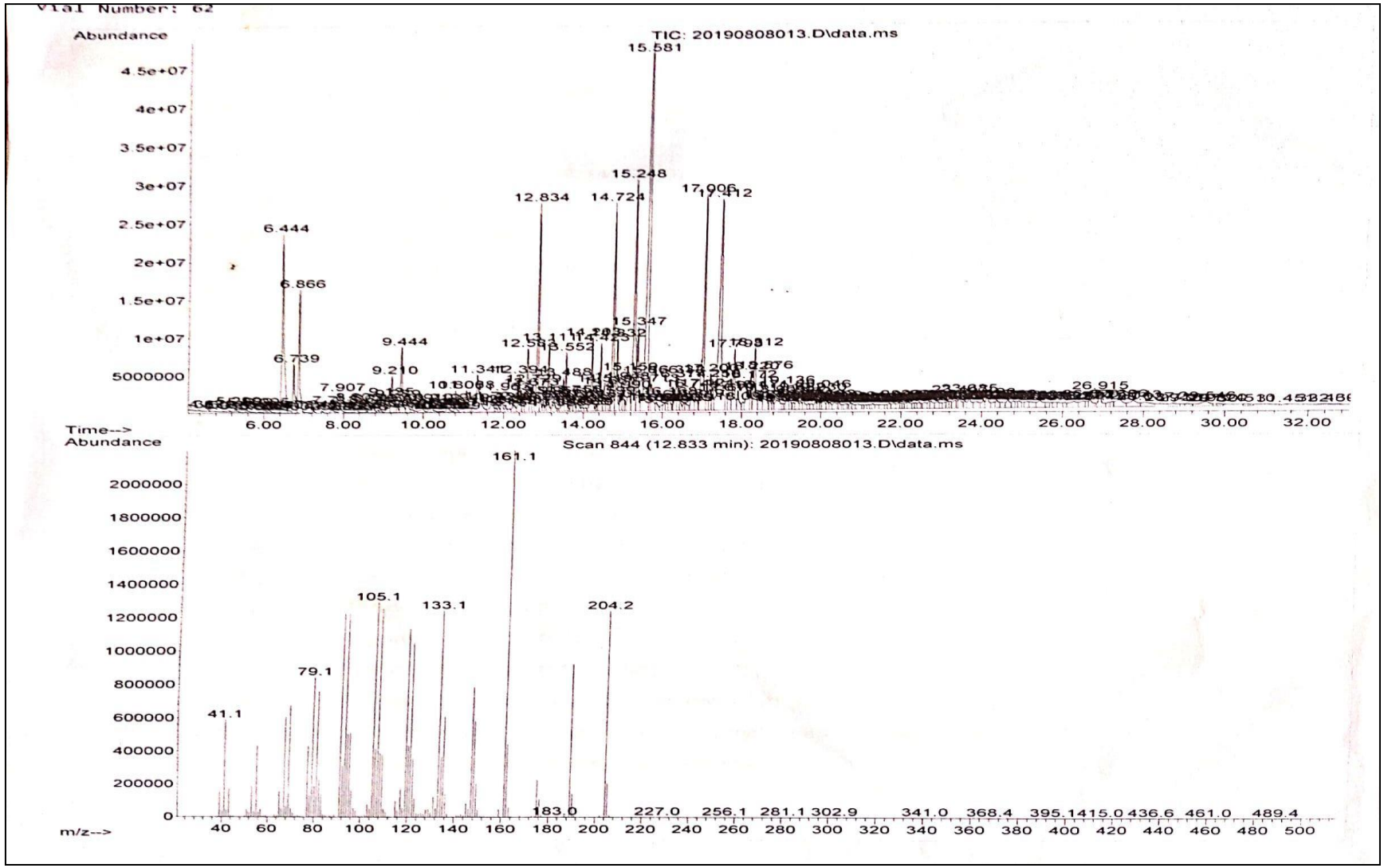

Figure 4.4: GC – MS chromatogram of MeOH stalk extract of *Eucalyptus saligna*

4.3.1.5 Compounds from DCM leaves extract of *Senna didymobotyra*

As indicated in table 4.8 and figure 4.5, the DCM leaves extract of the *Senna didymobotyra* contained 34 chemical compounds identified by NIST2011 database. The results indicate that this plant has diverse constituents of the chemical compounds which contribute to the observed bioassay activities. The chemical structure, molecular weight and their percentage abundance is as indicated in table 4.8. The peak area and retention time of each of the compound identified in the plant sample is as indicated in figure 4.5.

Table 4.8: Coumpounds present in DCM stalk extract of *Senna didymobotyra*

Sr No.	RT (Min)	Chemical name	Chemical formula	Molecular weight	% Peak area
1	4.8548	1, 3 – Cyclohexadiene, 1 – methyl – 4 – (1 – methylethyl) – (MT)	$C_{10}H_{16}$	136.2340	1.007
2	5.1481	3 – Carene (MT)	$C_{10}H_{16}$	136.2340	1.204
3	5.2559	Benzene, 1, 2, 3, 5 – tetramethyl – (AH)	$C_{10}H_{14}$	134.2182	0.349
4	5.3077	.alpha. – Pinene(MT)	$C_{10}H_{16}$	136.2340	1.036
5	5.5833	Camphene (MT)	$C_{10}H_{16}$	136.2340	1.132
6	5.7367	Benzene, 1 – ethyl – 2 – methyl – (AH)	C_9H_{12}	120.1916	0.692
7	5.8756	Benzene, 1, 3, 5 – trimethyl -	C_9H_{12}	120.1916	0.352
8	6.0145	Bicyclo[3. 1. 1]heptane, 6, 6 – dimethyl – 2 – methylene – (1S) -(MT)	$C_{10}H_{16}$	136.2340	0.877
9	6.1544	Benzonitrile (AH)	C_7H_5N	103.1213	0.362
10	6.3347	Benzofuran (F)	C_8H_6O	118.1326	0.137
11	6.4901	1H – Indene, 2, 3 – dihydro – 5 – methyl – (AH)	$C_{10}H_{12}$	132.2023	0.575
12	6.7388	Benzene, 1 – methyl – 3 – (1 – methylethyl) – (MT)	$C_{10}H_{14}$	134.2182	1.021
13	6.8031	Limonene (MT)	$C_{10}H_{16}$	136.2340	1.211
14	6.8673	Eucalyptol (MT)	$C_{10}H_{18}O$	154.2493	0.798
15	6.8995	Benzene, 1 – ethyl – 2, 4 – dimethyl -	$C_{10}H_{14}$	134.2182	0.326
16	7.0850	Benzene, propyl -*	C_9H_{12}	120.1916	0.492
17	7.2383	Benzene, tert – butyl – (AH)*	$C_{10}H_{14}$	134.2182	0.339
18	7.8321	Undecane	$C_{11}H_{24}$	156.3083	0.347
19	8.3057	Bicyclo[2. 2. 1] heptan – 2 – ol, 1, 3, 3 – trimethyl -	$C_{10}H_{18}O$	154.2493	0.638
20	9.2291	1 – Dodecanol	$C_{12}H_{26}O$	186.3342	0.843
21	9.3483	Dodecane	$C_{12}H_{26}$	170.3348	0.567
22	10.4136	Pentadecane	$C_{15}H_{32}$	212.4146	0.495
23	10.7929	Tridecane (HC)	$C_{13}H_{28}$	184.3614	0.822
24	12.0582	1 – Hexadecanol	$C_{16}H_{34}O$	242.4406	0.358
25	12.1588	Tetradecane	$C_{14}H_{30}$	198.3880	0.657
26	12.4883	1, 4 – Methanoazulene, decahydro – 4, 8, 8 – trimethyl – 9 –methylene – [1S – (1 alpha., 3a. be((TTP)	$C_{15}H_{24}$	204.3511	0.647
27	13.7112	Phenol, 2, 4 – bis (1, 1 – dimethylethyl) – (PC)	$C_{14}H_{22}O$	206.3239	0.753
28	14.6646	Hexadecane	$C_{16}H_{34}$	226.4412	0.536

Sr No.	RT (Min)	Chemical name	Chemical formula	Molecular weight	% Peak area
29	18.1020	1, 2 – Benzenedicarboxylic acid, bis (2 – methylpropyl) ester (FAD)	$C_{16}H_{22}O_4$	278.3435	1.016
30	18.2243	Hexadecanoic acid, methyl ester (FAD)	$C_{17}H_{34}O_2$	270.4507	0.948
31	18.5860	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.3435	0.274
32	19.8710	9, 12 – Octadecadienoic acid (Z, Z) – methyl ester	$C_{19}H_{34}O_2$	294.4721	0.985
33	23.6826	Bis (2 – ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.5561	0.423
34	7.0850	N – Benzyl – 2 – phenethylamine*	$C_{15}H_{17}N$	211.3022	0.365

Legend: AH stands for Aromatic hydrocarbon, FAD for Fatty acid derivatives, PC for phenolic compounds, TTP for triterpenoids, MT for monoterpenoids, KD for ketone derivatives, PS for phytosterols and SD for sugar derivatives. * for the phytocompounds absent in MeOH *Senna didymobotyra* leaves extract.

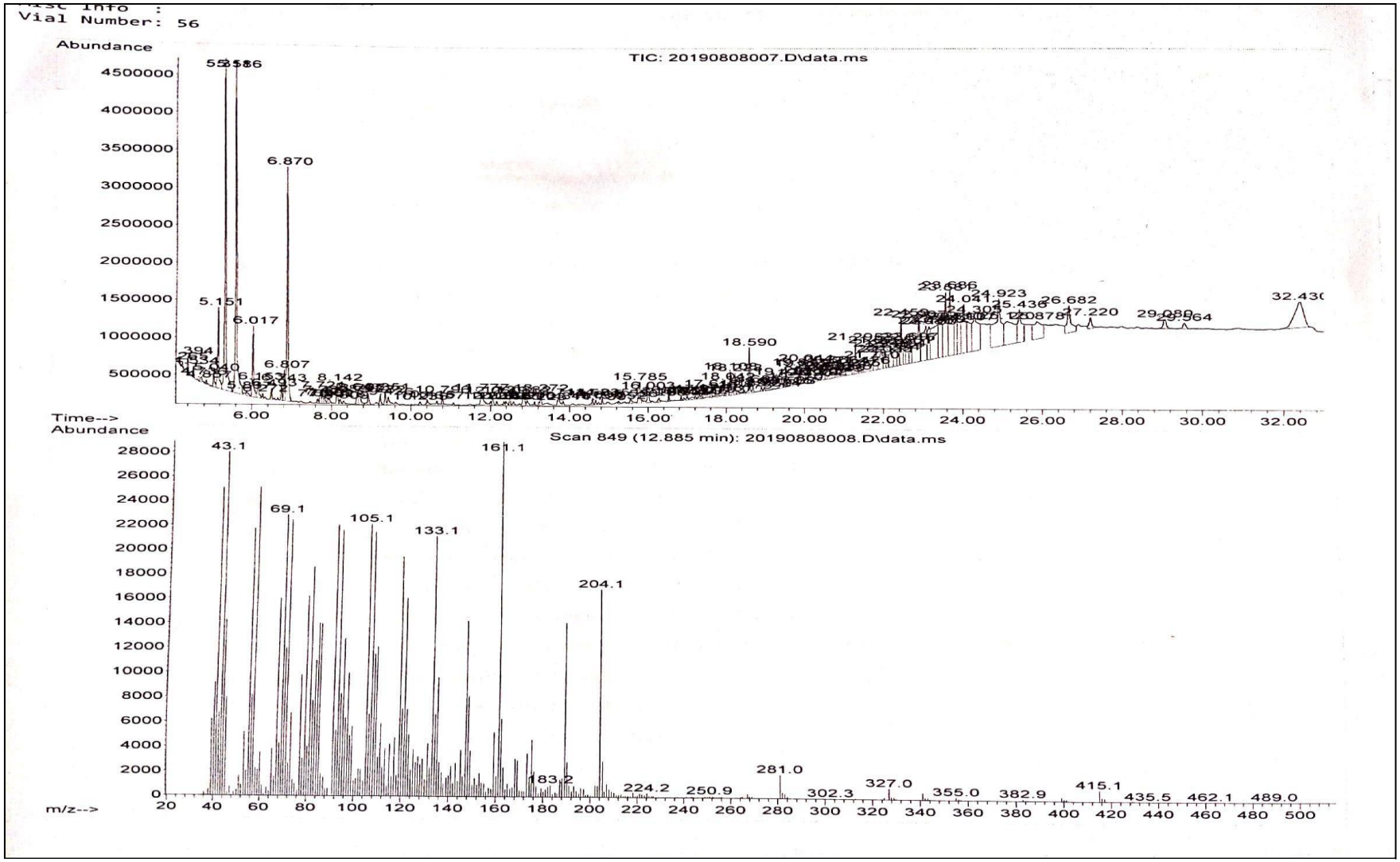

Figure 4.5: GC – MS chromatogram of DCM leave extract of *Senna didymobotyra*

4.3.1.6 Compounds from MeOH leaves extract of *Senna didymobotyra*

As indicated in table 4.9 and figure 4.6, the MeOH leaves extract of the *Senna didymobotyra* contained 55 chemical compounds identified by NIST2011 database. The results indicate that this plant has diverse constituents of the chemical compounds which contribute to the observed bioassay activities. The chemical structure, molecular weight and their percentage abundance is as indicated in table 4.9. The peak area and retention time of each of the compound identified in the plant sample is as indicated in figure 4.6.

Table 4.9: Compounds present in MeOH stalk extract of *Senna didymobotyra*

Sr No.	RT (Min)	Chemical name	Chemical formula	Molecular weight	% Peak area
1	13.7019	Phenol, 2 – 4 – bis (1, 1 – dimethylethyl) – (PC)	$C_{14}H_{22}O$	206.3239	0.483
2	9.4385	Benzyl Alcohol*	C_7H_8O	108.1378	0.623
3	4.6963	2 – Propenoic acid, butyl ester*	$C_7H_{12}O_2$	128.1690	0.261
4	4.8829	Ethanol, 2 – butoxy -*	$C_6H_{14}O_2$	118.1742	0.731
6	5.1357	3 – Carene(MT)	$C_{10}H_{16}$	136.2340	1.369
7	5.2964	.alpha. – Pinene(MT)	$C_{10}H_{16}$	136.2340	0.623
8	5.5741	Camphene(MT)	$C_{10}H_{16}$	136.2340	0.049
9	5.7306	Benzene, 1 – ethyl – 2 – methyl –(AH)	C_9H_{12}	120.1916	1.372
10	5.8695	Benzene, 1, 3, 5 – trimethyl – (AH)	C_9H_{12}	120.1916	0.603
11	6.0073	Bicyclo[3. 1. 1] heptane, 6, 6 – dimethyl – 2 – methylene – (1S) – (MT)	$C_{10}H_{16}$	136.2340	0.691
12	6.1493	Benzonitrile (AH)	C_7H_5N	103.1213	0.390
13	6.3275	Benzofuran (F)	C_8H_6O	118.1326	0.152
14	6.4850	1H – Indene, 2, 3 – dihydro – 5 – methyl –(AH)	$C_{10}H_{12}$	132.2023	0.477
15	6.7058	Benzene, 1, 2, 4, 5 – tetramethyl –(AH)*	$C_{10}H_{14}$	134.2182	0.623
16	6.7369	Benzene, 1 – methyl – 3 – (1 – methylethyl) – (MT)	$C_{10}H_{14}$	134.2182	0.946
17	6.8011	Limonene (MT)	$C_{10}H_{16}$	136.2340	1.197
18	6.8654	Eucalyptol (MT)	$C_{10}H_{18}O$	154.2493	0.975
19	7.0820	Benzene, 1, 3 – diethyl - *	$C_{10}H_{14}$	134.2182	0.711
20	7.1358	Benzene, 1 – methyl – 2 – propyl -*	$C_{10}H_{14}$	134.2182	0.758
21	7.2374	Benzene, 1 – ethyl – 3, 5 – dimethyl -*	$C_{10}H_{14}$	134.2182	0.039
22	7.2477	1, 3 – Cyclohexadiene, 1 – methyl – 4 – (1 – methylethyl) – (MT)	$C_{10}H_{16}$	136.2340	0.623
23	7.5783	Benzene, 1 – ethyl – 2, 4 – dimethyl -	$C_{10}H_{14}$	134.2182	0.858
24	7.8302	Undecane	$C_{11}H_{24}$	156.3083	1.476
25	8.2416	Benzene, 1, 2, 3, 5 – tetramethyl – (AH)	$C_{10}H_{14}$	134.2182	1.039
26	8.3058	Bicyclo[2. 2. 1] heptan – 2 – ol, 1, 3, 3 – trimethyl -	$C_{10}H_{18}O$	154.2493	0.864
27	9.2116	3 – Cyclohexen – 1 – ol, 4 – methyl -1 – (1 – methylethyl) –(MT)*	$C_{10}H_{16}O$	151.2493	0.147
28	9.3494	Dodecane	$C_{12}H_{26}$	170.3348	0.016
29	9.4385	Benzyl Alcohol*	C_7H_8O	108.1378	0.916

Sr No.	RT (Min)	Chemical name	Chemical Formula	Molecular weight	% abundance
30	9.4479	3 – Cyclohexene – 1 – methanol, .alpha., .alpha. 4 – trimethyl – (MT)*	$C_{10}H_{18}O$	154.2493	0.838
31	10.3785	Decane, 4 – methyl -*	$C_{11}H_{24}$	156.3083	0.616
32	10.7920	Tridecane	$C_{13}H_{28}$	184.3614	0.838
33	10.8023	1, 3 – Benzodioxole, 5 – (2 – propenyl) -*	$C_{10}H_{10}O_2$	162.1852	0.258
34	11.8158	Pentadecane, 2, 6, 10, 14 – tetramethyl -*	$C_{19}H_{40}$	268.5209	0.388
35	12.0542	1 – Tetradecanol*	$C_{14}H_{30}O$	214.3874	0.536
36	12.1547	Tetradecane	$C_{14}H_{30}$	198.3880	1.036
37	12.8294	1, 4 – Methanoazulene, decahydro – 4, 8, 8 – trimethyl – 9 – methylene -, [1S – (1 .alpha., 3a. be((TTP)	$C_{15}H_{24}$	204.3511	0.946
38	13.4418	Pentadecane	$C_{15}H_{32}$	212.4146	1.116
39	13.7019	Phenol, 2, 4 – bis (1, 1 – dimethylethyl) – (PC)	$C_{14}H_{22}O$	206.3239	0.758
40	13.7413	Dodecanoic acid, methyl ester*	$C_{13}H_{26}O_2$	214.3443	1.145
41	14.5766	1 – Hexadecene*	$C_{16}H_{32}$	224.4253	1.369
42	14.5766	1 – Hexadecanol	$C_{16}H_{34}O$	242.4406	0.483
43	14.6595	Hexadecane	$C_{16}H_{34}$	226.4412	0.623
44	16.0907	Methyl tetradecanoate*	$C_{15}H_{30}O_2$	242.3975	0.731
45	16.8399	Tetradecanoic acid, ethyl ester*	$C_{16}H_{32}O_2$	256.4241	0.623
46	16.8461	1 – Octadecanol*	$C_{18}H_{38}O$	270.4937	0.049
47	16.9156	Octadecane*	$C_{18}H_{38}$	254.4943	0.016
48	17.6099	1, 2 – Benzenedicarboxylic acid, bis (2 – methylpropyl) ester (FAD)	$C_{16}H_{22}O_4$	278.3435	1.372
49	18.2296	Hexadecanoic acid, methyl ester (FAD)	$C_{17}H_{34}O_2$	270.4507	0.691
50	18.5840	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.3435	0.390
51	18.8939	Hexadecanoic acid, ethyl ester (FAD)	$C_{18}H_{36}O_2$	284.4772	0.152
52	19.8763	9, 12 – Octadecadienoic acid (Z, Z) -, methyl ester	$C_{19}H_{34}O_2$	294.4721	0.477
53	20.1675	Octadecanoic acid, methyl ester*	$C_{19}H_{38}O_2$	298.5038	0.623
54	23.6869	Bis(2 – ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.5561	1.197
55	26.9181	Squalene (TTP)*	$C_{30}H_{50}$	410.7180	0.975

Legend: AH stands for Aromatic hydrocarbon, FAD for Fatty acid derivatives, PC for phenolic compounds, TTP for triterpenoids, MTfor monoterpenoids, KD for ketone derivatives, PS for phytosterols and SD for sugar derivatives. * for the phytocompounds absent in DCM *Senna didymobotyra* leaves extract

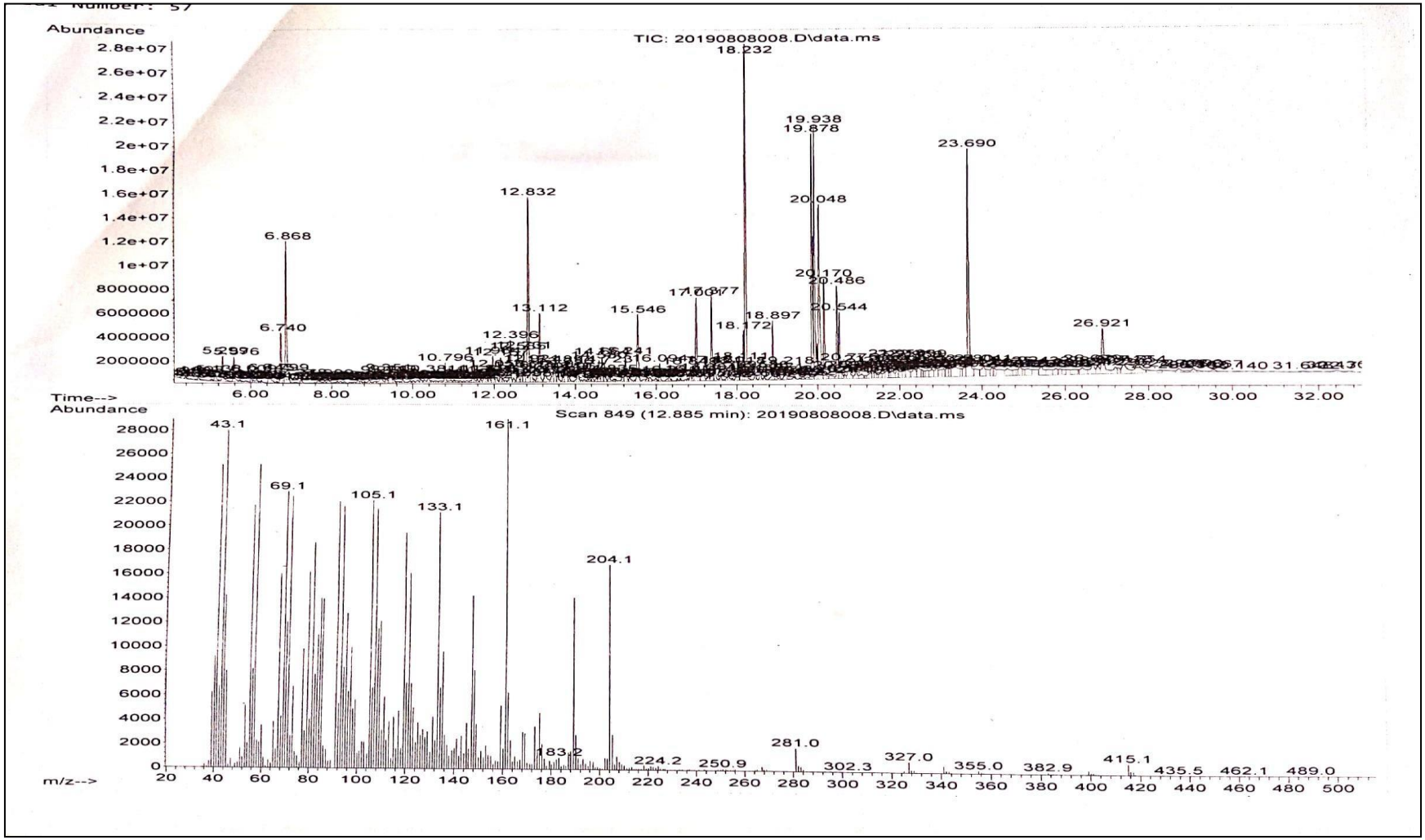

Figure 4.6: GC – MS chromatogram of MeOH stalk extract of *Senna didymobotyra*

4.3.1.7 Compounds from DCM stalk extract of *Bidens pilosa*

As indicated in table 4.10 and figure 4.7, the DCM stalk extract of the *Bidens pilosa* contained 34 chemical compounds as identified by NIST2011 database. The result indicates that this plant had diverse constituents of the chemical compounds which may contribute to various bioassay activities. The chemical structure, molecular weight, retention time, peak area and their percentage peak area abundance was as indicated in table 4.10 and figure 4.7.

Table 4.10: Compounds present in DCM stalk extract of *Bidens pilosa*

Sr No.	RT (Min)	Chemical name	Molecular formula	Molecular weight	% Abundance
1	22.5333	Vitamin E acetate (Vit)*	$C_{31}H_{52}O_3$	472.74	0.623
2	4.8402	1, 3 – Cyclohexadiene, 1 – methyl – 4 – (1 – methylethyl) – (MT)*	$C_{10}H_{16}$	136.2340	0.483
3	5.1346	3 – Carene (MT)	$C_{10}H_{16}$	136.23	1.168
4	5.2444	Benzene, 1, 2, 3, 5 – tetramethyl – (AH)	$C_{10}H_{14}$	134.22	0.261
5	5.2983	.alpha, - Pinene (MT)*	$C_{10}H_{16}$	136.23	0.731
6	5.5719	Camphene (MT)	$C_{10}H_{16}$	136.23	1.369
7	5.7294	Benzene, 1 – ethyl – 2 – methyl – (AH)	C_9H_{12}	120.19	0.049
8	5.7823	Benzene, 1 – ethyl – 4 – methyl – (AH)*	C_9H_{11}	119.18	0.016
9	6.0061	Bicyclo[3. 1. 1] heptane, 6, 6 – dimethyl – 2 – methylene -, (1S) – (MT)* (-) – β-Pinene	$C_{10}H_{16}$	136.2340	1.372
10	6.1481	Benzonitrile (AH)*	C_7H_5N	103.04	0.603
11	6.2072	Benzene, tert – butyl – (AH)	$C_{10}H_{14}$	134.2182	0.691
12	6.2642	Decane (AH)*	$C_{10}H_{22}$	142.29	0.390
13	6.3294	Benzofuran (F)	C_8H_6O	118.13	0.152
14	6.4838	1H – Indene, 2, 3 – dihydro – 5 methyl – (AH)	$C_{10}H_{12}$	132.346	0.477
15	6.6041	Decane, 4 – methyl – (AH)*	$C_{11}H_{24}$	156.313	0.946
16	6.7357	Benzene, 1 – methyl – 3 – (1 – methylethyl) – (MT)	$C_{10}H_{14}$	134.218	1.197
17	6.7854	1 – Hexanol, 2 – ethyl – (FAl)*	$C_8H_{18}O$	130.231	0.975
18	6.7999	Limonene (MT)	$C_{10}H_{16}$	136.23	0.858
19	6.8642	Eucalyptol (MT)*	$C_{10}H_{18}O$	154.249	1.476
20	7.0808	Benzene, 1, 3 – diethyl -*	$C_{10}H_{14}$	134.2182	0.711
21	7.2725	Decane, 2 – methyl -*	$C_{11}H_{24}$	156.31	0.758
22	7.8311	Undecane (AH)*	$C_{11}H_{24}$	156.31	0.039
23	9.2094	3 – Cyclohexen – 1 – ol, 4 – methyl – 1 – (1 – methylethyl) (MT)*	$C_{12}H_{20}O_2$	196.29	0.079
24	9.3493	Dodecane (AH)	$C_{12}H_{26}$	170.33	0.331

Sr No.	RT (Min)	Chemical name	Chemical formula	Molecular weight	% Abundance
25	9.4456	3 – Cyclohexene – 1 – methanol, .alpha., .alpha. 4 – trimethyl – (MT)	$C_{10}H_{18}O$	154.25	0.841
26	10.7918	Tridecane (H)	$C_{13}H_{28}$	184.367	1.012
27	11.8167	Pentadecane, 2, 6, 10, 14 – tetramethyl – (DT)*	$C_{19}H_{40}$	268.5	0.704
28	12.1577	Tetradecane	$C_{14}H_{30}$	198.39	0.269
29	12.5826	1, 4 – Methanoazulene, decahydro – 4, 8, 8 – trimethyl – 9 – methylene -, [1S – (1 .alpha.,3a. be (TTP)	$C_{15}H_{24}$	204.3511	0.504
30	17.6097	1,2 – Benzenedicarboxylic acid, bis (2 – methylpropyl) ester (FAD)	$C_{16}H_{22}O_4$	278.3435	1.132
31	18.5828	Dibutyl phthalate(Oil)	$C_{16}H_{22}O_4$	278.34	0.958
32	18.8917	Hexadecanoic acid, ethyl ester (FAD)	$C_{18}H_{36}O_2$	284.484	1.314
33	22.4576	Hexanedioic acid, bis (2 – ethylhexyl) ester - (FAD)	$C_{22}H_{42}O_4$	370.5665	1.304
34	23.6815	Bis (2 – ethylhexyl) phthalate (Phthalate)	$C_{24}H_{38}O_4$	390.564	0.949

Legend: AH stands for Aromatic hydrocarbon, FAD for Fatty acid derivatives, MT for monoterpenoids, Vit for vitamin derivative, DTfor diterpenoids, F for furan, FAl for fatty alcohol, Vit for vitamins and TTD for triterpenoids, * for phytocompounds absent in MeOHstalks extract of *B. pilosa.*

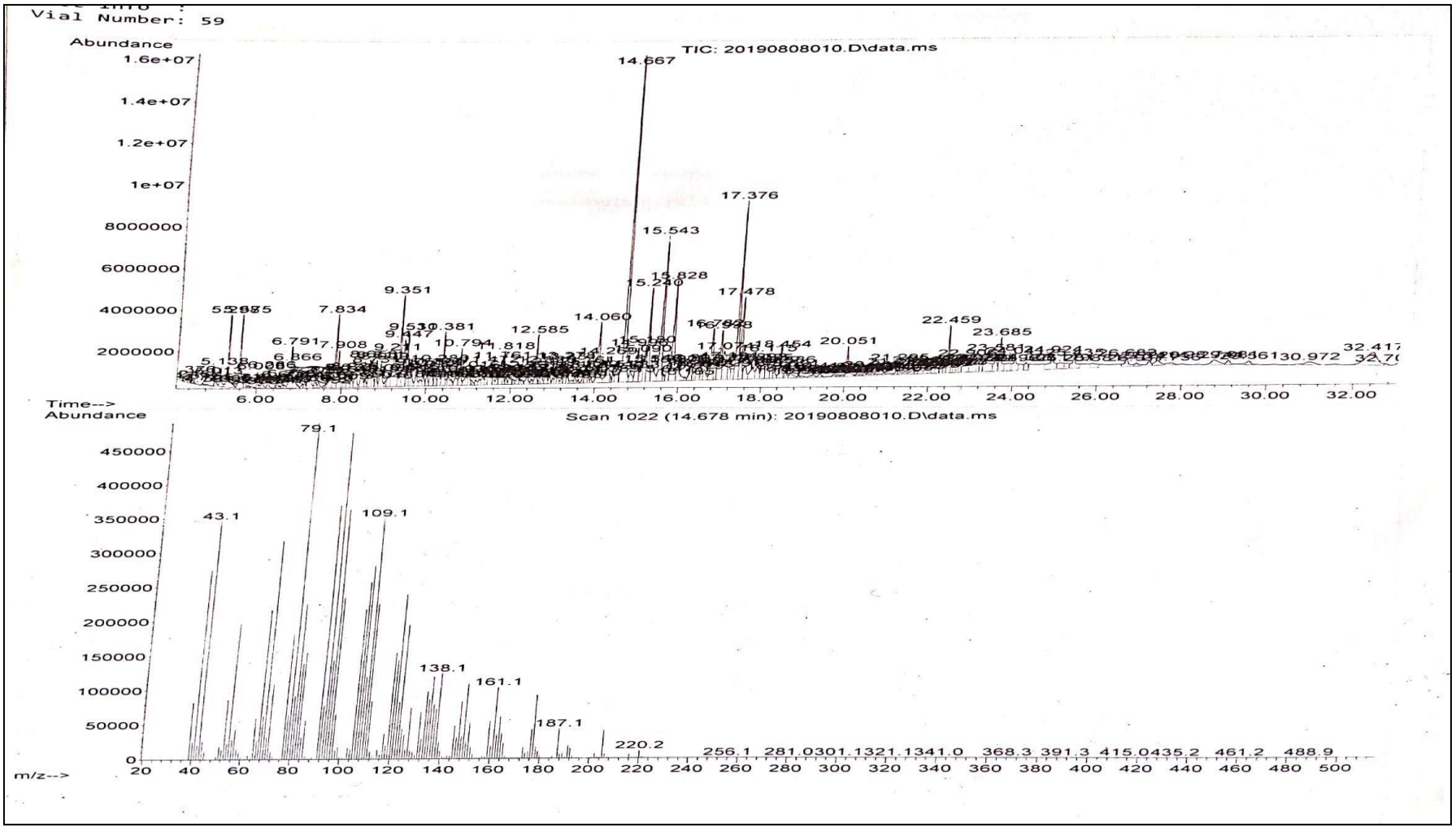

Figure 4.7: GC – MS chromatogram of DCM stalk extract of *Bidens pilosa*

4.3.1.8 Compounds from MeOH stalk extract of *Bidens pilosa*

As indicated in table 4.11 and figure 4.8, the methanolic stalk extract of the *Bidens pilosa* contained 55 chemical compounds as identified by NIST2011 database. The result indicates that this plant has diverse constituents of the chemical compounds which contribute to the observed bioassay activities. The chemical formula, molecular weight and their percentage abundance is as indicated in table 4.11. The peak area and retention time of each of the compound identified in the plant sample is as indicated in figure 4.8.

Table 4.11: Compounds present in MeOH stalk extract of *Bidens pilosa*

Sr No.	RT (Min)	Chemical name	Chemical formula	Molecular weight	% Abundance
1	4.2075	2 – Butanone (K)*	C_4H_8O	72.11	0.412
2	4.6925	2 – Propenoic acid, butyl ester*	$C_7H_{12}O_2$	128.1690	0.261
3	5.2925	3 – Carene (MT)	$C_{10}H_{16}$	136.24	0.143
4	5.5702	Camphene	$C_{10}H_{16}$	136.24	0.347
5	5.7277	Benzene, 1 – ethyl – 2 – methyl – (toluene)	C_9H_{12}	120.19	0.556
6	5.8676	Benzene, 1, 3, 5 – trimethyl – (benzene derivative)*	C_9H_{12}	120.19	0.753
7	6.3257	Benzofuran (phytochemicals with furan ring)	C_8H_6O	118.135	0.049
8	6.4843	1H – Indene, 2, 3 – dihydro – 5 – methyl -	$C_{10}H_{12}$	132.2023	0.872
9	6.7993	Limonene (MT)	$C_{10}H_{16}$	136.238	0.681
10	7.0397	Phenol, 3 – methyl – (PC)*	C_7H_8O	108.1378	0.477
11	7.1351	Benzene, 1 – methyl – 2 – propyl -*	C10H14	134.22	0.453
12	7.2366	Benzene, 1, 2, 4, 5 – tetramethyl – (Alkylbenzene)*	$C_{10}H_{14}$	134.2182	0.946
13	7.2366	Benzene, 1 – methyl – 4 – (1 – methylethyl) – (MT) also known as ρ-cymene)*	$C_{10}H_{14}$	134.21	1.197
14	7.2708	Decane, 2 – methyl –	$C_{11}H_{24}$	156.3083	0.975
15	7.5289	Benzene, 1 – ethyl – 3, 5 – dimethyl -*	$C_{10}H_{14}$	134.2182	0.711
16	7.5776	Benzene, 1 – methyl – 3 – (1 – methylethyl) -	$C_{10}H_{14}$	134.2182	0.758
17	7.6740	Benzene, 1 – ethyl – 2, 4 – dimethyl -*	$C_{10}H_{14}$	134.2182	0.039
18	7.8294	Undecane	$C_{11}H_{24}$	156.31	0.435
19	8.2408	Benzene, 1, 2, 3, 5 – tetramethyl – (AH)	$C_{10}H_{14}$	134.2182	0.487
20	8.3051	Phenylethyl Alcohol (found in essential oils)*	$C_8H_{10}O$	122.16	0.987
21	9.2274	1 – Dodecanol*	$C_{12}H_{26}O$	186.34	1.039
22	9.2999	Benzene, tert – butyl – (AH)	$C_{10}H_{14}$	134.2182	0.226
23	9.4450	3 – Cyclohexene – 1 – methanol., .alpha., .alpha. 4 – trimethyl – (MT alcohol)	$C_{10}H_{18}O$	154.2493	0.838
24	10.6275	Benzene, pentamethyl -*	$C_{11}H_{16}$	148.24	0.798
25	10.7922	Tridecane (H)	$C_{13}H_{28}$	184.36	0.546
26	10.7985	1, 3 – Benzodioxole, 5 – (2 – propenyl) -*	$C_{10}H_{10}O_2$	162.1852	0.679
27	11.0731	Phenol, 2 – methyl – 5 – (1 – methylethyl) -*	$C_{10}H_{14}O$	150.2176	0.916

Sr No.	RT (Min)	Chemical name	Chemical Formula	Molecular weight	% Abundance
28	12.0534	1 – Tetradecanol*	$C_{14}H_{30}O$	214.393	0.616
29	12.1539	Tetradecane	$C_{14}H_{30}$	198.39	0.958
30	12.7405	Diphenylmethane *	$C_{13}H_{12}$	168.234	0.258
31	12.8265	1, 4 – Methanoazulene, decahydro – 4, 8, 8 – trimethyl – 9 – methylene -, [1S – (1. Alpha., 3a. be (TTP)	$C_{15}H_{24}$	204.3511	0.758
32	13.2680	Hexadecane*	$C_{16}H_{3}$	226.41	1.054
33	13.4390	Pentadecane	$C_{15}H_{32}$	212.421	1.006
34	13.6960	Phenol, 2, 4 – bis (1, 1 – dimethylethyl) -*	$C_{14}H_{22}O$	206.32	0.946
35	13.7364	Dodecanoic acid, methyl ester*	$C_{13}H_{26}O_2$	214.3443	1.116
36	13.9312	Benzene, 1, 1’ – ethylidenebis -*	$C_{14}H_{14}$	182.2610	0.657
37	14.5758	1 – Hexadecene*	$C_{16}H_{32}$	224.432	1.145
38	16.0899	Methyl tetradecanoate (FA)*	$C_{15}H_{30}O_2$	242.3975	0.953
39	16.8464	1 – Hexadecanol*	$C_{16}H_{34}O$	242.44	0.483
40	16.9148	Octadecane*	$C_{18}H_{38}$	254.5	0.243
41	17.6102	1, 2 – Benzenedicarboxylic acid, bis (2 – methylpropyl) ester (FAD)	$C_{16}H_{22}O_4$	278.3435	1.168
42	18.2247	Hexadecanoic acid, methyl ester*	$C_{17}H_{34}O_2$	270.45	0.876
43	18.5833	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	0.261
44	18.8942	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.48	0.731
45	19.8724	9, 12 – Octadecadienoic acid (Z, Z) -, methyl ester*	$C_{19}H_{34}O_2$	294.4721	0.049
46	19.9844	9 – Octadecenoic acid (Z) -, methyl ester*	$C_{19}H_{36}O$	296.4879	0.016
47	20.1688	Octadecanoic acid, methyl ester*	$C_{18}H_{36}O_2$	284.4772	1.372
48	21.7078	Tricosane*	$C_{23}H_{48}$	324.63 g	0.691
49	22.4612	Hexanedioc acid, bis (2 – ethylhexyl) ester	$C_{22}H_{42}O_4$	370.5665	0.390
50	23.7068	Bis (2 – ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.564	0.477
51	26.9204	Squalene (TTP)*	$C_{30}H_{50}$	410.73	0.946
52	7.0397	Benzyl Alcohol*	$C_{7}H_{8}O$	108.14	0.858
53	11.0731	Thymol (MT)*	$C_{10}H_{14}O$	150.22	1.476
54	12.7405	Didesmethyldiphenhydramine*	$C_{17}H_{21}NO$	255.35	0.711

Legend: AH stands for Aromatic hydrocarbon, FAD for Fatty acid derivatives, P for phenolics, K for ketone, PS for phytosterols and SD for sugar derivatives, * for phytocompounds absent in DCM stalks extract of *B. pilosa.*

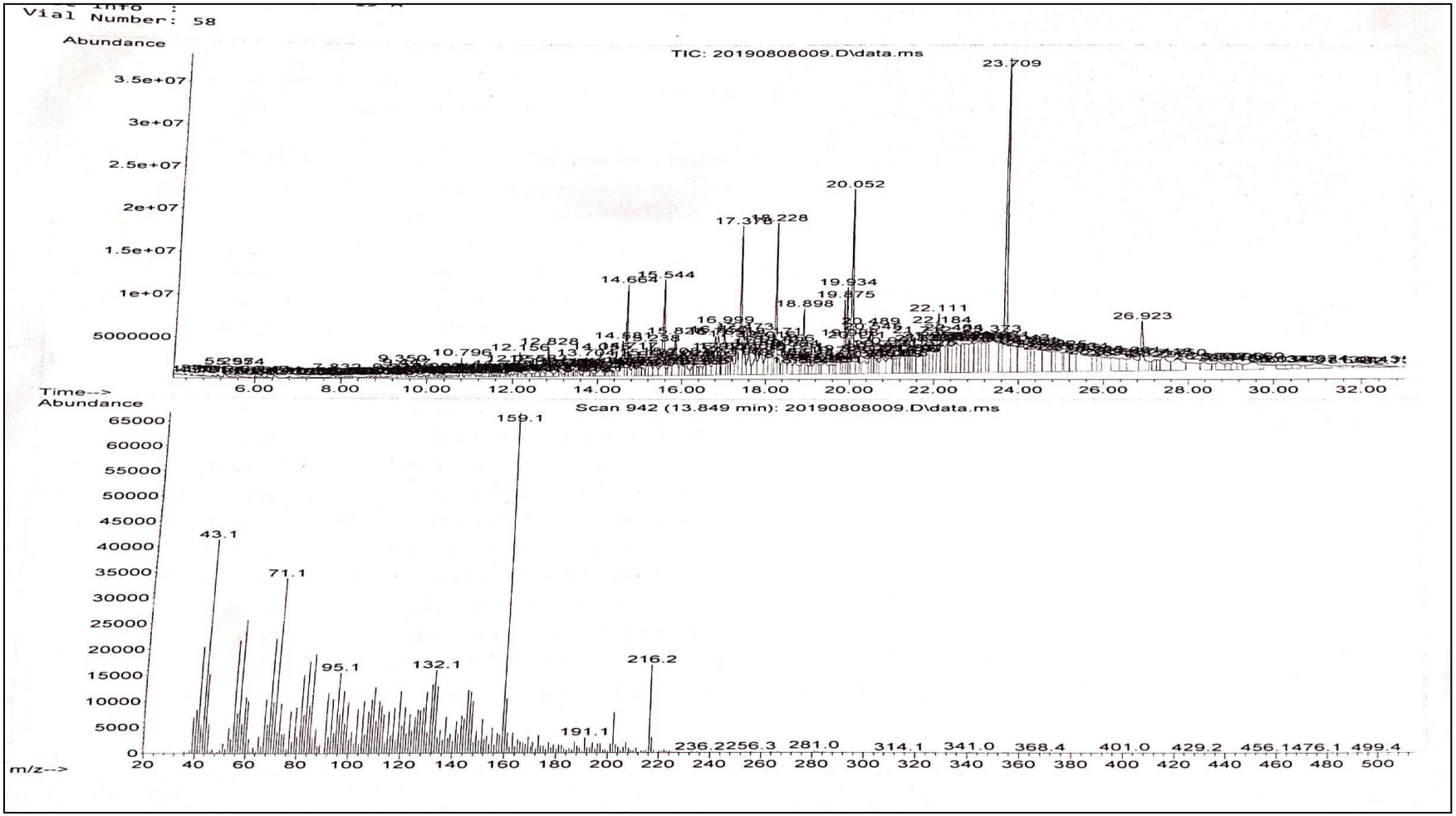

Figure 4.8: GC – MS chromatogram of MeOH stalk extract of *Bidens pilosa*

4.3.1.9 DCM *Mangifera indica* stem bark extract

As indicated in table 4.12 and figure 4.9, the DCM stem bark extract of the *Mangifera indica* contained 43 chemical compounds identified by NIST2011 database. The result indicates that this plant has diverse constituents of the chemical compounds which contribute to the observed bioassay activities. The chemical formula, molecular weight and their percentage abundance is as indicated in table 4.12. The peak area and retention time of each of the compound identified in the plant sample is as indicated in figure 4.9.

Table 4.12: Compounds present in DCM stalk extract of *Mangifera indica*

Sn No.	RT (Min)	Chemical Name	Chemical formula	Molecular weight	% Peak area
1	11.1069	Thymol (MT)*	$C_{10}H_{14}O$	150.22	0.483
2	13.7029	Phenol, 2, 4 – bis (1, 1 – dimethylethyl) – (PC)	$C_{14}H_{22}O$	206.32	1.168
3	5.1336	3 – Carene (MT)	$C_{10}H_{16}$	136.24	0.731
4	5.2942	.alpha. – Pinene (MT)	$C_{10}H_{16}$	136.23	1.369
5	5.5709	Camphene(MT)	$C_{10}H_{16}$	136.23	
6	5.7295	Benzene, 1 – ethyl – 2 – methyl – (AH)	C_9H_{12}	120.1916	0.016
7	5.7854	Benzene, 1 – ethyl – 4 – methyl –(AH)*	C_9H_{12}	120.1916	1.372
8	5.8683	Benzene, 1, 3, 5 – trimethyl – (AH)*	C_9H_{12}	120.1916	0.691
9	6.0062	Bicyclo[3. 1. 1]heptane, 6, 6 – dimethyl – 2 – methylene -, (1S) – (MT)	$C_{10}H_{16}$	136.2340	0.390
10	6.1492	Benzonitrile (AH)*	C_7H_5N	103.04	0.152
11	6.3264	Benzofuran (F)*			0.477
12	6.4839	1H – Indene, 2, 3 – dihydro – 5 – methyl – (AH)	$C_{10}H_{12}$	132.2023	
13	6.7357	Benzene, 1 – methyl – 3 – (1 – methylethyl) – (MT)	$C_{10}H_{14}$	134.2182	0.946
14	6.8000	Limonene (MT)	$C_{10}H_{16}$	136.23	1.197
15	7.1347	Benzene, 1 – methyl – 2 propyl -*	$C_{10}H_{14}$	134.2182	0.858
16	7.2373	Benzene, 1 – ethyl – 2, 4 – dimethyl -	$C_{10}H_{14}$	134.2182	1.476
17	7.8301	Undecane*	$C_{11}H_{24}$	156.31	0.039
18	9.2281	1 – Dodecanol*	$C_{12}H_{26}O$	186.34	
19	9.3483	Dodecane	$C_{12}H_{26}$	170.33	
20	9.4478	3 – Cyclohexene – 1 – methanol, .alpha., .alpha. 4 – trimethyl – (MT)*	$C_{10}H_{18}O$	154.2493	
21	10.7919	Tridecane	$C_{13}H_{28}$	184.37	
22	10.8054	1,3 – benzodioxole, 5 – (2 – propenyl) -*	$C_{10}H_{10}O_2$	162.1852	1.039
23	11.1069	Phenol, 2 – methyl – 5 – (1 – methylethyl) – (PC)*	$C_{10}H_{14}O$	150.2176	
24	11.8147	Pentadecane, 2, 6, 10, 14 – tetramethyl – (DT)*	$C_{19}H_{40}$	268.5209	0.838
25	12.0551	1 – Tetradecanol*	$C_{14}H_{30}O$	214.393	
26	12.1567	Tetradecane*	$C_{14}H_{30}$	198.39	

Sn No.	RT (Min)	Chemical Name	Chemical formula	Molecular weight	% Peak area
27	12.8262	1,4 – Methanoazulene, decahydro – 4, 8, 8 – trimethyl – 9 – methylene - , [1S – (1.alpha., 3a. be((TTP)	$C_{15}H_{24}$	204.3511	
28	13.4438	Pentadecane*	$C_{15}H_{32}$	212.421	
29	13.7029	Phenol, 2, 4 – bis (1, 1 – dimethylethyl) – (PC)	$C_{14}H_{22}O$	206.32	0.916
30	14.5786	1 – Hexadecene*	$C_{16}H_{32}$	224.42	
31	14.6605	Hexadecane	$C_{16}H_{34}$	226.41	
32	16.0916	Methyl tetradecanoate*	$C_{15}H_{30}O_2$	242.4	0.616
33	16.8450	1 – Octadecanol	$C_{18}H_{38}O$	270.49	0.324
34	16.9144	Octadecane*	$C_{18}H_{38}$	254.5	0.258
35	17.6088	1,2–Benzenedicarboxylic acid,bis(2– methylpropyl) ester (FAD)	$C_{16}H_{12}O_4$	278.34	0.758
36	18.2202	Hexadecanoic acid, methyl ester (FAD)	$C_{17}H_{34}O_2$	270.4507	
37	18.5829	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	
38	18.8907	Hexadecanoic acid, ethyl ester (FAD)*	$C_{18}H_{36}O_2$	284.4772	
39	18.8969	1 – Hexadecanol	$C_{16}H_{34}O$	242.44	1.036
40	19.8690	9, 12 – Octadecadienoic acid (Z, Z) -, methyl ester*	$C_{19}H_{34}O_2$	294.4721	1.116
41	19.9291	9 – Octadecenoic acid (Z) -, methyl ester*	$C_{19}H_{36}O_2$	296.4879	1.145
42	20.1623	Octadecanoic acid, methyl ester*	$C_{18}H_{36}O_2$	284.4772	0.564
43	23.6806	Bis (2 – ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.564	0.725

Legend: AH stands for Aromatic hydrocarbon, FAD for Fatty acid derivatives, P for phenolics, PS for phytosterols and SD for sugar derivatives and * for the phytocompounds absent in MeOH *M. indica* stem bark extract

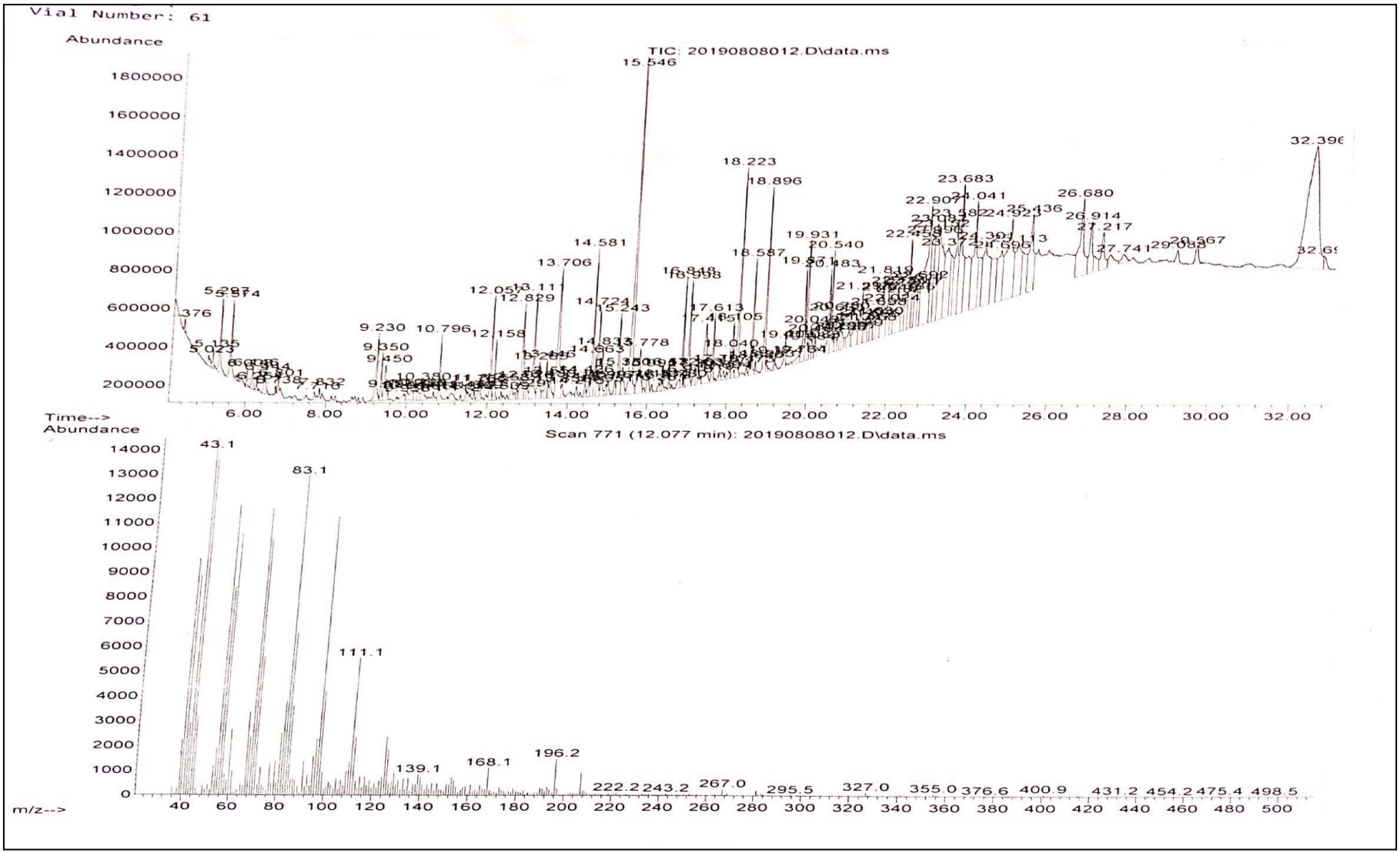

Figure 4.9: GC – MS chromatogram of DCM stem bark extract of *Mangifera indica*

4.3.1.10 Compounds from MeOH stem bark extract *of Mangifera indica*

As indicated in table 4.13 and figure 4.10, the MeOH stem bark extract of the *Mangifera indica* contained 32 chemical compounds identified by NIST2011 database. The result indicates that this plant has diverse constituents of the chemical compounds which contribute to the observed bioassay activities. The chemical formula, molecular weight and their percentage abundance is as indicated in table 4.12. The peak area and retention time of each of the compound identified in the plant sample is as indicated in figure 4.10.

Table 4.13: Compounds present in MeOH stalk of *Mangifera indica*

Sr No	RT (Min)	Chemical name	Chemical formula	Molecular weight	% Abundance
1	5.3044	.alpha.-pinene (MT)	$C_{10}H_{16}$	136.23	1.168
2	5.3044	3–Carene (MT)	$C_{10}H_{16}$	136.24	0.974
3	5.5801	Camphene (MT)	$C_{10}H_{16}$	136.23	
4	5.7356	Benzene, 1 – ethyl – 2 – methyl – (AH)	C_9H_{12}	120.1916	0.694
5	5.8102	Benzaldehyde (A)*	C_7H_6O	106.12	0.342
6	6.0133	Bicyclo[3. 1. 1]heptane, 6, 6 – dimethyl – 2 – methylene- (1S)- ,(MT)	$C_{10}H_{16}$	136.23	0.049
7	6.1521	Benzonitrile (AH)	C_7H_5N	103.04	0.018
8	6.3345	Benzofuran (F)	C_8H_6O	118.1	0.342
9	6.4889	1H – Indene, 2, 3 – dihydro – 5 – methyl – (AH)	$C_{10}H_{12}$	132.2023	0.683
10	6.7377	Benzene, 1 – methyl – 3 – (1 – methylethyl)- (MT)	$C_{10}H_{14}$	134.2182	0.591
11	6.8030	Limonene (MT)	$C_{10}H_{16}$	136.23	1.309
12	6.8973	Benzene, 1 – ethyl – 2, 4 – dimethyl -	$C_{10}H_{14}$	134.2182	0.252
13	7.0900	Benzene, propyl -*	C_9H_{12}	120.1916	0.467
14	7.8341	Undecane	$C_{11}H_{24}$	156.31	0.497
15	9.2279	1 – Dodecanol	$C_{12}H_{26}O$	186.34	1.136
16	9.3471	Dodecane (EO)	$C_{12}H_{26}$	170.33	0.719
17	10.7928	Tridecane (EO)	$C_{13}H_{28}$	184.37	0.859
18	12.0571	1 – Tetradecane*	$C_{14}H_{28}$	196.3721	0.961
19	12.4851	1,4 – Methanoazulene, decahydro – 4, 8, 8 – trimethyl – 9 – methylene -,TTP)	$C_{15}H_{24}$	204.3511	1.129
20	13.7048	Phenol, 2, 4 – bis (1, 1 – dimethylethyl) – (PC)	$C_{14}H_{22}O$	206.3239	1.039
21	14.5795	1 – Hexadecanol	$C_{16}H_{34}O$	242.44	0.386
22	14.5795	Hexadecane	$C_{16}H_{34}$	226.41	0.821
23	16.8459	1 – Octadecanol	$C_{18}H_{38}O$	270.4937	0.868
24	18.0988	1, 2 – Benzenedicarboxylic acid, bis (2 – methylpropyl) ester (FAD)	$C_{16}H_{22}O_4$	278.3435	0.919
25	18.2221	Hexadecanoic acid, methyl ester (FAD)	$C_{17}H_{34}O_2$	270.4507	1.006
26	18.5838	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	0.116
27	23.6814	Bis (2 – ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.564	0.273
28	7.0900	N – Benzyl – 2 – phenethylamine*	$C_{15}H_{17}N$	211.3022	0.558

Legend: AH stands for Aromatic hydrocarbon, FAD for Fatty acid derivatives, PC for phenolic compounds, TTP for triterpenoids,EO for essential oils, MT for monoterpenoids, PS for phytosterols and SD for sugar derivatives and * for the phytocompounds absent in DCM *M. indica* stem bark extract.

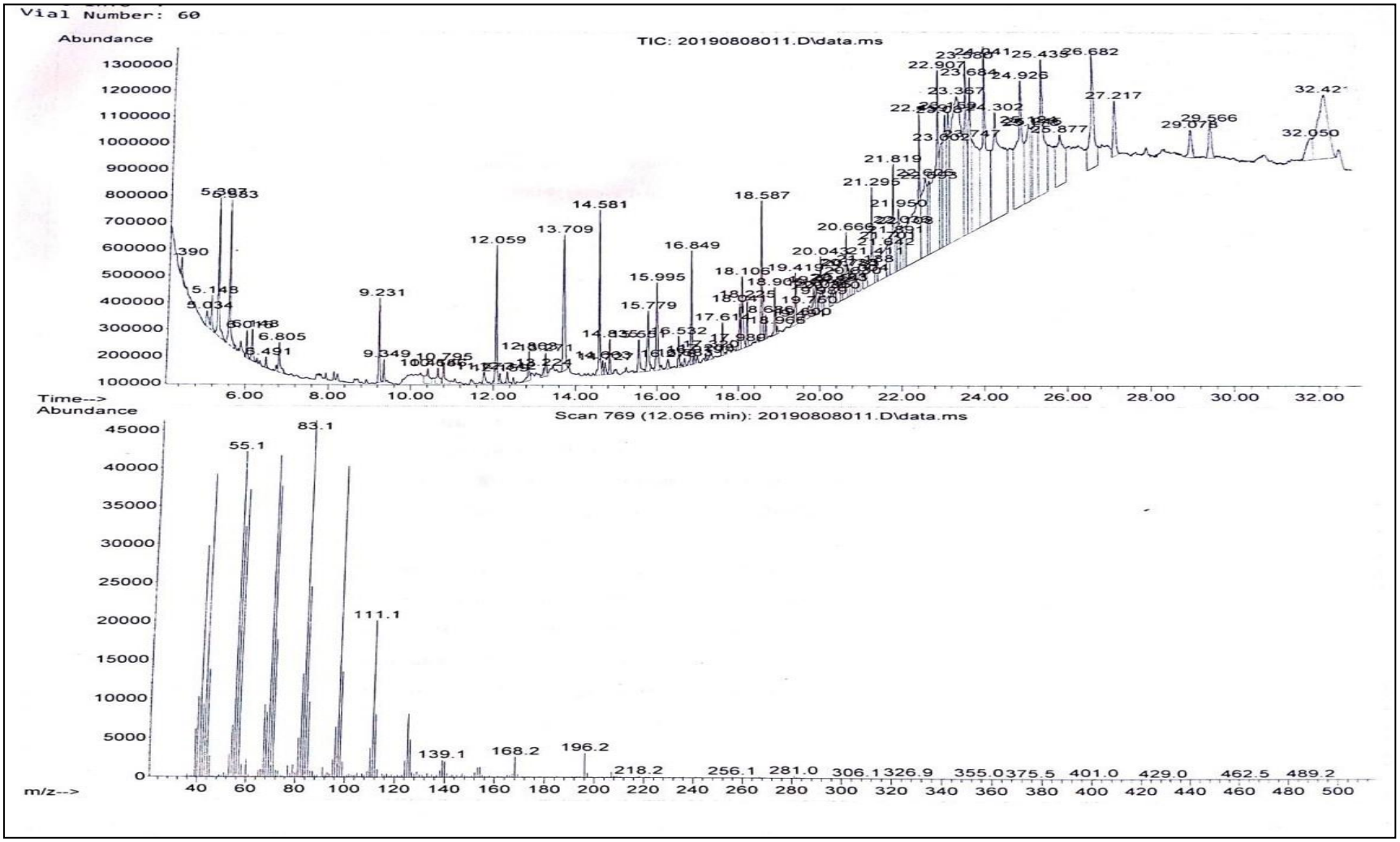

Figure 4.10: GC – MS chromatogram of MeOH stalk extract of *Mangifera indica*

4.4 Antipyretic activities of the plant extracts

4.4.1 Antipyretic activities of DCM leaf extract of *Eucalyptus saligna*

One hour following the administration of DCM leaf extract of the *E. saligna*, there was a significant difference in the rectal temperature in relation to animal models treated with a conventional antipyretic drug (Aspirin) and varying doses of the DCM extract of the *E. saligna* (50, 100 and 150 mg/Kg) with a P value=0.00 at $p<0.05$. The percentage change from the initial rectal temperature for the rat model in normal, negative and positive and experimental tests A, B and C was -0.21, -0.31, 0.52, 0.31, 0.51 and 0.98% respectively (Table 4.14 and 4.15).

The study results indicated that two hours following administration of DCM *E. saligna* leaves extract, there was a significant difference in the rectal temperature of the animal models in all the groups B and C with a P =0.00 at $p <0.01$ (Table 4.14 and 4.15). The percentage change in rectal temperaturechange in normal, negative and positive and the experimental test A, B and C was -0.16, -0.10, 1.09, 0.62, 1.08 and 1.09% respectively (Table 4.14 and 4.15). The three different dosages of the DCM leaf extract of *E. saligna* were effective in management of fever after 2 hours. The leaf extract dosage of 50 and 100 mg/Kg had no significant difference with the conventional drug (Aspirin). It is only the highest dosage of DCM left extract (150 mg/Kg) which had a significance percentage change in the rectal temperature when compared to the conventional drug at $p < 0.05$ (Table 4.14 and 4.15).

Three hours following administration of DCM leaf extract of the *E. saligna*, there was a

significant difference in the rectal temperature among the animals in normal, negative and positive control test group and experimental test groups A, B and C P=0.00 at $p<0.05$ (Table 4.14 and 4.15). The percentage change in rectal temperature change in normal, negative, positive and experiment tests A, B and C was -0.21, -0.16, 1.97, 1.19, 1.75 and 2.72% respectively (Table 4.14 and 4.15). The three dosages of DCM leaf extract of the *E. saligna* had some antipyretic activity. The rectal temperature in group treated with conventional drug and 50 mg/Kg (low dose) of DCM leaf *E. saligna* leaf extract had no significance different ($p<0.05$) (Table 4.14 and 4.15).

After the administration of the DCM *E. saligna* leaves extract, there was a significant difference in the rectal temperature among the animals in normal, negative and positive and experiment test groups A, B and C with P=0.00 after four hours at $p < 0.05$ (Table 4.14). The percentage change in rectal temperature in rats groups labeled:- normal, negative positive and the experiment test groups A, B and C is -0.32, -0.05, 2.9, 1.85, 2.57 and 3.44% respectively (Table 4.14 and 4.15). The highest dosage of 150 mg/Kg had better antipyretic activity than the lower doses of 50 and 100 mg/Kg. It had better antipyretic activity than the conventional drug (Aspirin) used in this experiment (Table 4.14 and 4.15).

Table 4.14: Antipyretic activity of DCM leaves extract of *Eucalyptus saligna* in percentage

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test	DMSO	100.21+0.27^{c} (-0.21)	100.16+0.23^{c} (-0.16)	100.21+0.19^{c} (-0.21)	100.32+0.13^{c} (-0.32)
Negative test	Turpentine+ DMSO	100.31+0.13^{c} (-0.31)	100.10+0.06^{c} (-0.10)	100.16+0.17^{c} (-0.16)	99.95+0.18^{c} (0.05)
Positive test	Turpentine+ Aspirin	99.48+0.08^{ab} (0.52)	98.91+0.17^{b} (1.09)	98.03+0.38^{ab} (1.97)	97.10+0.49^{ab} (2.9)
Experimental test A	Turpentine+ *E .saligna* 50mg/kg	99.69+0.05^{ab} (0.31)c	99.38+0.10^{b} (0.62)c	98.81+0.13^{b} (1.19)b	98.15+0.19^{b} (1.85)c
Experimental test B	Turpentine+ *E. saligna* 100mg/kg	99.48+0.08^{bc} (0.51)	98.92+0.16^{b} (1.08)	98.25+0.22^{ab} (1.75)	97.43+0.40^{ab} (2.57)
Experimental test C	Turpentine+ *E. saligna* 150mg/kg	99.03+0.15^{a} (0.98)	98.15+0.15^{a} (1.09)	97.28+0.13^{a} (2.72)	96.57+0.13^{a} (3.44)
	F- Value	11.06	24.25	28.47	28.76
	P Value	0.00	0.00	0.00	0.00

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Data was analyzed by one – way ANOVA andTurkey's test ($p < 0.05$). Aspirin = 100 mg/kg dose, Turpentine =20%concentrated.

Table 4.15: Time effects on antipyretic activity of DCM leave extract of *Eucalyptus saligna*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	0.053	0.286	(-0.795, 0.902)	0.19	0.998
		3	-0.000	0.286	(-0.849, 0.848)	-0.00	1.000
		4	-0.107	0.286	(-0.955, 0.742)	-0.37	0.981
	2	3	-0.054	0.286	(-0.902, 0.795)	-0.19	0.998
		4	-0.160	0.286	(-1.008, 0.688)	-0.56	0.942
	3	4	-0.106	0.286	(-0.955, 0.742)	-0.37	0.982
Negative control	1	2	0.206	0.225	(-0.463, 0.875)	0.91	0.798
		3	0.154	0.225	(-0.515, 0.823)	0.68	0.902
		4	0.360	0.225	(-0.309, 1.029)	1.60	0.415
	2	3	-0.052	0.225	(-0.721, 0.617)	-0.23	0.995
		4	0.154	0.225	(-0.515, 0.823)	0.68	0.901
	3	4	0.206	0.225	(-0.463, 0.876)	0.92	0.797
Positive control	1	2	0.570	0.304	(-0.333, 1.473)	1.87	0.289
		3	1.450	0.304	(0.547, 2.353)	4.77	0.002
		4	2.384	0.304	(1.481, 3.287)	7.84	0.000
	2	3	0.880	0.304	(-0.023, 1.783)	2.89	0.057
		4	1.815	0.304	(0.912, 2.717)	5.97	0.000
	3	4	0.935	0.304	(0.032, 1.837)	3.07	0.042
Experimental group A	1	2	0.309	0.130	(-0.075, 0.694)	2.39	0.133
		3	0.876	0.130	(0.491, 1.261)	6.76	0.000
		4	1.545	0.130	(1.160, 1.930)	11.92	0.000
	2	3	0.566	0.130	(0.181, 0.951)	4.37	0.004
		4	1.236	0.130	(0.851, 1.620)	9.53	0.000
	3	4	0.669	0.130	(0.284, 1.054)	5.16	0.001
Experimental group B	1	2	0.566	0.219	(-0.083, 1.215)	2.59	0.095
		3	1.235	0.219	(0.586, 1.884)	5.65	0.001
		4	2.056	0.219	(1.407, 2.705)	9.41	0.000
	2	3	0.669	0.219	(0.020, 1.318)	3.06	0.043
		4	1.491	0.219	(0.842, 2.140)	6.82	0.000
	3	4	0.821	0.219	(0.172, 1.470)	3.76	0.013
Experimental group C	1	2	0.872	0.160	(0.397, 1.347)	5.45	0.001
		3	1.743	0.160	(1.268, 2.218)	10.89	0.000
		4	2.460	0.160	(1.985, 2.935)	15.37	0.000
	2	3	0.871	0.160	(0.396, 1.346)	5.44	0.001
		4	1.588	0.160	(1.113, 2.064)	9.92	0.000
	3	4	0.717	0.160	(0.242, 1.192)	4.48	0.004

4.4.2 Antipyretic activities of methanolic leaf extract of *E. saligna*

The study results indicated that one hour following administration of methanolic *E. saligna* leaves extract, there was no significant difference in the rectal temperature of the animal models treated with a conventional antipyretic drug (Aspirin) and varying dosage of the methanolic extract of the *E. saligna* (50, 100 and 150 mg/Kg) but the rectal temperature change was statistically different between the negative and positive control test groups and the experimental test groups with a $P=0.00$ at $p<0.05$. The percentage change from the initial rectal temperature for the rat model in normal, negative, and positive tests and experiment tests A, B and C was 0.37, -0.36, 0.52, 0.57, 0.56 and 0.64%, respectively (Table 4.16 and 4.17).

After two hours following *E. saligna* methanolic leaves extract administration, the rectal temperature change was statistically different between all the experimental study groups with a $P=0.00$, $p<0.05$. The percentage rectal temperature change in normal, negative, positive and experiment test groups A, B and C was 0.37, -0.36, 0.88, 1.18, 1.28 and 1.39% respectively (Table 4.16). The three different dosages of the methanolic leaves extract of *E. saligna* were effective in management of fever after 2 hours. The rectal temperature change was not statistically different between the groups treated with 50 and 100 mg/Kg dose levels of the plant extract and the conventional drug (Aspirin). The highest dosage of methanolic leaves extract (150 mg/Kg) had a higher antipyretic efficancy change in the rectal temperature when compared to the conventional drug (Table 4.16 and 4.17).

Three hours following *E. saligna* methanolic leaves extract administration, the rectal temperature change between the animals in the group labeled:- normal, negative and positive control test group and experimental test groups A, B and C were statistically different with a P=0.00, p<0.05. (Table 4.16 and 4.17). The percentage rectal temperature change in in the groups labeled:- normal, negative, positive and the experiment test groups A, B and C was 0.48, -0.21, 1.09, 1.90, 2.20 and 2.16% respectively (Table 4.16 and 4.17). The three dosages of methanolic leaves extract of the *E. saligna* had a higher antipyretic activity potential than the conventional drug (Aspirin) (Table 4.16 and 4.17).

Four hours following administration of *E. saligna* methanolic leaves extract, there was a significant difference in the rectal temperature change among the animals in normal, negative and positive control test groups and experimental test groups A, B and C P=0.00, p<0.05 (Table 4.16 and 4.17). The percentage change in rectal temperature change in groups labeled:- normal, negative, positive and experiment test groups A, B and C was 0.53, 0.00, 1.35, 2.82, 3.38 and 3.13% respectively (Table 4.16). The three dosages of methanolic leaves extracts of *E. saligna* had better antipyretic activity than the conventional drug (Aspirin) used in this experiment (Table 4.16 and 4.17).

Table 4.16: Antipyretic activities of methanolic leaves extract of *E. saligna* in percentage

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	99.63+0.26^{a} (0.37)	99.63+0.37^{ab} (0.37)	99.53+0.28^{ab} (0.48)	99.47+0.15^{a} (0.53)
Negative test control	Turpentine+ DMSO	100.36+0.06^{b} (-0.36)	100.36+0.16^{a} (-0.36)	100.21+0.21^{a} (-0.21)	100.00+0.23^{a} (0.00)
Positive test control	Turpentine+ Aspirin	99.48+0.12^{a} (0.52)	99.12+0.13^{bc} (0.88)	98.91+0.19^{bc} (1.09)	98.65+0.10^{b} (1.35)
Experimental test A	Turpentine+ *E .saligna* 50mg/kg	99.44+0.13^{a} (0.57)	98.82+0.24^{bc} (1.18)	98.10+0.30^{cd} (1.90)	97.18+0.27^{c} (2.82)
Experimental test B	Turpentine+ *E. saligna* 100mg/kg	99.44+0.05^{a} (0.56)	98.72+0.23^{bc} (1.28)	97.80+0.20^{d} (2.20)	96.62+0.14^{c} (3.38)
Experimental test C	Turpentine+ *E. saligna* 150mg/kg	99.38+0.12^{a} (0.62)	98.61+0.16^{c} (1.39)	97.84+0.20^{d} (2.16)	96.87+0.13^{c} (3.13)
	F. Value	7.28	8.71	18.75	65.32
	P. Value	0.00	0.00	0.00	0.00

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different.Data was analyzed using one – way ANOVA and Turkey's test ($p < 0.05$). Aspirin = 100 mg/kg dose, Turpentine =20% concentrated.

Table 4.17: Time effects on antipyretic activity of MeOH leave extract of *Eucalyptus saligna*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	-0.003	0.274	(-0.816, 0.811)	-0.01	1.000
		3	0.104	0.274	(-0.709, 0.917)	0.38	0.980
		4	0.158	0.274	(-0.655, 0.972)	0.58	0.937
	2	3	0.107	0.274	(-0.707, 0.920)	0.39	0.979
		4	0.161	0.274	(-0.652, 0.974)	0.59	0.934
	3	4	0.054	0.274	(-0.759, 0.868)	0.20	0.997
Negative control	1	2	-0.001	0.131	(-0.391, 0.389)	-0.01	1.000
		3	0.153	0.131	(-0.237, 0.543)	1.16	0.659
		4	0.358	0.131	(-.0.032, 0.748)	2.73	0.075
	2	3	0.154	0.131	(-0.236, 0.544)	1.17	0.655
		4	0.359	0.131	(-0.031, 0.749)	2.74	0.075
	3	4	0.205	0.131	(-0.185, 0.595)	1.56	0.433
Positive control	1	2	0.364	0.118	(0.014, 0.714)	3.09	0.041
		3	0.571	0.118	0.221, 0.921)	4.84	0.002
		4	0.831	0.118	(0.481, 1.181)	7.05	0.000
	2	3	0.207	0.118	(-0.143, 0.557)	1.75	0.340
		4	0.467	0.118	(0.117, 0.817)	3.96	0.009
	3	4	0.260	0.118	(-0.090, 0.610)	2.21	0.179
Experimental group A	1	2	0.616	0.218	(-0.031, 1.262)	2.83	0.064
		3	1.333	0.218	(0.687, 1.980)	6.12	0.000
		4	2.257	0.218	(1.610, 2.903)	10.36	0.000
	2	3	0.718	0.218	(0.071, 1.365)	3.30	0.028
		4	1.641	0.218	(0.994, 2.288)	7.54	0.000
	3	4	0.923	0.218	(0.276, 1.570)	4.24	0.005
Experimental group B	1	2	0.715	0.116	(0.370, 1.059)	6.16	0.000
		3	1.637	0.116	(1.292, 1.982)	14.11	0.000
		4	2.815	0.116	(2.471, 3.160)	24.26	0.000
	2	3	0.922	0.116	(0.578, 1.267)	7.95	0.000
		4	2.101	0.116	(1.756, 2.445)	18.10	0.000
	3	4	1.178	0.116	(0.834, 1.523)	10.15	0.000
Experimental group C	1	2	0.771	0.116	(0.427, 1.115)	6.66	0.000
		3	1.541	0.116	(1.197, 1.885)	13.30	0.000
		4	2.516	0.116	(2.172, 2.860)	21.71	0.000
	2	3	0.770	0.116	(0.426, 1.114)	6.64	0.000
		4	1.744	0.116	(1.400, 2.089)	15.06	0.000
	3	4	0.975	0.116	(0.631, 1.319)	8.41	0.000

4.4.3 Antipyretic activities of DCM bark extract of *Mangifera indica*

The study results indicated that one hour upon DCM stem bark extract administration of the *M. indica*, there was no significant difference in the rectal temperature of the animal models treated with a conventional antipyretic drug (Aspirin) and varying dosage of the DCM extract of the *M. indica* (50, 100 and 150 mg/Kg) with a P value =0.40, P<0.05. The percentage change from the initial rectal temperature for the rat model in the groups labeled:- normal, negative, positive and experiment test groups A, B and C was 0.16, 0.15,0.62, 0.46, 0.57 and 0.67%, respectively (Table 4.18 and 4.19).

After two hours upon the administration of the DCM stem bark extract of the *M. indica*, there was a significant difference in the rectal temperature of the animal models in the gropus labeled:- normal, negative, positive and experiment test groups A, B and C with a P=0.00, $p < 0.05$ (Table 4.18 and 4.19). The percentage change in rectal temperature of the experimental animals in the groups labeled:- normal, negative, positive and the experiment test groups A, B and C was -0.17, 0.36, 1.44, 0.87, 1.34 and 1.59%, respectively (Table 4.18 and 4.19). The three different dosages of the DCM stem bark extract of *M. indica* were effective in management of fever after 2 hours. The stem bark extract dosage of 100 and 150 mg/Kg had no significance difference with the conventional drug (Aspirin). It was only the lower dosage of DCM left extract (50 mg/Kg) which had a significance lower percentage change in the rectal temperature when compared to the conventional drug (Table 4.18 and 4.19).

Three hours following administration of DCM stem bark extract of the *M. indica*, there

was a significant difference in the rectal temperature among the animals in normal, negative and positive test control groups and experimental test groups A, B and C P=0.00, P < 0.05 (Table 4.18 and 4.19). The percentage change in rectal temperature change of the experimental animals in the groups labeled:- normal, negative, positive and the experiment test groups A, B and C was -0.16, 0.93, 2.21, 1.49, 2.26 and 2.56%, respectively (Table 4.18 and 4.19). The three dosages of *M. indica* DCM stem bark extract had some antipyretic activity. The rectal temperature in group treated with conventional drug and 100mg/Kg of the DCM stem bark extract of the *M. indica* had no significance different (Table 4.15). *Mangifera Indica* DCM stem bark extract dosage of 150 mg/Kg had higher antipyretic activity than the conventional antipyretic drug (Aspirin) (Table 4.18 and 4.19).

Four hours following administration of DCM stem bark extract of the *M. indica*, there was a significant difference in the rectal temperature among the animals in normal, negative and positive test control groups and experimental test groups A, B and C P=0.00, P < 0.05 (Table 4.18 and 4.19). The percentage change in rectal temperature change in the experimental animals in the groups labeled:- normal, negative, positive and the experiment test groups A, B and C was - 0.65, 1.34, 3.03, 2.32, 3.09 and 3.23%, respectively (Table 4.18 and 4.19). There was no significant difference in the antipyretic activity of the conventional antipyretic drug (Aspirin)and the 100mg/Kg dosage of the *M. indica* stem bark extract. The highest dosage of 150 mg/Kg of *M. indica* extract had better antipyretic activity than the lower doses of 50, 100 mg/Kg and the conventional drug (Aspirin) used in this experiment (Table 4.18 and 4.19).

Table 4.18: Antipyretic activities of DCM stem bark extract of *Mangifera indica* in perecentage

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	99.84+0.24^{a} (0.16)	100.16+0.30^{a} (-0.17)	100.16+0.22^{a} (-0.16)	100.65+0.18^{a} (-0.65)
Negative test control	Turpentine+ DMSO	99.84+0.16^{a} (0.15)	99.64+0.25^{ab} (0.36)	99.07+0.19^{b} (0.93)	98.66+0.2^{b} (1.34)
Positive test control	Turpentine+ Aspirin	99.38+0.10^{a} (0.62)	98.56+0.17^{c} (1.44)	97.79+0.13^{cd} (2.21)	96.97+0.1^{cd} (3.03)
Experimental test A	Turpentine+ *M. indica* 50mg/kg	99.54+0.05^{a} (0.46)	99.13+0.06^{bc} (0.87)	98.51+0.10^{bc} (1.49)	97.68+0.8^{c} (2.32)
Experimental test B	Turpentine+ *M. indica* 100mg/kg	99.44+0.12^{a} (0.57)	98.66+0.20c (1.34)	97.74+0.24^{cd} (2.26)	96.91+0.3^{cd} (3.09)
Experimental test C	Turpentine+ *M. indica* 150mg/kg	99.33+0.06^{a} (0.67)	98.41+0.09^{c} (1.59)	97.44+0.13^{d} (2.56)	96.77+0.2^{d} (3.23)
	F value	2.79	12.04	33.83	52.71
	P value	0.40	0.000	0.000	0.000

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Data was analyzed using one – way ANOVA and Turkey's test ($p < 0.05$). Aspirin = 100 mg/kg dose, Turpentine =20% concentrated.

Table 4.19: Time effects on antipyretic activity of DCM leave extract of *Mangifera indica*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	-0.324	0.172	(-0.84, 0.19)	-1.88	0.286
		3	-0.323	0.172	(-0.83, 0.19)	-1.88	0.288
		4	-0.807	0.172	(-1.32, -0.30)	-4.69	0.003
	2	3	0.001	0.172	(-0.51, 0.51)	0.00	1.000
		4	-0.484	0.172	(-0.10, 0.03)	-2.81	0.066
	3	4	-0.484	0.172	(-0.10, 0.03)	-2.81	0.065
Negative control	1	2	0.206	0.131	(-0.18, 0.59)	1.57	0.429
		3	0.775	0.131	(0.39, 1.16)	5.92	0.000
		4	1.187	0.131	(0.80, 1.58)	9.07	0.000
	2	3	0.569	0.131	(0.18, 0.96)	4.35	0.005
		4	0.982	0.131	(0.59, 1.37)	7.50	0.000
	3	4	0.413	0.131	(0.02, 0.80)	3.15	0.036
Positive control	1	2	0.822	0.125	(0.45, 1.19)	6.56	0.000
		3	1.593	0.125	(1.22, 1.97)	12.72	0.000
		4	2.415	0.125	(2.04, 2.79)	19.29	0.000
	2	3	0.771	0.125	(0.40, 1.14)	6.16	0.000
		4	1.594	0.125	(1.22, 1.97)	12.73	0.000
	3	4	0.822	0.125	(0.45, 1.19)	6.57	0.000
Experimental group A	1	2	0.4116	0.079	(0.18 0.65)	5.23	0.001
		3	1.0294	0.079	(0.80, 1.26)	13.07	0.000
		4	1.8528	0.079	(1.62, 2.09)	23.52	0.000
	2	3	0.6178	0.079	(0.38, 0.85)	7.84	0.000
		4	1.4412	0.079	(1.21, 1.68)	18.30	0.000
	3	4	0.8234	0.079	(0.59, 1.06)	10.45	0.000
Experimental group B	1	2	0.772	0.147	(0.34, 1.21)	5.25	0.001
		3	1.698	0.147	(1.26, 2.13)	11.56	0.000
		4	2.521	0.147	(2.09, 2.96)	17.16	0.000
	2	3	0.926	0.147	(0.49, 1.36)	6.31	0.000
		4	1.750	0.147	(1.31, 2.19)	11.91	0.000
	3	4	0.823	0.147	(0.39, 1.26)	5.60	0.001
Experimental group C	1	2	0.923	0.110	(0.60, 1.25)	8.40	0.000
		3	1.898	0.110	(1.57, 2.22)	17.27	0.000
		4	2.564	0.110	(2.24, 2.89)	23.34	0.000
	2	3	0.975	0.110	(0.65, 1.30)	8.87	0.000
		4	1.640	0.110	(1.31, 1.97)	14.93	0.000
	3	4	0.666	0.110	(0.34, 0.99)	6.06	0.000

4.4.4 Antipyretic activities of methanolic stem bark extract of *M. indica*

One hour upon the administration of stem bark methanolic extract of the *M. indica*, there was a significant difference in the rectal temperature of the animal models treated with a conventional antipyretic drug (Aspirin) and varying dosage of the methanolic stem bark extract of *M. indica* (50, 100 and 150 mg/Kg) with a P value=0.00, $p<0.05$. The methanolic stem bark extract dose (50 mg/Kg) antipyretic potential was significantly different in relation to the conventional drug (Aspirin). The percentage change from the initial rectal temperature for the rat model in the experimental study groups labeled:- normal, negative, positive andexperiment test A, B and C was -0.16, -0.26, 0.67, 0.46, 0.61 and 0.82%, respectively (Table 4.20 and 4.21).

After two hours upon the administration of the methanolic stem bark extract of *M. indica*, there was a significant difference in the rectal temperature of the animal models in the experimental groups labeled:- normal, negative, positive test and test groups A, B and C with a P=0.00 at $p < 0.05$ but the rectal temperature change between the groups labeled: positive and test groups A, B and C were not significantly different (Table 4.20 and 4.21). The percentage change in rectal temperature change in the experimental animals models categorized in the group labeled:- normal, negative, positive and the test groups A, B and C was - 0.16, -0.31, 1.75, 1.23, 1.28 and 1.69%, respectively (Table 4.20, 4.21). The three different dosages of the methanolic *M. indica* stem bark extract were effective in management of fever after 2 hours (Table 4.20, 4.21).

Three hours following methanolic *M. indica* stem bark extract administration, there was a

significant difference in the rectal temperature among the animals in normal, negative and positive control test group and experimental test groups A, B and C with a P=0.00, $p < 0.05$ (Table 4.20 and 4.21). The percentage change in rectal temperature change in the animals models in the groups labeled as:- normal, negative and positive and test groups A, B and C was –0.27, -0.16, 3.00, 2.36, 2.45 and 2.87%, respectively (Table 4.20, and 4.21). The rectal temperature in group treated with conventional drug and 50, 100 and 150 mg/Kg of the MeOH stem bark extract of *M. indica* had no significance different (Table 4.20 and 4.21).

Four hours following administration of the methanolic *M. indica* stem bark extract, there was a significant difference in the rectal temperature among the animals in normal, negative, positive control test group and experimental test groups A, B and C with P=0.00, $p < 0.05$ (Table 4.20, 4.21). The percentage change in rectal temperature change in the animal models in the group labeled as:- normal, negative and positive and test groups A, B and C was -0.32, 0.21, 3.91, 3.13, 3.93 and 4.10%, respectively (Table 4.16). The three dosages of 50, 100 and 150 mg/Kg did not have any significant difference in antipyretic potential with the conventional drug (Aspirin) used in this experiment (Table 4.20 and 4.21).

Table 4.20: Antipyretic activities of *Mangifera indica* MeOH stem bark extract

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	100.16+0.16^{ab} (-0.16)	100.16+0.11^{a} (-0.16)	100.27+0.23^{a} (-0.27)	100.32+0.16^{a} (-0.32)
Negative test control	Turpentine+ DMSO	100.26+0.26^{a} (-0.26)	100.31+0.05^{a} (-0.31)	100.15+0.10^{a} (-0.16)	99.79+0.0^{a} (0.21)
Positive test control	Turpentine+ Aspirin	99.33+0.13^{c} (0.67)	98.25+0.28^{b} (1.75)	97.01+0.29^{b} (3.00)	96.09+0.22^{c} (3.91)
Experimental test A	Turpentine+ *M. indica* 50mg/kg	99.54+0.05^{bc} (0.46)	98.77+0.15^{b} (1.23)	97.64+0.12^{b} (2.36)	96.87+0.14^{b} (3.13)
Experimental test B	Turpentine+ *M. indica* 100mg/kg	99.39+0.10^{c} (0.61)	98.73+0.14^{b} (1.28)	97.55+0.17^{b} (2.45)	96.07+0.24^{c} (3.93)
Experimental test C	Turpentine+ *M. indica* 150mg/kg	99.18+0.05^{c} (0.82)	98.31+0.10^{b} (1.69)	97.13+0.14^{b} (2.87)	95.90+0.11^{c} (4.10)
F value		9.54	35.52	64.97	140.76
P value		0.000	0.000	0.000	0.000

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Data was analyzed using one – way ANOVA and Turkey's test ($p < 0.05$). Aspirin = 100 mg/kg dose, Turpentine =20% concentrated.

Table 4.21: Time effects on antipyretic activity of MeOH stem bark extract of *Mangifera indica*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	0.003	0.301	(-0.891, 0.896)	0.01	1.000
		3	-0.104	0.301	(-0.998, 0.789)	-0.35	0.985
		4	-0.158	0.301	(-1.051, 0.735)	-0.53	0.951
	2	3	-0.107	0.301	(-1.001, 0.786)	-0.36	0.984
		4	-0.161	0.301	(-1.054, 0.733)	-0.53	0.949
	3	4	-0.054	0.301	(-0.947, 0.840)	-0.18	0.998
Negative control	1	2	0.359	0.203	(-0.2444, 0.963)	1.77	0.333
		3	0.978	0.203	(0.375, 1.581)	4.82	0.002
		4	2.164	0.203	(1.561, 2.767)	10.66	0.000
	2	3	0.619	0.203	(0.016, 1.222)	3.05	0.044
		4	1.805	0.203	(1.202, 2.408)	8.89	0.000
	3	4	1.186	0.203	(0.583, 1.789)	5.84	0.000
Positive control	1	2	1.082	0.157	(0.615, 1.548)	6.89	0.000
		3	2.317	0.157	(1.851, 2.784)	14.76	0.000
		4	3.244	0.157	(2.777, 3.710)	20.66	0.000
	2	3	1.236	0.157	(0.769, 1.702)	7.87	0.000
		4	2.162	0.157	(1.696, 2.628)	13.77	0.000
	3	4	0.926	0.157	(0.460, 1.392)	5.90	0.000
Experimental group A	1	2	0.769	0.120	(0.412, 1.125)	6.40	0.000
		3	1.898	0.120	(1.542, 2.255)	15.81	0.000
		4	2.667	0.120	(2.311, 3.024)	22.21	0.000
	2	3	1.130	0.120	(0.773, 1.486)	9.41	0.000
		4	1.899	0.120	(1.542, 2.255)	15.81	0.000
	3	4	0.769	0.120	(0.412, 1.126)	6.40	0.000
Experimental group B	1	2	0.663	0.122	(0.302, 1.025)	5.45	0.001
		3	1.838	0.122	(1.476, 2.200)	15.10	0.000
		4	3.318	0.122	(2.956, 3.679)	27.25	0.000
	2	3	1.175	0.122	(0.813, 1.536)	9.65	0.000
		4	2.655	0.122	(2.293, 3.016)	21.81	0.000
	3	4	1.480	0.122	(1.118, 1.841)	12.16	0.000
Experimental group C	1	2	0.870	0.128	(0.489, 1.252)	6.77	0.000
		3	2.047	0.128	(1.665, 2.428)	15.93	0.000
		4	3.276	0.128	(2.895, 3.658)	25.50	0.000
	2	3	1.177	0.128	(0.795, 1.558)	9.16	0.000
		4	2.406	0.128	(2.025, 2.788)	18.73	0.000
	3	4	1.230	0.128	(0.848, 1.611)	9.57	0.000

4.4.5 Antipyretic activities of DCM stalk extract of *Bidens pilosa*

One hour upon administration of the DCM stalk extract of *Bidens pilosa*, there was a significant difference in the rectal temperature of the animal models treated with a conventional antipyretic drug (Aspirin), varying dosage of the DCM extract of the *B. pilosa* (50, 100 and 150 mg/Kg), negative and positive test control group with a P value = 0.00 at $p < 0.05$ (Table 4.22 and 4.23). The percentage change from the initial rectal temperature for the rat model in normal, negative and positive test control groups and experimental test groups A, B and C was 0.00, -0.41, 0.67, 0.56, 0.67 and 0.67%, respectively (Table 4.22 and 4.23).

After two hours upon the administration of the DCM stalk extract of the *B. pilosa*, there wasa significant difference in the rectal temperature of the animal models in the groups labeled:- normal, negative, positive and test groups A, B and C with a P = 0.00 at $p < 0.05$ (Table 4.22, 4.23). The percentage change in rectal temperature change in the normal, negative and positive test control groups and test groups A, B and C was 0.32, -0.57, 1.54, 1.49, 1.49 and 1.39%, respectively (Table 4.22 and 4.23). The three different dosages of the DCM stalk extract of *B. pilosa* were effective in management of fever after 2 hours. The DCM stalk extract dosage of 50, 100 and 150 mg/Kg had no significant difference in relation to the aspirin ($p < 0.05$) (Table 4.22 and 4.23).

After three hours upon DCM stalk extract of the *B. pilosa* administration, there was a significant difference in the rectal temperature among the animals in normal, negative and positive control test group and experimental test groups A, B and C with P=0.00 at

$p < 0.05$ (Table 4.22 and 4.23). The percentage change in rectal temperature change in animal models in the groups labeled:- normal, negative, positive and test groups A, B and C was 0.26, -0.52, 2.51, 2.51, 2.37 and 2.41%, respectively (Table 4.22 and 4.23). The three dosages of DCM leaf extract of the *B. pilosa* had some antipyretic activity. The rectal temperature in group treated with conventional drug and three dose levels of *B. pilosa* DCM stalk extract (50, 100 and 150 mg/Kg) had no significant difference ($p < 0.05$) (Table 4.22 and 4.23).

After four hours following administration of *B. pilosa* DCM stalk extract, there was a significant difference in the rectal temperature among the animals in the group labeled:- normal, negative, positive and test groups A, B and C with P=0.00 ($p < 0.05$) (Table 4.22 and 4.23). The percentage change in rectal temperature change in the normal, negative and positive control test group and the experimental test groups A, B and C was 0.10, -0.31, 3.59, 3.39, 2.99 and 3.63%, respectively (Table 4.22 and 4.23). The three dosages of DCM stalk extract of the *B. pilosa* had some antipyretic activity. The rectal temperature in group treated with conventional drug and three dose levels of the DCM stalkextract of the *B. pilosa* had no significance different (Table 4.22 and 4.23).

Table 4.22: Antipyretic activities of DCM stalks extract of *Bidens pilosa* in percentage

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	100.00+0.12^{b} (0.00)	99.69+0.32^{b} (0.32)	99.74+0.46^{b} (0.26)	99.90+0.54^{b} (0.10)
Negative test control	Turpentine+ DMSO	100.41+0.13^{b} (-0.41)	100.57+0.10^{c} (-0.57)	100.52+0.08^{b} (-0.52)	100.31+0.05^{b} (-0.31)
Positive test control	Turpentine+ Aspirin	99.34+0.13^{a} (0.67)	98.46+0.16^{a} (1.54)	97.49+0.24^{a} (2.51)	96.42+0.27^{a} (3.59)
Experimental test A	Turpentine+ *B. pilosa* 50mg/kg	99.44+0.05^{a} (0.56)	98.51+0.09^{a} (1.49)	97.49+0.15^{a} (2.51)	96.61+0.28^{a} (3.39)
Experimental test B	Turpentine+ *B. pilosa* 100mg/kg	99.33+0.10^{a} (0.67)	98.51+0.17^{a} (1.49)	97.63+0.20^{a} (2.37)	97.01+0.17^{a} (2.99)
Experimental test C	Turpentine+ *B. pilosa* 150mg/kg	99.33+0.15^{a} (0.67)	98.61+0.13^{a} (1.39)	97.59+0.06^{a} (2.41)	96.36+0.16^{a} (3.63)
	F Value	14.98	24.14	32.74	40.17
	P Value	0.000	0.000	0.000	0.000

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significantdifferent. Data was analyzed using one – way ANOVA and Turkey's test ($p < 0.05$). Aspirin = 100 mg/kg body weight dose, Turpentine = 20% concentrated.

Table 4.23: Time effects on antipyretic activity of DCM *Bidens pilosa* stalks extract

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	0.316	0.300	(-0.574, 1.206)	1.05	0.722
		3	0.261	0.300	(-0.629, 1.151)	0.87	0.820
		4	0.100	0.300	(-0.790, 0.990)	0.33	0.987
	2	3	-0.055	0.300	(-0.945, 0.835)	-0.18	0.998
		4	-0.216	0.300	(-1.106, 0.674)	-0.72	0.887
	3	4	-0.161	0.300	(-1.051, 0.729)	-0.54	0.948
Negative control	1	2	-0.154	0.147	(-0.592, 0.284)	-1.05	0.727
		3	-0.102	0.147	(-0.540, 0.335)	-0.69	0.897
		4	0.104	0.147	(-0.334, 0.542)	0.71	0.893
	2	3	0.052	0.147	(-0.386, 0.490)	0.35	0.984
		4	0.258	0.147	(-0.179, 0.696)	1.75	0.341
	3	4	0.206	0.147	(-0.231, 0.644)	1.40	0.522
Positive control	1	2	0.871	0.150	(0.425, 1.318)	5.80	0.000
		3	1.844	0.150	(1.398, 2.291)	12.27	0.000
		4	2.920	0.150	(2.474, 3.367)	19.43	0.000
	2	3	0.973	0.150	(0.526, 1.419)	6.47	0.000
		4	2.049	0.150	(1.603, 2.495)	13.63	0.000
	3	4	1.076	0.150	(0.630, 1.523)	7.16	0.000
Experimental group A	1	2	0.924	0.161	(0.446, 1.401)	5.74	0.000
		3	1.950	0.161	(1.472, 2.428)	12.12	0.000
		4	2.822	0.161	(2.344, 3.299)	17.54	0.000
	2	3	1.026	0.161	(0.549, 1.504)	6.38	0.000
		4	1.898	0.161	(1.420, 2.376)	11.80	0.000
	3	4	0.872	0.161	(0.394, 1.349)	5.42	0.001
Experimental group B	1	2	0.8242	0.0965	(0.5377, 1.1107)	8.54	0.000
		3	1.7003	0.0965	(1.4138, 1.9868)	17.63	0.000
		4	2.3192	0.0965	(2.0327, 2.6057)	24.04	0.000
	2	3	0.8760	0.0965	(0.5895, 1.1625)	9.08	0.000
		4	1.4950	0.0965	(1.2085, 1.7815)	15.50	0.000
	3	4	0.6190	0.0965	(0.3325, 0.9055)	6.42	0.000
Experimental group C	1	2	0.719	0.144	(0.293, 1.146)	5.01	0.002
		3	1.746	0.144	(1.319, 2.172)	12.16	0.000
		4	2.977	0.144	(2.550, 3.403)	20.73	0.000
	2	3	1.027	0.144	(0.600, 1.453)	7.15	0.000
		4	2.258	0.144	(1.831, 2.684)	15.72	0.000
	3	4	1.231	0.144	(0.805, 1.657)	8.57	0.000

4.4.6 Antipyretic activity of *Bidens pilosa* methanolic stalks extract

One hour following administration of the methanolic stalk extract of the *Bidens pilosa*, there was a significant difference in the rectal temperature of the animal models treated with a conventional antipyretic drug (Aspirin), normal control, negative control and varying dosage of the methanolic stalks extract of the *Bidens pilosa* (50, 100 and 150 mg/Kg) with a P value=0.00, at $p < 0.05$. The percentage change from the initial rectal temperature for the rat model in groups labeled:- normal, negative, positive and test groups A, B and C was 0.10, -0.36,0.62, 0.82, 0.77 and 0.67%, respectively (Table 4.24 and 4.25).

After two hours upon administring methanolic stalks extract of the *B. pilosa*, there was a significant difference in the rectal temperature percentage change of the animal models in groups labeled:- normal, negative,positive and test groups A, B and C with a P=0.00 at $p < 0.05$ (Table 4.24 and 4.25). The percentage change in rectal temperature change in groups:- normal, negative, positive and test groups A, B and C was 0.10, -0.62, 1.89, 1.54, 1.54 and 1.64%, respectively (Table 4.24 and 4.25). The three different dosages of the methanolic stalk extract of *B. pilosa* were effective in management of fever after 2 hours. The stalks extract dosage of 50, 100 and 150 mg/Kg had no significance different with the conventional drug (Aspirin) (Table 4.24 and 4.25).

Three hours after the administration of the methanolic stalks extract of the *B. pilosa*, there was a significant difference in the rectal temperature among the animals in normal, negative and positive control test group and experimental test groups A, B and C, P=0.00

at $p < 0.05$ (Table 4.24 and 4.25). The percentage change in rectal temperature change in groups labeled:- normal, negative, positive and test groups A, B and C was -0.11, -0.46, 2.17, 2.46, 2.37 and 2.62%, respectively (Table 4.24 and 4.25). The three dosages of methanol stalk extract of the *B. pilosa* had some antipyretic activity. The rectal temperature in group treated with conventional drug and three dose levels of the methanolic stalks extract of the *B. pilosa* had no significant difference (Table 4.24 and 4.25).

Four hours following administration of methanolic stalks extract of the *B. pilosa*, there was a significant difference in the rectal temperature change among the animals in all experimental groups P=0.00 at $p < 0.05$ (Table 4.24 and 4.25). The percentage change in rectal temperature change in animal model groups labeled:- normal, negative, positive and test groups A, B and C was - 0.32, -0.21, 1.45, 2.72, 3.14 and 2.77%, respectively (Table 4.24, and 4.25). The methanolic *B. pilosa* stalks extract at three dose levels had higher antipyretic activity thanthe conventional drug (Aspirin) (Table 4.24 and 4.25).

Table 4.24: Antipyretic activities of MeOH stalks of *Bidens pilosa* in percentage

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	99.90+0.23^{ab} (0.10)	99.90+0.25^{a} (0.10)	100.11+0.11^{a} (-0.11)	100.32+0.23^{a} (-0.32)
Negative test control	Turpentine+ DMSO	100.36+0.10^{a} (-0.36)	100.62+0.10^{a} (-0.62)	100.46+0.10^{a} (-0.46)	100.21+0.10^{a} (-0.21)
Positive test control	Turpentine+ Aspirin	99.38+0.13^{b} (0.62)	98.81+0.10^{b} (1.89)	97.83+0.16^{b} (2.17)	98.56+0.56^{b} (1.45)
Experimental test A	Turpentine+ *B. pilosa* 50mg/kg	99.18+0.18^{b} (0.82)	98.46+0.28^{b} (1.54)	97.54+0.35^{b} (2.46)	97.28+0.40^{c} (2.72)
Experimental test B	Turpentine+ *B. pilosa* 100mg/kg	99.23+0.11^{b} (0.77)	98.46+0.29^{b} (1.54)	97.64+0.26^{b} (2.37)	96.86+0.29^{c} (3.14)
Experimental test C	Turpentine+ *B. pilosa* 150mg/kg	99.33+0.06^{b} (0.67)	98.36+0.22^{b} (1.64)	97.38+0.20^{b} (2.62)	97.28+0.15^{c} (2.77)
	F. value	7.77	18.06	38.71	29.28
	P. value	0.00	0.00	0.00	0.00

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Data was analyzed using one – way ANOVA and Turkey's test ($p < 0.05$). Aspirin = 100 mg/kg dose, Turpentine =20% concentrated.

Table 4.25: Time effects on antipyretic activity of MeOH *Bidens pilosa* stalk extract

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	-0.000	0.203	(-0.601, 0.601)	-0.00	1.000
		3	-0.213	0.203	(-0.815, 0.388)	-1.05	0.723
		4	-0.427	0.203	(-1.028, 0.174)	-2.11	0.205
	2	3	-0.213	0.203	(-0.815, 0.388)	-1.05	0.723
		4	-0.427	0.203	(-1.028, 0.174)	-2.11	0.205
	3	4	-0.214	0.203	(-0.815, 0.388)	-1.05	0.722
Negative control	1	2	-0.257	0.102	(-0.559, 0.046)	-2.52	0.107
		3	-0.102	0.102	(-0.405, 0.200)	-1.00	0.751
		4	0.155	0.102	(-0.148, 0.457)	1.52	0.458
	2	3	0.155	0.102	(-0.148, 0.457)	1.52	0.458
		4	0.411	0.102	(0.109, 0.714)	4.04	0.008
	3	4	0.257	0.102	(-0.046, 0.559)	2.52	0.107
Positive control	1	2	0.567	0.361	(-0.507, 1.640)	1.57	0.431
		3	1.548	0.361	(0.474, 2.621)	4.28	0.005
		4	0.825	0.361	(-0.248, 1.899)	2.28	0.157
	2	3	0.981	0.361	(-0.093, 2.054)	2.71	0.077
		4	0.258	0.361	(-0.815, 1.332)	0.71	0.889
	3	4	-0.722	0.361	(-1.796, 0.351)	-2.00	0.242
Experimental group A	1	2	0.718	0.284	(-0.126, 1.563)	2.53	0.106
		3	1.642	0.284	(0.798, 2.486)	5.78	0.000
		4	1.899	0.284	(1.055, 2.743)	6.68	0.000
	2	3	0.924	0.284	(0.080, 1.768)	3.25	0.031
		4	1.181	0.284	(0.336, 2.025)	4.15	0.006
	3	4	0.257	0.284	(-0.587, 1.101)	0.90	0.803
Experimental group B	1	2	0.770	0.179	(0.240, 1.301)	4.31	0.005
		3	1.594	0.179	(1.063, 2.124)	8.92	0.000
		4	2.366	0.179	(1.835, 2.896)	13.25	0.000
	2	3	0.824	0.179	(0.293, 1.354)	4.61	0.003
		4	1.596	0.179	(1.065, 2.126)	8.93	0.000
	3	4	0.772	0.179	(0.241, 1.302)	4.32	0.005
Experimental group C	1	2	0.975	0.187	(0.418, 1.531)	5.20	0.001
		3	1.950	0.187	(1.394, 2.507)	10.41	0.000
		4	2.106	0.187	(1.550, 2.662)	11.24	0.000
	2	3	0.976	0.187	(0.419, 1.532)	5.21	0.001
		4	1.132	0.187	(0.575, 1.688)	6.04	0.000
	3	4	0.156	0.187	(-0.400, 0.712)	0.83	0.838

4.4.7 Antipyretic activities of dichlomethane leave extract of *Senna didymobotyra*

One hour afollowing administration of the DCM *S. didymobotyra* leaf extract, there was a significant difference in the rectal temperature change among the animals in all the groups P=0.01at $p < 0.05$ (Table 4.26 and 4.27). The percentage change in rectal temperature change in in animal models in the groups labeled:- normal, negative, positive and test groups A, B and C was 0.27, -0.15, 1.60, 0.72, 0.77 and 0.77%, respectively (Table 4.26 and 4.27). The three dosages of DCM leaf extract of the *S. didymobotyra* had some antipyretic activity. The rectal temperature change in group treated with conventional drug (Aspirin), and three dose levels of the DCM leaf extract of the *S. didymobotyra* were statistically different at $p < 0.05$ (Table 4.26 and 4.27). The conventional drug (Aspirin) had a higher antipyretic activity when compared to three dosages of *S. didymobotyra* dichloromethane leaves extract (Table 4.26, 4.27).

Two hours following administration of DCM *S. didymobotyra* leaves extract, there was a significant difference in the rectal temperature change of the animal models in all the groups with a F value = 18.46 and P = 0.00 at $p < 0.05$ (Table 4.26 and 4.27). The percentage change in rectal temperature change in animal models in the group labeled:- normal, negative, positive and test groups A, B and C was 0.11, -0.31, 4.13, 1.65, 1.65 and 1.74%, respectively (Table 4.26 and 4.27). The three different dosages of the DCM leaves extract of *S. didymobotyra* were effective in management of fever after 2 hours. The leaves extract dosage of 50, 100 and 150 mg/Kg had an antipyretic activity with statistically different from thaty of the animals treated with conventional drug (aspirin) ($p < 0.05$) (Table 4.26 and 4.27).

Three hours after the administration of DCM *S. didymobotyra* leaves extract, there was a rectal temperature change among the animals in all the groups were statistically different with P=0.00 at a significance level of $p < 0.05$ (Table 4.26 and 4.27). The percentage change in rectal temperature change in animal model in the groups labeled:- normal, negative, positive and test groups A, B and C was 0.21, -0.46, 3.51, 2.47, 2.42 and 2.86%, respectively (Table 4.26 and 4.27). The three dosages of DCM leaves extract of the *S. didymobotyra* had some antipyretic activity. The rectal temperature change in group treated with conventional drug, 50, 100 and 150 mg/Kg of the *S. didymobotyra* DCM leaves extract were not statistically different (Table 4.26 and 4.27).

Four hours following administration of DCM leaves extract of the *S. didymobotyra*, rectal temperature change was statistically different among the animals in all the test groups with a calculated P=0.00 at a significance level of $p < 0.05$ (Table 4.26 and 4.27). The percentage change in rectal temperature in the animal model groups labeled:- normal, negative, positive and test groups A, B and C was 0.37, -0.31, 4.13, 3.24, 3.09 and 4.03%, respectively (Table 4.26 and 4.27). The rectal temperature in group treated with conventional drug and three dose levels of DCM leaves extract of the *S. didymobotyra* were not statistically different ($p < 0.05$) (Table 4.26 and 4.27).

Table 4.26: Antipyretic activities of DCM leave extract of *Senna didymobotyra* in percentage

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	99.73+0.29^{a} (0.27)	99.89+0.14^{ab} (0.11)	99.79+0.31^{a} (0.21)	99.63+0.34^{a} (0.37)
Negative test control	Turpentine+ DMSO	100.15+0.06^{a} (-0.15)	100.31+0.05^{a} (-0.31)	100.46+0.05^{a} (-0.46)	100.31+0.05^{a} (-0.31)
Positive test control	Turpentine+ Aspirin	98.40+0.60^{b} (1.60)	95.87+0.86^{d} (4.13)	96.49+0.72^{b} (3.51)	95.87+0.66^{b} (4.13)
Experimental test A	Turpentine+ *S. didymobotyra* 50mg/kg	99.28+0.09^{ab} (0.72)	98.35+0.10^{bc} (1.65)	97.53+0.10^{b} (2.47)	96.76+0.23^{b} (3.24)
Experimental test B	Turpentine+ *S. didymobotyra* 100mg/kg	99.23+0.08^{ab} (0.77)	98.35+0.06^{bc} (1.65)	97.58+0.10^{b} (2.42)	96.91+0.08^{b} (3.09)
Experimental test C	Turpentine+ *S. didymobotyra* 150mg/kg	99.24+0.11^{ab} (0.77)	98.26+0.17^{c} (1.74)	97.14+0.19^{b} (2.86)	95.97+0.14^{b} (4.03)
	F value	4.36	18.46	22.19	34.46
	P value	0.01	0.00	0.00	0.00

Values indicated as Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Data analyzed using one – way ANOVA and Turkey's test ($p < 0.05$). Aspirin = 100 mg/kg dose, Turpentine =20% concentrated.

Table 4.27: Time effects on antipyretic activity of DCM leave extract of *Senna didymobotyra*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	-0.27	0.25	(-1.02, 0.49)	-1.05	0.722
		3	0.04	0.25	(-0.71, 0.79)	0.16	0.999
		4	0.05	0.25	(-0.70, 0.80)	0.21	0.997
	2	3	0.31	0.25	(-0.45, 1.06)	1.21	0.632
		4	0.32	0.25	(-0.44, 1.07)	1.26	0.601
	3	4	0.01	0.25	(-0.74, 0.77)	0.05	1.000
Negative control	1	2	-0.15	0.09	(-0.41, 0.10)	-1.78	0.328
		3	-0.31	0.09	(-0.57, -0.05)	-3.56	0.018
		4	-0.15	0.09	(-0.41, 0.10)	-1.78	0.328
	2	3	-0.15	0.09	(-0.41, 0.10)	-1.78	0.329
		4	0.00	0.09	(-0.26, 0.26)	0.00	1.000
	3	4	0.15	0.09	(-0.10, 0.41)	1.78	0.329
Positive control	1	2	2.53	0.53	(0.97, 4.10)	4.80	0.002
		3	1.91	0.53	(0.34, 3.48)	3.62	0.016
		4	2.53	0.53	(0.97, 4.10)	4.80	0.002
	2	3	-0.62	0.53	(-2.19, 0.95)	-1.18	0.650
		4	0.00	0.53	(-1.57, 1.57)	0.00	1.000
	3	4	0.62	0.53	(-0.94, 2.19)	1.18	0.649
Experimental group A	1	2	0.93	0.12	(0.58, 1.27)	8.06	0.000
		3	1.73	0.12	(1.41, 2.09)	15.23	0.000
		4	2.52	0.12	(2.18, 2.86)	21.94	0.000
	2	3	0.82	0.12	(0.48, 1.16)	7.17	0.000
		4	1.59	0.12	(1.25, 1.94)	13.88	0.000
	3	4	0.77	0.12	(0.43, 1.11)	6.71	0.000
Experimental group B	1	2	0.88	0.08	(0.65, 1.11)	11.34	0.000
		3	1.65	0.08	(1.42, 1.88)	21.34	0.000
		4	2.32	0.08	(2.09, 2.52)	30.01	0.000
	2	3	0.77	0.08	(0.54, 1.00)	10.00	0.000
		4	1.44	0.08	(1.21, 1.67)	18.67	0.000
	3	4	0.67	0.08	(0.44, 0.90)	8.67	0.000
Experimental group C	1	2	0.97	0.15	(0.52, 1.42)	6.40	0.000
		3	2.09	0.15	(1.64, 2.54)	13.80	0.000
		4	3.27	0.15	(2.82, 3.72)	21.54	0.000
	2	3	1.12	0.15	(0.67, 1.57)	7.40	0.000
		4	2.30	0.15	(1.85, 2.75)	15.14	0.000
	3	4	1.18	0.15	(0.72, 1.63)	7.74	0.000

4.4.8 Antipyretic activities of methanolic leaves extract of *S. didymobotyra*

One hour following administration of methanolic leaves extract of the *S. didymobotyra*, the rectal temperature change between all experimental groups were statistically different with a P value=0.00; $p < 0.05$ (Table 4.28 and 4.29). The percentage change from the initial rectal temperature for the rat model in groups labeled:- normal, negative, positive and test groups A, B and C were:- 0.70, -0.10, 1.09, 1.08, 0.98 and 0.67%, respectively. (Table 4.28 and 4.29). The average rectal temperature change of the animals treated with 50 mg/Kg of leaves extract of *S. didymobotyra* had no statistically significant difference with those of the group treated with conventional drug (Aspirin) (Table 4.28 and 4.29).

The ractal temperature change was statistically different two our after the administration of the methanol *S. didymobotyra* leaves extract between all the experimental animal groups with a P=0.00 at a significance level of $p < 0.05$ (Table 4.28 and 4.29). The percentage change in rectal temperature change in animal model groups labeled:- normal, negative, positive and test groups A, B and C was 0.32, -0.26, 3.38, 1.85, 1.95 and 1.60%, respectively (Table 4.28 and 4.29). The three different dosages of the methanolic leaves extract of *S. didymobotyra* were effective in management of fever after 2 hours in relation to negative control. The leaves extract dose levels of 50, 100 and 150 mg/Kg rectal temperature change values were statistically difference in relation to results observed in the animals treated with the conventional drug (Aspirin). The three dosage levels of methanolic leaves extract of the *S. didymobotyra* had less antipyretic activity in relation to the conventional drug (Aspirin) (Table 4.28 and 4.29).

Three hours after the administration of the methanolic leaves extract of *S. didymobotyra* rectal temperature change was statistaically different among the animals in normal, negative and positive control test group and experimental test groups A, B and C P=0.00, $p < 0.05$ (Table 4.28 and 4.29). The percentage change in rectal temperature change in animal model groups labeled:- normal, negative, positive and test groups A, B and C is 0.37, -0.36, 3.17, 2.72, 2.57 and 2.47%, respectively (Table 4.28 and 4.29). The three dosages of methanolic leaves extract of *S. didymobotyra* had some antipyretic activity. The rectal temperature in group treated with conventional drug and dose levels of the methanolic leaves extract of the *S. didymobotyra* had no statistical different (Table 4.28 and 4.29).

Four hours after following administration of methanolic *S. didymobotyra* leaves extract, rectal temperature change among the animals in normal, negative and positive control test group and experimental test groups A, B and C, were statistically different with P=0.00 at $p < 0.05$ (Table 4.28 and 4.29). The percentage rectal temperature change in animal model in the groups labeled:- normal,negative, positive and test groups A, B and C was 0.21, 0.00, 3.80, 3.90, 3.44 and 3.14%, respectively (Table 4.28 and 4.29). The rectal temperature change in group treated with conventional drug and all three dose level of methanolic *S. didymobotyra* leaves extract were not statistically different (Table 4.28 and 4.29).

Table 4.28: Antipyretic activities of MeOH leave extract of *Senna didymobotyra* in percentage

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	99.31+0.14^{ab} (0.70)	99.68+0.16^{a} (0.32)	99.63+0.14^{a} (0.37)	99.79+0.43^{a} (0.21)
Negative test control	Turpentine+ DMSO	100.10+0.06^{a} (-0.10)	100.26+0.00^{a} (-0.26)	100.36+0.0^{a} (-0.36)	100.00+0.0^{a} (0.00)
Positive test control	Turpentine+ Aspirin	98.91+0.58^{b} (1.09)	96.62+0.52^{c} (3.38)	96.83+0.58^{b} (3.17)	96.20+0.26^{b} (3.80)
Experimental test A	Turpentine+ *S. didymobotyra* 50mg/kg	98.92+0.12^{b} (1.08)	98.15+0.09^{b} (1.85)	97.28+0.10^{b} (2.72)	96.10+0.2^{b} (3.90)
Experimental test B	Turpentine+ *S. didymobotyra* 100mg/kg	99.02+0.13^{ab} (0.98)	97.43+0.11^{b} (1.95)	97.43+0.11^{b} (2.57)	96.56+0.19^{b} (3.44)
Experimental test C	Turpentine+ *S. didymobotyra* 150mg/kg	99.33+0.10^{ab} (0.67)	97.53+0.13^{b} (1.60)	97.53+0.13^{b} (2.47)	96.86+0.15^{b} (3.14)
	F Value	2.99	30.96	31.95	60.36
	P Value	0.031	0.000	0.000	0.000

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Data was analyzed using one – way ANOVA and Turkey's test ($p < 0.05$). Aspirin = 100 mg/kg dose, Turpentine =20% concentrated.

Table 4. 29: Time effects on antipyretic activity of MeOH leave extract of *Senna didymobotyra*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	-0.38	0.38	(-1.52, 0.77)	-0.98	0.766
		3	-0.32	0.38	(-1.46, 0.82)	-0.83	0.837
		4	-0.48	0.38	(-1.62, 0.66)	-1.25	0.607
	2	3	0.05	0.38	(-1.09, 1.20)	0.14	0.999
		4	-0.11	0.38	(-1.25, 1.04)	-0.28	0.992
	3	4	-0.16	0.38	(-1.30, 0.98)	-0.42	0.974
Negative control	1	2	-0.15	0.08	(-0.38, 0.08)	-1.98	0.248
		3	-0.26	0.08	(-0.49, -0.03)	-3.32	0.027
		4	0.10	0.08	(-0.13, 0.33)	1.33	0.561
	2	3	-0.10	0.08	(-0.33, 0.13)	-1.33	0.560
		4	0.25	0.08	(0.03, 0.49)	3.31	0.027
	3	4	0.36	0.08	(0.13, 0.59)	4.65	0.003
Positive control	1	2	2.29	0.67	(0.30, 4.28)	3.42	0.023
		3	2.08	0.67	(0.09, 4.07)	3.10	0.040
		4	2.71	0.67	(0.72, 4.70)	4.04	0.008
	2	3	-0.22	0.67	(-2.20, 1.77)	-0.32	0.988
		4	0.41	0.67	(-1.58, 2.40)	0.62	0.925
	3	4	0.63	0.67	(-1.36, 2.62)	0.94	0.785
Experimental group A	1	2	0.77	0.08	(0.53, 1.01)	9.60	0.000
		3	1.64	0.08	(1.40, 1.88)	20.48	0.000
		4	2.82	0.08	(2.59, 3.06)	35.18	0.000
	2	3	0.87	0.08	(0.63, 1.11)	10.88	0.000
		4	2.05	0.08	(1.82, 2.29)	25.59	0.000
	3	4	1.18	0.08	(0.94, 1.42)	14.71	0.000
Experimental group B	1	2	0.98	0.15	(0.54, 1.41)	6.65	0.000
		3	1.59	0.15	(1.16, 2.03)	10.84	0.000
		4	2.46	0.15	(2.03, 2.90)	16.79	0.000
	2	3	0.62	0.15	(0.18, 1.05)	4.20	0.006
		4	1.49	0.15	(1.05, 1.92)	10.14	0.000
	3	4	0.87	0.15	(0.44, 1.31)	5.94	0.000
Experimental group C	1	2	0.93	0.12	(0.58, 1.27)	7.98	0.000
		3	1.80	0.12	(1.46, 2.15)	15.52	0.000
		4	2.47	0.12	(2.13, 2.82)	21.29	0.000
	2	3	0.88	0.12	(0.53, 1.22)	7.54	0.000
		4	1.55	0.12	(1.20, 1.89)	13.30	0.000
	3	4	0.67	0.12	(0.33, 1.02)	5.77	0.000

4.4.9 Antipyretic activities of DCM *P. africana* stem bark extract

One hour following administration of DCM leaves extract of the *P. africana*, there was a significant difference in the rectal temperature change of the animal models in all the experimental groups with a P=0.00, $p < 0.05$ (Table 4.30 and 4.31). The percentage change in rectal temperature in animal models labeled:- normal, negative, positive and the test groups A, B and C was -0.27, -0.31, 0.67, 0.82, 0.82 and 0.98%, respectively (Table 4.30, 4.31). The three different dosages of DCM stem bark extract of *P. africana* were effective in management of fever after 1 hour. The stem bark extract dosage of 50, 100 and 150 mg/Kg did not exhibit rectal temperature change statistically with the one exhibited by the conventional drug (Aspirin) (Table 4.30, 4.31).

Two hours post administration of DCM stem bark extract of the *P. africana*, there was a significant difference in the rectal temperature change of the animal models in group labeled:- normal, negative, positive and test groups A, B and C with a P=0.00 ($p < 0.05$) (Table 4.30 and 4.31). The percentage change in rectal temperature in the animal models in the groups labeled:- normal, negative, positive, test A, test B and test C was -0.16, -0.31, 1.08, 1.64, 1.80 and 1.80%, respectively (Table 4.30 and 4.31). The three different dosages of the DCM stem bark extract of *P. africana* were effective in management of fever after 2 hours.

Three hours post administration of DCM *P. africana*, stem bark extract, there was a significant difference in the rectal temperature change among the animals models in all groups with calculated P=0.00 ($p < 0.05$) (Table 4.30 and 4.31). The percentage change in rectal temperature in animal models in groups labeled:- normal, negative, positive, test A,

test B and test C was - 0.81, -0.26, 1.60, 2.47, 2.87 and 2.52%, respectively (Table 4.30 and 4.31). The three dosages of DCM leaves extract of the *P. africana* had better antipyretic activity than the conventional drug (Aspirin).The highest dosage of 150mg/Kg had better antipyretic activity than the lower doses of 50 and 100 mg/Kg (Table 4.30 and 4.31).

Four hours after the administration of DCM *P. africana* stem bark extract, there was a significant difference in the rectal temperature change among the animals in all experimental groups with calculated $P = 0.00$, at a significance level $p < 0.05$ (Table 4.30, 4.31). The percentage change in rectal temperature in animal models in groups labeled:- normal, negative, positive, test A, test B and test C was - 0.54, 0.05, 2.67, 3.34, 3.34 and 3.09%, respectively (Table 4.30, 4.31). The three dosages of DCM *P. africana* stem bark extract had better antipyretic activity than the conventional drug (Aspirin) though there was no significant difference in the antipyretic activity of the conventional drug (Aspirin) and three plant extract doses (Table 4.30 and 4.31).

Table 4. 30: Antipyretic activities of DCM stem bark extract of *Prunus africana* in percentage

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	100.27+0.15^{a} (-0.27)	100.16+0.28^{a} (-0.16)	100.81+0.39^{a} (-0.81)	100.54+0.34^{a} (-0.54)
Negative test control	Turpentine+ DMSO	100.31+0.10^{a} (-0.31)	100.31+0.05^{a} (-0.31)	100.26+0.08^{a} (-0.26)	99.95+0.10^{a} (0.05)
Positive test control	Turpentine+ Aspirin	99.33+0.21^{b} (0.67)	98.92+0.22^{b} (1.08)	98.41+0.21^{b} (1.60)	97.33+0.34^{b} (2.67)
Experimental test A	Turpentine+ *P. africanus* 50mg/kg	99.18+0.15^{b} (0.82)	98.36+0.17^{b} (1.64)	97.53+0.15^{bc} (2.47)	96.66+0.25^{b} (3.34)
Experimental test B	Turpentine+ *P. africanus* 100mg/kg	99.18+0.13^{b} (0.82)	98.20+0.25^{b} (1.80)	97.13+0.38^{bc} (2.87)	96.66+0.12^{b} (3.34)
Experimental test C	Turpentine+ *P. africanus* 150mg/kg	99.02+0.05^{b} (0.98)	98.20+0.14^{b} (1.80)	97.48+0.12^{c} (2.52)	96.91+0.10^{b} (3.09)
	F Value	17.69	24.10	38.44	56.10
	P Value	0.000	0.000	0.000	0.000

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Data was analyzed using one – way ANOVA and Turkey's test ($p < 0.05$). Aspirin = 100 mg/kg dose, Turpentine =20% concentrated.

Table 4.31: Time effects on antipyretic activity of DCM stem bark extract of *Prunus africana*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	0.11	0.24	(-0.62, 0.83)	0.43	0.971
		3	-0.54	0.24	(-1.26, 0.19)	-2.20	0.179
		4	-0.27	0.24	(-0.10, 0.46)	-1.10	0.696
	2	3	-0.64	0.24	(-1.37, 0.08)	-2.63	0.089
		4	-0.38	0.24	(-1.10, 0.35)	-1.54	0.448
	3	4	0.27	0.24	(-0.46, 0.99)	1.10	0.698
Negative control	1	2	0.00	0.10	(-0.30, 0.30)	0.01	1.00
		3	0.05	0.10	(-0.25, 0.36)	0.51	0.956
		4	0.36	0.10	(0.06, 0.67)	3.54	0.018
	2	3	0.05	0.10	(-0.25, 0.35)	0.50	0.957
		4	0.36	0.10	(0.06, 0.66)	3.54	0.019
	3	4	0.31	0.10	(0.01, 0.61)	3.03	0.045
Positive control	1	2	0.41	0.17	(-0.10, 0.92)	2.40	0.130
		3	0.92	0.17	(0.42, 1.43)	5.41	0.001
		4	2.00	0.17	(1.50, 2.51)	11.73	0.000
	2	3	0.51	0.17	(0.01, 1.02)	3.01	0.046
		4	1.59	0.17	(1.09, 2.10)	9.33	0.000
	3	4	1.08	0.17	(0.57, 1.59)	6.32	0.000
Experimental group A	1	2	0.82	0.12	(0.47, 1.17)	6.96	0.000
		3	1.65	0.12	(1.29, 2.00)	13.92	0.000
		4	2.52	0.12	(2.17, 2.87)	21.30	0.000
	2	3	0.82	0.12	(0.47, 1.17)	6.96	0.000
		4	1.70	0.12	(1.35, 2.05)	14.35	0.000
	3	4	0.87	0.12	(0.52, 1.22)	7.39	0.000
Experimental group B	1	2	0.98	0.35	(-0.06, 2.03)	2.76	0.072
		3	2.05	0.35	(1.00, 3.10)	5.79	0.000
		4	2.52	0.35	(1.46, 3.57)	7.11	0.000
	2	3	1.07	0.35	(0.02, 2.13)	3.03	0.045
		4	1.54	0.35	(0.49, 2.59)	4.35	0.005
	3	4	0.46	0.35	(-0.59, 1.52)	1.31	0.573
Experimental group C	1	2	0.82	0.10	(0.52, 1.13)	8.04	0.000
		3	1.54	0.10	(1.24, 1.85)	15.08	0.000
		4	2.11	0.10	(1.81, 2.41)	20.61	0.000
	2	3	0.72	0.10	(0.42, 1.03)	7.04	0.000
		4	1.29	0.10	(0.98, 1.59)	12.57	0.000
	3	4	0.57	0.10	(0.26, 0.87)	5.53	0.001

4.4.10 Antipyretic activities of methanolic stem bark extract of *P. africana*

One hour upon the administration of the *P. africana* methanolic stem bark extract there was a significant difference in the rectal temperature change of the animal models in groups labeled:- normal, negative, positive, test A, test B and test C with a P=0.014 at a significance level of $p < 0.05$ (Table 4.32 and 4.33). The percentage change in rectal temperature in animal model groups labeled:- normal, negative, positive, test A, test B and test C was 0.90, -0.21, 0.57, 0.92, 0.77 and 1.03%, respectively (Table 4.32 and 4.33). The three different dosages of the methanolic stem bark had antipyretic activity better than the conventional drug (Aspirin) with no statistically significance difference. The methanolic stem bark extract dosage of 100 mg/Kg did not indicate any significant difference with the conventional drug (Aspirin). It is only 50 and 150 mg/Kg doses of *P. africana* methanolic stem bark extract which had a significant difference in percentage change in the rectal temperature in relation to the conventional drug at a significance level of $p < 0.05$ (Tables 4.32 and 4.33).

Two hours upon the administering *P. Africana* stem bark methanolic extract, there was a significant difference in the rectal temperature change of the animal models in groups labeled as:- normal, negative, positive, test A, B and C with a P=0.00 at a significance level of $p < 0.05$ (Table 4.32 and 4.33). The percentage change in rectal temperature change in groups labeled:- normal, negative, positive, test A, B and C was 0.26, -0.41, 1.39, 2.00, 1.70 and 1.80%, respectively (Tables 4.32 and 4.33). The three different dose levels of methanolic *P. africana* stem bark extract were effective in management of fever after 2 hours.

Three hours after administration of the methanolic *P. africana*, stem bark extract, there was a significant difference in the rectal temperature among the animals in test groups involved with calculated P=0.00 at a significance level of $p < 0.05$ (Tables 4.32 and 4.33). The percentage change in rectal temperature in animal model groups labeled:- normal, negative, positive, test A, B andC was - 0.17, - 0.31, 2.32, 2.87, 2.41 and 2.47%, respectively (Table 4.32 and 4.33). The three dosages of methanolic *P. africana* stem bark extract had some antipyretic activity.

Four hours following injecting the animals with methanolic *P africana*, stem bark extract, there was a significant difference in the rectal temperature change among the animals in normal, negative and positive control test group and experimental test groups A, B and C P=0.00; $p < 0.05$ (Tables 4.32 and 4.33). The percentage change in rectal temperature change in animal model labeled:- normal, negative, positive, test A, B and C was 0.42, 0.00, 3.45, 3.53, 3.19 and 3.24%, respectively (Tables 4.32 and 4.33). The lowest dosage of 50 mg/Kg had better antipyretic activity than the other doses of 100and 150 mg/Kg.

Table 4. 32: Antipyretic activities of MeOH stem bark extracts of *Prunus africana* in percentage

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	99.10+0.44^{b} (0.90)	99.74+0.39^{a} (0.26)	100.16+0.38^{a} (-0.17)	99.58+0.52^{a} (0.42)
Negative test control	Turpentine+ DMSO	100.21+0.13^{a} (-0.21)	100.41+0.13^{a} (-0.41)	100.31+0.10^{a} (-0.31)	100.00+0.08^{a} (0.00)
Positive test control	Turpentine+ Aspirin	99.43+0.12^{ab} (0.57)	98.61+0.17^{b} (1.39)	97.68+0.23^{b} (2.32)	96.55+0.22^{b} (3.45)
Experimental test A	Turpentine+ *P. africanus* 50mg/kg	99.08+0.13^{b} (0.92)	98.01+0.24^{b} (2.00)	97.13+0.28^{b} (2.87)	96.47+0.29^{b} (3.53)
Experimental test B	Turpentine+ *P. africanus* 100mg/kg	99.23+0.16^{ab} (0.77)	98.31+0.17^{b} (1.70)	97.59+0.20^{b} (2.41)	96.81+0.20^{b} (3.19)
Experimental test C	Turpentine+ *P. africanus* 150mg/kg	98.97+0.27^{b} (1.03)	98.20+0.22^{b} (1.80)	97.53+0.21^{b} (2.47)	96.77+0.15^{b} (3.24)
	F Value	3.61	16.92	33.49	34.12
	P Value	0.014	0.000	0.000	0.000

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Data was analyzed using one – way ANOVA and Turkey's test ($p < 0.05$). Aspirin = 100 mg/kg dose, Turpentine =20% concentrated.

Table 4. 33: Time effects on antipyretic activity of MeOH stem bark extract of *Prunus africana*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	-0.64	0.21	(-1.26, -0.01)	-3.03	0.045
		3	-1.06	0.21	(-1.69, -0.44)	-5.04	0.001
		4	-0.48	0.21	(-1.11, 0.15)	-2.28	0.158
	2	3	-0.42	0.21	(-1.05, 0.20)	-2.02	0.236
		4	0.16	0.21	(-0.47, 0.78)	0.75	0.875
	3	4	0.58	0.21	(-0.04, 1.21)	2.77	0.071
Negative control	1	2	-0.21	0.01	(-0.50, 0.09)	-2.07	0.217
		3	-0.10	0.01	(-0.40, 0.19)	-1.04	0.731
		4	0.21	0.01	(-0.09, 0.50)	2.06	0.219
	2	3	0.10	0.01	(-0.19, 0.40)	1.03	0.735
		4	0.41	0.01	(0.12, 0.71)	4.13	0.007
	3	4	0.31	0.01	(0.013, 0.60)	3.10	0.040
Positive control	1	2	0.82	0.15	(0.39, 1.26)	5.63	0.001
		3	1.75	0.15	(1.32, 2.19)	11.98	0.000
		4	2.89	0.15	(2.45, 3.32)	19.73	0.000
	2	3	0.93	0.15	(0.49, 1.36)	6.35	0.000
		4	2.06	0.15	(1.63, 2.50)	14.10	0.000
	3	4	1.13	0.15	(0.70, 1.57)	7.75	0.000
Experimental group A	1	2	1.08	0.12	(0.71, 1.44)	8.70	0.000
		3	1.95	0.12	(1.58, 2.31)	15.75	0.000
		4	2.61	0.12	(2.24, 2.98)	21.14	0.000
	2	3	0.87	0.12	(0.50, 1.24)	7.05	0.000
		4	1.54	0.12	(1.17, 1.90)	12.44	0.000
	3	4	0.67	0.12	(0.30, 1.03)	5.39	0.000
Experimental group B	1	2	0.93	0.11	(0.61, 1.24)	8.64	0.000
		3	1.64	0.11	(1.33, 1.96)	15.35	0.000
		4	2.42	0.11	(2.10, 2.73)	22.55	0.000
	2	3	0.72	0.11	(0.40, 1.04)	6.72	0.000
		4	1.49	0.11	(1.17, 1.81)	13.91	0.000
	3	4	0.77	0.11	(0.45, 1.09)	7.20	0.000
Experimental group C	1	2	0.77	0.15	(0.31, 1.23)	5.00	0.002
		3	1.44	0.15	(0.98, 1.90)	9.34	0.000
		4	2.21	0.15	(1.75, 2.66)	14.33	0.000
	2	3	0.67	0.15	(0.21, 1.13)	4.34	0.005
		4	1.44	0.15	(0.98, 1.89)	9.33	0.000
	3	4	0.77	0.15	(0.31, 1.23)	4.99	0.002

4.5 Anti-inflammatory activities of the plant extracts

4.5.1 Anti- inflammatory activities of DCM leave extract of *E. saligna*

Anti-inflammatory potential of *E. saligna* was established after inducing inflammation to animal models using carrageenan. An hour after plant test samples and standard drug administration (diclofenac), there was a significant difference (P=0.00) in paw edema inhibition in groups labeled as:- normal, negative, positive, test A, B and C was -0.81, -4.68, 1.72, 6.48, 2.34 and 5.32%, respectively at a statistical significance level of $p < 0.05$ (Table 4.34). The DCM leaves extracts of *E. saligna* elucidated better anti-inflammatory pharmacological activity in relation to experimental standard drug (diclofenac) (Tables 4.34 and 4.35). The dose level of 50mg/Kg indicated better anti- inflammatory pharmacological activity compared to experimental standard drug (diclofenac) and the plant extracts (100 and 150 mg/Kg).

Two hours following plant sample and standard drug (diclofenac) intraperitoneal administration, the percentage paw edema inhibition in all the animal models in the experimental groups labeled:- normal, negative, positive, test A, B and C was -1.71, -.68, -4.30, 12.86, 6.46 and 10.04%, respectively with a statistically significant difference in the percentage inhibition of paw edema at P value = 0.00, $p < 0.05$ (Tables 4.34 and 4.35). The DCM leaves extracts of *E. saligna* elucidated better anti-inflammatory activity in relation to the standard drug (diclofenac) (Tables 4.34 and 4.35). The lowest dose of 50 mg/Kg body weight indicated better anti-inflammatory pharmacological activity compared to standard drug (diclofenac) and the plant extracts (100 and 150 mg/Kg).

Anti-inflammatory pharmacological activity was statistically different between the three dose levels of the plant extracts (Tables 4.34 and 4.35).

Three hours after intraperitoneal injection of the plant test products samples and the standard drug, the percentage paw edema inhibition the experimental animal models in the groups labeled:- normal, negative, positive, test A, B and C were -0.77 -4.66, 3.52, 19.89, 10.02 and 14.11%, respectively. The paw edema inhibition between all these experimental groups was statistically difference with P value=0.00, $p < 0.05$ (Tables 4.34 and 4.35). The DCM leaves extracts of *E. saligna* had better pharmacological anti-inflammatory activity in relation to the standard drug (diclofenac) (Table 4.23). The dose level of 50 mg/Kg indicated better anti-inflammatory pharmacological activity in relation to standard drug (diclofenac) and some plant sample extracts (100 and 150 mg/Kg) (Tables 4.34 and 4.35).

Four hours following intraperitoneal administration plant sample products and the standard drug (diclofenac), the percentage paw edema inhibition in paw edema in the test experimental animal models in the groups labeled:- normal, negative, positive, test A, B and C were -0.84, -2.91, 6.44, 20.52, 15.98 and 17.56%, respectively. The paw edema inhibitions were statistically different with $P = 0.00$ at a significance level of $p < 0.05$ (Tables 4.34 and 4.35). The DCM *E. saligna* leaves extract elucidated better anti-inflammatory pharmacological activity in relation to the standard drug (diclofenac) (Tables 4.34 and 4.35). The least anti- inflammatory activity was observed in dose level of 50 mg/Kg.

Table 4.34: Anti-inflammatory activities of DCM leave extract of *Eucalyptus saligna*

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	100.81+0.16^{ab} (-0.81)	101.71±2.17^{ab} (-1.71)	100.77±2.02^{ab} (-0.77)	100.84±1.56^{ab} (-0.84)
Negative test control	DMSO + Carrageenan	104.68+0.74^{a} (-4.68)	104.68±1.22^{a} (-4.68)	104.66±0.72^{a} (-4.66)	102.91±1.30^{a} (-2.91)
Positive test control	Carrageenan+ diclofenac	98.28+1.12^{bc} (1.72)	104.30±6.65^{a} (-4.30)	96.48±1.65^{bc} (3.52)	93.56±2.80^{b} (6.44)
Experimental test A	Carrageenan+ *E. saligna* 50 mg/kg	93.52+0.60^{d} (6.48)	87.14±1.92^{c} (12.86)	80.11±1.96^{e} (19.89)	79.48±1.39^{c} (20.52)
Experimental test B	Carrageenan+ *E. saligna* 100 mg/kg	97.66+0.58^{bc} (2.34)	93.54±1.04^{abc} (6.46)	89.98±1.46^{cd} (10.02)	84.02±0.71^{c} (15.98)
Experimental test C	Carrageenan+ *E. saligna* 150 mg/kg	94.68±0.63^{cd} (5.32)	89.96±1.55^{bc} (10.04)	85.89±1.39^{de} (14.11)	82.44±1.86^{c} (17.56)
	F Value	9.27	34.48	52.69	55.29
	P Value	0.000	0.000	0.000	0.000

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Data was analyzed using one – way ANOVA and Turkey's test ($p < 0.05$). Figures enclosed in the brankets are indicating paw edema inhibition percentage.

Table 4.35: Time effects on antiinflammation activitiy of DCM leave extract of *Eucalyptus saligna*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	-0.90	1.51	(-5.38, 3.58)	-0.60	0.931
		3	0.03	1.51	(-4.45, 4.51)	0.02	1.000
		4	-0.03	1.51	(-4.51, 4.45)	-0.02	1.000
	2	3	0.93	1.51	(-3.55, 5.41)	0.62	0.924
		4	0.87	1.51	(-3.61, 5.35)	0.58	0.937
	3	4	-0.06	1.51	(-4.55, 4.42)	-0.04	1.000
Negative control	1	2	-0.00	1.17	(-3.48, 3.48)	-0.00	1.000
		3	0.02	1.17	(-3.46, 3.50)	0.01	1.000
		4	1.76	1.17	(-1.72, 5.24)	1.51	0.464
	2	3	0.02	1.17	(-3.46, 3.50)	0.02	1.000
		4	1.77	1.17	(-1.71, 5.25)	1.51	0.463
	3	4	1.75	1.17	(-1.73, 5.23)	1.49	0.472
Positive control	1	2	-6.02	4.13	(-18.30, 6.26)	-1.46	0.491
		3	1.81	4.13	(-10.47, 14.09)	0.44	0.971
		4	4.72	4.13	(-7.56, 17.00)	1.14	0.672
	2	3	7.83	4.13	(-4.45, 20.11)	1.89	0.281
		4	10.74	4.13	(-1.54, 23.02)	2.60	0.094
	3	4	2.92	4.13	(-9.36, 15.20)	0.71	0.893
Experimental group A	1	2	6.39	1.50	(1.92, 10.85)	4.25	0.005
		3	13.42	1.50	(8.96, 17.88)	8.93	0.000
		4	14.04	1.50	(9.58, 18.50)	9.35	0.000
	2	3	7.03	1.50	(2.57, 11.49)	4.68	0.003
		4	7.66	1.50	(3.19, 12.12)	5.10	0.001
	3	4	0.62	1.50	(-3.84, 5.09)	0.42	0.975
Experimental group B	1	2	4.12	1.02	(1.08, 7.16)	4.03	0.008
		3	7.69	1.02	(4.65, 10.72)	7.52	0.000
		4	13.65	1.02	(10.61, 16.68)	13.35	0.000
	2	3	3.57	1.02	(0.53, 6.60)	3.49	0.020
		4	9.53	1.02	(6.49, 12.56)	9.32	0.000
	3	4	5.96	1.02	(2.92, 9.00)	5.83	0.000
Experimental group C	1	2	4.71	1.36	(0.67, 8.76)	3.46	0.021
		3	8.79	1.36	(4.74, 12.83)	6.45	0.000
		4	12.24	1.36	(8.19, 16.28)	8.98	0.000
	2	3	4.07	1.36	(0.03, 8.12)	2.99	0.048
		4	7.52	1.36	(3.48, 11.57)	5.52	0.001
	3	4	3.45	1.36	(-0.60, 7.49)	2.53	0.105

4.5.2 Anti- inflammatory activities of methanolic leaves extract of *E. saligna*

An hour following intraperitoneal test products samples and standard drug (diclofenac) injection, the inhibition percentage of paw edema in the tests animal models in groups labeled:- normal,negative, positive and test A, B and C were -0.09, -3.45, 7.55, 4.09, 2.89 and 2.37%, respectively. This indicated a significant difference in percentage paw diameter inhibition between the all experimental groups with a calculated P value=0.00, at a significance level of $p < 0.05$ (Tables 4.36 and 4.37). Methanolic leaves extracts of *E. saligna* had lower anti-inflammatory activity than the conventional drug (diclofenac) (Table 4.36). The lowest dose level of 50 mg/Kg indicated higher anti-inflammatory activity in relation to the plantextract two dose levels (100 and 150 mg/Kg).

Two hours following intraperitoneal administration experimental test products and the standard anti-inflammatory drug (diclofenac) , the perecentage inhibition of the paw edema in the test animal models groups labeled:- normal, negative, positive, test A, B and C were 0.68%, -4.06, 12.75, 9.40, 8.13 and 6.53%, respectively (Tables 4.36 and 4.37). This indicated a significant difference in paw edema inhibition between all the study groups a P value=0.00,at a statistical significance level of $p<0.05$ (Tables 4.36 and 4.37). Methanolic leaves extracts of *E. saligna* had lower anti- inflammatory activity than the conventional drug (diclofenac) (Tables 4.36 and 4.37). The low dose of 50 mg/Kg indicated higher anti-inflammatory activity in relation to the plant extract higher dose levels (100 and 150mg/Kg). This indicated a significant difference in the anti-inflammatory pharmacological activity between different dose levels of the plant extract in the study (50, 100 and 150 mg/Kg), (Tables 4.36 and 4.37).

Three hours after the administration of the methanolic plants extracts and the conventional drug (diclofenac), the percentage inhibition of the paw among experimental animals in the groups labeled:- normal, negative, positive and test A, B and C were -0.92, -2.90, 19.10, 15.87, 13.38 and 10.07%, respectively (Tables 4.36 and 4.37). This indicated a significant difference in size reduction of paw diameter between the animals in the study groups with calculated P value=0.00, at a significance level of $p < 0.05$ (Tables 4.36 and 4.37). Methanolic leaves extracts of *E. saligna* had lower anti-inflammatory pharmacological activity in relation to standard drug (diclofenac) (Tables 4.36 and 4.37). The low dose of 50 mg/Kg indicated higher anti- inflammatory activity in relation to the plant extract high dose levels (100 and 150 mg/Kg. This indicated a statistically significant difference in the anti-inflammatory pharmacological activity between the three plant extracts dose levels (50, 100 and 150 mg/Kg) (Tables 4.36 and 4.37).

Four hours following intraperitoneal administration of test sample plant products and the standard anti-inflammatory drug (diclofenac), the percentage inhibition of the paw edema in the study experimental mice in groups labeled as:- normal,negative, positive, test A, B and C was 0.60, -0.59, 28.37, 19.97, 18.62 and 15.99%, respectively (Tables 4.36 and 4.37). This indicated a significant difference in the paw edema inhibiton between all the animal study groups with a P value=0.00, at a statistical significance level of $p < 0.05$ (Tables 4.36 and 4.37). Methanolic leaves extracts of *E. saligna* had lower anti-inflammatory pharmacological activity in relation to standard anti-inflammatory drug (diclofenac) (Tables 4.36 and 4.37).

Table 4.36: Anti-inflammatory activities of MeOH leave extract of *Eucalyptus saligna*

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	100.09±1.20^{ab} (-0.09)	99.32±1.48^{b} (0.68)	100.92±1.45^{a} (-0.92)	99.40±2.22^{a} (0.60)
Negative test control	DMSO + Carrageenan	103.45±0.61^{a} (-3.45)	104.06±0.78^{a} (-4.06)	102.90±0.96^{a} (-2.90)	100.59±0.58^{a} (-0.59)
Positive test control	Carrageenan+ diclofenac	92.45±0.69^{c} (7.55)	87.25±1.05^{d} (12.75)	80.90±1.75^{d} (19.10)	71.63±2.28^{c} (28.37)
Experimental test A	Carrageenan+ *E. saligna* 50 mg/kgbw	95.91±1.46^{bc} (4.09)	90.60±1.06^{cd} (9.40)	84.13±1.46^{cd} (15.87)	80.03±1.32^{b} (19.97)
Experimental test B	Carrageenan+ *E. saligna* 100 mg/kgbw	97.11±1.60^{bc} (2.89)	91.87±0.57^{c} (8.13)	86.62±0.73^{bc} (13.38)	81.38±1.21^{b} (18.62)
Experimental test C	Carrageenan+ *E. saligna* 150 mg/kgbw	97.63±1.44^{bc} (2.37)	93.47±1.13^{c} (6.53)	89.93±0.77^{b} (10.07)	84.01±0.80^{b} (15.99)
	F Value	9.27	34.48	52.69	55.29
	P Value	0.000	0.000	0.000	0.000

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Data was analyzed using one – way ANOVA and Turkey's test ($p < 0.05$). Figures enclosed in the brankets are indicating paw edema inhibition percentage.

Table 4.37: Time effects on anti-inflammatory activity of MeOH *Eucalyptus saligna* leave extract

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	0.77	1.41	(-3.42, 4.96)	0.54	0.947
		3	-0.83	1.41	(-5.02, 3.36)	-0.59	0.933
		4	0.68	1.41	(-3.51, 4.87)	0.48	0.961
	2	3	-1.60	1.41	(-5.79, 2.59)	-1.13	0.677
		4	-0.09	1.41	(-4.27, 4.10)	-0.06	1.000
	3	4	1.51	1.41	(-2.67, 5.70)	1.07	0.711
Negative control	1	2	-0.61	0.79	(-2.96, 1.75)	-0.76	0.869
		3	0.55	0.79	(-1.80, 2.91)	0.70	0.896
		4	2.86	0.97	(0.51, 5.22)	3.61	0.016
	2	3	1.16	0.97	(-1.20, 3.52)	1.46	0.488
		4	3.47	0.97	(1.11, 5.82)	4.37	0.004
	3	4	2.31	0.97	(-0.05, 4.66)	2.91	0.055
Positive control	1	2	5.20	1.55	(0.58, 9.81)	3.34	0.026
		3	11.55	1.55	(0.58, 9.81)	7.44	0.000
		4	20.82	1.55	(6.94, 16.17)	13.40	0.000
	2	3	6.36	1.55	(16.21, 25.43)	4.09	0.007
		4	15.63	1.55	(1.75, 10.97)	10.06	0.000
	3	4	9.27	1.55	(11.01, 20.24)	5.97	0.000
Experimental group A	1	2	5.313	0.72	(3.17, 7.46)	7.35	0.000
		3	11.79	0.72	(9.64, 13.93)	16.31	0.000
		4	15.89	0.72	(13.74, 18.03)	21.99	0.000
	2	3	6.47	0.72	(4.33, 8.62)	8.96	0.000
		4	10.57	0.72	(8.43, 12.72)	14.64	0.000
	3	4	4.10	0.72	(1.96, 6.25)	5.68	0.001
Experimental group B	1	2	5.24	1.10	(1.97, 8.52)	4.76	0.002
		3	10.49	1.10	(7.21, 13.76)	9.52	0.000
		4	15.73	1.10	(12.46, 19.00)	14.27	0.000
	2	3	5.24	1.10	(1.97, 8.52)	4.76	0.002
		4	10.49	1.10	(7.21, 13.76)	9.52	0.000
	3	4	5.24	1.10	(1.97, 8.52)	4.76	0.002
Experimental group C	1	2	4.15	0.83	(1.70, 6.61)	5.02	0.001
		3	7.70	0.83	(5.25, 10.16)	9.31	0.000
		4	13.62	0.83	(11.17, 16.08)	16.46	0.000
	2	3	3.55	0.83	(1.09, 6.01)	4.29	0.005
		4	9.47	0.83	(7.01, 11.93)	11.44	0.000
	3	4	5.92	0.83	(3.46,8.38)	7.15	0.000

4.5.3 Anti-inflammatory activity of DCM stem bark extract of *M. indica*

An hour following intraperitoneal administration of plant sample products and the standard inti-inflammatory drug (diclofenac), the percentage inhibition of the paw edema in experimental mice in groups labeled:- normal,negative, positive, test A, B and C was 1.81, -4.16, 5.18, 4.61, 5.92 and 6.38%, respectively. This indicated a significant difference in the reduction of paw edema between the experimental study group animal models with a F value=12.59; P value=0.00; $p < 0.05$ (Tables 4.38 and 4.39). The DCM stem bark extracts of *M. indica* dose level of 50 mg/Kg had lower anti-inflammatory activity and which was statistically difference to the standard anti-inflammatory drug (diclofenac). The anti-inflammatory pharmacological activity of DCM *M. indica* extracts higher dose dose levels (100 and 150 mg/Kg) had a higher and statistically different anti-inflammatory activity in relation to the conventional drug (diclofenac) (Tables 4.38 and 4.39).

Two hours following intraperitoneal administration of experimental plant extracts test products and the standard anti-inflammatory drug (diclofenac), the percentage inhibition of the paw edema in the study experimental mice in groups labeled:- normal, negative, positive, test A, B and C was 0.83, -5.37, 11.53, 9.23, 8.24 and 11.09%, respectively (Tables 4.38 and 4.39).This indicated a significant difference in the percentage inhibition of the paw edema between all experimental animal models in different groups with a calculated F value=38.06; P value=0.00 at $p < 0.05$ (Tables 4.38 and 4.39). The DCM stem bark extracts of *M. indica* had anti-inflammatory pharmacological activity which was not statistically different from anti-inflammatory activity of the conventional drug

(diclofenac) activity (Tables 4.38 and 4.39).

Three hours following intraperitoneal administration of test plant products samples and the standard anti-inflammatory drug (diclofenac), the percentage inhibition of the paw edema in experimental animal model labeled:- normal,negative, positive, test A, B and C was 2.61, -4.75, 15.53, 16.08, 13.57 and 17.50%, respectively (Tables 4.38 and 4.39). This indicated a significant difference in paw edema inhibition between all the study groups with calculated F = 56.76 and P value=0.00 at $p < 0.05$ (Table 4.25). There was no significant difference between the anti-inflammatory activity of the three DCM *M. indica* stem bark extract dose levels and the conventional drug (diclofenac) (Tables 4.38 and 4.39).

Four hours following intraperitoneal administration experimental plant extracts test products and the standard anti-inflammatory drug (diclofenac), the percentage inhition of paw edema in study animal model (mice) in the groups labeled:- normal, negative, positive, test A, B and C was 0.03, -2.41, 20.71, 21.86, 18.89 and 23.56%, respectively (Table 4.38 and 4.39). This indicated a significant difference on paw edema inhibition between all the study groups with a calculated F value=70.50 and P value = 0.00 at $p < 0.05$ (Table 4.25). The DCM stem bark extracts of *M. indica* at a dosage level of 150 mg/Kg had a higher and statistically significant different anti-inflammatory activity in relation to the conventional drug (diclofenac) (Tables 4.38 and 4.39).

Table 4.38: Anti – inflammatory activity of DCM stem bark extract of *Mangifera indica*

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	98.19±1.64^{c} (1.81)	99.17±0.83^{c} (0.83)	97.39±1.07^{c} (2.61)	99.97±1.24^{c} (0.03)
Negative test control	DMSO + Carrageenan	104.16±1.17^{a} (-4.16)	105.37±1.18^{a} (-5.37)	104.75±0.78^{a} (-4.75)	102.41±1.13^{a} (-2.41)
Positive test control	Carrageenan+ diclofenac	94.82±1.40^{ab} (5.18)	88.47±1.35^{b} (11.53)	84.47±0.78^{b} (15.53)	79.29±1.16^{b} (20.71)
Experimental test A	Carrageenan+ *M. indica* 50 mg/kg bw	95.39±0.68^{bc} (4.61)	90.77±1.06^{b} (9.23)	83.92±1.98^{b} (16.08)	78.14±1.90^{b} (21.86)
Experimental test B	Carrageenan+ *M. indica* 100 mg/kg bw	94.08±0.07^{b} (5.92)	91.76±1.39^{b} (8.24)	86.43±1.41^{b} (13.57)	81.11±1.89^{b} (18.89)
Experimental test C	Carrageenan+ *M. indica* 150 mg/kg bw	93.62±1.03^{b} (6.38)	88.91±0.45^{b} (11.09)	82±0.50^{b} (17.50)	76.64±0.51^{bc} (23.56)
	F value	12.59	38.06	56.76	70.50
	P value	0.00	0.00	0.00	0.00

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Analysis was done using one – way ANOVA and Turkey's test ($p < 0.05$). Figures enclosed in the brankets are indicating paw edema inhibition percentage.

Table 4.39: Time effects on anti-inflammatory activity of DCM stem bark extract of *Mangifera indica*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	-0.98	1.51	(-5.47, 3.52)	-0.65	0.915
		3	0.80	1.51	(-3.69, 5.30)	0.53	0.950
		4	-1.78	1.51	(-6.27, 2.71)	-1.18	0.653
	2	3	1.78	1.51	(-2.71, 6.27)	1.18	0.653
		4	-0.80	1.51	(-5.30, 3.69)	-0.53	0.950
	3	4	-2.58	1.51	(-7.07, 1.91)	-1.71	3.62
Negative control	1	2	-1.22	1.03	(-4.28, 1.85)	-1.18	0.651
		3	-0.59	1.03	(-3.66, 2.47)	-0.57	0.938
		4	1.75	1.03	(-1.32, 4.81)	1.69	0.368
	2	3	0.62	1.03	(-2.44, 3.69)	0.60	0.929
		4	2.96	1.03	(-0.10, 6.03)	2.87	0.059
	3	4	2.34	1.03	(-0.72, 5.40)	2.27	0.160
Positive control	1	2	6.35	0.836	(3.870, 8.84)	7.60	0.000
		3	10.37	0.836	(7.871, 12.84)	12.38	0.000
		4	15.53	0.836	(13.05, 18.02)	18.57	0.000
	2	3	4.002	0.836	(1.518, 6.49)	4.79	0.000
		4	9.180	0.836	(6.70, 11.66)	10.98	0.000
	3	4	5.178	0.836	(2.70, 7.66)	6.19	0.000
Experimental group A	1	2	4.62	1.55	(0.02, 9.23)	2.98	0.049
		3	11.47	1.55	(6.86, 16.08)	7.39	0.000
		4	17.25	1.55	(12.65, 21.86)	11.12	0.000
	2	3	6.85	1.55	(2.24, 11.45)	4.41	0.004
		4	12.63	1.55	(8.02, 17.24)	8.14	0.000
	3	4	5.79	1.55	(1.18, 10.39)	3.73	0.013
Experimental group B	1	2	2.32	1.23	(-1.33, 5.97)	1.89	0.284
		3	7.65	1.23	(4.00, 11.30)	6.22	0.000
		4	12.97	1.23	(9.31, 16.62)	10.54	0.000
	2	3	5.33	1.23	(1.68, 8.99)	4.33	0.005
		4	10.65	1.23	(6.99, 14.30)	8.65	0.000
	3	4	5.31	1.23	(1.66, 8.97)	4.32	0.005
Experimental group C	1	2	4.713	0.556	(3.06, 6.365)	8.48	0.000
		3	11.126	0.556	(9.47, 12.78)	20.01	0.000
		4	16.982	0.556	(15.33, 18.63)	30.54	0.000
	2	3	6.412	0.556	(4.76, 8.06)	11.53	0.000
		4	12.269	0.556	(10.62, 13.92)	22.06	0.000
	3	4	5.857	0.556	(4.21, 7.508)	10.53	0.000

4.5.4 Anti-inflammatory activities of *M. indica* stem bark MeOH extract

An hour following intraperitoneal administration of study plant sample products and standard anti-inflammatory drug (diclofenac), the paw edema percentage inhibition in all experimental animals in groups labeled:- normal,negative, positive, test A, B and C was 2.27, -1.98, 5.03, 4.76, 6.32 and 4.76%, respectively (Tables 4.40 and 4.41). This indicated a statistical significance difference in paw edema inhibition between all the study groups with a calculated F value = 12.59; P value=0.00; $p < 0.05$ (Tables 4.40, and 4.41). Administration of methanolicstem bark *M. indica* extracts at three dose levels (50, 100 and 150 mg/Kg) elucidated anti- inflammatory activity with statistical significant difference with conventional drug (diclofenac) at $p < 0.05$ though the edema inhibition activity associated with low and high dose level of plant extracts (50 and 150 mg/Kg) of the *M. indica* stem bark extract was slightly lower than that of the conventional drug (diclofenac). The edema inhibition activity of the 100 mg/Kg was higher and had statistically significant different anti-inflammatory activity with the conventional drug (diclofenac) (Tables 4.40 and 4.41).

Two hours upon administration of plant test products and the conventional drug (diclofenac), the percentage inhibition of the paw edema in the study animal model (mice) in the groups labeled:- normal, negative, positive, test A, B and C was 0.00, -3.78, 10.65, 8.92, 11.48 and 11.25%, respectively (Tables 4.40 and 4.41). This indicated a statistical significance difference in the paw edema inhibition between all the study group animals with a calculated F value=49.25; P value=0.00 at $p < 0.05$ (Tables 4.40 and 4.41). The methanolic stem barks *M. indica* extracts had anti-inflammatory

pharmacological activity not statistically different in relation to standard anti-inflammatory drug (diclofenac) activity (Table 4.40, 4.41).

Three hours following administration of test products and the conventional drug (diclofenac), the percentage inhibition of the paw edema in all of the experimental animal model (mice) grouped as:- normal, negative, positive, test A, B and C was 2.31, -3.78, 17.41, 16.09, 20.09 and 17.21%, respectively. This indicated a statistically significance difference in edema inhibition between experimental groups with a calculated F = 75.32 and P value = 0.00 at a significance level of $p < 0.05$ (Tables 4.40 and 4.41).

Four hours following intraperitoneal paw injection with plant extract test products and the conventional drug (diclofenac), the percentage inhibition of paw edema in the animal model grouped as:- normal, negative, positive, test A, B, and C was 1.53, -1.19, 24.14, 20.89, 25.29 and 23.17%, respectively (Tables 4.40 and 4.41). This indicates a statistical significance difference in the percentage paw edema inhibition between all the animal model test groups with a calculated F value =105.15 and P value=0.00 at $p < 0.05$ (Tables 4.40 and 4.41).

Table 4.40: Anti-inflammatory activities of MeOH stem bark extract of *Mangifera indica*

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	97.73±1.66^{c} (2.27)	100.00±0.00^{c} (0.00)	97.69±0.94^{c} (2.31)	98.47±1.66^{c} (1.53)
Negative test control	DMSO + Carrageenan	101.98±0.83^{a} (-1.98)	103.78±0.56^{a} (-3.78)	103.78±0.56^{a} (-3.78)	101.19±0.73^{a} (-1.19)
Positive test control	Carrageenan+ diclofenac	94.97±0.51^{ab} (5.03)	89.35±0.93^{b} (10.65)	82.59±0.90^{b} (17.41)	75.86±0.79^{b} (24.14)
Experimental test A	Carrageenan+ *M. indica* 50 mg/kg bw	95.24±0.69^{bc} (4.76)	91.08±1.22^{b} (8.92)	83.91±1.59^{b} (16.09)	79.11±1.33^{b} (20.89)
Experimental test B	Carrageenan+ *M. indica* 100 mg/kg bw	93.68±0.57^{b} (6.32)	88.52±0.87^{b} (11.48)	79.91±1.16^{b} (20.09)	74.71±1.03^{b} (25.29)
Experimental test C	Carrageenan+ *M. indica* 150 mg/kg bw	95.24±0.73^{b} (4.76)	88.75±1.38^{b} (11.25)	82.79±1.32^{b} (17.21)	76.83±1.26^{bc} (23.17)
	F Value	12.59	49.23	75.32	105.15
	P Value	0.00	0.00	0.00	0.00

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Analysis was done using one – way ANOVA and Turkey's test ($p < 0.05$). Figures enclosed in the brankets are indicating paw edema inhibition percentage.

Table 4.41: Time effects on anti-inflammatory activity of MeOH stem bark extract of *Mangifera indica*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	-2.27	1.36	(-6.32, 1.77)	-1.67	0.379
		3	0.04	1.36	(-4.01, 4.08)	0.03	1.000
		4	-0.74	1.36	(-4.78, 3.30)	-0.54	0.946
	2	3	2.31	1.36	(-1.73, 6.35)	1.70	0.367
		4	1.53	1.36	(-2.51, 5.57)	1.13	0.681
	3	4	-0.78	1.36	(-4.82, 3.26)	-0.57	0.939
Negative control	1	2	-1.80	0.77	(-4.08, 0.48)	-2.35	0.142
		3	-1.80	0.77	(-4.08, 0.48)	-2.35	0.142
		4	0.78	0.77	(-1.50, 3.06)	1.02	0.742
	2	3	-0.00	0.77	(-2.28, 2.28)	-0.00	1.000
		4	2.58	0.77	(0.30, 4.86)	3.37	0.025
	3	4	2.58	0.77	(0.30, 4.86)	3.37	0.025
Positive control	1	2	5.62	0.84	(3.12, 8.12)	6.68	0.000
		3	12.38	0.84	(9.89, 14.88)	14.73	0.000
		4	19.11	0.84	(16.62, 21.61)	22.73	0.000
	2	3	6.77	0.84	(4.27, 9.26)	8.05	0.000
		4	13.50	0.84	(10.99, 15.99)	16.05	0.000
	3	4	6.73	0.84	(4.23, 9.23)	8.00	0.000
Experimental group A	1	2	4.16	0.83	(1.69, 6.62)	5.01	0.002
		3	11.33	0.83	(8.86, 13.79)	13.65	0.000
		4	16.13	0.83	(13.66, 18.59)	19.43	0.000
	2	3	7.17	0.83	(4.71, 9.64)	8.64	0.000
		4	11.97	0.83	(9.51, 14.44)	14.42	0.000
	3	4	4.80	0.83	(2.33, 7.26)	5.78	0.000
Experimental group B	1	2	5.16	0.91	(2.47, 7.85)	5.70	0.001
		3	13.77	0.91	(11.08, 16.46)	15.21	0.000
		4	18.97	0.91	(16.28, 21.65)	20.94	0.000
	2	3	8.61	0.91	(5.92, 11.29)	9.51	0.000
		4	13.80	0.91	(11.11, 16.49)	15.24	0.000
	3	4	5.19	0.91	(2.51, 7.88)	5.74	0.000
Experimental group C	1	2	6.49	0.87	(3.90, 9.09)	7.44	0.000
		3	12.45	0.87	(9.86, 15.04)	14.27	0.000
		4	18.41	0.87	(15.82, 21.00)	21.09	0.000
	2	3	5.96	0.87	(3.37, 8.55)	6.83	0.000
		4	11.92	0.87	(9.32, 14.51)	13.65	0.000
	3	4	5.96	0.87	(3.37, 8.55)	6.83	0.000

4.5.5 Anti-inflammatory activities of DCM stalk extract of *B. pilosa*

Edema inhibition activity of DCM stalk extract of *B. pilosa* was demonstrated by use carrageenan edema test in albino mice. An hours following administering plant sample extract and the standard anti-inflammatory drug(diclofenac), percentage inhibition of the paw edema among the study animal groups categorized as:- normal, negative, positive, test A, B and C was -1.01, -2.29, 5.80, 3.50, 7.50 and 8.15%, respectively. This indicates a statistical significancedifference in the percentage inhibition of the paw edema between all study groups of the animal model with a calculated F value =13.96; P value=0.00; $p < 0.05$ (Tables 4.42 and 4.43). The methanolic stem bark extracts of *M. indica* three dose levels had anti-inflammatory activity with no statistical significant difference with that of the standard anti-inflammatory drug (diclofenac). The anti-inflammatory pharmacological activity the lowest dose (50 mg/Kg) of the *B. pilosa* stalk extract was slightly lower than that of standard anti-inflammatory drug (diclofenac) with a statistical significance different at $p < 0.05$ (Tables 4.42 and 4.43).

Two hours following intraperitoneal administration of both plant sample extracts and the standard anti-inflammatory drug (diclofenac), percentage inhibition of the paw edema in animal study model groups labeled:- normal,negative, positive, test A, B and C was 0.77, -2.30, 12.19, 9.81, 14.44 and 15.13%, respectively (Table 4.27). This indicated a statistical significance difference in percentage inhibition of paw edema between all the study animal models in different groups with a calculated F value=47.83; P value=0.00 at $p < 0.05$ (Tables 4.42 and 4.43). The DCM stalk extracts of *B. pilosa* at 100 mg/Kg dose level had anti-inflammatory activity with no statistical different in relation to

conventional drug (diclofenac) (Tables 4.42 and 4.43). The edema inhibition activity at 50 mg/Kg dose level was lower and with statistical significant difference in relation to the conventional drug (diclofenac). The inflammatory activity inhibition of theplant extract at 150 mg/Kg dose level was higher and with statistical significant difference in relation to the conventional drug (diclofenac) (Tables 4.42 and 4.43).

Three hours following administration of plant experimental products and conventional drug (diclofenac), the percentage inhibition of the paw edema in animal models grouped as:- normal, negative, positive, test A, B and C was 1.60, -1.75, 19.76, 15.59, 20.79 and 21.51%, respectively (Tables 4.42 and 4.43). This indicates a statistical significance difference in percentage paw edema inhibition between the six groups of the animal model with a calculated F value=94.95 and P value=0.00 at $p < 0.05$ (Table 4.27). Edema inhibition for doses of 100 and 150 mg/Kg body weight was higher than for conventional drug (diclofenac) while for the dosage level of 50mg/Kg it was lower (Tables 4.42 and 4.43).

Four hours following intraperitoneal administration of all study plant test products and standard anti-inflammatory drug (diclofenac), the percentage inhibition of the paw edema in experimental animal model (mice) is groups labeled:- normal, negative, positive, test A, B and C was -0.90, -0.02, 25.58, 19.05, 26.57 and 27.33%, respectively (Tables 4.42, and 4.43). This indicates a statistical significance difference edema inhibition by the test products and the standard drug between all the study groups with a calculated F value = 127.11 and P value = 0.00 at $p < 0.05$ (Tables 4.42 and 4.43). Edema inhibition activity

of 100 mg/Kg was lower and with a statistical significance difference to the conventional drug (diclofenac) (Tables 4.42 and 4.43).

Table 4.42: Anti-inflammatory effects of DCM stalk extract of *Bidens pilosa*

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	101.01±2.05^{ab} (-1.01)	99.23±1.52^{a} (0.77)	98.40±0.98^{a} (1.60)	100.90±1.55^{a} (-0.90)
Negative test control	DMSO + Carrageenan	102.29±0.57^{a} (-2.29)	102.30±0.58^{a} (-2.30)	101.75±1.17^{a} (-1.75)	100.02±0.92^{a} (-0.02)
Positive test control	Carrageenan+ Diclofenac	94.20±0.92^{c} (5.80)	87.81±0.44^{bc} (12.19)	80.24±1.00^{bc} (19.76)	74.42±1.00^{c} (25.58)
Experimental test A	Carrageenan+ *B. pilosa* 50mg/kg bw	96.50±0.64^{bc} (3.50)	90.20±0.61^{b} (9.81)	84.41±0.56^{b} (15.59)	80.95±1.02^{b} (19.05)
Experimental test B	Carrageenan+ *B. pilosa* 100mg/kg bw	92.50±1.14^{c} (7.50)	85.56±1.54^{bc} (14.44)	79.21±1.61^{c} (20.79)	73.43±1.59^{c} (26.57)
Experimental test C	Carrageenan+ *B. pilosa* 150mg/kg bw	91.85±1.11^{c} (8.15)	84.87±1.11^{c} (15.13)	78.49±0.70^{c} (21.51)	72.67±0.71^{c} (27.33)
	F value	12.96	47.83	94.95	127.11
	P value	0.00	0.00	0.00	0.00

Values are in terms of Mean ± SEM. Column statistical comparison are made and different superscripts indicates significant different. Analysis was done using one – way ANOVA and Turkey's test ($p < 0.05$). Figures enclosed in the brankets are indicating paw edema inhibition percentage.

Table 4.43: Time effects on anti-inflammatory activity of DCM stalk extract of *Bidens pilosa*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	1.78	1.08	(-1.42, 4.97)	1.65	0.388
		3	2.61	1.08	(-0.58, 5.81)	2.43	0.124
		4	0.11	1.08	(-3.09, 3.30)	0.10	1.000
	2	3	0.83	1.08	(-2.36, 4.03)	0.77	0.864
		4	-1.67	1.08	(-4.87, 1.53)	-1.55	0.440
	3	4	-2.50	1.08	(-5.70, 0.69)	-2.33	0.146
Negative control	1	2	-0.02	1.06	(-3.16, 3.13)	-0.02	1.000
		3	0.54	1.06	(-2.60, 3.68)	0.51	0.955
		4	2.27	1.06	(-0.87, 5.41)	2.15	0.194
	2	3	0.55	1.06	(-2.59, 3.70)	0.52	0.952
		4	2.29	1.06	(-0.86, 5.43)	2.16	0.190
	3	4	1.73	1.06	(-1.41, 4.87)	1.64	0.396
Positive control	1	2	6.39	1.04	(3.31, 9.48)	6.16	0.000
		3	13.96	1.04	(10.88, 17.04)	13.45	0.000
		4	19.78	1.04	(16.70, 22.86)	19.06	0.000
	2	3	7.57	1.04	(4.49, 10.65)	7.29	0.000
		4	13.39	1.04	(10.31, 16.47)	12.90	0.000
	3	4	5.82	1.04	(2.74, 8.90)	5.61	0.001
Experimental group A	1	2	6.31	0.85	(3.79, 8.82)	7.45	0.000
		3	12.09	0.85	(9.58, 14.61)	14.29	0.000
		4	15.56	0.85	(13.04, 18.07)	18.38	0.000
	2	3	5.79	0.85	(3.27, 8.30)	6.83	0.000
		4	9.25	0.85	(6.74, 11.76)	10.93	0.000
	3	4	3.46	0.85	(0.95, 5.98)	4.09	0.007
Experimental group B	1	2	6.94	1.32	(3.02, 10.86)	5.26	0.001
		3	13.29	1.32	(9.37, 17.21)	10.07	0.000
		4	19.08	1.32	(15.15, 23.00)	14.45	0.000
	2	3	6.35	1.32	(2.43, 10.27)	4.81	0.002
		4	12.13	1.32	(8.21, 16.06)	9.19	0.000
	3	4	5.78	1.32	(1.86, 9.70)	4.38	0.004
Experimental group C	1	2	6.98	0.56	(5.30, 8.65)	12.37	0.000
		3	13.36	0.56	(11.69, 15.04)	23.71	0.000
		4	19.18	0.56	(17.50, 20.85)	34.02	0.000
	2	3	6.39	0.56	(4.71, 8.06)	11.33	0.000
		4	12.20	0.56	(10.53, 13.88)	21.65	0.000
	3	4	5.82	0.56	(4.14, 7.49)	10.32	0.000

4.5.6 Anti-inflammatory activities of MeOH stalks extract of *B. pilosa*

An hour following intraperitoneal administration standard anti-inflamatory drug (diclofenac) and plant extract samples in all animal model, the results in groups indicated as:- normal, negative, positive, test A, B and C was 3.42, -2.84, 2.25, 5.18, 3.48 and 6.34%, respectively (Tables 4.44 and 4.45). This indicated a statistical significance difference edema inhibition between all the study groups with a calculated F value=10.44; P value=0.00; $p < 0.05$ (Tables 4.44 and 4.45). Methanolic stalks extracts of *B. pilosa* dose levels of 50, 100 and 150 mg/Kg body weight had anti-inflammatory activity with no statistical significant difference with that of the standard anti-inflammatory drug (diclofenac) (Tables 4.44 and 4.45). Edema inhibition by 50, 100 and 150 mg/Kg of the *B. pilosa* stalks extract was slightly higher than that of the conventional drug (diclofenac) (Table 4.44, 4.45).

Two hours following intraperitoneal administration of experimental plant sample extract and the standard anti-inflammatory drug (diclofenac), the percentage inhibition of paw edema in groups categorized as:- normal, negative, positive, test A, B and C was 0.88, -2.84, 7.99, 10.93, 9.30 and 14.36%, respectively (Tables 4.44 and 4.45). This indicates a statistical significance difference in percentage inhibition of paw edema between these groups with a calculated F value=25.86; P value=0.00 at $p < 0.05$ (Tables 4.44 and 4.45). Methanolic stalks extracts of *B. pilosa* had anti-inflammatory activity which had statistical significance different in relation to the conventional drug (diclofenac) (Tables 4.44 and 4.45). The three dose levels of methanolic stalks extracts of *B. pilosa* exhibited better anti-inflammatory activity in relation to standard anti-inflammatory drug

(diclofenac) (Tables 4.44 and 4.45).

Three hours following intraperitoneal administration both plant extract and standard anti-inflammatory drug(diclofenac), percentage inhibition of paw edema animal model (mice) categorized as:- normal,negative, positive, test A, B and C was -0.03, -0.57, 14.82, 18.38, 14.53 and 19.54%, respectively (Tables 4.44 and 4.45). This indicated a statistical significance difference in the percentage inhibition of paw edema between the study animal model groups with a calculated F=50.49 and P value=0.00 at $p < 0.05$ (Tables 4.44 and 4.45).

Four hours following the dministration of plant test products and the standard anti-inflammatory drug(diclofenac), the percentage inhibition of paw edema in experimental animal model (mice) in the study groups categorized as:- normal, negative, positive and test groups A, B and C was 1.74, 0.00, 20.58, 21.25, 19.76 and 27.02%, respectively (Table 4.44, 4.45). This indicated a statistical significance difference of perecentage inhibition of paw edema between all the study groups with a calculated F value=80.08 and P value=0.00 at a significance level of $p < 0.05$ (Tables 4.44 and 4.45). The edema percentage inhibition of 150 mg/kg body weight dose for *B. pilosa* methanolic stalks extract was higher and hada statistical significance difference with that of 50, 100 mg/Kg dose level and the conventional anti-inflammatory drug (diclofenac) (Tables 4.44 and 4.45).

Table 4.44: Anti-inflammatory effects of MeOH stalk extract of *Bidens pilosa*

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	96.58±1.68^{b} (3.42)	99.12±2.55^{a} (0.88)	100.03±1.90^{a} (-0.03)	98.26±1.07^{a} (1.74)
Negative test control	DMSO + Carrageenan	102.84±0.03^{a} (-2.84)	102.84±0.03^{a} (-2.84)	100.57±0.57^{a} (-0.57)	100.00±0.00^{a} (0.00)
Positive test control	Carrageenan+ Diclofenac	97.75±1.04^{b} (2.25)	92.01±0.96^{b} (7.99)	85.18±1.90^{b} (14.82)	79.42±2.57^{b} (20.58)
Experimental test A	Carrageenan+ *B. pilosa* 50 mg/kg bw	94.82±0.58^{b} (5.18)	89.07±0.60^{bc} (10.93)	81.62±0.56^{b} (18.38)	78.75±0.55^{b} (21.25)
Experimental test B	Carrageenan+ *B. pilosa* 100 mg/kg bw	96.52±1.11^{b} (3.48)	90.70±1.10^{bc} (9.30)	85.47±0.90^{b} (14.53)	80.24±0.45^{b} (19.76)
Experimental test C	Carrageenan+ *B. pilosa* 150 mg/kg bw	93.66±0.62^{b} (6.34)	85.64±0.86^{c} (14.36)	80.46±1.04^{b} (19.54)	72.98±1.15^{c} (27.02)
	F Value	10.44	25.86	50.49	80.08
	P Value	0.00	0.00	0.00	0.00

Values are in terms of Mean ± SEM. Column statistical comparison were made and different superscripts indicates significant different. Analysis was done using one – way ANOVA and Turkey's test ($p < 0.05$). Figures enclosed in the brackets are indicating paw edema inhibition percentage.

Table 4.45: Time effects on anti-inflammatory activity of MeOH stalk extract of *Bidens pilosa*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	-2.54	1.33	(-6.50, 1.42)	-1.91	0.276
		3	-3.45	1.33	(-7.41, 0.51)	-2.59	0.096
		4	-1.68	1.33	(-5.64, 2.28)	-1.26	0.604
	2	3	-0.91	1.33	(-4.87, 3.05)	-0.68	0.902
		4	0.86	1.33	(-3.10, 4.83)	0.65	0.914
	3	4	1.77	1.33	(-2.19, 5.74)	1.33	0.563
Negative control	1	2	0.00	0.40	(-1.20, 1.20)	0.00	1.000
		3	2.27	0.40	(1.07, 3.47)	5.63	0.001
		4	2.84	0.40	(1.65, 4.04)	7.05	0.000
	2	3	2.27	0.40	(1.07, 3.47)	5.63	0.001
		4	2.84	0.40	(1.65, 4.04)	7.05	0.000
	3	4	0.57	0.40	(-0.63, 1.77)	1.42	0.513
Positive control	1	2	5.74	1.73	(0.60, 10.87)	3.32	0.027
		3	12.57	1.73	(7.43, 17.70)	7.27	0.000
		4	18.32	1.73	(13.19, 23.45)	10.60	0.000
	2	3	6.83	1.73	(1.70, 11.96)	3.95	0.009
		4	12.58	1.73	(7.45, 17.72)	7.28	0.000
	3	4	5.75	1.73	(0.62, 10.89)	3.33	0.027
Experimental group A	1	2	5.750	0.49	(4.29, 7.22)	11.66	0.000
		3	13.20	0.49	(11.73, 14.66)	26.76	0.000
		4	16.07	0.49	(14.60, 17.54)	32.58	0.000
	2	3	7.45	0.49	(5.98, 8.91)	15.10	0.000
		4	10.32	0.49	(8.86, 11.79)	20.93	0.000
	3	4	2.88	0.49	(1.41, 4.34)	5.83	0.000
Experimental group B	1	2	5.82	0.57	(4.12, 7.52)	10.18	0.000
		3	11.05	0.57	(9.35, 12.75)	19.32	0.000
		4	16.28	0.57	(14.58, 17.98)	28.47	0.000
	2	3	5.23	0.57	(3.53, 6.93)	9.15	0.000
		4	10.46	0.57	(8.76, 12.16)	18.29	0.000
	3	4	5.23	0.57	(3.53, 6.93)	9.15	0.000
Experimental group C	1	2	8.02	0.96	(5.18, 10.86)	8.39	0.000
		3	13.20	0.96	(10.36, 16.04)	13.81	0.000
		4	20.68	0.96	(17.84, 23.52)	21.64	0.000
	2	3	5.18	0.96	(2.34, 8.02)	5.42	0.001
		4	12.67	0.96	(9.82, 15.50)	13.25	0.000
	3	4	7.48	0.96	(4.64, 10.32)	7.83	0.000

4.5.7 Anti-inflammatory activities of DCM leaves extract of *S. didymobotyra*

An hour following intraperitoneal administration of plant test products and standard anti-inflammatory drug (diclofenac) the percentage inhibition of paw edema amongst the experimental study groups of the animal models categorized as:- normal, negative, positive, test A, B and C was -1.66, -2.86, 7.24, 6.23, 5.58 and 6.75%, respectively (Tables 4.46 and 4.47). This indicates a statistical significance difference in percentage inhibition between the study groups with a calculated F value=15.06; P value=0.00; $p < 0.05$ (Tables 4.46 and 4.47). The DCM leaves extracts of *S. didymobotyra* dose levels of 50, 100 and 150 mg/Kg body had anti-inflammatory activity with no statistical significant difference with that of the standard anti-inflammatory drug (diclofenac) (Tables 4.46 and 4.47). The percentage inhibition of paw edema associated with 50 and 150 mg/Kg dose level of the *S. didymobotyra* leaves extract was slightly lower than that of the conventional drug (diclofenac) (Tables 4.46 and 4.47).

Two hours following intraperitoneal administration of *S. didymobotyra* extract and the standard anti-inflammatory drug (diclofenac) the perecentage inhibition of induced paw edema in all experimental animal model (mice) groups categorized as:- normal, negative, positive, test A, B and C was -0.83, -2.86, 16.72, 12.39, 12.87 and 16.25%, respectively (Table 4.46, 4.47). This indicated a statistical significance difference in percentage inhibition of paw edema between these group with a calculated F value=52.80; P value=0.00 at $p < 0.05$ (Tables 4.46 and 4.47). The DCM leaves extracts of *S. didymobotyra* was associated with anti-inflammatory activity though the pharmacological effect was not statistically different in relation to standard anti-inflammatory drug

(diclofenac) (Tables 4.46 and 4.47).

Three hours post intraperitoneal administration of *S. didymobotyra* extracts and the standard anti-inflammatory drug (diclofenac), percentage inhibition of the paw edema study animal model (mice) categorized as:- normal, negative, positive, test A, B and C was -1.63, -2.88, 25.09, 20.27, 21.90 and 23.55%, respectively (Tables 4.46 and 4.47). This indicated a statistical significance difference in percentage inhibition of paw edema between all the study groups with a calculated F=97.69 and P value=0.00 at $p < 0.05$ (Tables 4.46 and 4.47). The statistical significantdifferent between edema inhition activity of three doses of DCM leaves extract of *S. didymobotyra* and the conventional drug (diclofenac) (Tables 4.46 and 4.47).

Four hours post intraperitoneal administration *S. didymobotyra* extract and the standard anti-inflammatory drug (diclofenac), the percentage inhibition of the paw edema inhibition in study animal model (mice) categorized as:- normal, negative, positive, test A, B and C was -0.06, -1.75, 31.26, 25.38, 28.61 and 29.73%, respectively (Tables 4.46 and 4.47). This indicated a statistical significance difference in percentage inhibition following edema induction between all animal study groups with a calculated F value=140.45 and P value=0.00 at $p < 0.05$ (Tables 4.46 and 4.47). There was statistical significant different between edema inhibition activity of the three different doselevels of DCM leaves extract of *S. didymobotyra* and the conventional drug (diclofenac) at $p < 0.05$ (Tables 4.46, and 4.47). The 50 mg/kg body weight dose level exhibited lower anti- inflammatory activity in relation to other dose levels and the conventional drug (Tables 4.46 and 4.47).

Table 4.46: Anti-inflammatory activities of DCM leave extract of *Senna didymobotyra*

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	101.86±1.59^{a} (-1.66)	100.83±0.83^{a} (-0.83)	101.63±1.00^{a} (-1.63)	100.06±1.27^{a} (-0.06)
Negative test control	DMSO + Carrageenan	102.86±0.06^{a} (-2.86)	102.86±0.06^{a} (-2.86)	102.88±0.96^{a} (-2.88)	101.75±1.20^{a} (-1.75)
Positive test control	Carrageenan+ Diclofenac	92.76±1.66^{b} (7.24)	83.28±1.90^{b} (16.72)	74.91±1.57^{b} (25.09)	68.74±0.82^{c} (31.26)
Experimental test A	Carrageenan+ *S. didymobotyra* 50 mg/kg bw	93.77±0.59^{b} (6.23)	87.61±1.02^{b} (12.39)	79.73±1.66^{b} (20.27)	74.62±1.60^{b} (25.38)
Experimental test B	Carrageenan+ *S. didymobotyra* 100 mg/kg bw	94.42±1.46^{b} (5.58)	87.13±1.28^{b} (12.87)	78.10±1.32^{b} (21.90)	71.39±1.63^{bc} (28.61)
Experimental test C	Carrageenan+ *S. didymobotyra* 150 mg/kg bw	93.25±0.70^{b} (6.75)	83.75±1.25^{b} (16.25)	76.45±1.21^{b} (23.55)	70.27±1.13^{bc} (29.73)
F Value		15.06	52.80	97.69	140.45
P Value		0.00	0.00	0.00	0.00

Values are in terms of Mean ± SEM. Column statistical comparison were made and different superscripts indicates significant different. Analysis was done using one – way ANOVA and Turkey's test ($p < 0.05$). Figures enclosed in the brackets are indicating paw edema inhibition percentage.

Table 4.47: Time effects on anti-inflammatory activity of DCM *Senna didymobotyra* leaves extract

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	0.83	1.51	(-3.67, 5.33)	0.55	0.945
		3	0.03	1.51	(-4.47, 4.53)	0.02	1.000
		4	1.60	1.51	(-2.90, 6.10)	1.06	0.721
	2	3	-0.80	1.51	(-5.30, 3.70)	-0.53	0.951
		4	0.77	1.51	(-3.73, 5.27)	0.51	0.956
	3	4	1.57	1.51	(-2.93, 6.07)	1.04	0.732
Negative control	1	2	0.00	0.91	(-2.72, 2.72)	0.00	1.000
		3	-0.02	0.91	(-2.73, 2.70)	-0.02	1.000
		4	1.11	0.91	(-1.61, 3.83)	1.21	0.631
	2	3	-0.02	0.91	(-2.73, 2.70)	-0.02	1.000
		4	1.11	0.91	(-1.61, 3.83)	1.21	0.631
	3	4	1.13	0.91	(-1.59, 3.84)	1.23	0.619
Positive control	1	2	9.48	1.32	(5.57, 13.39)	7.21	0.000
		3	17.85	1.32	(13.94, 21.75)	13.57	0.000
		4	24.02	1.32	(20.12, 27.93)	18.27	0.000
	2	3	8.37	1.32	(4.46, 12.27)	6.36	0.000
		4	14.54	1.32	(10.64, 18.45)	11.06	0.000
	3	4	6.18	1.32	(2.27, 10.08)	4.70	0.003
Experimental group A	1	2	6.16	1.28	(2.36, 9.97)	4.81	0.002
		3	14.04	1.28	(10.24, 17.84)	10.96	0.000
		4	19.15	1.28	(15.35, 22.96)	14.95	0.000
	2	3	7.88	1.28	(4.07, 11.68)	6.15	0.000
		4	12.99	1.28	(9.19, 16.80)	10.14	0.000
	3	4	5.11	1.28	(1.31, 8.92)	3.99	0.008
Experimental group B	1	2	7.29	1.14	(3.91, 10.67)	6.40	0.000
		3	16.33	1.14	(12.94, 19.71)	14.33	0.000
		4	23.03	1.14	(19.65, 26.41)	20.22	0.000
	2	3	9.04	1.14	(5.65, 12.42)	7.93	0.000
		4	15.74	1.14	(12.36, 19.12)	13.82	0.000
	3	4	6.70	1.14	(3.32, 10.09)	5.88	0.000
Experimental group C	1	2	9.50	0.86	(6.937, 12.058)	11.01	0.000
		3	16.80	0.86	(14.24, 19.36)	19.49	0.000
		4	22.98	0.86	(20.42, 25.54)	26.65	0.000
	2	3	7.307	0.86	(4.75, 9.87)	8.47	0.000
		4	13.49	0.86	(10.92, 16.05)	15.64	0.000
	3	4	6.18	0.86	(3.62, 8.74)	7.17	0.000

4.5.8 Anti-inflammatory activity of Methanolic leaves extract of *S. didymobotyra*

Edema inhibition activity of methanolic *S. didymobotyra* leaves extract was demonstrated after inducing edema in mice using carrageenan. An hour post intraperitoneal administration *S. didymobotyra* extract and the standard anti-inflammatory drug (diclofenac), percentage inhibition of paw edema in animal model groups labeled:- normal, negative, positive, test A, B and C was -0.83, -2.87, 6.24, 5.72, 3.43 and 6.26%, respectively (Tables 4.48 and 4.49). This indicated a statistical significance difference in percentage inhibition of paw edema between these animal model study groups with a calculated F value=12.62; P value=0.00; $p < 0.05$ (Tables 4.48 and 4.49). The methanolic leaves extracts of *S. didymobotyra* dose levels of 50 and 150 mg/Kg had anti-inflammatory activity with no statistical significant difference with that of the conventional drug (diclofenac). The anti-inflammatory pharmacological effect of plant extracts at low dose level (50 mg/Kg) was slightly lower compared to the same activity of the standard drug (diclofenac) and had a statistical significance difference at $p < 0.05$ (Tables 4.48 and 4.49).

Two hours post administration of plant extracts test products and conventional drug (diclofenac), the percentage inhibition of paw edema inhibition animal study model (mice) categorized as:- normal, negative, positive, test A, B and C was 0.00, -3.46, 13.05, 11.43, 9.72 and 13.10%, respectively (Tables 4.48 and 4.49). This indicated a statistical significance difference in the percentage inhibition of paw edema between animals in different study groups with a calculated F value=27.42; P value=0.00 at $p < 0.05$ (Tables 4.48 and 4.49). Methanolic leaves extracts of *S. didymobotyra* had anti-

inflammatory pharmacological activity with no statistical significance different when compared to the standard anti-inflammatory drug (diclofenac) at $p < 0.05$ (Tables 4.48 and 4.49).

Three hours post administration of plant extracts test products and conventional drug (diclofenac), the percentage inhibition of the induced paw edema in the study animal model (mice) categorized as:- normal, negative, positive, test A, B and C was -2.57, -2.88, 19.31, 18.28, 17.18 and 19.94%, respectively (Tables 4.48 and 4.49). This indicates a statistical significance difference in the percentage inhibition of paw edema between the animal model study groups with a calculated F=45.36 and P value=0.00 at $p < 0.05$ (Tables 4.48 and 4.49).

Four hours post the administration plant extract samples and and the standard anti-inflammatory drug (diclofenac), the percentage inhibition of paw edema in study animal model categorized as:- normal, negative, positive, test A, B and C was 0.00, -1.16, 25.55, 26.83, 25.82 and 26.83%, respectively (Tables 4.48 and 4.49). This indicated a statistical significance difference in percentage inhibition of paw edema between the animal model study groups with a calculated F value=90.21 and P value=0.00 at $p < 0.05$ (Tables 4.48 and 4.49). There was no statistical significant difference between the anti-inflammatory pharmacological effects of the methanolic *S. didymobotyra* leaves extract in all three dose levels and the standard anti-inflammatory drug (diclofenac) (Tables 4.48 and 4.49).

Table 4.48: Anti-inflammatory activities of MeOH leaves extract of *Senna didymobotyra*

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	100.83±0.83^{ab} (-0.83)	100.00±0.00^{a} (0.00)	102.57±1.05^{a} (-2.57)	100.00±1.26^{a} (0.00)
Negative test control	DMSO + Carrageenan	102.87±0.03^{a} (-2.87)	103.46±0.61^{a} (-3.46)	102.87±0.93^{a} (-2.88)	101.16±0.71^{a} (-1.16)
Positive test control	Carrageenan+ Diclofenac	93.76±1.05^{c} (6.24)	86.95±0.60^{b} (13.05)	80.69±1.00^{b} (19.31)	74.45±1.13^{b} (25.55)
Experimental test A	Carrageenan+ *S. didymobotyra* 50 mg/kg bw	94.28±0.91^{c} (5.72)	88.57±0.91^{b} (11.43)	81.72±1.41^{b} (18.28)	73.17±2.04^{b} (26.83)
Experimental test B	Carrageenan+ *S. didymobotyra* 100 mg/kg bw	96.57±1.92^{bc} (3.43)	90.28±2.80^{b} (9.72)	82.82±2.80^{b} (17.18)	74.18±1.90^{b} (25.82)
Experimental test C	Carrageenan+ *S. didymobotyra* 150 mg/kg bw	93.74±1.05^{c} (6.26)	86.90±1.39^{b} (13.10)	80.06±1.86^{b} (19.94)	73.17±1.29^{b} (26.83)
	F Value	12.62	27.42	45.36	90.21
	P Value	0.00	0.00	0.00	0.00

Values are in terms of Mean ± SEM. Column statistical comparison were made and different superscripts indicates significant different. Analysis was done using one – way ANOVA and Turkey's test ($p < 0.05$). Figures enclosed in the brackets are indicating paw edema inhibition percentage.

Table 4.49: Time effects on anti-inflammatory activity of MeOH *Senna didymobotyra* leave extract

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	0.83	1.26	(-2.89, 4.56)	0.66	0.909
		3	-1.74	1.26	(-5.47, 1.99)	-1.39	0.531
		4	0.83	1.26	(-2.89, 4.56)	0.66	0.909
	2	3	-2.57	1.26	(-6.30, 1.16)	-2.05	0.224
		4	0.00	1.26	(-3.73, 3.73)	0.00	1.000
	3	4	2.57	1.26	(-1.16, 6.30)	2.05	0.224
Negative control	1	2	-0.59	0.69	(-2.64, 1.47)	-0.85	0.830
		3	0.00	0.69	(-2.06, 2.06)	0.00	1.000
		4	1.72	0.69	(-0.34, 3.77)	2.48	0.114
	2	3	0.59	0.69	(-1.47, 2.64)	0.85	0.830
		4	2.30	0.69	(0.25, 4.36)	3.33	0.027
	3	4	1.72	0.69	(-0.34, 3.78)	2.48	0.114
Positive control	1	2	6.81	0.70	(4.74, 8.88)	9.76	0.000
		3	13.07	0.70	(10.99, 15.14)	18.72	0.000
		4	19.31	0.70	(17.23, 21.38)	27.66	0.000
	2	3	6.26	0.70	(4.18, 8.33)	8.96	0.000
		4	12.50	0.70	(10.42, 14.57)	17.90	0.000
	3	4	6.24	0.70	(4.17, 8.31)	8.94	0.000
Experimental group A	1	2	5.72	0.93	(2.96, 8.48)	6.15	0.000
		3	12.56	0.93	(9.80, 15.32)	13.51	0.000
		4	21.12	0.93	(18.36, 23.88)	22.72	0.000
	2	3	6.84	0.93	(4.08, 9.60)	7.36	0.000
		4	15.40	0.93	(12.64, 18.16)	16.57	0.000
	3	4	8.56	0.93	(5.80, 11.32)	9.21	0.000
Experimental group B	1	2	6.29	1.03	(3.22, 9.36)	6.08	0.000
		3	13.75	1.03	(10.68, 16.83)	13.29	0.000
		4	22.39	1.03	(19.32, 25.47)	21.64	0.000
	2	3	7.46	1.03	(4.39, 10.54)	7.21	0.000
		4	16.11	1.03	(13.03, 19.18)	15.56	0.000
	3	4	8.64	1.03	(5.57, 11.71)	8.35	0.000
Experimental group C	1	2	6.85	1.02	(3.83, 9.86)	6.74	0.000
		3	13.68	1.02	(10.66, 16.70)	13.46	0.000
		4	20.58	1.02	(17.56, 23.59)	20.25	0.000
	2	3	6.83	1.02	(3.81, 9.85)	6.72	0.000
		4	13.73	1.02	(10.71, 16.75)	13.51	0.000
	3	4	6.90	1.02	(3.88, 9.91)	6.79	0.000

4.5.9 Anti-inflammatory activities of DCM stem bark extract of *P. africana*

An hour post intraperitoneal administration of both extracted samples and and the standard anti-inflammatory drug (diclofenac), percentage inhibition in paw edema in animal model categorized as:- normal, negative, positive, test A, B and C was -0.03, -1.18, 6.24, 3.02, 4.53 and 5.87%, respectively (Tables 4.50 and 4.51). This indicates a statistical significance difference in percentage inhibition of paw edema between animals in differents study groupswith a calculated F value=11.29; P value = 0.00; $p < 0.05$ (Tables 4.50 and 4.51). The DCM stem bark extractsof *P. africana* dosages of 50 mg/Kg had an edema inhibition activity with statistical significant difference with that of the standard anti-inflammatory drug (diclofenac). Anti- inflammatory pharmacological effects two doses (100 and 150 mg/Kg) of *P. africana* stem bark extract was not statistically significant different with that of the conventional drug (diclofenac) $p < 0.05$ (Tables 4.50 and 4.51).

Two hours following intraperitoneal administration of the plant sample extract under test and the standard anti-inflammatory drug (diclofenac), the percentage inhibition of induced paw edema in the study animal model in different groups categorized as:- normal, negative, positive, test A, B and C was 2.37, -2.89, 14.50, 8.99, 7.95 and 14.09%, respectively (Tables 4.50 and 4.51). This indicates a statistical significance difference in percentage inhibition of induced paw edema between animals in different study groups with a calculated F value=49.17; P value=0.00 at $p < 0.05$ (Tables 4.50 and 4.51). *P. Africana* stem bark DCM extracts of high dose (150 mg/Kg) had anti-inflammatory pharmacological effect with no statistical significance difference with the standard anti-

inflammatory drug (diclofenac) (Tables 4.50 and 4.51). Edema inhibition activity following administration of both low and highest dose (50 and 100 mg/Kg) of *P. africana* stem bark extract was statistically significant different with that of the conventional drug (diclofenac) (Tables 4.50 and 4.51).

Three hours post administration of plant sample extract test products and the standard anti-inflammatory drug (diclofenac), percentage inhibition of induced paw edema in animal models (mice) groups categorized as:- normal, negative, positive, test A, B and C was -0.83, -2.32, 20.89, 14.40, 14.78 and 21.76%, respectively (Tables 4.50 and 4.51). This indicates a statistical significance difference in percentage inhibition of paw edema between experimental animal groups with a calculated F=114.46 and P value=0.00 at a significance level of $p < 0.05$ (Tables 4.50 and 4.51). The DCM stem bark extracts of *P. africana* of 150 mg/Kg dose level had anti-inflammatory activity and had no statistical significant different in relation to the standard anti-inflammatory drug (diclofenac) (Tables 4.50 and 4.51). The anti-inflammatory pharmacological effect both 50 and 100 mg/Kg dose level of *P. africana* stem bark extract was statistically significant different with that of the conventional drug (diclofenac) (Tables 4.50 and 4.51).

Four hours post intraperitoneal administration of both standard anti-inflammatory drug (diclofenac) and test plant sample extracts, the percentage inhibition of the induced paw edema in the experimental animal models categorized as:- normal, negative, positive, test A, B and C was 0.66, 0.00, 26.71, 18.04, 24.48 and 28.23%, respectively (Tables 4.50 and 4.51). This indicates a statistical significance difference in the percentage inhibition

of induced paw edema between the animal model study groups with a calculated F value=140.28 and P value=0.00 at $p < 0.05$ (Tables 4.50 and 4.51). Low dose level (50 mg/Kg) of *P.africana* DCM stem bark extract had anti-inflammatory pharmacological activity statistically significantly different in relation to the conventional drug (diclofenac) (Tables 4.50 and 4.51).

Table 4.50:Anti-inflammatory effects of DCM bark extracts of *Prunus africana*

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	100.03±1.29^{ab} (-0.03)	97.63±0.97^{b} (2.37)	100.83±0.83^{a} (-0.83)	99.34±1.57^{a} (0.66)
Negative test control	DMSO + Carrageenan	101.18±0.72^{a} (-1.18)	102.89±0.04^{a} (-2.89)	102.32±0.58^{a} (-2.32)	100.00±0.00^{a} (0.00)
Positive test control	Carrageenan+ Diclofenac	93.66±1.32^{c} (6.24)	85.50±1.45^{d} (14.50)	79.11±1.19^{c} (20.89)	73.29±1.15^{c} (26.71)
Experimental test A	Carrageenan+ *P. africanus* extract 50mg/Kg bw	96.98±0.05b^{c} (3.02)	91.01±0.83^{c} (8.99)	85.60±0.88^{b} (14.40)	81.96±0.72^{b} (18.04)
Experimental test B	Carrageenan+ *P. africanus* extract 100mg/Kg bw	95.47±0.63^{c} (4.53)	92.05±0.96^{c} (7.95)	85.22±1.17^{b} (14.78)	75.52±1.57^{c} (24.48)
Experimental test C	Carrageenan+ *P. africanus* extract 150mg/Kg bw	94.13±0.89^{c} (5.87)	85.91±0.98^{d} (14.09)	78.24±1.11^{c} (21.76)	71.77±0.59^{c} (28.23)
	F ValueP Value	11.29 0.00	49.17 0.00	114.46 0.00	140.28 0.00

Values are in terms of Mean ± SEM. Column statistical comparison were made and different superscripts indicates significant different.Analysis was done using one – way ANOVA and Turkey's test ($p < 0.05$). Figures enclosed in the brackets are indicating paw edema inhibition percentage.

Table 4.51:Time effects on antipyretic activity of DCM stem bark extract of *Prunus africana*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	2.40	1.31	(-1.50, 6.30)	1.83	0.307
		3	-0.80	1.31	(-4.70, 3.10)	-0.61	0.927
		4	0.69	1.31	(-3.21, 4.59)	0.53	0.951
	2	3	-3.20	1.31	(-7.10, 0.70)	-2.44	0.122
		4	-1.71	1.31	(-5.61, 2.19)	-1.30	0.576
	3	4	1.49	1.31	(-2.41, 5.39)	1.14	0.675
Negative control	1	2	-1.72	0.60	(-3.50, 0.07)	-2.85	0.061
		3	-1.14	0.60	(-2.93, 0.64)	-1.90	0.278
		4	1.18	0.60	(-0.61, 2.96)	1.96	0.256
	2	3	0.57	0.60	(-1.22, 2.37)	0.95	0.779
		4	2.89	0.60	(1.11, 4.68)	4.81	0.002
	3	4	2.32	0.60	(0.54, 4.11)	3.86	0.011
Positive control	1	2	8.16	0.43	(6.87, 9.45)	18.79	0.000
		3	14.55	0.43	(13.26, 15.84)	33.51	0.000
		4	20.37	0.43	(19.08, 21.66)	46.91	0.000
	2	3	6.390	0.43	(5.10, 7.68)	14.72	0.000
		4	12.21	0.43	(10.92, 13.50)	28.12	0.000
	3	4	5.82	0.43	(4.53, 7.11)	13.40	0.000
Experimental group A	1	2	5.98	0.81	(3.57, 8.38)	7.38	0.000
		3	11.38	0.81	(8.98, 13.79)	14.06	0.000
		4	15.03	0.81	(12.62, 17.43)	18.55	0.000
	2	3	5.41	0.81	(3.00, 7.81)	6.68	0.000
		4	9.05	0.81	(6.64, 11.45)	11.17	0.000
	3	4	3.64	0.81	(1.24, 6.05)	4.50	0.004
Experimental group B	1	2	3.42	0.80	(1.05, 5.79)	4.28	0.005
		3	10.25	0.80	(7.88, 12.62)	12.84	0.000
		4	19.95	0.80	(17.58, 22.32)	24.99	0.000
	2	3	6.84	0.80	(4.47, 9.21)	8.56	0.000
		4	16.53	0.80	(14.16, 18.90)	20.71	0.000
	3	4	9.70	0.80	(7.33, 12.07)	12.25	0.000
Experimental group C	1	2	8.22	0.68	(6.20, 10.24)	12.08	0.000
		3	15.89	0.68	(13.87, 17.91)	23.35	0.000
		4	22.36	0.68	(20.34, 24.38)	32.86	0.000
	2	3	7.67	0.68	(5.65, 9.69)	11.27	0.000
		4	14.14	0.68	(12.12, 16.16)	20.78	0.000
	3	4	6.47	0.68	(4.45, 8.49)	9.51	0.000

4.5.10 *P. africana* methanol stem bark anti-inflammatory activity

An hour post intraperitoneal administration of study plant sample extracts and the standard anti-inflammatory (diclofenac), percentage inhibition of the induced paw edema in the animal models categorized as:- normal, negative, positive, test A, B and C was -2.64, -0.59, 5.18, 8.56, 5.75 and 6.34%, respectively (Tables 4.52 and 4.53). This indicates a statistical significance difference in the percentage inhibition of edema between the study groups with a calculated F value=14.63; P value=0.00; $p < 0.05$ (Tables 4.52 and 4.53). The methanolic stem bark extracts of *P. africana* three dose levels (50, 100 and 150 mg/Kg) were as effective as the standard anti-inflammatory drug (diclofenac) (Tables 4.52 and 4.53).

Two hours following intraperitoneal administration of plant sample extracts and the standard anti-inflammatory drug (diclofenac), percentage inhibition of the induced paw edema in animal model groups categorized as:- normal, negative, positive, test A, B and C was -0.90, -2.87, 12.63, 13.11, 12.63 and 15.61%, respectively (Tables 4.52 and 4.53). This indicates a statistical significance difference in the percentage inhibition of the induced paw edema between experimental groups with a calculated F value=36.84; P value=0.00 at $p < 0.05$ (Tables 4.52 and 4.53). The methanolic stem bark extracts of *P. africana* three different dose levels had similar anti-inflammatory activity with the standard drug (diclofenac) (Tables 4.52 and 4.53).

Three hours post following intraperitoneal administration study plant sample extracts and the standard anti-inflammatory drug (diclofenac), the percentage inhibition of the induced

paw in the study animal model categorized as:- normal, negative, positive, tests A, B and C was -1.70, -2.87, 21.28, 21.13, 20.66 and 23.27%, respectively (Tables 4.52 and 4,53). This indicates a statistical significance difference in the percentage inhibition of the induced paw edema between the study animal model groups with a calculated F=91.01 and P value=0.00 at $p < 0.05$ (Table 4.32). There was no statistical significant between the edema inhibition activity of the three methanolic *P. africana* stem bark extract dosages and the conventional drug (diclofenac) (Tables 4.52 and 4.53).

Four hours post intraperitoneal administration study plant samples and the standard anti-inflammatory drug (Diclofenac), percentage inhibition of the induced paw edema in the animal model categorized as:- normal, negative, positive, test A, B and C was -1.70, -1.16, 27.03, 28.53, 26.41 and 30.27%, respectively (Tables 4.52 and 4.53). This indicates a statistical significance difference in the percentage inhibition of paw edema between the animal study groups with a calculated F value=111.39 and P value=0.00 at $p < 0.05$ (Tables 4.52 and 4.53). The difference between edema inhibition effects of methanolic stem bark extract of *P. africana* stem bark in three doses and the conventional drug (diclofenac) were not statistically significant (Tables 4.52 and 4.53).

Table 4.52: Anti-inflammatory effects of MeOH bark extract of *Prunus africana*

Groups	Treatment	1hr	2hr	3hr	4hr
Normal test control	DMSO	102.64±1.66[a] (-2.64)	100.90±1.55[a] (-0.90)	101.70±1.04[a] (-1.70)	101.70±1.04[a] (-1.70)
Negative test control	DMSO + Carrageenan	100.59±0.59[a] (-0.59)	102.87±0.03[a] (-2.87)	102.87±0.03[a] (-2.87)	101.16±0.71[a] (-1.16)
Positive test control	Carrageenan+ Diclofenac	94.82±0.58[b] (5.18)	87.37±0.60[b] (12.63)	78.72±0.81[b] (21.28)	72.97±0.84[b] (27.03)
Experimental test A	Carrageenan+ *P. africanus* extract 50mg/Kg bw	91.44±0.86[b] (8.56)	86.89±1.04[b] (13.11)	78.87±1.41[b] (21.13)	71.47±1.84[b] (28.53)
Experimental test B	Carrageenan+ *P. africanus* extract 100mg/Kg bw	94.25±1.91[b] (5.75)	87.37±0.60[b] (12.63)	79.34±1.57[b] (20.66)	73.59±1.55[b] (26.41)
Experimental test C	Carrageenan+ *P. africanus* extract 150mg/Kg bw	93.66±1.91[b] (6.34)	84.39±2.52[b] (15.61)	76.73±1.97[b] (23.27)	69.73±2.12[b] (30.27)
	F Value	14.63	36.84	91.01	111.39
	P Value	0.00	0.00	0.00	0.00

Values are in terms of Mean ± SEM. Column statistical comparison were made and different superscripts indicates significant different. Analysis was done using one – way ANOVA and Turkey's test ($p < 0.05$). Figures enclosed in the brackets are indicating paw edema inhibition percentage.

Table 4.53: Time effects on anti-inflammatory activitiy of MeOH stem bark extract of *Prunus africana*

Treatment	Difference in time levels		Difference of means	SE of differences	Simultaneous 95% CI	T-value	Adjusted p – value
Normal control	1	2	1.74	1.11	(-1.55, 5.03)	1.57	0.430
		3	0.94	1.11	(-2.35, 4.23)	0.85	0.831
		4	0.94	1.11	(-2.35, 4.23)	0.85	0.831
	2	3	-0.80	1.11	(-4.09, 2.49)	-0.72	0.866
		4	-0.80	1.11	(-4.09, 2.49)	-0.72	0.886
	3	4	-0.00	1.11	(-3.29, 3.29)	-0.00	1.000
Negative control	1	2	-2.29	0.57	(-3.99, -0.59)	-4.00	0.008
		3	-2.29	0.57	(-3.99, -0.59)	-4.00	0.008
		4	-0.57	0.57	(-2.27, 1.13)	-1.00	0.753
	2	3	-0.00	0.57	(-1.70, 1.70)	-0.00	1.000
		4	1.72	0.57	(0.02, 3.41)	3.00	0.048
	3	4	1.72	0.57	(0.02, 3.41)	3.00	0.048
Positive control	1	2	7.45	0.78	(5.14, 9.76)	9.57	0.000
		3	16.11	0.78	(13.79, 18.42)	20.69	0.000
		4	21.86	0.78	(19.54, 24.17)	28.08	0.000
	2	3	8.66	0.78	(6.35, 10.97)	11.12	0.000
		4	14.41	0.78	(12.10, 16.72)	18.51	0.000
	3	4	5.75	0.78	(3.44, 8.06)	7.39	0.000
Experimental group A	1	2	4.56	1.44	(0.29, 8.82)	3.17	0.035
		3	12.58	1.44	(8.31, 16.84)	8.75	0.000
		4	19.97	1.44	(15.71, 24.24)	13.91	0.000
	2	3	8.02	1.44	(3.75, 12.28)	5.58	0.001
		4	15.42	1.44	(11.15, 19.68)	10.73	0.000
	3	4	7.40	1.44	(3.13, 11.66)	5.15	0.001
Experimental group B	1	2	6.88	1.13	(3.52, 10.23)	6.09	0.000
		3	14.91	1.13	(11.56, 18.27)	13.21	0.000
		4	20.66	1.13	(17.31, 24.02)	18.31	0.000
	2	3	8.04	1.13	(4.68, 11.39)	7.12	0.000
		4	13.79	1.13	(10.43, 17.14)	12.21	0.000
	3	4	5.75	1.13	(2.40, 9.10)	5.09	0.001
Experimental group C	1	2	9.27	1.05	(6.16, 12.38)	8.85	0.000
		3	16.93	1.05	(13.82, 20.04)	16.15	0.000
		4	23.93	1.05	(20.82, 27.04)	22.83	0.000
	2	3	7.66	1.05	(4.55, 10.77)	7.31	0.000
		4	14.66	1.05	(11.55, 17.77)	13.99	0.000
	3	4	7.00	1.05	(3.89, 10.11)	6.68	0.000

4.6 Analgesic activities of the plant extracts

4.6.1 Analgesic activity of DCM leaves extract of *Eucalyptus saligna*

Formalin test model was carried out to establish analgesic activity of dichloromethane (DCM) stem bark extract of *E. saligna* in both early and late phases of pain management. The negative control group experienced pain throughout the experiment after pain induction. The early phase analgesic activity of the DCM leaves extract of *E. saligna* at a three dose levels effectively reduced paw licking time by 13.41, 21.61 and 26.55%, respectively (Table 4.54). Early phase analgesic bioactivity of the DCM *E. saligna* leaves extracts at dose levels (50 and 150 mg/kg) were statistically significant different in relation to standard drug (Diclofenac) ($p > 0.05$; Table 4.54). Early phase analgesic bioactivities of the DCM leaves extract of *E. saligna* at dose level 100mg/kg was not statistically significant different in relation to standard drug (Diclofenac) ($p < 0.05$; Table 4.54). The highest dose of 150mg/kg body weight was more effective in management of pain in the late phase than standard analgesic drug ($p < 0.005$; Table 4.54).

Percentage pain inhibition in the late phase after administration of DCM leaves extracts of *E. saligna* at three dose levels of (50, 100 and 150 mg/kg) was 20.86, 29.22 and 37.04%, respectively (Table 4.54). Antinociceptive effectiveness of DCM leaves extract at dose levels was statistically significant in relation to standard drug (Diclofenac) ($p > 0.05$; Table 4.54).

Table 4.54: Analgesic effects of DCM stem bark extract of *Eucalyptus saligna*

Group	Treatment	1st phase	2nd phase
Normal test	DMSO	0.00±0.00^{d} (100.00)	0.00±0.00^{e} (100.00)
Negative test	DMSO+ Formalin	100.00±0.00^{a} (0.00)	100.00±0.00^{a} (0.00)
Positive test	Diclofenac+ Formalin	79.34±4.46^{bc} (20.66)	54.55±3.00^{d} (45.45)
Test A	*E. saligna* extract (50 mg/Kg)+ Formalin	86.59±2.35^{b} (13.41)	79.14±2.81^{b} (20.86)
Test B	*E. saligna* extract (100 mg/Kg)+ Formalin	78.39±0.44^{bc} (21.61)	70.78±5.22^{bc} (29.22)
Test C	*E. saligna* extract (150 mg/Kg)+ Formalin	73.45±2.56^{c} (26.55)	62.96±4.65^{cd} (37.04)
	F Value	232.61	104.16
	P Value	0.000	0.000

Values are in terms of Mean ± SEM for per group each with five animals. Comparison along the column was statistically calculated using one way ANOVA and Turkey's test and superscript indicates statistical significant different ($p < 0.05$). Figures in brackets indicates percentage paw licking inhibition.

4.6.2 Analgesic activity of MeOH leaves extract of *Eucalyptus saligna*

Early phase analgesic activity of the methanolic (MeOH) leaves extract of *E. saligna* at a three dose levels effectively reduced pain by 12.15, 18.66 and 21.83%, respectively (Table 4.55). The early phase analgesic bioactivity of the methanolic (MeOH) leaves extract from *E. saligna* in two dose levels of 50 and 150 mg/kg body were statistically significant different in relation to standard drug (diclofenac) ($p > 0.05$; Table 4.55). Early phase analgesic activity of methanolic (MeOH) leaves extract of *E. saligna* of 100mg/kg dosage was not statistically significant different in relation to standard drug (diclofenac) ($p < 0.05$; Table 4.55). The highest dose of 150mg/kg body of methanolic leaves extract of *E.saligna* was more effective in management of pain in the early phase than the reference analgesic (diclofenac) ($p < 0.005$; Table 4.55).

The percentage pain inhibition at late phase after administration of methanol leaves extracts for *E. saligna* at dosage of 50, 100 and 150 mg/kg was 17.43, 28.72 and 42.79%, respectively (Table 4.55). The analgesic effectiveness of MeOH leaves extract of *E. saligna* in three dose levels was statistically significant in relation to standard drug (diclofenac) ($p < 0.05$; Table 4.55).

Table 4.55: Analgesic effects of MeOH leaves extract of *Eucalyptus saligna*

Group	Treatment	1st phase	2nd phase
Normal control	DMSO	0.00±0.00^{d} (100.00)	0.00±0.00^{e} (100.00)
Negative test	DMSO+ Formalin	100.00±0.00^{a} (0.00)	100.00±0.00^{a} (0.00)
Positive test	Diclofenac + Formalin	84.09±4.13^{bc} (15.91)	60.23±3.62^{d} (39.77)
Test A	*E. saligna* extract (50 mg/Kg)+ Formalin	87.85±5.11^{b} (12.15)	82.57±6.12^{b} (17.43)
Test B	*E. saligna* extract (100 mg/Kg)+ Formalin	81.34±3.64^{bc} (18.66)	71.28±1.29^{bc} (28.72)
Test C	*E. saligna* extract (150 mg/Kg)+ Formalin	78.17±4.84^{c} (21.83)	57.21±3.77^{cd} (42.79)
	F Value	97.51	105.35
	P Value	0.000	0.000

Values are in terms of Mean ± SEM for per group each with five animals. Comparison along the column was statistically calculated using one way ANOVA and Turkey's test and superscript indicates statistical significant different ($p < 0.05$). Figures in brackets indicates percentage paw licking inhibition.

4.6.3 Analgesic activity of DCM stem bark *Mangifera indica* extract

The early phase analgesic activity of DCM stem bark extract of *M. indica* at a dose levels effectively reduced pain by 14.75, 17.72 and 28.51%, respectively (Table 4.56). The early phase analgesic activity of the DCM stem bark extract of *M. indica* in two dosage levels of 50 and 100mg/kg were less and statistically significant different in relation to conventional drug (Diclofenac) ($p > 0.05$; Table 4.56). The early phase analgesic effectiveness of the DCM stem bark extract of *M. indica* of 150 mg/kg body weight dosage was higher with no statistical significant different in relation to standard drug (Diclofenac)($p < 0.05$; Table 4.56).

The percentage pain inhibition in the late phase after administration of dichloromethane (DCM) *Mangifera indica* stem bark extracts dose levels: 50, 100 and 150 mg/kg body were 14.77, 35.56 and 43.10, respectively (Table 4.56). The analgesic effectiveness ofthe DCM *M. indica* stem bark extract at 50 mg/Kg dosages level was very minimal and had a statistical significant difference in relation to the standard drug (Diclofenac) ($p < 0.05$; Table 4.56). There was no statistical significant different in analgesic activity observed between DCM of *M. indica* extract at higher dose levels (100 and 150 mg/Kg) and diclofenac at $p < 0.05$ (Table 4.56).

Table 4.56: Analgesic effects of DCM stem bark extract of *Mangifera indica*

Group	Treatment	1st phase	2nd phase
Normal test	DMSO	0.00±0.00[c] (100.00)	0.00±0.00[c] (100.00)
Negative test	DMSO+ Formalin	100.00±0.00[a] (0.00)	100.00±0.00[a] (0.00)
Positive test	Diclofenac+ Formalin	73.16±3.26[b] (26.84)	50.00±4.52[b] (50.00)
Test A	*M. indica* extract (50 mg/Kg)+ Formalin	85.23±6.25[ab] (14.75)	85.25±5.50[a] (14.77)
Test B	*M. indica* extract (100 mg/Kg)+ Formalin	82.28±6.25[ab] (17.72)	64.44±3.84[b] (35.56)
Test C	*M. indica* extract (150 mg/Kg)+ Formalin	71.49±3.23[b] (28.51)	56.90±7.41[b] (43.10)
	F. Value	71.13	59.49
	P. Value	0.000	0.000

Values are in terms of Mean ± SEM for per group each with five animals. Comparison along the column was statistically calculated using one way ANOVA and Turkey's test and superscript indicates statistical significant different ($p < 0.05$). Figures in brackets indicates percentage paw licking inhibition.

4.6.4 Analgesic activity of MeOH *Mangifera indica* stems bark extract

Early phase analgesic activity the methanolic *M. indica* stem bark extract at all three dosage levels administered effectively reduced pain by 12.61, 23.13 and 23.73%, respectively (Table 4.57). Early phase analgesic effectiveness of the methanolic *M. indica* stem bark extract at three dose level were less and statistically had a significant different in relation to standard drug (Diclofenac) ($P<0.05$; Table 4.57). The highest dose of 150 mg/kg methanolic *M. indica* stem bark extract was more effective in management of pain in the early phase but the efficancy could not match with reference analgesic drug (Diclofenac) ($p<0.05$; Table 4.57).

Percentage pain inhibition in the late phase after administration of *M. indica* methanolic bark extracts of dose levels: 50, 100 and 150 mg/kg were 30.13, 43.98 and 48.13%, respectively (Table 4.57). The antinociceptive effectiveness of the methanolic *M. indica* stem bark extract in three dose levels were less and statistically had a significant different in relation to the standard drug (Diclofenac) ($p<0.05$; Table 4.57).

Table 4. 57: Analgesic effects of MeOH stem bark extract of *Mangifera indica*

Group	Treatment	1st phase	2nd phase
Normal test	DMSO	0.00±0.00^{d} (100.00)	0.00±0.00^{e} (100.00)
Negative test	DMSO+ Formalin	100.00±0.00^{a} (0.00)	100.00±0.00^{a} (0.00)
Positive test	Diclofenac+ Formalin	64.83±1.90^{c} (35.17)	39.83±4.54^{d} (60.17)
Test A	*M. indica* extract (50 mg/Kg)+ Formalin	87.39±4.32^{ab} (12.61)	69.87±5.01^{b} (30.13)
Test B	*M. indica* extract (100 mg/Kg)+ Formalin	76.87±3.92^{bc} (23.13)	56.02±3.43^{bc} (43.98)
Test C	*M. indica* extract (150 mg/Kg)+ Formalin	76.27±6.80^{bc} (23.73)	51.87±3.96^{cd} (48.13)
	F. Value	88.27	89.97
	P. Value	0.00	0.00

Values are in terms of Mean ± SEM for per group each with five animals. Comparison along the column was statistically calculated using one way ANOVA and Turkey's test and superscript indicates statistical significant different ($p < 0.05$). Figures in brackets indicates percentage paw licking inhibition.

4.6.5 Analgesic activity of dichloromethane (DCM) stalk extract of *Bidens pilosa*

The early phase analgesic activity of DCM stalk extract of *B. pilosa* at three administered doses (50, 100 and 150 mg/Kg) effectively offered substancial analgesic effects by 17.41, 23.32 and 34.51%, respectively (Table 4.58). Early phase analgesic activities ofthe DCM *B. pilosa* stalk extract at low administered doses (50 and 100 mg/Kg) were less and had a statistical significance different in relation to pain management with standard drug (Diclofenac) ($P > 0.05$; Table 4.58). Early phase analgesic effectiveness of the DCM leaves extract of B. pilosa of 150 mg/kg dosage was a little bit less but with no statistical significant different to standard drug (Diclofenac) ($P>0.05$; Table 4.58).

The percentage pain inhibition in the late phase after administration of DCM stalk extracts of B. pilosa at three doses: 50, 100 and 150 mg/Kg were 26.19, 26.97 and 35.31%, respectively (Table 4.58). The antinociceptive effectiveness of the DCM stalk extract of B. pilosa in two doses 50 and 100 mg/Kg were less and had a statistical significance different in their effectiveness in management of induced pain in phase in relation to the standard drug (Diclofenac) ($P<0.05$; Table 4.58). There was no statistical significant different between the pain management efficacy of 150 mg/kg dose level of DCM *B. pilosa* stalk extract and the conventional drug (Diclofenac) in the early phase ($p<0.05$; Table 4.58).

Table 4.58: Analgesic effects of DCM stalk extract of *Bidens pilosa*

Group	Treatment	1st phase	2nd phase
Normal test	DMSO	0.00±0.00[d] (100.00)	0.00±0.00[d] (100.00)
Negative test	DMSO+ Formalin	100.00±0.00[a] (0.00)	100.00±0.00[a] (0.00)
Positive test	Diclofenac + Formalin	54.45±4.54[c] (45.55)	54.15±3.27[c] (45.85)
Test A	*B. pilosa* extract (50 mg/Kg)+ Formalin	82.59±1.55[b] (17.41)	73.81±4.17[b] (26.19)
Test B	*B. pilosa* extract (100 mg/Kg)+ Formalin	76.68±1.04[b] (23.32)	73.03±5.12[b] (26.97)
Test C	*B. pilosa* extract (150 mg/Kg)+ Formalin	65.49±4.94[bc] (34.51)	64.69±4.67[c] (35.31)
	F. Value	75.97	199.83
	P. Value	0.000	0.000

Values are in terms of Mean ± SEM for per group each with five animals. Comparison along the column was statistically calculated using one way ANOVA and Turkey's test and superscript indicates statistical significant different ($p < 0.05$). Figuresin brackets indicates percentage paw licking inhibition.

4.6.6 Analgesic activity of methanolic (MeOH) stalk extract of *B. pilosa*

The early phase analgesic activity of methanolic stalk extract of *B. pilosa* at three doses ranging from 50, 100 and 150 mg/Kg effectively inhibited nociception by 25.19, 34.77 and 38.37%, respectively (Table 4.59). The early phase analgesic effectiveness of the methanolic *B. pilosa* extract of stalks in dose levels of 50 and 100 mg/Kg had a statistically significant different in relation to conventional analgesic drug (diclofenac) ($P < 0.05$; Table 4.59). Thetwo dosage levels had lower antinociceptive activity in relation to the standard drug (diclofenac). Early phase analgesic effectiveness of the methanolic *B. pilosa* stalks extract at 150 mg/Kg dosage was not statistically significant difference in relation to standard drug (diclofenac) ($P<0.05$; Table 4.38). The highest dose of 150 mg/Kg was as effective as the standard analgesic drug (diclofenac) ($P<0.05$; Table 4.59).

Percentage pain inhibition in late phase after administration of methanolic *B. pilosa* stalk extract of at three administered doses (50, 100 and 150 mg/Kg) was 28.36, 44.07 and 46.16%, respectively(Table 4.59). Analgesic effectiveness of methanol stalk extract of *B. pilosa* in three dose level had a statistically significant difference in relation to standard analgesic drug (diclofenac) ($P<0.05$; Table 4.59). Three doses of plant extract administered were less effective in management of pain in relation to the conventional drug (diclofenac).

Table 4.59: Analgesic effects of MeOH stalk extract of *Biden pilosa*

Group	Treatment	1st phase	2nd phase
Normal test	DMSO	0.00±0.00[d] (100.00)	0.00±0.00[d] (100.00)
Negative test	DMSO+ Formalin	100.00±0.00[a] (0.00)	100.00±0.00[a] (0.00)
Positive test	Diclofenac+ Formalin	52.78±1.55[c] (47.22)	46.77±6.23[c] (53.23)
Test A	*B. pilosa* extract (50 mg/Kg)+ Formalin	74.81±2.29[b] (25.19)	71.64±5.39[b] (28.36)
Test B	*B. pilosa* extract (100 mg/Kg)+ Formalin	65.23±3.90[b] (34.77)	55.93±3.61[b] (44.07)
Test C	*B. pilosa* extract (150 mg/Kg)+ Formalin	61.63±2.92[c] (38.37)	53.84±5.11[bc] (46.16)
	F Value	117.46	79.48
	P Value	0.000	0.000

Values are in terms of Mean ± SEM for per group each with five animals. Comparison along the column was statistically calculated using one way ANOVA and Turkey's test and superscript indicates statistical significant different ($p < 0.05$). Figures in brackets indicates percentage paw licking inhibition.

4.6.7 Analgesic activity of DCM leaves extract of *Senna didymobotyra*

The early phase analgesic activity of DCM leaves extract of *S. didymobotyra* at a dosage levels (50, 100 and 150 mg/Kg) effectively inhibited formaldehyde generated nociception by 31.90, 32.75 and 48.40%, respectively (Table 4.60). Early phase analgesic effectiveness of the DCM *S. didymobotyra* leaves extract in dose levels of 50 and 100 mg/kg was statistically significant difference in relation to standard drug (diclofenac) ($P<0.05$; Table 4.60). Early phase antinociceptive activity of the DCM leaves extract of *S. didymobotyra* of 150 mg/Kg dose was less than that of diclofenac but it had no statistical significant different ($P<0.05$; Table 4.60).

The percentage pain inhibition in the late phase after administration of dichloromethane leaves extracts of S. didymobotyra dose levels: 50, 100 and 150 mg/Kg were 28.24, 40.62 and 50.23%, respectively (Table 4.60). The effectiveness of the three dose levels was lower than that of the standard drug (diclofenac). The antinociceptive effectiveness of the DCM leaves extract of *S. didymobotyra* in three dose levels was statistically significant difference in relation to the standard drug (diclofenac) ($P<0.05$; Table 4.60).

Table 4.60: Analgesic effects of DCM leave extract of *Senna didymobotyra*

Group	Treatment	1st phase	2nd phase
Normal test	DMSO	0.00±0.00[d] (100.00)	0.00±0.00[e] (100.00)
Negative test	DMSO+ Formalin	100.00±0.00[a] (0.00)	100.00±0.00[a] (0.00)
Positive test	Diclofenac + Formalin	46.60±4.70[c] (53.40)	37.85±2.84[d] (62.15)
Test A	*S. didymobotyra* extract (50 mg/Kg)+ Formalin	71.76±3.44[b] (28.24)	68.10±3.70[b] (31.90)
Test B	*S. didymobotyra* extract (100 mg/Kg)+ Formalin	67.25±3.62[b] (32.75)	59.38±1.75[c] (40.62)
Test C	*S. didymobotyra* extract (150 mg/Kg)+ Formalin	51.60±3.79[c] (48.40)	49.77±3.99[c] (50.23)
	F ValueP	103.28	174.28
	Value	0.000	0.000

Values are in terms of Mean ± SEM for per group each with five animals. Comparison along the column was statistically calculated using one way ANOVA and Turkey's test and superscript indicates statistical significant different ($p < 0.05$). Figures in brackets indicates percentage paw licking inhibition.

4.6.8 Analgesic activity of methanolic (MeOH) leaves extract of *Senna didymobotyra*

The early phase analgesic activity of methanolic leaves extract of *S. didymobotyra* at dose levels of 50, 100 and 150 mg/Kg effectively reduced pain by 28.36, 34.77 and 38.37%, respectively (Table 4.61). Early phase analgesic effectiveness of the DCM *S. didymobotyra* leaves extract at a dose level of 50mg/Kg body was statistically significant different in relation to the standard drug (diclofenac) ($p<0.05$; Table 4.61). Early phase analgesic effectiveness of the DCM leaves extract of *S. didymobotyra* dosage levels of 100 and 150 mg/Kg were not statistically significant difference in relation to standard drug (diclofenac) ($p<0.05$; Table 4.61).

The percentage pain inhibition in late phase after administration of methanolic *S. didymobotyra* leaves extracts at three dose levelsof 50, 100 and 150 mg/Kg were 25.19, 44.07 and 46.16%, respectively (Table 4.61). The antinociceptive effectiveness of the methanolic leaves extract in two low doses (50 and 100 mg/Kg) were lower compared to standard analgesic drug (diclofenac) and indicated a statistically significant difference in relation to the standarddrug (diclofenac) ($p<0.05$; Table 4.61). There was no statistical significance in the antinociceptive activity of methanolic leaves extract of *S. didymobotyra* at 150 mg/Kg doseand the standard analgesic drug (diclofenac) at $p<0.05$.

Table 4.61: Analgesic effects of MeOH leaves extract of *Senna didymobotyra*

Group	Treatment	1st phase	2nd phase
Normal test	DMSO	0.00±0.00[d] (100.00)	0.00±0.00[d] (100.00)
Negative test	DMSO+ Formalin	100.00±0.00[a] (0.00)	100.00±0.00[a] (0.00)
Positive test	Diclofenac + Formalin	52.78±1.55[c] (47.22)	46.77±6.23[c] (53.23)
Test A	*S. didymobotyra* extract (50 mg/Kg)+ Formalin	74.64±5.39[b] (25.36)	71.81±2.29[b] (28.19)
Test B	*S. didymobotyra* extract (100 mg/Kg)+ Formalin	65.23±3.90[c] (34.77)	55.93±3.61[bc] (44.07)
Test C	*S. didymobotyra* extract (150 mg/Kg)+ Formalin	61.63±2.92[c] (38.37)	53.84±5.11[c] (46.16)
	F Value	389.71	98.14
	P Value	0.000	0.000

Values are in terms of Mean ± SEM for per group each with five animals. Comparison along the column was statistically calculated using one way ANOVA and Turkey's test and superscript indicates statistical significant different ($p < 0.05$). Figures in brackets indicates percentage paw licking inhibition.

4.6.9 Analgesic activity of DCM stems bark extract of *Prunus africana*

The percentage pain inhibition in the early phase after administering DCM *P. africana* stem bark extracts of at 50, 100 and 150 mg/Kg dose levels were 33.84, 46.96 and 50.00%, respectively (Table 4.62). The antinociceptive activity of DCM *P. africana* extract at low doses (50 and 100 mg/Kg) had a statistically significant difference in relation to standard drug (diclofenac) ($p<0.05$; Table 4.62). The highest dose level of 150mg/Kg was not statistically significant difference in the efficancy in management of pain in the early phase in relation to reference analgesic drug (diclofenac) ($p<0.05$; Table 4.62).

Late phase analgesic activity of the DCM *P. africana* stem bark extract in all the three doses 50, 100 and 150 mg/Kg effectively acted against formalin generated pain by 43.65, 61.41 and 66.93%, respectively (Table 4.62). Late phase analgesic effectiveness of the DCM extract of *P. africana* low dose (50 mg/Kg) statistically had a significant difference result in relation to standard drug (diclofenac) ($p<0.05$; Table 4.62). Late phase antinociceptive effectiveness of the DCM *P. africana* stem bark extract at dose levels of 100 and 150 mg/Kg was not statistically significant difference in relation to standard drug (diclofenac) ($p<0.05$; Table 4.62).

Table 4.62: Analgesic effects of DCM stem bark extract of *Prunus africana*

Group	Treatment	1st phase	2nd phase
Normal test	DMSO	0.00±0.00^{d} (100.00)	0.00±0.00^{d} (100.00)
Negative test	DMSO+ Formalin	100.00±0.00^{a} (0.00)	100.00±0.00^{a} (0.00)
Positive test	Diclofenac+ Formalin	45.05±3.09^{c} (54.95)	39.78±1.90^{c} (60.22)
Test A	*P. africana* extract (50 mg/Kg)+ Formalin	66.16±4.07^{b} (33.84)	56.35±1.24^{b} (43.65)
Test B	*P. africana* extract (100 mg/Kg)+ Formalin	53.04±4.18^{bc} (46.96)	38.59±3.02^{c} (61.41)
Test C	*P. africana* extract (150 mg/Kg)+ Formalin	50.00±4.56^{c} (50.00)	33.07±1.51^{c} (66.93)
	F Value	98.14	389.71
	P Value	0.000	0.000

Values are in terms of Mean ± SEM for per group each with five animals. Comparison along the column was statistically calculated using one way ANOVA and Turkey's test and superscript indicates statistical significant different ($p < 0.05$). Figures in brackets indicates percentage paw licking inhibition.

4.6.10 Analgesic activity of methanolic (MeOH) leaves extract of *Prunus africana*

The early phase analgesic activity of methanolic P. africana stem bark extract at all the three dose levelsof 50, 100 and 150 mg/Kg effectively inhibited formalin induced pain by 23.79, 42.62 and 52.62%, respectively (Table 4.63). The early phase antinociceptive effectiveness of methanolic *P. africana* stem bark extract in dose levels of 50mg/Kg was statistically significant difference in relation to standard drug (diclofenac) ($p < 0.05$; Table 4.63). Early phase analgesic effectiveness of the methanolic *P. africana* extract higher dose levels (100 and 150 mg/Kg) did not statistically offer any analgesic activity with significant different in relation to standard drug (diclofenac) ($p<0.05$; Table 4.63). The highest dose of 150 mg/Kg effectiveness in management of pain was higher in early phase than the reference analgesic(diclofenac) ($p<0.05$; Table 4.63).

The percentage pain inhibition in late phase after administration of methanolic *P. Africana* stem bark at dose levels: 50, 100 and 150 mg/Kg were 30.84, 43.67 and 54.76%, respectively (Table 4.63). Antinociceptive pharmacological effectiveness of methanolic *P. africana*extract in at three dose levels were statistically significant difference in relation to the standard analgesic drug (diclofenac) ($p<0.05$; Table 4.63). Late phase antinociceptive effectiveness of the methanolic *P. africana* extract at higher dose level (150 mg/Kg) indicated results with no statistically significant difference in relation to the standard drug (diclofenac)($p<0.05$; Table 4.63).

Table 4.63: Analgesic effects of MeOH stem bark extract of *Prunus africana*

Group	Treatment	1st phase	2nd phase
Normal test	DMSO	0.00±0.00^{d} (100.00)	0.00±0.00^{e} (100.00)
Negative test	DMSO+ Formalin	100.00±0.00^{a} (0.00)	100.00±0.00^{a} (0.00)
Positive test	Diclofenac + Formalin	57.85±5.08^{c} (42.15)	34.50±2.17^{d} (65.50)
Test A	*P. africana* extract (50 mg/Kg)+ Formalin	76.21±3.90^{b} (23.79)	69.16±4.47^{b} (30.84)
Test B	*P. africana* extract (100 mg/Kg)+ Formalin	57.38±3.77^{c} (42.62)	56.33±2.08^{c} (43.67)
Test C	*P. africana* extract (150 mg/Kg)+ Formalin	47.38±2.32^{c} (52.62)	45.24±2.77^{d} (54.76)
	F ValueP	110.17	185.34
	Value	0.000	0.000

Values are in terms of Mean ± SEM for per group each with five animals. Comparison along the column was statistically calculated using one way ANOVA and Turkey's test and superscript indicates statistical significant different ($p < 0.05$). Figures in brackets indicates percentage paw licking inhibition.

4.7 Toxicological effects of plant extracts in study animals

4.7.1 Effects of DCM and MeOH extracts on body weights of the study animals

Oral administration of both DCM and Methanol plant extracts to the rats was associated with negative effect in relation to the weight gain as indicated in the table 4.64. The rate of body weight gain in 1^{st}, 2^{nd}, 3^{rd} and 4^{th} weeks of all the study animal groups orally administered with methanol and dichloromethane plant extracts did not differ significantly with that of the normal control group ($P<0.01$; Table 4.64). The average weight gain in the first week ranged from 3.84±1.96 to 4.48±1.61% and 8.77±1.88 to 9.62±1.82% in the 4^{th} week (Table 4.64). Weekly percentage change in body weight gain indicates that in all of the experimental groups there was a positive change (Table 4.64). The weekly percentage changes in body weight of the normal control group was however higher (9.62±1.82) in relations to other study groups and lowest in the group orally administered with methanolicstem bark extract of *M. indica* (8.77±1.88) (Table 4.64).

Table 4.64: Effects of oraladministration of plant extracts on body weights of the rats

TREATMENT	Solvent	Percentage Change in Weekly Body Weights				
		Week 1	Week 2	Week 3	Week 4	Average % Δ in body weight/Week
Normal Control		4.48±1.61[a]	7.60±1.70[a]	10.80±1.91[a]	15.60±2.04[a]	9.62±1.82[a]
M. indica stem bark extract	MeOH	4.19±1.52[a]	6.97±1.85[a]	9.89±2.12[a]	14.01±2.03[a]	8.77±1.88[a]
	DCM	4.45±0.92[a]	7.01±0.25[a]	10.01±1.16[a]	14.71±1.03[a]	9.05±0.84[a]
E. saligna leaves extract	MeOH	3.98±1.59[a]	6.76±1.26[a]	9.94±1.07[a]	15.14±1.30[a]	8.96±1.31[a]
	DCM	3.96±1.78[a]	6.98±1.81[a]	10.19±1.26[a]	14.91±2.11[a]	9.01±1.74[a]
B. pilosa stalks extract	MeOH	4.25±0.45[a]	7.21±0.53[a]	10.53±1.41[a]	15.24±0.85[a]	9.31±0.81[a]
	DCM	4.16±1.15[a]	7.35±0.45[a]	10.19±1.26[a]	14.91±1.03[a]	9.15±0.97[a]
S. didymobotyra leaves extract	MeOH	3.94±1.90[a]	7.04±1.61[a]	9.79±2.16[a]	15.11±1.73[a]	8.97±1.85[a]
	DCM	3.84±1.96[a]	7.19±0.91[a]	10.29±1.44[a]	14.98±2.13[a]	9.07±1.61[a]
P. africana stem bark extract	MeOH	4.12±0.87[a]	6.94±1.31[a]	9.89±1.46[a]	15.01±1.95[a]	8.99±1.40[a]
	DCM	4.04±0.93[a]	6.67±1.26[a]	10.11±2.01[a]	15.08±1.16[a]	8.98±1.34[a]
F Value		0.06	0.12	0.11	0.17	0.07
P Value		1.000	0.999	1.000	0.997	1.000

Means having similar lowercase letter superscript in the same column are not significantly different ($P < 0.01$). Plants extract dose = 200mg/Kg

4.7.2 Effects of DCM and MeOH extracts on organ weights of the rats

The study results demonstrated that the average organ weights for the liver, kidneys, spleen, heart, lungs and brain of the rats treated with DCM and MeOH extract of *S. didymobotyra* leaves, *E. saligna* leaves, *B. pilosa* stalks, *M. indica* and *P. africana* stem bark extract was lower than that of the normal control group except in the group treated with DCM and methanolic extract of the *E. saligna* leaves extract. The average liver weight of the animals administered with *B. pilosa* methanolic stalks extract was also higher than that of the normal control group animals (Table 4.65). The results indicated that there was no statistical significant different in the organ weights of the animals receiving different treatments ($P<0.01$) (Table 4.65).

Table 4.65: Effects of oral administration of plant extracts at a dose levels of 200 mg/Kg body weight on organ weights of the rats

TREATMENT		Organ weights (g)					
(mg/kg bw)	Solvent	Liver	Kidneys	Spleen	Heart	Lungs	Brain
Normal Control		10.33±0.44[a]	1.89±0.20[a]	1.31±0.10[a]	0.99 ±0.12[a]	2.82 ±0.32[a]	2.17±0.22[a]
M. indica stem bark extract	MeOH	9.31±1.47[a]	1.85±0.24[a]	1.12±0.16[a]	0.94±0.13[a]	2.26±0.41[a]	2.02±0.33[a]
	DCM	9.02±1.37[a]	1.65±0.30[a]	0.92±0.27[a]	0.93±0.15[a]	2.12±0.31[a]	1.96±0.24[a]
E. saligna leaves extract	MeOH	10.85±1.71[a]	1.10±0.18[a]	0.99±0.24[a]	0.95±0.27[a]	1.98±0.48[a]	1.93±0.35[a]
	DCM	11.55±1.23[a]	0.98±0.20[a]	0.95±0.25[a]	0.96±0.25[a]	1.97±0.32[a]	1.92±0.45[a]
B. pilosa stalks extract	MeOH	10.70±1.70[a]	1.01±0.93[a]	1.03±0.21[a]	0.99±0.32[a]	1.94±0.36[a]	1.05±0.33[a]
	DCM	9.93±1.44[a]	1.89±0.28[a]	1.10±0.12[a]	0.89 ±0.16[a]	2.05 ±0.22[a]	2.07±0.23[a]
S. didymobotyra leaves extract	MeOH	9.31±1.47[a]	1.85±0.20[a]	1.11±0.36[a]	0.82±0.11[a]	2.06±0.32[a]	1.95±0.23[a]
	DCM	10.22±1.73[a]	1.65±0.08[a]	0.72±0.74[a]	0.93±0.30[a]	2.12±0.23[a]	2.06±0.12[a]
P. africana stem bark extract	MeOH	9.56±1.71[a]	1.05±0.18[a]	0.99±0.34[a]	0.95±0.27[a]	1.98±0.28[a]	2.03±0.45[a]
	DCM	9.55±1.03[a]	1.21±0.15[a]	1.05±0.22[a]	0.96±0.25[a]	1.95±0.36[a]	1.95±0.35[a]
	F Value	0.88	2.25	0.85	0.11	1.00	1.55
	P Value	0.564	0.055	0.585	1.000	0.474	0.189

Means followed by similar lower-case letters within respective columns are not statistically different ($P < 0.01$).

4.7.3 Effects of plant extracts on ALT, AST, ALP, GGT, Creatinine and blood urea nitrogen in study rats

The biochemical parameters such as AST, GGT, blood urea nitrogen and creatinine did not vary significantly in different groups orally administered with different plants extracts at $P<0.01$ (Table 4.66). The results indicated that the serum levels of the ALT and ALP varied significantly between the different groups treated with different dichloromethane and methanol plant extract ($P<0.01$; Table 4.66). The levels of serum ALT, ALP, GGT, blood urea nitrogen and creatinine was lowest in the normal control groups than the groups treated with both methanolic and dichlomethanolic plants extracts. The levels of the serum AST was only lower in relation to the normal control group only to the groups orally receiving methanolic and dichlomethanolic extract of *M. indica, E. saligna* and *P. africana*. The dichlomethanolic extract of *B. pilosa* also indicated lower levels of serum AST in relation to the normal control group (Table 4.66).

Table 4.66: Effects of plants extracts of ALT, AST, ALP, GGT, creatinine and blood urea nitrogen

TREATMENT		Serum biochemical parameters					
(200 mg/kg bw)	**Solvent**	**AST**	**ALT**	**ALP**	**GGT**	**Urea**	**Creatinine**
Normal Control		49.49±8.54^{a}	42.83±5.62^{b}	62.04±2.59^{b}	4.86±1.48^{a}	6.32±2.13^{a}	70.86±3.35^{a}
M. indica stem bark extract	MeOH	43.41±4.37^{a}	50.85±3.43^{ab}	65.12±3.06^{ab}	5.60±1.14^{a}	6.59±1.53^{a}	73.59±5.53^{a}
	DCM	45.12±2.73^{a}	54.65±1.08^{b}	66.27±2.74^{ab}	6.10±2.01^{a}	7.09±1.16^{a}	76.56±3.98^{a}
E. saligna leaves extract	MeOH	47.45±4.81^{a}	51.03±2.78^{ab}	63.96±1.54^{ab}	5.99±0.28^{a}	7.35±2.28^{a}	74.35±5.65^{a}
	DCM	46.12±3.52^{a}	49.21±1.23^{ab}	67.56±2.52^{ab}	6.57±0.42^{a}	6.92±0.35^{a}	77.53±3.05^{a}
B. pilosa stalks extract	MeOH	50.70±4.13^{a}	51.70±2.93^{a}	68.36±3.41^{ab}	5.94±0.26^{a}	7.05±0.31^{a}	71.53±3.13^{a}
	DCM	48.63±6.24^{a}	50.08±2.15^{ab}	71.32±3.21^{a}	4.98 ±1.72^{a}	6.70±0.18^{a}	72.07±3.90^{a}
S. didymobotyra leaves extract	MeOH	53.93±4.17^{a}	52.85±2.43^{a}	69.12±1.26^{ab}	6.16±1.23^{a}	6.59±0.53^{a}	74.95±3.53^{a}
	DCM	51.82±2.73^{a}	54.65±3.18^{a}	70.27±3.47^{a}	5.34±0.81^{a}	6.65±0.23^{a}	71.65±4.19^{a}
P. africana stem bark extract	MeOH	48.56±2.17^{a}	55.03±2.18^{a}	67.96±2.54^{ab}	5.09±0.80^{a}	7.03±0.35^{a}	71.35±5.05^{a}
	DCM	47.75±2.23^{a}	54.12±1.10^{a}	69.56±2.52^{ab}	6.05±0.43^{a}	6.92±0.65^{a}	74.05±3.25^{a}
	F Value	1.33	4.60	3.25	0.75	0.20	0.86
	P Value	0.275	0.001	0.010	0.674	0.994	0.578

Means followed by similar lower-case letters within respective columns are not statistically different ($p > 0.01$). ALT = alaninetransaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; bw = body weight

4.7.4 Effects plant extracts of on total protein, albumin, total bilirubin, direct bilirubin and indirect bilirubin

The results indicated that no significant difference between serum total protein, albumin, total bilirubin, direct bilirubin and indirect bilirubin among the normal control group and the experimental groups orally treated with either methanolic and dichloromethane extracts of either *Mangifera indica*, *Eucalyptus saligna*, *Bidens pilosa*, *Senna didymobotyra* and *Prunus africana*. Though there was no significant difference between total protein and albumin, the serum levels of these two parameters was higher in the normal control groups ($P<0.01$; Table 4.67).

The levels total bilirubin was lower in normal control test group than the experimental group while the serum levels of direct bilirubin was only lower than that of normal control group in the groups receiving DCM *Mangifera indica* extract. The levels of indirect bilirubin were lower in relation to normal control group among groups receiving methanolic *Mangifera indica* extract (1.83±0.87), DCM leaves extract of *Eucalyptus saligna* (1.30±0.05) and DCM stem bark extract of *Prunus africana* (1.81±0.05) (Table 4.67).

Table 4.67: Effects of plant extracts on total protein, albumin, total bilirubin, direct bilirubin and indirect bilirubin

TREATMENT (200 mg/kgbw)	Solvent	Serum biochemical parameters				
		Total Protein	**Albumin**	**Total Bilirubin**	**Direct Bilirubin**	**Indirect Bilirubin**
Normal Control		78.42±8.91[a]	45.34±3.81[a]	6.01±0.87[a]	4.08±1.19[a]	1.93±0.46[a]
M. indica stem bark extract	MeOH	73.01±3.29[a]	43.24±2.85[a]	6.18±1.18[a]	4.35±1.90[a]	1.83±0.87[a]
	DCM	68.93±0.73[a]	41.65±3.80[a]	6.27±1.74[a]	3.98±1.11[a]	2.29±0.40[a]
E. saligna leaves extract	MeOH	70.85±4.32[a]	38.96±2.95[a]	6.86±1.38[a]	4.76±1.67[a]	2.10±1.67[a]
	DCM	76.75±2.93[a]	41.21±1.60[a]	7.05±1.52[a]	5.75±1.06[a]	1.30±0.05[a]
B. pilosa stalks extract	MeOH	69.43±4.57[a]	43.48±3.19[a]	7.58±2.87[a]	5.45±1.46[a]	2.13±1.03[a]
	DCM	74.34±3.65[a]	39.89±2.25[a]	7.31±1.65[a]	5.01 ±0.27[a]	2.30±0.10[a]
S. didymobotyra leaves extract	MeOH	70.43±4.91[a]	40.48±3.14[a]	6.85±1.97[a]	4.43±1.46[a]	2.42±1.03[a]
	DCM	73.12±3.73[a]	42.65±1.24[a]	6.72±1.74[a]	5.11±0.11[a]	2.61±0.10[a]
P. africana stem bark extract	MeOH	69.94±6.14[a]	44.48±2.92[a]	6.85±1.67[a]	4.49±1.46[a]	2.36±1.03[a]
	DCM	73.45±3.54[a]	42.21±2.19[a]	6.56±1.52[a]	4.75±0.06[a]	1.81±0.05[a]
	F Value	1.28	1.42	0.23	0.61	0.62
	P Value	0.301	0.235	0.990	0.792	0.783

Means followed by similar lower-case letters within respective columns are not statistically different ($P < 0.01$). bw = body weight

4.7.5 Effects of plant extracts of on erythrocytes and related parameters in study rats

The results indicated that treatment with methanolic and dichloromethane extract of *E. saligna, P. africana, B. pilosa, M. indica and S. didymobotyra* increased the levels of red blood cells (RBCs), mean cell haemoglobin (MCH), haemoglobin (Hb), mean cell volume (MCV), packed cell volume (PCV), red cell distribution width (RDW) and mean cell haemoglobin concentration (MCHC) (Table 4.68). The extract-treated rats did not show significantly higher levels of all these paramaters in relation to those observed in the normal control group ($P<0.01$; Table 4.68).

Table 4.68: Effects of plant extracts on erythrocytes and related parameters in study rats

TREATMENT (200 mg/kgbw)	Solvent	Erythrocytes and related parameters						
		RBC ($x10^6$/µL)	Hb (g/dL)	PCV (%)	MCV (fL)	RDW	MCH (pg)	MCHC (%)
Normal Control		5.40±0.63[a]	14.16±1.29[a]	39.12±1.88[a]	87.22±3.84[a]	13.02±0.20[a]	26.82±0.97[a]	30.06±1.43[a]
M. indica stem bark extract	MeOH	6.07±0.67[a]	15.06±1.25[a]	41.28±2.03[a]	87.30±1.94[a]	14.00±0.19[a]	29.52±1.70[a]	31.10±1.61[a]
	DCM	5.82±0.37[a]	15.56±0.58[a]	40.12±0.47[a]	92.12±0.81[a]	15.32±1.08[a]	31.56±0.10[a]	34.11±1.39[a]
E. saligna leaves extract	MeOH	6.73±1.31[a]	15.82±0.34[a]	41.28±2.09[a]	88.99±2.59[a]	13.19±1.96[a]	30.50±0.99[a]	34.00±0.76[a]
	DCM	5.95±0.33[a]	17.12±0.10[a]	42.65±1.25[a]	93.15±1.16[a]	16.12±1.98[a]	30.42±1.45[a]	33.45±2.45[a]
B. pilosa stalks extract	MeOH	6.89±0.97[a]	15.92±0.60[a]	43.78±2.19[a]	90.92±2.14[a]	15.22±1.33[a]	29.58±1.81[a]	34.26±1.53[a]
	DCM	4.33±2.44[a]	17.18±0.45[a]	44.03±1.11[a]	92.75 ±1.27[a]	14.20±0.20[a]	28.17±2.12[a]	30.38±2.65[a]
S. didymobotyra leaves extract	MeOH	6.34±1.41[a]	16.44±1.38[a]	39.58±0.77[a]	93.46±3.46[a]	14.00±0.19[a]	26.98±1.83[a]	30.90±2.27[a]
	DCM	5.92±1.37[a]	14.96±2.38[a]	40.92±2.67[a]	88.63±3.11[a]	15.32±1.08[a]	31.20±1.18[a]	32.81±2.59[a]
P. africana stem bark extract	MeOH	6.90±1.91[a]	15.84±1.39[a]	43.21±1.37[a]	90.70±2.46[a]	13.19±1.96[a]	29.88±1.23[a]	33.10±1.49[a]
	DCM	7.55±1.33[a]	14.22±1.17[a]	42.85±1.35[a]	91.85±3.06[a]	16.12±1.98[a]	30.72±1.85[a]	31.75±2.25[a]
	F Value	1.31	2.59	2.34	2.44	1.84	2.88	1.49
	P Value	0.286	0.030	0.047	0.039	0.111	0.019	0.208

Means followed by similar lower-case letters within respective columns are not statistically different (p>0.01). bw = body weight

4.7.6 Effects of plant extracts oral administration on white blood cells and differential leucocytes counts in study rats

The blood levels of white blood cells (WBC) were significantly different among different groups treated with both methanol and dichloromethane extracts in relation to the normal control ($P<0.01$; Table 4.69). The white blood cells level was not significantly different between the group orally treated with methanolic stem bark extract of *M. indica* and the normal control group ($P<0.01$; Table 4.69). The levels of white blood cells in both methanol and dichlomethane extracts of *M. indica* stem bark, *E. saligna* leaves extracts, *B. pilosa* stalks extract, *S. didymobotyra* leaves extract were not statistically different ($P<0.01$; Table 4.69). The WBC levels among the groups orally administered with methanolic and dichlomethane extracts of *P. africana* stem bark extract were statistically different ($P<0.01$; Table 4.69). The differential leucocyte counts for lymphocytes, monocytes, neutrophils, eosinophils and basophils among the normal control group and both methanolic and dichloromethane extracts of *B. pilosa*, *E. saligna*, *M. indica*, *S. didymobotyra* and *P. africana* were not statistically significant ($P<0.01$; Table 4.48) thoughthe levels were slightly lower in the normal control ($P<0.01$; Table 4.69).

Table 4.69: Effects of plants extracts on white blood cells and differential leucocytes counts in study rats

TREATMENT (200 mg/kgbw)	Solvent	White blood cells and differential leucocytes counts					
		WBC ($x10^3/\mu L$)	LYM ($x10^3/\mu L$)	MON ($x10^3/\mu L$)	NEU ($x10^3/\mu L$)	EOS ($x10^3/\mu L$)	BAS ($x10^3/\mu L$)
Normal Control		10.52±1.96^{c}	5.69±0.76^{a}	1.65±0.31^{a}	2.64±0.59^{a}	2.15±0.21^{a}	0.43±0.23^{a}
M. indica stem bark extract	MeOH	11.28±1.29^{c}	6.37±0.76^{a}	2.02±0.13^{a}	2.85±0.72^{a}	2.41±0.32^{a}	0.58±0.52^{a}
	DCM	11.45±1.75^{bc}	7.65±1.38^{a}	1.89±0.74^{a}	3.12±0.56^{a}	2.68±0.28^{a}	0.65±0.23^{a}
E. saligna leaves extract	MeOH	12.39±1.98^{abc}	7.81±0.62^{a}	1.94±0.65^{a}	2.98±0.61^{a}	3.16±0.19^{a}	0.50±0.19^{a}
	DCM	12.84±2.54^{abc}	8.21±1.46^{a}	1.85±0.52^{a}	2.85±0.38^{a}	2.61±0.61^{a}	0.82±0.24^{a}
B. pilosa stalks extract	MeOH	13.69±2.42^{abc}	6.43±2.75^{a}	1.90±0.52^{a}	2.76±0.65^{a}	2.33±0.21^{a}	0.52±0.23^{a}
	DCM	14.32±2.98^{abc}	7.89±0.14^{a}	2.03±0.29^{a}	2.98 ±0.73^{a}	3.11±0.23^{a}	1.07±0.12^{a}
S. didymobotyra leaves extract	MeOH	20.43±6.14^{ab}	8.47±1.92^{a}	2.08±0.49^{a}	3.41±0.64^{a}	2.43±0.34^{a}	0.78±0.43^{a}
	DCM	18.78±2.93^{abc}	6.51±0.80^{a}	1.97±0.74^{a}	2.82±0.76^{a}	2.52±0.38^{a}	0.65±0.35^{a}
P. africana stem bark extract	MeOH	19.32±4.15^{abc}	7.48±1.32^{a}	2.58±0.89^{a}	3.01±0.64^{a}	2.65±0.61^{a}	0.81±0.23^{a}
	DCM	20.65±2.72^{a}	9.21±1.25^{a}	1.95±0.52^{a}	2.75±0.39^{a}	2.83±0.42^{a}	0.57±0.14^{a}
	F Value	4.87	1.79	0.48	0.35	2.09	1.23
	P. Value	0.001	0.123	0.886	0.955	0.072	0.326

Means followed by similar lower-case letters within respective columns are not statistically different (p>0.01); bw = body weight

4.7.7 Effects of plant extracts on platelet count and other related parameters in study rats

The results indicated that the platelet count was lowest in the normal control group (402.86±20.50) and this was not significantly different from the average platelet count of the animals treated with both methanolic and dichlomethane extracts of *M. indica, E. saligna, B. pilosa, S. didymobotyra* and *P. africana* ($p<0.01$; Table 4.70). The MPV values were slightly higher in animals treated with both methanol and dichloromethane plant parts extracts except for animals treated with dichlomethane (6.92±2.41) and methanol 6.16±1.28) extract of *M. indica* stem bark, respectively. This was also observed in the animals treated with dichloromethane extracts of the *S. didymobotyra* leaves (6.51±2.08; Table 4.70). The results also indicates PDW values were slightly higher than that of the normal control group (20.87±1.74) with no statistical significant difference ($P<0.01$; Table 4.70) except in methanolic and dichloromethane *M. indica* stem bark extract treated animals with a PDW values of 19.50±1.94 and 18.34±1.74, respectively (Table 4.70).

Table 4.70: Effects of Plants extracts on platelet count and related variants in study rats

TREATMENT (200 mg/kgbw)	Platelet Count and Related Variants			
	Solvent	**PLT (x10^3/µL)**	**MPV (fL)**	**PDW**
Normal Control	DCM	402.86±20.50^a	7.28±1.73^a	20.87±1.74^a
M. indica stem bark extract	MeOH	434.80±51.60^a	6.92±2.41^a	19.50±1.94^a
	DCM	523.46±23.37^a	6.16±1.28^a	18.34±1.74^a
E. saligna leaves extract	MeOH	476.32±42.87^a	7.36±1.19^a	21.78±1.93^a
	DCM	501.21± 75.33^a	8.02±1.10^a	22.43±1.52^a
B. pilosa stalks extract	MeOH	543.45±53.72^a	8.16±1.44^a	21.48±2.29^a
	DCM	495.62±28.54^a	7.29±1.45^a	20.98±2.17^a
S. didymobotyra leaves extract	MeOH	512.34±80.21^a	7.84±1.92^a	23.18±2.17^a
	DCM	496.34±34.09^a	6.51±2.08^a	21.27±2.74^a
P. africana stem bark extract	MeOH	542.21±61.34^a	7.48±1.37^a	23.85±2.77^a
	DCM	503.59±54.32^a	7.89±2.10^a	22.56±2.52^a
	F Value	2.07	0.42	1.59
	P Value	0.075	0.924	0.174

Means followed by similar lower-case letters within respective columns are not statistically different ($p < 0.01$); bw = body weight.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

The plant extract yield in dichloromethane and methanol solvents varied in both percentage peak abundance and also in terms of the number of phytocompounds obtained. In all the plants sampled, the numbers of phytocompounds identified were higher in methanol than in dichloromethane. This indicates that methanol is more efficient in phytochemical extraction in all the plants than dichloromethane. This means that most of the phytochemicals identified in the plant samples were polar molecules.

The qualitative biochemical evaluation of the phytochemical compounds presents in both methanol and dichloromethane plant sample extracts indicated presence of tannins, flavonoids, saponins, coumarins, anthocyanidins and steroids. These phytocompounds in plants serve different pharmacological roles. This means that the plants under the study can serve a vital role in development of new pharmacologically active substances because they have various bio-active compounds because the identified phytochemicals are associated with hypolipidemic, hypoglycemic and antioxidants activities. Different studies have indicated that natural herbs or plant based components are used or can be used in management of various diseases. Various drugs used in management of different disease conditions are directly or indirectly derived from plant sources. Different plants contain phytochemicals with antibacterial, anti-allergic, anti-inflammatory and anticancer activityand are used in drug development (Ekam and Ebong, 2007).

The leaves extracts of the *S. didymobotyra* contained terpenoids, steroids, tannins, glycosides, alkaloids, saponins, phenols, quinones and terpenoids. This result concurs with what was observed by Kitonde *et al.* (2014). Korir *et al*. (2012) also found that *Senna didymobotyra* leaves contain phenols, terpenoids, alkaloids, quinones and flavonoids.The cardiotonic activities, antimicrobial properties and insecticidal activity of the *Senna didymobotyra* leaves extracts is associated with steroids found in it (Ngbede *et al*., 2008; Anpin Raja *et al*., 2011). The steroids found in *Senna didymobotyra* leaves extracts is of great value in nutritional value of the plant part, cosmetic purposes and also has some pharmacological value hence it is utilized as a herbal medicine (Anpin-Raja *et al*., 2011).

The terpenoids extracts from the plant parts are associated various pharmacological activities like edema inhibition, antioxidants, antimicrobial and antiplasmodial activities. It is also known to inhibit the biosynthesis of cholesterol (Mahato and Sen, 1997). The main constituent of the *Senna didymobotyra* leaves extract was alkaloids. Alkaloids are used in management of constipation. Alkaloids have a stimulating laxative and irritant effects to the large intestine (Mengs *et al*., 2001). Decoction or infusion of the *Senna didymobotyra* roots, stems or leaves extracts are used for purgative and laxative activities in cases of abdominal pains. High doses of plants parts rich in alkaloids are used as an emetic (Singh *et al*., 2003).

Tannins are plants secondary metabolites. They include protocatechic or gallic acid glycosides. They are useful in management of hemorrhage and diarrhea because of their stringent property which makes them capable of precipitating mucus, proteins and

vasoconstriction (Kokwaro, 2009). This makes medicine men to use plants rich in tannins for antidiarrheal purposes. Tannins have antiparasitic pharmacological effects (Bajal, 1988).

The saponins in plants extracts can be used as mild detergents and as intracellular histochemistry stain. They facilitate intercellular physiological functioning of body proteins like the antibodies (Trease and Evans, 1989). Saponins are commonly used in management of hypercholesterolemia, inflammation, cancer, obesity and hyperglycemia (Trease and Evans, 1989). Plants rich in saponins can also be used in management of ulcers, wounds and bleeding because they facilitate red blood cell precipitation and coagulation (Maobe *et al*., 2013). Saponins are capable of binding to both cholesterol and glucose molecules. Saponins are also known to inhibit inflammation (Maobe *et al*., 2013). In India, plants rich in saponins are commonly used by the local communities to treat wounds (Foster and Duke, 1990), this is associated with the ability of saponins to facilitate the process of blood clotting hence leading to enteric ulcers and wood healing (Chiej, 1984). Saponins are also associated with prevention of hypercholesterolemia, anti-diabetic, antibiotic and anti-inflammatory activity (Just *et al*., 1988). Saponins helps in improving the immune response of the animals and that why they are commonly used in the developments of the veterinary vaccines because of their adjuvant activity (Parekh and Chands, 2007; Tsuzuki *et al*., 2007; Elumalai *et al*., 2011; Narayami *et al*., 2012).

Flavonoids are capable of scavenging free radicals like the hydrogen peroxide and peroxides. This makes them to have antioxidants activities hence they can be used to inhibit oxidation process associated with degenerative diseases (Samatha *et al*., 2012).

Flavonoids have anti-diabetic pharmacological activity. Flavonoids inhibit synthesis of off flavours associated with fat oxidation (Marjorie, 1999). Flavonoids are capable of complexing with the extracellular soluble proteins to form a bacterial cell wall complex hence the flavonoids have antibacterial activity (Yadav and Agarwala, 2011). Plants synthesize flavonoids when attacked by microbial infections. Flavonoids have a wide range antibacterial activity (Ghasemzadeh and Ghasemzadeh, 2011). Flavonoids are associated with altered biochemical reactions in the body in cases of virus attack, exposure to carcinogenic substances or chemicals and allergens. This makes the plants rich in flavonoid to possess anti-allergic, antibacterial, anticancer and anti-inflammatory activity (Ekam and Ebong, 2007).

Glycosides are secondary organic metabolites found in plants. In plants and animals, glycosides have a non-carbohydrate moiety which binds to the sugar molecule (Anthoney *et al*., 2014). Glycoside is a term used to mean a compound formed by combining a sugar with compound other than the sugar. The compounds are formed by a formation of a glycosidic bond. Cardiac glycosides were used traditionally as heart drugs or as arrow poisons. Cardiac glycosides under regulated therapeutic dose strengthen the heart and help it to function properly. Cardiac glycosides inhibit the Na^+/K^+ - ATPase leading to increased sodium ions levels in cardiac myocytes. This leads to increased calcium ions levels hence increasing the force of cardiac contraction (Newman *et al*., 2008). Currently cardiac glycosides are associated with anticancer properties triggering the need to carrying out clinical trials to establish the role of cardiac glycosides in the management of cancer (Ngule *et al*., 2013).

Alkaloids are cyclic secondary metabolites compounds. They contain nitrogen in a state of negative oxidation. Alkaloids affect the nervous system chemical transmission. Several studies support that alkaloids possess cytotoxic, anti-inflammatory and antiprotozoal activities (Karou, 2006). Presence of alkaloids justifies the medicinal value of most of the plants. Alkaloids are isolated and tested for medicinal purposes from different plants. Most of the alkaloids isolated from the plants are known to have active antiplasmodial activity hence can be used in the management of malaria. Most of the antimalarial drugs are derived from quinoline a derivative extracted from cinchona species bark (Okwu and Josiah, 2006; Ameyawn and Duker–Eshon, 2009). Alkaloids are also associated with antibacterial, antispasmodic and analgesic activity (Karou, 2006). Alkaloids are also used in management of hypertension related headache (Ayitey, 1977). Alkaloids physiologically plays great role as an analgesic and a sedative. They are associated with stress reduction and they can act as antidepressants. Alkaloids are very poisonous if taken in large amounts because they are known to stimulate, excite and cause nerve cell disorders (Jisika *et al*., 1992; Obochi, 2006).

Phenols are secondary metabolites isolated from the plants. They have diverse human health functions like increasing the nutrient uptake, enzyme activity, allelopathy, facilitates formation of structural components and proteins synthesis (Wu *et al*., 2000). Phenolic compounds are associated with both biological and pharmacological activities such antimicrobial activity (Mungole *et al*., 2010; Anpin Raja *et al*., 2011). Medicinal plants rich in phenolic compounds are associated with excellent antioxidant activity

(Narayana *et al*., 2001). The phenolic compounds are also associated with curing of stomach and kidney problems. Phenolic compounds are also known to have strong anti-inflammatory properties (Shirwaikar *et al*., 2003). They help protects plants against invading agents. They are commonly found in flower pigments (Jones *et al*., 1994).

The other objective of the study was to determine antipyretic activity of dichloromethane and methanolic plant parts extracts of *P. africana* stem bark, *E. saligna* leaves, *S. didymobotyra* leaves, *B.pilosa* stalks and *M. indica*. The antipyretic activities of the plant parts extracts were evaluated using pyrexia induced male rats in which pyrexia was induced through intraperitoneal administration of distilled turpentine. Turpentine is commonly used in fever experimental studies because it is a pyrogen whose fever induction mechanism involves initiation, synthesis and release from host phagocytic cells of endogenous pyrogens like pro-inflammatory cytokines. The released pyrogens leads to increased concentration of prostaglandins E_2 in the hypothalamus thermoregulatory center hence leading to elevated body temperature (Gitahi *et al*., 2015). Turpentine causes localized inflammation associated with strong acute fever in animals after administration (Leon, 2002). Pyrexia was induced in rats by injecting them with turpentine (20 mg/kg body weight) and those with a rectal temperature change of greater or equal to 0.8^0C after 1 hourby injecting turpentine were considered for study as describe by Taran *et al*. (1984) and Grover, (1990).

Exogenous and endogenous pyrogens stimulate fever production. Exogenous pyrogens are products of bacteria cell wall breakdown such as lipopolysaccharide. Endogenous

pyrogens associated with fever include cytokines. Cytokines like the interleukins-1, 6 and tumor necrotic factor – α (TNF-α) acts as fever signaling molecules by activating the cyclooxygenase – 2 pathway leading to increased production of prostaglandin E_2 (PGE_2). Prostaglandin E_2 (PGE_2) production leads to activation of the prostaglandin E_2 (PGE_2) hypothalamic receptors (Dinarello, 2004). Lipopolysaccharides (LPS) leads to fever by binding to toll – like receptor 4 (TLR4) found in neutrophils, macrophages and dendritic cells stimulating increased synthesis of prostaglandin E_2 (PGE_2). Prostaglandin E_2 (PGE_2) is capable of crossing the blood brain barrier and this leads to fever initiation (Evans *et al*., 2015).

Exogenous pyrogens including amphetamines, lipopolysaccharides (LPS), Sulphur and turpentine induce fever because they are capable of acting on the immune cells such as the macrophages and monocytes leading to release of proinflammatory cytokines like interleukin – 1 (IL-1), interleukin – 6 (IL-6) and tumour necrosis factor α (TNF-α). These proinflammatory cytokines are endogenous pyrogens (Soszynski and Krajewska, 2002). Turpentine regulates IL-1 interaction with IL-1 type receptor 1(IL-1r1) leading to increased IL-6 production which is followed by fever induction (Leon, 2002). Turpentine is good for pyrexia studies because it is not easy for experimental animals to develop tolerance against turpentine in relation to other pyrogen (Soszynski and Krajewska, 2002).

Four hours after the test period, it was observed that both methanolic and dichloromethane extracts of *B. pilosa* stalk , *S. didymobotyra* leaves, *E. saligna* leaves, *P. africana* and *M. indica* stem bark was able to produce reasonable antipyretic activity in turpentine fever

induced rats. Methanolic extract of *M. indica* stem bark at a dose level of 150 mg/kg, illustrated greatest rectal temperature change of 4.10% which compared very well will the rectal temperature change (3.91%) associated with the conventional drug (Aspirin) while the dichloromethane leaves extracts of the *E. saligna* at a dose level of 50 mg/kg demonstrated least rectal temperature reduction (1.85%). These study finding were in supports of other previous studies results which associates other medicinal plants with antipyretic activity in laboratory animal tests. Similar studies demonstrates that *P. kotschyi* methanolic leaves extract is associated with effective antipyretic activity in rats after inducing fever using amphetamine and brewer's yeast (Akuodorl *et al*., 2013). Abdur *et al*. (2014) also demonstrated that *Diaspyros lotus* various organic fractions and crude methanolic extracts is associated with appreciable antipyretic activity in mice after intraperitoneal administration with brewer's yeast.

The Non–steroidal agents like aspirin or paracetamol are drugs of choice in routine management of febrile conditions. After intensive investigation it is clearly known that NSAIDs have antipyretic activity because they are capable of blocking the enzyme cyclooxygenase pathway by inhibiting synthesis of prostaglandins (Blandizzi *et al*., 2009). Generally, non-steroidal anti-inflammatory drugs antipyretic activity is associated with hypothalamus prostaglandin biosynthesis inhibition (Clark and Cumby, 1975).

Prostaglandin biosynthesis inhibition results from both inhibition of the enzymes prostaglandin synthase and cyclooxygenase which are involved in biosynthesis of PGE_2 in the hypothalamus (Gege-Adebayo *et al*., 2013). The DCM and MeOH extract of

Eucalyptus saligna, Senna didymobotyra, Bidens pilosa, Prunus africana and *Mangifera indica* was as effective as aspirin in this study, thus suggesting mimicry of aspirin action by the bioactive components of the extract. Elgorashi *et al.* (2009) established that the DCM stem bark extract of *A. nubica, A. senegal and A. nilotica* had high COX-2 inhibition properties selectivity. Therefore, the antipyretic activities of both methanolic and dichloromethane extracts of *B. pilosa* stalks , *S. didymobotyra* leaves, *E. saligna* leaves, *P. africana* and *M. indica* stem bark may be associated with hypothalamus prostaglandin synthesis inhibition as a results of inhibiting both the enzymes prostaglandin synthase and/or cyclooxygenase. However, various alternative mechanisms involved in blocking fevershould not be ignored.

The DCM extracts of the *S. didymobotyra* leaves, *B. pilosa* stalks, *M. indica* stem bark, *E. saligna* leaves and methanolic extracts of *M. indica* stem bark and *E. saligna* after intraperitoneal administration to turpentine fever induced rats demonstrated a dose dependent response. This is consistent with dose dependent antipyretic activity response in brewer's yeast fever induced rats associated with alkaloid extract fraction of *H. zeylanica* (Reanmongkol *et al*., 1994). This was also observed in study by Amiya *et al*. (2010) which demonstrated dose dependent antipyretic activity of the *Capparis zeylanica* Linn extract in experimental rats where fever was induced using brewer's yeast. Antipyretic dose dependent activities of both methanolic and dichloromethane extracts of *B. pilosa* stalks, *S. didymobotyra* leaves, *E. saligna* leaves, *P. africana* and *M. indica* stem bark could be associated with passive diffusion across the cell membrane of the plants active compounds in the peritoneal cavity.

The *Eucalyptus saligna, Senna didymobotyra, Bidens pilosa, Prunus africana* and *Mangifera indica* DCM and MeOH extracts were associated with fever reduction in time and dose–dependent state. This may be as a result of low doses, leading to low plasma concentration of pharmacologically active phytocomponents. Time–dependent antipyretic activity can be associated with time taken for the bioactive phytochemicals to be transported across the peritoneum cavity (Koech *et al*., 2017). The dose and time dependent antipyretic activities exhibited are in consistent with results observed in study on antipyretic activities of ethyl acetate and methanolic extracts of *Acacia hydaspica* on brewer's yeast in Sprague Dawley rats (Afsar *et al*., 2015). Pharmacological activities against pyrexia of methanolic: dichloromethane of *Carissa edulis* root barks extract on pyretic turpentine induced rats was also dose–dependent manner (Gitahi *et al*., 2015). Similarly, *Kigelia africana* and *acacia hockii* was associated with dose–dependent antipyretic activities on turpentine–induced pyrexia in rats (Kamau *et al.,* 2016). The study used dose range is equal to one used by Akuodorl *et al*. (2013) and Abdur *et al*., (2014). Ethanolic *Pseudocedrella kotschyi* antipyretic activities was evaluated using three dose levels and was very effective (Akuodorl *et al*., 2013) and the *Diospyros lotus* methanolic extracts was evaluated in 50 and 100mg/kg (Abdur *et al*., 2014).

It was observed that lower doses of DCM extracts of the *S. didymobotyra* leaves, *B. pilosa* stalks, *M. indica* stem bark, *E. saligna* leaves and methanolic extracts of *M. indica* stem bark and *E. saligna* at three dose levels were less effective in relation to higher dose level (150mg/kg). This may be due to by inactivation and rapid metabolic breakdown of active

compounds at lower concentrations. This may also be due to active principle(s) insufficient concentration at lower doses.

The plant methanolic and DCM extracts for *S. didymobotyra, E. saligna, M. indica, P. africana* and *B. pilosa* did not inhibit effectively the rectal temperature in first two hours as compared to 3rd and 4th hours. The best methanolic activity against pyrexia was 1.08 and 2.00% in the 1st and 2nd hours and 2.87 and 4.10% in the 3rd and 4th hours, respectively while the highest dichloromethane antipyretic activity in the 1st and 2nd hours was 0.98 and 1.80% and 2.87 and 4.03% in the 3rd and 4th hours, respectively. The observation could be explained by the fact that active phytocompounds undergoes some biotransformation for them to achieve the antipyretic activity.

After the fourth hour of study, both DCM and MeOH extracts of *Eucalyptus saligna, Senna didymobotyra, Bidens pilosa, Prunus africana* and *Mangifera indica* showed an appreciable antipyretic activity. The high dose of 150 mg/kg demonstrated highest rectal temperature reduction after 4 hours. This is an indicator that the plant samples contain bioactive principles with the ability of crossing the brain blood barrier and have the capability of inhibiting the biosynthesis of prostaglandins or other substances that stimulate production of antipyretic substances like glucocorticoids or arginine vasopressin (Leon *et al.*, 1999; Nthiga *et al*., 2016b). This suggests that the plants extracts mimics the action of the aspirin and offers a better or almost equal prostaglandins biosynthesis blockage. This also suggests that the plants extracts may be inhibiting other fever blockage mechanisms efficiently. Sudden or more rectal temperature decline after treatment with plants samples

may suggests some extracts advantage over the conventional drug (aspirin).

Methanolic and dichloromethane extracts of *S. didymobotyra, E. saligna, B. pilosa, P. africana* and *M. indica* qualitative and quantitative phytochemical analysis is associated with one or more phytocompounds known to possess antipyretic potential like alkaloids, flavonoids, phenolics, saponins, steroids and terpenoids. These phytochemicals prevent prostaglandins or increases body antipyretic components by inhibiting the enzyme cyclooxygenase action (Okokon and Nwafor, 2010). Steroids, alkaloids, tannins and terpenoids are the known to predominantly inhibit PG synthase and flavonoids inhibit peroxidation of the arachidonic acid and inhibition of the tumor necrosis factor–α. Tumornecrotic factor–α stimulates PGE_2 synthesis resulting to fever.

Flavonoids exhibit antipyretic activity by suppressing TNF–α (Adesokan *et al*., 2008). They also reduce prostaglandins levels by inhibiting the peroxidation of arachidonic acid hence reducing fever (Taiwe *et al*., 2011). Presence of flavonoids in both methanolic and dichloromethane extracts of *S. didymobotyra, E. saligna, B. pilosa, P. africana* and *M. indica* may be the antipyretic activity contributory factor.

Alkaloid presence in both methanolic and dichloromethane extracts *S. didymobotyra, E. saligna, B. pilosa, P. africana* and *M. indica* may be responsible for antipyretic activity observed in this study. Alkaloids isolated from the *Hunteria zeylanica* indicated some antipyretic activity in experimental animals studies (Reanmongkol *et al*., 1994). Saponins isolated from both methanolic and dichloromethane of *S. didymobotyra, E. saligna, B. pilosa, P. africana* and *M. indica* plant extracts may also be associated with prostaglandin synthesis

inhibition. Additionally, saponins inhibit both the cyclooxygenase and phospholipase A_2 enzymes which are involved in pyrexia development (Kumar *et al*., 2015; Ashfaq *et al*., 2016; Kamau *et al*., 2016). Saponins synergistically act to exhibit some antipyretic activity in experimental animals studies (Zakaria *et al*., 2007). Related antipyretic activity study in rats associated *Asparagens racemosus* ethanolic extracts to thesaponins present (Vasundra and Divya, 2013).

Most of the antipyretics are capable of down regulating the activity of the enzyme cyclooxygenase. Other antipyretic drugs acts by reducing the production of the proinflammatory markers leading to enhanced anti-inflammatory signals associated with either tissues injury or hypothalamic antipyretic signals. The extracts of *S. didymobotyra, M. indica, B. pilosa, E. saligna and P. africana* indicated some antipyretic activity. This is a clear indication that the phytochemical compounds found in dichloromethane and methanol extracts of this plants parts interfere with the activity of the cyclooxygenase and also proinflammatory cytokines like the interleukins–1, Interleukin–6 (Il-6) and the Tumor necrotic factor–α (TNF–α) release hence the antipyretic activity. Conventional drugs mostly used to manage inflammation have a number toxicological adverse effect (Deghrigue *et al.,* 2015). Herbal medications are mostly accepted as a result of their potency, little or no adverse effects, low cost, availability and general accceptancy (Malviya *et al*., 2011).

Methanolic and dichloromethane extracts of *S. didymobotyra, E. saligna, P. africana, B. pilosa* and *M. indica* anti-inflammatory activity was demonstrated using animal model (albino mice) after paw edema induction by use of carrageenan. Carrageenan is a common agent for evaluating anti-inflammatory activity of various pharmacological

agents especially when using experimental laboratory animals (Jain *et al*., 2001; Paschapur *et al*., 2009). Carrageenan is prepared by addition of sulphate group to a polysaccharide obtained either from moss or sea weed to get a sulphated polysaccharide (Necas and Bartosikova, 2013). Carrageenan leads to severe inflammation following subcutaneous injection especially in the hind paw of mice or rat especially when evaluating anti-inflammatory activity of natural product (Dirosa *et al*. 1971; John and Nodine, 1999). Edema associated with carrageenan administration occurs in both early/late phases. Early phase results from the secretion of cytokinins, histamine and serotonin and the late phase is induced by both production and release prostaglandins, reactive oxygen species, proteolytic and lysosomal enzymes (Ashfar *et al*., 2015; Samriti *et al*., 2016). DCM and methanolic extract of *S. didymobotyra, E. saligna, P. africana, B. pilosa* and *M. indica* in this study illustrated strong edema inhibition activities in carrageenan stimulated inflammation in animal model by reduction of paw diameter at early/late phases.

The anti-inflammatory pharmacological effects of plant extracted products may result into inhibition of the mediator involved inflammatory markers biosynthetic pathway either early and/or late phases hence inhibiting edema. The study results are in agreement with findings from others studies involved in evaluation of medicinal plants anti-inflammatory activity in animal models. Tukappa *et al*., (2015) studies on the *in vitro* and *in vivo* edema inhibition and toxicological analysis of *Rumex vesicarius* linn methanolic stem bark extract in inflammation induced Wistar albino rats indicated some anti-inflammatory activities. Additionally, Mwangi *et al*. (2015) demonstrated positive anti-

pharmacological inflammatory effect using leaves extract of *Caesalphinia volkensi* and *Myenus obscura* using both methanol and dichloromethanesolvent for extraction after the product was administered in animal models. Therefore, there is a possibility that both DCM and methanolic extracts of *S. didymobotyra* leaves, *E. saligna* leaves, *P. africana* stem bark, *B. pilosa* stalks and *M. indica* stem bark are able to inhibit prostaglandins biosynthesis hence inhibiting inflammatory process initiation. This is a clearly indicates that the plant compounds can inhibit the cyclo-oxygenase action hence inhibiting metabolism of arachidonic acid to synthesize prostaglandins.

Dichloromethane leaves extracted product from *S. didymobotyra*, stalks extract of *B. pilosa*, and both dichloromethane and methanolic *P. africana* stem bark products indicated a dose dependent edema inhibition response. Dichloromethane *M. indica* stem bark products, leaves extracts of *E. saligna* and methanolic leaves extract of *S. didymobotyra*, stalks extract of *B. pilosa*, stem bark extract of *M. indica* and leaves extract of *E. saligna* demonstrated a non – dose dependent anti-inflammatory response. The pharmacological activity of plant extracts followed a dose dependent edema inhibition response from 1st to 4th hour. The inhibition of paw edema was higher at the 4th hour in relation to the 3rd, 2ndand 1st hours. The percentage paw edema inhibition at the 4th hour may be associated decreased absorption rate of pharmacologically active phytochemicals at the 1st hour. In the 4th hour, there might be increased plasma concentration of the pharmacologically active compounds responsible for anti-inflammatory activities. In the 1st-3rd hours, anti-inflammatory activities might be lower because the phytochemical are not biotransformed to generate active pharmacological

agents (Jarko *et al*., 2008).

The plant extracts were administered in three dose levels (50, 100, and 150 mg/kg). The dose level is similarone used by Ashfar *et al.* (2015); Mwangi *et al.* (2015); Mworia *et al.* (2015 and Kamau *et al*. (2016). In dose dependent anti-inflammatory response cases, a plant extracts at a dose level of 150mg/Kg demonstrated better edema inhibition activity the two lower doses (50 and 100 mg/Kg). This may be associated with inadequate active phytochemicals in the plasma concentration and this may be lead to increased metabolism and excretion from the body. The anti-inflammatory effects demonstrated in this study indicate some level of similarities with results observed by Mwangi *et al*. (2015) in a study to evaluate dichloromethane: methanolic anti-inflammatory activity of *Mytenus obscura* and *Caesalpinia volkensi* leaves extract. Likewise, Mbiri *et al.* (2016) study, demonstrated similar dose dependent anti-inflammatory response in methanolic stem bark of *T. brownii* extract.

The non–dose dependent anti-edema activity associated with dichloromethane stem bark extract of *M. indica*, leaves extracts of *E. saligna* and methanolic leaves extract of *S. didymobotyra*, stalks extract of *B. pilosa*, stem bark extract of *M. indica* and leaves extract of *E. saligna* demonstrated a non–dose dependent anti-inflammatory response may be associated with increased antagonistic metabolites levels leading to competitive inhibition response in anti-inflammatory activities. This resulted to lower plant extract doses exhibiting betteranti-inflammatory response than higher doses.

Phytochemical screening of methanolic and dichloromethane *S. didymobotyra* leaves, *E. saligna* leaves, *P. africana* stem bark, *B. pilosa* stalks and *M. indica* stem bark demonstrated the presence of various secondary metabolites (Table 3.2). These secondary metabolites are reported to inhibit prostaglandins synthesis pathway (Kumar *et al*., 2013). Flavonoids affect the activities of the cyclo-oxygenase, phospholipase, TNF-α and lipo-oxygenase that are crucial catalysts for arachidonic acid anabolism (Chi *et al.,* 2001; Jang *et al*., 2002). Saponins are associated with inflammation inhibition and they can also play a role in inhibition of inflammation (Vasudevan *et al*., 2007). Saponins and flavonoids synergistically can inhibit enzymes catalyzing the biochemical pathways involved in generation of inflammatory mediators. Enzymes inhibited by saponins and flavonoids include:- lipoxygenase, nitric oxide synthase and cyclooxygenase (Ashfaq *et al.,* 2016). Inflammation process leads to production of free radicals (*Samriti et al*., 2016)commonly generated in the carrageenan test late phase (Samanta *et al*., 2013). Phytochemicals like tannins, flavonoids, phenolic compounds and terpenoids are capable of scavenging these free radicals since they are potential antioxidants hence facilitating the process of wound healing (Ghasemzadeh and Ghasemzadeh, 2011; Abayomi *et al*., 2013). The phytochemical presence in both dichloromethane and methanol of the *S. didymobotyra* leaves, *E. saligna* leaves, *P. africana* stem bark, *B. pilosa* stalks and *M. indica* extracts include:- alkaloids, cardiac glycosides, saponins, terpenoids, steroids and flavonoids can be able to work alone, or in either additively and / or synergistically to inhibit inflammatory process. To maximize the pharmacological activity of the herbal medicines one can combine two or more herbs to get a concortion associated with better results (Salawu *et al*., 2008; Agbaje andAjidahum, 2011).

In this study, plants extracts were demonstrated some positive anti-inflammatory activities. This might be attributed to availability of pharmacological active anti-inflammatory agents in these plants. Sometimes the higher doses of the plant extracts exhibited lower anti- inflammatory activity in relation to the lower dose, this can be attributed to increased levels of the antagonistic metabolites which could competitively inhibit the anti-inflammatory activities of the active metabolites. Low dose administration of the formalin to the paw of the mice induces edema by facilitating the release of substance P (neuro-peptide) which leads to the mediation of the neurogenic inflammation. High doses of formalin induce edema by triggering the release of histamine, 5-hydroxytryptamine, prostanoids and substance P (Damas and Liegeois, 1999). In this study, high of formalin was administered to the animal model, this implicate the release of histamine, 5-hydroxytryptamine, prostanoids and substance P in the inflammatory induction process.

The first and second hour anti-inflammatory activities of both DCM and MeOH plants extracts was less than that observed in the 3rd and 4th hour at the end of study time. This indicates that the pharmacologically active phytocompounds undergoes biotransformation before gaining some pharmacologically anti-inflammatory activities or takes more time before passing acrossthe cell membrane in peritoneum cavity.

In this study, less polar fraction of the plants was extracted using DCM and the most polar fraction was extracted using methanol. This indicates that both the polar and the non-

polar fractions of the secondary metabolites in herbal plants involved in the study were extracted for anti-inflammatory effects. The secondary metabolites edema inhibition pharmacological activity was related with edema inhibition of the conventional drug (diclofenac). *B.pilosa, S. didymobotyra, M. indica, P. africana* and *E. saligna* contained several secondary plant metabolites like the flavonoids and alkaloids. In this study of plant extracts, qualitative analyses indicate that several phytochemical compounds were found in plants under investigation. These phytochemical compounds including flavonoids and alkaloids are associated with some anti-inflammatory activies (Ferrandiz and Alcaraz,1991).

Alkaloids which form the major class of phytochemicals in plant is attributed to a number of pharmacological effects (Barbosa–Filho *et al.,* 2006); including the edema inhibition activity (Souto *et al.,* 2010). The anti-inflammatory activities associated with these plant sample extracts can be associated with the availability of these secondary metabolites while acting singly or in a synergistic manner. According to this study, in some cases herbal plants extracts indicated a better anti-inflammatory activity than the standard anti-inflammatory drug (Diclofenac). This can be associated to the facts that the conventional drug (diclofenac) was solution was prepared using normal saline instead of the vehicle like DMSO. Dimethyl sulfoxide (DMSO) exhibits some anti-inflammatory activity individually (Smith *et al*., 1998).

Analgesic activity evaluation of dichloromethane and methanol extracts of *B. pilosa* stalks, *E. saligna* leaves, *M. indica* stem bark, *P. africana* stem bark and *S. didymobotyra*

leaves paw licking test was induced by subcutaneous injection of formalin in the hind paw. Formalin induced paw licking test is mostly applicable for antinociceptive activities evaluation because of its high specificity and reliability for antinociceptive responses (Shibata *et al*., 1989; Tjolsen *et al.,* 1992; Bolegave *et al.,* 2015). This method is considered to be ideal and suitable for testing clinical and chronic pain (Tjolsen and Hole, 1997). Formalin when administered intraperitoneally induces paw licking behavior of intensive nociception in two well distinct phases because of different pain mediators involvement (Hunskaar et al., 1985; Hunskaar and Hole, 1987; Rosland, 1991; Correa and Calixa, 1995; Seguin *et al*., 1995; Santos and Calixto, 1997).

The prophylactic analgesic activities of both DCM and MeOH extract of *M. indica, S. didymobotyra, E. saligna, P. africana* and *B. pilosa* was evaluated through formalin-induced paw licking test (Bolegave *et al*., 2015). This was used to observe the analgesic activities of plant extracts through the post–treatment which resembles a natural clinical condition (Chang *et al*., 2012). Formalin induced pain exists in two distinct phases (Rezaee–Als *et al.,* 2014).

The nociception first phase which starts five minutes after the administration of formalin is also referred to as neurogenic phase. It involves directs stimulation of the nociceptors and the afferent C and Aδ fiber by the chemicals (Santos and Calixto, 1997). Opioid agonists like morphine are capable of inhibiting neurogenic phase (Shibata *et al*., 1989; Hunskaar and Hole, 1987; Correa and Calixa, 1993; Rajnarayana *et al*., 2001; Manjunatha *et al*., 2006). Second nociception phase occurs between 15–30 minutes following formalin

injection. The second phase is associated with inflammatory pain due inflammatory response associated with the release of various proinflammatory mediators like brandykinin, histamine, prostaglandins and serotonin (Hunskaar and Hole, 1987; Yaksh *et al*., 2001; Ferreira *et al*., 2004). Centrally acting analgesics drugs like narcotics are capable of equally inhibiting both phases while drugs acting peripherally like non–steroidal anti-inflammatory drugs like (aspirin) and steroids such as dexamethasone and hydrocortisone only suppresses the late phase pain (Adzu *et al*., 2003; Paschapur *et al.*, 2009; Couto *et al*., 2011; Adebayo *et al*., 2014).

In this study, methanolic and dichloromethane extracts of *B. pilosa* stalks, *E. saligna* leaves, *M. indica* stem bark, *P. africana* stem bark and *S. didymobotyra* leaves indicated significant analgesic activities by reduced paw licking time. The highest early phase antinociceptive activity was 52.62% and it was observed in methanolic stem bark extract of *Prunus africana* while the highest late phase antinociceptive activity was 66.93% and it was observed in dichloromethane stem bark *Prunus africana* extract. The lowest early and late phase antinociceptive activity was 12.51 and 14.75% they were observed in methanolic and dichloromethane stem bark extract of *Mangifera indica* respectively. These finding indicate direct effects of active phytocompounds on nociceptors blockage and inhibition of both or either release or synthesis of inflammatory and pain mediators like the prostaglandins. The early and late phases of pain experience were not inhibited equally. For example, the highest antinociceptive activity of plants extracts was 52.62 and 66.93% a clear indicator of different pain activity inhibition. This clearly suggests that the methanolic and dichloromethane plant parts extract contains both peripherally and centrally acting

antinociceptive phytocompounds.

The analgesic activities could be associated with either synthesis and / or release of the active pain mediators or nociceptor blockers (Mahdi and Vihid–reza, 2008; Vyas *et al*., 2016). The result concurs with results from various studies evaluating medicinal plants extracts antinociceptive activities. Both methanolic and dichloromethane extracts of *B. pilosa* stalks, *E. saligna* leaves, *M. indica* stem bark, *P. africana* stem bark and *S. didymobotyra* leaves indicated a reduced inhibition in paw licking in mice both in early and late phases. This is conciding with results obtained by Mei *et al*. (2011) while investigating analgesic activity of *Radix Aconiti carmichaeli* aqueous extracts in animal models.

The the research study employed dose ranges of 50, 100 and 150 mg/Kg which concurred with dosages in other concluded studies (Norma *et al*., 2013; Santanu *et al*., 2013; and Ishola *et al*., 2014). Dose ranges used by Ishola *et al*. (2014) to evaluate *Alafia barteli, Combretum mucronatum* and *Capparis thonningii* analgesic activity on hot plate, formalin and acetic acid pain induced tests were 50, 100 and 200 mg/Kg. To evaluate analgesic activity of *Cleome spinosa*, Norma *et al.* (2013) administered 50 and 100 mg/Kg dose levels in mice.However, when evaluating the analgesic activity of root bark extract of *Eugeniajambolana*, Santanu *et al*. (2013) used 100, 200, 300 and 400 mg/Kg dose levels.

Methanolic and dichloromethane extracts of *B. pilosa* stalks, *E. saligna* leaves, *M. indica*

stem bark, *P. africana* stem bark and *S. didymobotyra* leaves indicated less antinociceptive activity in lower dose (50 mg/Kg body weight) than the higher doses. This is a clear indication of increased clearance, metabolism and inactivation of active phytocompounds at lower dose concentration. In lower dose levels, the plasma concentration of active phytochemicals is also not sufficiently achieved. The dose–dependent analgesic activities is attributed to increased metabolism clearance of the pharmacologically active compounds in low doses. This could also be associated with the fact that at lower doses the achievement of pharmacologically active components plasma concentration is insufficient.

Some of the drugs are known to exert therapeutic activities up to a limited drug plasma concentration or drug dose range, meaning that if the drug dose range is exceeded, therapeutic activities reduces and this could be the reason behind the non–dose dependent analgesic activities (Nthiga *et al*., 2016b; Koech *et al*., 2017). The analgesic activities of *Cynanchum viminale* aqueous stem extract indicated analgesic activity in dose independent behavior in formalin pain induced mice both in early and late phase (Safari *et al.*, 2016b). However, *Harrisonia abyssinica* methanolic stem bark extract indicated antinociceptive activity in dose–dependent behavior in formalin pain induced mice both in acute and chronic phases (Nthiga *et al*., 2016b).

The analgesic activity of the both methanolic and dichloromethane extracts of *B. pilosa* stalks, *E. saligna* leaves, *M. indica* stem bark, *P. africana* stem bark and *S. didymobotyra* leaves may be associated with the one or more groups of phytochemicals present in the plant samples. Plants containing alkaloids, organic acids, flavonoids and saponins are

associated with significant analgesic activities. Numerous studies have associated various compounds isolated in the plant sample extracts with some antinociceptive activity. Alkaloids, flavonoids and saponins analgesic activities is associated with inhibition of the prostaglandins which are involved in pain perception (Salawu *et al*., 2008; Sani *et al*., 2013; Ashfaq *et al.,* 2016).

Saponins present in *Carissa edulis* are associated with some analgesic activity (Halimatu *et al*., 2010). Chido *et al*. (2010) also associated saponins identified in methanolic *Ficus platyphylla* stem bark extract with some analgesic activity. Moreover, *Bupleurum falcatum, Madhuca longifolia* and *Phytolacca Americana* saponins fractions are known to possess some significant analgesic activities (Chandel and Rastogi, 1980; Singh *et al*., 1992). Terpenoids are also associated with interference of intracellular signal transduction mechanisms and platelet aggregation inhibition (Mworia *et al*., 2015). Terpenoids presents in both methanolic and dichloromethane extracts of *B. pilosa* stalks, *E. saligna* leaves, *M. indica* stem bark, *P. africana* stem bark and *S. didymobotyra* leaves also could be associated with antinociceptive activity. Terpenoids present in *M. officinalis* extracts are associated with antinociceptive activities in animal models (Miladi–Gorgi *et al*., 2005).

Waqar *et al*. (2013) established that *Ziziphus oxyphylla* cyclopeptide alkaloids have significant potential to attenuate pain in both early and late phases. Alkaloids are also known to acts a good mood enhancer and in promotion of sense of well–being (Gurib *et al.,* 2013). Alkaloids isolated from *K. macrophylla* possess strong analgesic actions in early and late phase in studies involving animal model (Reanmongkol *et al*., 2005). Studies

by Mei *et al*. (2011) also established that Radix *Aconiti carmichaeli* contain high quantities of mesaconitine alkaloids associated with antinociceptive activity. Therefore Both methanolic and dichloromethane extracts of *B. pilosa* stalks, *E. saligna* leaves, *M. indica* stem bark, *P. africana* stem bark and *S. didymobotyra* leaves also could be associated with analgesic activity observed in laboratory animals.

Flavonoids isolated in plant extract samples could be responsible for the analgesic activities. For instance, flavonoids isolated in *M. officinalis* is associated with some antinociceptive activity in animal models (Miladi–Gorgi *et al*., 2005). Flavonoids targets prostaglandins hence attenuating pain perception by moderating the opioidergic mechanism (Rajnarayana *et al*., 2001; Anjaneyulu and Chopra, 2003; Manjunatha *et al.*, 2006).

Antipyretic, analgesic and anti-inflammatory compounds isolated in this study were: Vitamin E acetate, 3 – carene, alpha – pinene, camphene, β- pinene, limonene, eucalyptol (DCM *B. pilosa*); 3 – carene, camphene, β- pinene, limonene, phenol, eucalyptol, squalene, thymol (MeOH *B. pilosa*); thymol, 3 – carene, alpha, - pinene, camphene, limonene, eucalyptol (DCM *E. saligna*); .alpha – pinene, camphene, limonene, eucalyptol, borneol, squalene, thymol (MeOH *E. saligna*); alpha. pinene, 3 – carene, camphene, limonene (MeOH *M. indica*); thymol, 3 – carene, alpha – pinene, limonene (DCM *M. indica*); 3 – carene, .alpha. – pinene, camphene, limonene, eucalyptol, squalene, thymol (DCM *P. africana*); 3 – carene, .alpha. – pinene, camphene, limonene (MeOH P. africana); 3 – carene, .alpha, - pinene, camphene, limonene, eucalyptol (DCM

S. didymobotyra) and 3 – carene, .alpha. – pinene, camphene, limonene, eucalyptol, squalene (MeOH, *S. didymobotyra*) (Rajnarayana *et al*., 2001; Anjaneyulu and Chopra, 2003; Manjunatha *et al.,* 2006).

The percentage change in weekly and average percentage change in body weight per week was higher in normal control group than the groups treated with methanolic and dichloromethane plant extract though not significantly different. The decreased percentage change in body weight gain of the dichlomethane and methanolic extracts of *B. pilosa, S. didymobotyra, E. saligna, P. africana* and *M. indica* may be associated with anti-obesity effects of the phytochemicals acting singly or either as additives or synergies. Tannins identified in the plants extracts are associated with reduced food intake and decreased palatability (Zhang *et al*., 2015). Palatability reduction is associated with tannins astringency. Astringency sensation results from complexes formation between the salivary glycoproteins and the tannins leading to depressed feed intake.

Reduced digestibility influences feed intake negatively because it is associated with indigestion (Zhang *et al*., 2015). Tannins also influence fat digestion by inhibiting the formation of micelle in the small intestine hence inhibiting the activity of the α-glucosidase reducing the absorption of triacylglycerol (Kim *et al*., 2010). Reduced absorption of triglycerides results to reduced body weight due to reduced body fat mass (Thom, 2007). The presence of flavonoids in the plant extracts affects appetite by acting centrally to the hypothalamus leading to satiety signals release. Flavonoids delays gastric emptying by enhancing the sympathomimetic activity (Zheng *et al*., 2010). Flavonoids decreases hunger by increasing fullness filling resulting from prolonging the gastric emptying (Murray *et al*.,

2008).

The phytochemical analysis of the dichloromethane and methanolic plants GC-MS profile indicated long–chain polyunsaturated fatty acids presence. The poly–and mono–unsaturated fatty acids (PUFAs and MUFAs) increases release of satiety hormones like cholecystokinin (CCK) (Pasman *et al*., 2008). Increased levels of CCK delays the emptying of the gastric content leading to satiety feeling and appetite decrease (Lawton *et al*., 2000). The polyunsaturated fatty acids are associated with increased synthesis of HDLs (Guine *et al*., 2009). Plants fatty acids leads to decreased body weight also by stimulating the release of CCK, GLP-1 and peptide YY which leads to inhibition of the upper gastric motility leading to satiety effect indirectly (Burns *et al*., 2002; Diepvens *et al*., 2007).

Terpenoids like squalene promote body weight reduction by suppressing de novo synthesis of fatty acid, increasing the rate of lipid oxidation and reducing food intake (Gugler *et al*., 2013). Diterpenes increases the activity of the adenylate cyclase which leads to increased conversion of ATP to cAMP. Increased levels of cAMP leads to promotion of the lipolysis which is associated with increased basal metabolic rate. Increased basal metabolic rate promotes the degradation of the body protein and fat (Huang *et al*., 2016). Satiety leads to energy consumption suppression (Gugler *et al*., 2013). Squalene is also associated weight reduction and hypoglycemic activity because it enhances lipolysis and pancreatic islets cell regeneration (Elliot *et al*., 2000).

The GC-MS profile also indicates the presence of phytosterols in both dichloromethane

and methanolic plant extracts. Phytosterols are associated with decreased body weight because of both peripheral and central mechanisms. Phytosterols are known to induce adipose tissues lipogenesis (Atif *et al*., 2003). Through central mechanism, phytosterols are associated with decreased appetite because of the amplification of the hypothalamus signaling energy sensation function (Kuriyan *et al*., 2007).

The observed weight reduction in both methanolic and dichloromethane plants extracts of *E. saligna, S. didymobotyra, M. indica, B. pilosa* and *P. africana* is associated with the phytochemical synergistic activities regulating various pathways involved in lipid absorption, nutrient intake, energy expenditure, pre-adipocyte proliferation and differentiation decrease, increased lipolysis and decreased lipogenesis (Chandrasekaran *et al*., 2012). They also offer anti-inflammatory and anti-oxidant activities (Rayalam et al., 2008). The combinations of the various phytochemical compounds in the plants extracts results in increased bioavailability, synergistic effects and multiple molecular targets actions, offering better results over single chemical treatments (Gonzalez-Castejon and Rodriguez–Casado, 2011).

The organ weight of the animals orally treated with both methanolic and dichloromethane plants extract did not indicate any significant different in organ weight in relation to the normal control group ($p<0.01$). The organ weight of the animals treated with plant extracts was slightly lower than that of the normal control group. The results were comparable to previous study in which treatment of rats with *Moringa oleifera* for eight weeks resulted into a decreased body and organ weight (Bais *et al*., 2014). Decreased organ weight could be associated with decreased body weight. Appetite and hunger regulation is very important in homeostatic balance maintenance. Feed intake is suppressed

when satiety signals like the melanocyte–stimulating hormones and leptin is activated resulting to body weight reduction. Indirect satiety activation is moreover generated whenever there is increased release of cholecystokinin (CCK), glucagon–like peptide–1 (GLP–1) and peptide YY (PYY) leading to slow intestinal transit and emptying of the gastric content (Diepvens *et al*., 2007). These findings clearly indicate that the plant phytochemicals or their metabolites were not associated with either organ tissue atrophy or hypertrophy because the low organ weight is associated with reduction in body weight.

Treatment with methanolic and dichloromethane plants extracts did not lead to significant chance in the serum biochemical parameters such as AST, GGT, urea, creatinine, total protein, albumin, total bilirubin, direct bilirubin and indirect bilirubin though there was a significant change in serum levels of ALT and ALP at $p<0.01$. Alanine aminotransferase (ALT) catalyses transamination reaction between alanine and α-ketoglutarate leading to the formation of glutamate and pyruvate. High levels of alanine aminotransferase is presence in the hepatocytes cytosolic fluid though small levels are alsopresence in muscles, adipose and brain tissues. Increased levels of serum ALT are associated with liver tissue injury (Xing–Jiu *et al*., 2006; Liu *et al*., 2014).

The methanolic and dichloromethane plant extracts did not alter significantly the levels of serum AST indicating that probably the plants extracts had minimal adverse effect to the hepatocytes. Aspartate aminotransferase (AST) is involved in the transfer of amino group to α-ketoglutarate from the aspartate. AST is mainly found in liver but small amount is found in heart cells, red blood cells, muscle tissue, kidneys and pancreas. In cases of liver

damage, the levels of AST in the serum are elevated indicating an increased enzyme activity.AST is not tissue specific hence for valuable diagnosis of liver damage there is a need to monitor levels of other enzymes associated with liver tissue damage (Xing–Jiu *et al*., 2006). Both methanolic and dichloromethane plant extracts did not increase the serumlevels of AST activity indicating that the liver parenchyma cells were not affected adversely.

The enzyme gamma –glutamyl transferase majorly plays a great role in metabolism of glutathionylated and glutathione xenobiotics. Previously it has been used as major biochemical marker associated with liver dysfunction, alcohol consumption and biliary disorders (Emdin *et al*., 2005; Koenig and Seneff, 2015). Increased activity of gamma glutamyl transferase is also used as an early predictive biochemical marker for gestational diabetes, atherosclerosis, arteriosclerosis, plaque, heart failure and numerous hepatic disorders like viral hepatitis, several cancers and other infectious diseases associated with poor prognosis (Koenig and Seneff, 2015). Its increased activity is also related to increased risk of hemorrhagic stroke and coronary heart disease (Emdin *et al*., 2005).

The study results indicates that there was a significant change enzyme alkaline transferase activity level at $p<0.01$, an indicator of hepatocytes and associated injury. Alkaline phosphatase (ALP) is involved in hydrolysis of organic phosphate esters (Ren *et al*., 2015). The enzyme low substrate specificity is commonly increased in metastatic osteosarcoma hence a convenient and effective biomarker of the same (Ren *et al*., 2015). Elevated activity of ALP is also an important biomarker of vascular calcification (Lee *et*

al., 2015).

After administration of the methanolic and dichloromethane plants extracts orally, the levels of total bilirubin, direct bilirubin and indirect bilirubin was not significantly altered. Bilirubin is a main product of heme degradation in the hepatocytes after hemolysis of the red blood cells. It is a yellowish green pigment. It exists in the serum in the form of direct (conjugated) or indirect (un-conjugated) bilirubin. Total bilirubin is the sum total of direct and indirect bilirubin. Conjugation of bilirubin in the liver makes its more soluble leading to easy excretion in urine and bile. Bilirubin accumulation in the body causes jaundice and the pigments can be deposited in the basal ganglia especially in the neonates leading to a fatal condition referred to as kernicterus (Hardiker and Suchy, 2006; Guyton and Hall, 2006b; Walker *et al*., 2013). Insignificant variation in the serum levels of bilirubin after the administration of the plants extracts is a clear indicator that plants extracts were not associated with any hemolytic activity.

The study results were also not associated with any significance change in the serum levels of total protein and albumin after the administration of plant sample extracts orally. Total plasma protein is mainly composed of albumin and all the globulin fractions. Albumin and globulin fractions are synthesized mainly in the liver and the B-cellsrespectively. The main function of the total plasma protein is to maintain the plasma oncotic pressure ensuring that blood is retained within the vascular space. Any liver damage for example in cases of hepatitis and liver cirrhosis is associated with decreased levels of plasma proteins. Albumin is the main plasma protein which plays a key role in maintaining the plasma colloidal oncotic pressure. A decrease in albumin fraction is associated with

ascites development which is very fatal (Walker *et al*., 2013). The study results were not associated with any significance change in the levels of both albumin andtotal protein an indicator that there was no adverse effects to the hepatocytes. Considering all the parameters evaluated to test liver functions, there is evidence that oral administration of both methanolic and dichloromethane plant extracts was not associated with any hepatocyte damage.

Oral administration of methanolic and dichloromethane plant extracts to the animals did not affect the end-point heamatological parameters like erythrocytes count, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration, RDW (red cell distribution width), WBC (white blood cell) count and WBC (white blood cell) differential count, platelet count, MPV (mean platelet volume) and PDW (platelet distribution width) significantly at $p<0.01$.

The mean cell volume value is related to the red blood cells size and the mean corpuscular hemoglobin signifies the hemoglobin quantity per red blood cell. The mean corpuscular hemoglobin concentration value is used to indicate the hemoglobin amount in a unit volume and the red cell distribution width is a parameter that signifies the coefficient of the red blood cell volume distribution variation (Moreno *et al*., 2003). These red blood cells related indices are very useful in determination of the causes of anemias. In increased levels of RBC counts and Hb levels without a significance change in the values in PCV, MCV, RDW, MCH and MCHC was an indicator that the plant sample extract did not have any adverse effect in the functioning of the bone marrow, number of precursor cells, bone

marrow infiltration and nutrients absorptions like folate or vitamin B_{12} (Moreno *et al*., 2003).

The study results indicated a significant increased levels of total white blood cell counts in all the animals treated with both methanolic and dichloromethane plant parts extracts at $p<0.01$. The levels of lymphocytes, monocytes, neutrophils, eosinophils and basophils was higher in animals treated with plant sample extract with no significant different $p<0.01$. Increased levels white blood cells count is an indicator of increased body ability to respond well in cases of pathogenic microbial infections (Hoюorand *et al*., 2006). Increased neutrophil and monocytes levels is an indicator of immune system ability to protects the body in cases of bacteria infection; increased lymphocytes signifies enhanced antibody production hence improved protection against foreign organisms and viruses; increased levels of eosinophils signifies an improved ability to eliminate parasitic infection; increased levels of basophil levels is associated with improved allergic responses (Hoюorand *et al*., 2006). The increased levels of the total whiteblood cell counts and the related leucocytes differential counts implies an improved immune function able to categorically recognize a wide range of antigens, proper establishment of immunologic response memory, mount immunological attack and destruction of various types of antigens (Hoюorand *et al*., 2006). This result concurs with results observed when diabetic rats are treated with *Acalypha wilkesiana* leaves, leading to an improved hematopoietic system leading to increased levels of total white blood cell count, erythrocytes count, neutrophil count and mean cell volume (Ikewuchi *et al*., 2011).

The study indicated that the levels of platelet count, MPV and PDW did not significantly vary after treatment with different plant methanolic and dichlomethanolic extracts $p<0.01$. Platelets are very important in hemostasis processes (Nakashima and Rogers, 2014). Increased levels of platelets in the body can lead to hypercoagulable state causing thrombo-embolism and thrombosis especially in the coronary artery, brain and lung muscles. Arterial thrombosis is associated with increased rate of mortality, cardiac and neurological disorders. Thrombosis in cases of deep vein thrombosis is a painful and life threatening condition (Briere, 2007).

Essential thrombocythemia is an acquired disorder associated with myeloproliferation and chronic elevation of platelet quantity leading to increased cases of thrombosis and hemorrhage (Briere, 2007). Platelets mediates the coagulation process by forming a sticky plugs which helps in stopping bleeding due to physical injuries (Arika *et al*., 2016). Platelets are also associated with atherosclerosis development by hardening of arteries in cases of hyperglycemic states. Normal levels of platelets and related indices is an indicator of lack of bone marrow injury or hemolysis (Borazzoni *et al*., 2014). Normalization of the platelets and the associated indices after plant sample extract treatment is a clear indicator that the plants can be potential protective agents against hemophilia, coronary thrombosis, hyperglycemia and myocardial infarction (Borazzoni *et al*., 2014).

5.2 Conclusions

The study objectives revolved around determination of both methanolic and

dichloromethane phytochemical profile and the antiinflammation, antinociceptive, antipyretic and toxicity of *Senna didymobotyra*, *Eucalyptus saligna*, *Mangifera indica*, *Prunus africana* stem and *Bidens pilosa* extract.

Qualitative analysis of phytochemical compounds in the plants samples in both methanolic and dichloromethane extracts indicated positive results for flavonoids, alkaloids, terpenes, glycosides, tannins, cardiac glycosides, saponins, anthocyanin and betacyanin, quinones, phenols, coumarins and steroids. The GC-MS quantitative analysis of phytochemical compounds present in the plants samples indicated presence of different secondary metabolites and in a varying concentration some associated with with antiinflammation, antinociceptive and antipyretic activities.

The dichloromethane and methanolic extracts exhibited edema, pain and fever inhibition. These pharmacological activities can be associated with the secondary metabolites identified in the plant extracts. Therefore the study provides an avenue for further studies for established a herbal derived medicine for management of pyrexia, pain and inflammation.

In conclusion, the dichloromethane (DCM) and methanol (MeOH) extracts of *Eucalyptus saligna, Senna didymobotyra, Bidens pilosa, Prunus africana* and *Mangifera indica* indicated some anti-inflammatory, antinociceptive, and antipyretic activities *in vivo* validating their use in traditional medicine among the Meru communities. The three doses used for evaluation of plants extracts displayed significant anti-inflammatory effect in acute inflammation and significantly inhibited pain sensation through both peripheral and central mechanisms. Moreover, the herbal extract also exhibited appreciable antipyretic

effects.

On the other hand, the classes of phytochemicals indentified in the plants extracts such as tannins, alkaloids, phenolic compounds, glycosides and saponins are know to exhibit some anti-inflammatory, antinociceptive and antipyretic pharmacological activities. Some of the specific phytochemicals isolated from the plant samples associated with anti-inflammatory, analgesic and antipyretic activities include:- vitamin E acetate, 3 – carene, alpha – pinene, camphene, β- pinene, limonene, eucalyptol, phenol, squalene, thymol and borneol. Plants extracts under the study may serve as effective alternative and complementary treatment strategy to the conventional interventions. Therefore, the research results indicate the pharmacological role supporting use of *Eucalyptus saligna, Senna didymobotyra, Bidens pilosa, Prunus africana* and *Mangifera indica* stem bark extract for management of inflammation, pain and fever.

The plant extract both dichloromethane and methanolic extarcts of the five selected plants did not indicate any significance toxicological effects on the vital organs like the liver, kidney, brain, spleen and overall body weight. This indicates that the plants organic phytochemical components and their metabolites are not or less associated with any cytotoxic effects. The compounds use in development of analgesic, antipyretic and anti-inflammatory molecules can result to a product with less toxicological effect among the users.

5.3 Recommendations

5.3.1 Recommendations of the study

The dichloromethane and methanolic extract of *Eucalyptus saligna, Senna didymobotyra, Bidens pilosa, Prunus africana* and *Mangifera indica* may be used as an alternative and complementary candidate for synthesize of effective agents to manage diseases associated with pain, fever and inflammation.

5.3.2 Suggestions/recommendations for the research

i. Phytochemical separations and bioscreening of both methanolic and dichloromethane extract of *Eucalyptus saligna, Senna didymobotyra, Bidens pilosa, Prunus africana* and *Mangifera indica* to isolate and identify the phytocompounds associated with anti-inflammatory, antinociceptive and antipyretic activities. This could lead to discovery of novel anti-inflammatory, antinociceptive and antipyretic agents.

ii. Investigate mode of action of the anti-inflammatory, antinociceptive and antipyretic effects associated with methanolic and dichloromethane extracted phytocompounds from *Eucalyptus saligna, Senna didymobotyra, Bidens pilosa, Prunus africana* and *Mangifera indica*. This will positively influence management especially of diseases associated with inflammation, pain and fever.

iii. Bioscreening of dichloromethane and methanolic extract of *Eucalyptus saligna, Senna didymobotyra, Bidens pilosa, Prunus africana* and *Mangifera indica* to determine how they compare with conventional anti-inflammatory, analgesic and antipyretic drugs.

iv. Use of different routes of administering plant parts extracts and compare the convenience of different routes of the administration.

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APPENDICES

Appendix 1: Antinociceptive activity of MeOH extract of *P. africana*

Variable	C1	Mean	SE Mean	StDev
1st phase	Test A	79.65	3.77	8.43
	Test B	65.44	3.87	8.65
	Test C	52.05	1.93	4.31
	negative	115.81	3.48	7.78
	normal	0.000000	0.000000	0.000000
	Positive	39.74	1.83	4.10
2nd phase	Test A	112.37	4.05	9.06
	Test B	84.59	4.14	9.26
	Test C	70.20	4.23	9.46
	negative	148.35	6.04	13.51
	normal	0.000000	0.000000	0.000000
	positive	84.93	4.99	11.17
% phase 1	Test A	69.16	4.47	9.99
	Test B	56.33	2.08	4.64
	Test C	45.24	2.77	6.20
	Negative	100.00	0.000000	0.000000
	normal	0.000000	0.000000	0.000000
	positive	34.50	2.17	4.86
% 2nd phase	Test A	76.21	3.90	8.71
	Test B	57.38	3.77	8.42
	Test C	47.38	2.32	5.20
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	57.85	5.08	11.36
% inhibition Phase 1	Test A	30.84	4.47	9.99
	Test B	43.67	2.08	4.64
	Test C	54.76	2.77	6.20
	negative	0.00	0.00	0.00
	normal	100.00	0.00	0.00
	positive	65.50	2.17	4.86
% inhibition phase 2	Test A	23.79	3.90	8.71
	Test A	42.62	3.77	8.42
	Test C	52.62	2.32	5.20
	negative	0.00	0.00	0.00
	normal	100.00	0.000000	0.00
	positive	42.15	5.08	11.36

Appendix 2: Antinociceptive activity of DCM extract of *P. africana*

Variable	C1	Mean	SE Mean	StDev
phase 1	Test A	71.39	4.31	9.63
	Test B	57.00	3.89	8.69
	Test C	53.79	4.21	9.42
	negative	108.17	3.52	7.87
	normal	0.00	0.00	0.00
	positive	48.34	2.18	4.88
phase 2	Test A	92.79	3.75	8.39
	Test B	63.33	4.63	10.36
	Test C	54.57	3.56	7.96
	negative	164.62	5.25	11.74
	normal	0.00	0.00	0.00
	positive	65.14	1.78	3.99
% phase 1	Test A	66.16	4.07	9.11
	Test B	53.04	4.18	9.34
	Test C	50.00	4.56	10.19
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	45.05	3.09	6.90
% phase 2	Test A	56.35	1.24	2.78
	Test B	38.59	3.02	6.76
	Test C	33.07	1.51	3.38
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	39.78	1.90	4.26
% inhibition phase 1	Test A	33.84	4.07	9.11
	Test B	46.96	4.18	9.34
	Test C	50.00	4.56	10.19
	negative	0.00	0.00	0.00
	normal	100.00	0.00	0.00
	positive	54.95	3.09	6.90
% inhibition phase 2	Test A	43.65	1.24	2.78
	Test B	61.41	3.02	6.76
	Test C	66.93	1.51	3.38
	negative	0.00	0.00	0.00
	normal	100.00	0.00	0.00
	positive	60.22	1.90	4.26

Appendix 3: Antinociceptive activity of MeOH extract of *E. saligna*

Variable	C1	Mean	SE Mean	StDev
phase 1	Test A	81.59	6.15	13.76
	Test B	70.41	1.70	3.81
	Test C	56.43	3.43	7.66
	negative	98.76	1.37	3.06
	normal	0.00	0.00	0.00
	positive	59.35	3.06	6.84
phase 2	Test A	126.42	5.37	12.01
	Test B	117.14	3.09	6.90
	Test C	112.27	3.99	8.92
	Negative	144.65	4.35	9.73
	normal	0.00	0.00	0.00
	positive	120.99	3.28	7.34
% phase 1	Test A	82.57	6.12	13.68
	Test B	71.28	1.29	2.89
	Test C	57.21	3.77	8.42
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	60.23	3.62	8.09
% phase 2	Test A	87.85	5.11	11.43
	Test B	81.34	3.64	8.14
	Test C	78.17	4.84	10.83
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	84.09	4.13	9.23
% inhibition phase 1	Test A	17.43	6.12	13.68
	Test B	28.72	1.29	2.89
	Test C	42.79	3.77	8.42
	negative	0.00	0.00	0.00
	normal	100.00	0.00	0.00
	positive	39.77	3.62	8.09
% inhibition phase 2	Test A	12.15	5.11	11.43
	Test B	18.66	3.64	8.14
	Test C	21.83	4.84	10.83
	negative	0.00	0.00	0.00
	normal	100.00	0.00	0.00
	positive	15.91	4.13	9.23

Appendix 4: Antinociceptive activity of DCM extract of *E. saligna*

Variable	C1	Mean	SE Mean	StDev
phase 1	Test A	82.30	1.50	3.36
	Test B	73.55	4.57	10.21
	Test C	65.42	4.38	9.79
	negative	104.42	3.52	7.86
	normal	0.00	0.00	0.00
	positive	56.74	2.75	6.15
phase 2	Test A	128.45	4.07	9.09
	Test B	116.28	1.98	4.43
	Test C	108.90	3.73	8.34
	negative	148.35	2.53	5.65
	normal	0.00	0.00	0.00
	positive	117.36	5.18	11.58
% phase 1	Test A	79.14	2.81	6.28
	Test B	70.78	5.22	11.68
	Test C	62.96	4.65	10.41
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	54.55	3.00	6.70
% phase 2	Test A	86.59	2.35	5.26
	Test B	78.388	0.443	0.992
	Test C	73.45	2.56	5.73
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	79.34	4.46	9.98
% inhibition phase 1	Test A	20.86	2.81	6.28
	Test B	29.22	5.22	11.68
	Test C	37.04	4.65	10.41
	negative	0.00	0.00	0.00
	normal	100.00	0.00	0.00
	positive	45.45	3.00	6.70
% inhibition phase 2	Test A	13.41	2.35	5.26
	Test B	21.61	0.44	0.99
	Test C	26.55	2.56	5.73
	Negative	0.00	0.00	0.00
	Normal	100.00	0.00	0.00
	Positive	20.66	4.46	9.98

Appendix 5: Antinociceptive activity of DCM extract of *S. didymobotyra*

Variable	C1	Mean	SE Mean	StDev
phase 1	Test A	80.16	1.57	3.51
	Test B	66.57	1.98	4.42
	Test C	55.55	3.68	8.23
	negative	112.37	3.81	8.52
	normal	0.00	0.00	0.00
	positive	42.25	2.59	5.79
phase 2	Test A	110.77	3.56	7.95
	Test B	109.28	3.17	7.09
	Test C	83.61	3.37	7.54
	negative	163.96	7.65	17.10
	normal	0.00	0.00	0.00
	positive	75.27	5.01	11.20
% phase 1	Test A	71.76	3.44	7.68
	Test B	59.38	1.75	3.92
	Test C	49.77	3.99	8.93
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	37.85	2.84	6.36
% phase 2	Test A	68.10	3.70	8.28
	Test B	67.25	3.62	8.10
	Test C	51.60	3.79	8.48
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	46.60	4.70	10.52
% inhibition phase 1	Test A	28.24	3.44	7.68
	Test B	40.62	1.75	3.92
	Test C	50.23	3.99	8.93
	Negative	0.00	0.00	0.00
	normal	100.00	0.00	0.00
	Positive	62.15	2.84	6.36
% inhibition phase 2	Test A	31.90	3.70	8.28
	Test B	32.75	3.62	8.10
	Test C	48.40	3.79	8.48
	negative	0.00	0.00	0.00
	normal	100.00	0.00	0.00
	positive	53.40	4.70	10.52

Appendix 6: Antinociceptive activity of MeoH extract of *B. pilosa*

Variable	C1	Mean	SE Mean	StDev
phase 1	Test A	88.75	3.11	6.95
	Test B	66.32	4.32	9.66
	Test C	63.73	5.72	12.80
	negative	118.57	0.783	1.75
	normal	0.00	0.00	0.00
	positive	55.31	7.06	15.79
phase 2	Test A	136.27	9.82	21.95
	Test B	124.38	8.13	18.19
	Test C	117.30	5.40	12.08
	negative	190.41	1.64	3.66
	normal	0.00	0.00	0.00
	positive	100.51	3.26	7.29
% phase 1	Test A	74.81	2.29	5.13
	Test B	55.93	3.61	8.08
	Test C	53.84	5.11	11.42
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	46.77	6.23	13.93
% phase 2	Test A	71.64	5.39	12.05
	Test B	65.23	3.90	8.72
	Test C	61.63	2.92	6.53
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	52.78	1.55	3.46
% inhibition phase 1	Test A	25.19	2.29	5.13
	Test B	44.07	3.61	8.08
	Test C	46.16	5.11	11.42
	negative	0.00	0.00	0.00
	normal	100.00	0.00	0.00
	positive	53.23	6.23	13.93
% inhibition phase 2	Test A	28.36	5.39	12.05
	Test B	34.77	3.90	8.72
	Test C	38.37	2.92	6.53
	negative	0.00	0.00	0.00
	normal	100.00	0.00	0.00
	positive	47.22	1.55	3.46

Appendix 7: Antinociceptive activity of DCM extract of *B. pilosa*

Variable	C1	Mean	SE Mean	StDev
phase 1	Test A	87.57	5.58	12.48
	Test B	86.18	4.93	11.01
	Test C	77.79	6.60	14.76
	negative	118.48	2.27	5.07
	normal	0.00	0.00	0.00
	positive	64.36	5.09	11.38
phase 2	Test A	154.82	4.17	9.32
	Test B	143.72	3.25	7.27
	Test C	120.98	7.98	17.85
	negative	187.34	1.82	4.06
	normal	0.00	0.00	0.00
	positive	101.52	6.56	14.66
% phase 1	Test A	73.81	4.17	9.31
	Test B	73.03	5.12	11.46
	Test C	65.49	4.94	11.05
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	54.45	4.54	10.16
% phase 2	Test A	82.59	1.55	3.48
	Test B	76.68	1.04	2.32
	Test C	64.69	4.67	10.43
	negative	100.00	0.00	0.00
	normal	0.00	0.00	0.00
	positive	54.15	3.27	7.32
% inhibition phase 1	Test A	26.19	4.17	9.31
	Test B	26.97	5.12	11.46
	Test C	34.51	4.94	11.05
	Negative	0.000000	0.000000	0.000000
	normal	100.00	0.000000	0.000000
	Positive	45.55	4.54	10.16
% inhibition phase 2	Test A	17.41	1.55	3.48
	Test B	23.32	1.04	2.32
	Test C	35.31	4.67	10.43
	negative	0.000000	0.000000	0.000000
	normal	100.00	0.000000	0.000000
	Positive	45.85	3.27	7.32

Appendix 8: Antinociceptive activity of MeOH extract of *M. indica*

Variable	C1	Mean	SE Mean	StDev
phase 1	Test A	77.39	5.34	11.94
	Test B	61.88	2.73	6.11
	Test C	57.33	3.76	8.42
	Negative	111.00	3.43	7.66
	normal	0.000000	0.000000	0.000000
	Positive	44.26	5.35	11.96
phase 2	Test A	148.74	2.68	6.00
	Test B	131.38	7.15	16.00
	Test C	129.33	8.74	19.54
	negative	171.34	6.19	13.85
	normal	0.000000	0.000000	0.000000
	Positive	110.76	3.16	7.06
% phase 1	Test A	69.87	5.01	11.21
	Test B	56.02	3.43	7.66
	Test C	51.87	3.96	8.86
	negative	100.00	0.000000	0.000000
	normal	0.000000	0.000000	0.000000
	Positive	39.83	4.54	10.15
% phase 2	Test A	87.39	4.32	9.67
	Test B	76.87	3.92	8.78
	Test C	76.27	6.80	15.21
	negative	100.00	0.000000	0.000000
	normal	0.000000	0.000000	0.000000
	Positive	64.83	1.90	4.25
% inhibition phase 1	Test A	30.13	5.01	11.21
	Test B	43.98	3.43	7.66
	Test C	48.13	3.96	8.86
	negative	0.000000	0.000000	0.000000
	normal	100.00	0.000000	0.000000
	Positive	60.17	4.54	10.15
% inhibition phase 2	Test A	12.61	4.32	9.67
	Test B	23.13	3.92	8.78
	Test C	23.73	6.80	15.21
	negative	0.000000	0.000000	0.000000
	normal	100.00	0.000000	0.000000
	Positive	35.17	1.90	4.25

Appendix 9: Antinociceptive activity of DCM extract of *M. indica*

Variable	C1	Mean	SE Mean	StDev
phase 1	Test A	92.69	3.84	8.60
	Test B	70.32	3.57	7.99
	Test C	61.51	6.58	14.72
	negative	109.43	2.81	6.28
	normal	0.000000	0.000000	0.000000
	Positive	54.52	4.46	9.98
phase 2	Test A	123.40	6.61	14.79
	Test B	119.13	7.52	16.82
	Test C	104.36	6.20	13.85
	negative	145.71	3.42	7.64
	normal	0.000000	0.000000	0.000000
	positive	106.38	4.08	9.12
% phase 1	Test A	85.25	5.50	12.31
	Test B	64.44	3.84	8.59
	Test C	56.90	7.41	16.56
	negative	100.00	0.000000	0.000000
	normal	0.000000	0.000000	0.000000
	positive	50.00	4.52	10.11
% phase 2	Test A	85.23	6.25	13.98
	Test B	82.28	6.65	14.86
	Test C	71.49	3.23	7.23
	negative	100.00	0.000000	0.000000
	normal	0.000000	0.000000	0.000000
	Positive	73.16	3.26	7.29
% inhibition phase 1	Test A	14.75	5.50	12.31
	Test B	35.56	3.84	8.59
	Test C	43.10	7.41	16.56
	negative	0.000000	0.000000	0.000000
	normal	100.00	0.000000	0.000000
	Positive	50.00	4.52	10.11
% inhibition phase 2	Test A	14.77	6.25	13.98
	Test B	17.72	6.65	14.86
	Test C	28.51	3.23	7.23
	negative	0.000000	0.000000	0.000000
	normal	100.00	0.000000	0.000000
	Positive	26.84	3.26	7.29

Appendix 10: Anti-inflammation activity of MeOH extract of *S. didymobotyra*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	3.5000	0.0316	0.0707
	Test B	3.4800	0.0374	0.0837
	Test C	3.5000	0.0548	0.1225
	Negative	3.4800	0.0374	0.0837
	normal	2.4000	0.0447	0.1000
	Positive	3.5200	0.0374	0.0837
1 hour	Test A	3.3000	0.0447	0.1000
	Test B	3.3600	0.0678	0.1517
	Test C	3.2800	0.0490	0.1095
	negative	3.5800	0.0374	0.0837
	normal	2.4200	0.0490	0.1095
	positive	3.3000	0.0447	0.1000
2 hour	Test A	3.1000	0.0447	0.1000
	Test B	3.1400	0.0872	0.1949
	Test C	3.0400	0.0510	0.1140
	negative	3.6000	0.0316	0.0707
	normal	2.4000	0.0447	0.1000
	Positive	3.0600	0.0245	0.0548
3 hour	Test A	2.8600	0.0510	0.1140
	Test B	2.8800	0.0860	0.1924
	Test C	2.8000	0.0548	0.1225
	negative	3.5800	0.0490	0.1095
	normal	2.4600	0.0245	0.0548
	Positive	2.8400	0.0400	0.0894
4 hour	Test A	2.5600	0.0678	0.1517
	Test B	2.5800	0.0583	0.1304
	Test C	2.5600	0.0510	0.1140
	negative	3.5200	0.0374	0.0837
	normal	2.4000	0.0548	0.1225
	Positive	2.6200	0.0374	0.0837

Appendix 11: Anti-inflammation activity of DCM extract of *S. didymobotyra*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	3.5400	0.0510	0.1140
	Test B	3.5600	0.0510	0.1140
	Test C	3.5600	0.0510	0.1140
	Negative	3.5000	0.0707	0.1581
	normal	2.4800	0.0374	0.0837
	Positive	3.5800	0.0374	0.0837
1 hour	Test A	3.3200	0.0583	0.1304
	Test B	3.3600	0.0510	0.1140
	Test C	3.3200	0.0583	0.1304
	negative	3.6000	0.0707	0.1581
	normal	2.5200	0.0374	0.0837
	Positive	3.3200	0.0583	0.1304
2 hour	Test A	3.1000	0.0316	0.0707
	Test B	3.1000	0.0316	0.0707
	Test C	2.9800	0.0374	0.0837
	negative	3.6000	0.0707	0.1581
	normal	2.5000	0.0316	0.0707
	Positive	2.9800	0.0583	0.1304
3 hour	Test A	2.8200	0.0374	0.0837
	Test B	2.7800	0.0583	0.1304
	Test C	2.7200	0.0374	0.0837
	negative	3.6000	0.0707	0.1581
	normal	2.5200	0.0374	0.0837
	Positive	2.6800	0.0374	0.0837
4 hour	Test A	2.6400	0.0510	0.1140
	Test B	2.5400	0.0510	0.1140
	Test C	2.5000	0.0316	0.0707
	Negative	3.5600	0.0678	0.1517
	normal	2.4800	0.0200	0.0447
	Positive	2.4600	0.0245	0.0548

Appendix 12: Anti-inflammation activity of MeOH extract of *B. pilosa*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	3.4800	0.0374	0.0837
	Test B	3.4400	0.0510	0.1140
	Test C	3.4800	0.0374	0.0837
	negative	3.5200	0.0374	0.0837
	normal	2.3800	0.0800	0.1789
	Positive	3.4800	0.0583	0.1304
1 hour	Test A	3.3000	0.0447	0.1000
	Test B	3.3200	0.0583	0.1304
	Test C	3.2600	0.0510	0.1140
	negative	3.6200	0.0374	0.0837
	normal	2.3000	0.0949	0.2121
	positive	3.4000	0.0447	0.1000
2 hour	Test A	3.1000	0.0447	0.1000
	Test B	3.1200	0.0583	0.1304
	Test C	2.9800	0.0374	0.0837
	negative	3.6200	0.0374	0.0837
	normal	2.360	0.103	0.230
	Positive	3.2000	0.0316	0.0707
3 hour	Test A	2.8400	0.0245	0.0548
	Test B	2.9400	0.0510	0.1140
	Test C	2.8000	0.0447	0.1000
	negative	3.5400	0.0400	0.0894
	normal	2.3800	0.0860	0.1924
	Positive	2.9600	0.0245	0.0548
4 hour	Test A	2.7400	0.0245	0.0548
	Test B	2.7600	0.0400	0.0894
	Test C	2.5400	0.0510	0.1140
	negative	3.5200	0.0374	0.0837
	normal	2.3400	0.0927	0.2074
	positive	2.7600	0.0678	0.1517

Appendix 13: Anti-inflammation activity of DCM extract of *B. pilosa*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	3.4600	0.0510	0.1140
	Test B	3.4600	0.0245	0.0548
	Test C	3.4400	0.0245	0.0548
	negative	3.5000	0.0316	0.0707
	normal	2.3800	0.0583	0.1304
	positive	3.4400	0.0510	0.1140
1 hour	Test A	3.3400	0.0678	0.1517
	Test B	3.2000	0.0316	0.0707
	Test C	3.1600	0.0510	0.1140
	negative	3.5800	0.0374	0.0837
	normal	2.4000	0.0316	0.0707
	Positive	3.2400	0.0510	0.1140
2 hour	Test A	3.1200	0.0374	0.0837
	Test B	2.9600	0.0510	0.1140
	Test C	2.9200	0.0490	0.1095
	negative	3.5800	0.0200	0.0447
	normal	2.3600	0.0510	0.1140
	Positive	3.0200	0.0374	0.0837
3 hour	Test A	2.9200	0.0374	0.0837
	Test B	2.7400	0.0510	0.1140
	Test C	2.7000	0.0316	0.0707
	negative	3.5600	0.0245	0.0548
	normal	2.3400	0.0400	0.0894
	positive	2.7600	0.0510	0.1140
4 hour	Test A	2.8000	0.0447	0.1000
	Test B	2.5400	0.0510	0.1140
	Test C	2.5000	0.0316	0.0707
	negative	3.5000	0.0316	0.0707
	normal	2.4000	0.0548	0.1225
	Positive	2.5600	0.0510	0.1140

Appendix 14: Anti-inflammation activity of MeOH extract of *M. indica*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	3.3400	0.0510	0.1140
	Test B	3.4800	0.0374	0.0837
	Test C	3.3600	0.0510	0.1140
	negative	3.180	0.171	0.383
	normal	2.5000	0.0707	0.1581
	positive	3.5600	0.0927	0.2074
1 hour	Test A	3.1800	0.0374	0.0837
	Test B	3.2600	0.0400	0.0894
	Test C	3.2000	0.0548	0.1225
	negative	3.240	0.166	0.371
	normal	2.4400	0.0510	0.1140
	Positive	3.3800	0.0800	0.1789
2 hour	Test A	3.0400	0.0245	0.0548
	Test B	3.0800	0.0374	0.0837
	Test C	2.9800	0.0374	0.0837
	negative	3.300	0.179	0.400
	normal	2.5000	0.0707	0.1581
	Positive	3.1800	0.0800	0.1789
3 hour	Test A	2.8000	0.0316	0.0707
	Test B	2.7800	0.0374	0.0837
	Test C	2.7800	0.0374	0.0837
	Negative	3.300	0.179	0.400
	normal	2.4400	0.0510	0.1140
	Positive	2.9400	0.0812	0.1817
4 hour	Test A	2.6400	0.0245	0.0548
	Test B	2.6000	0.0447	0.1000
	Test C	2.5800	0.0374	0.0837
	Negative	3.220	0.183	0.409
	normal	2.4600	0.0678	0.1517
	Positive	2.7000	0.0707	0.1581

Appendix 15: Anti-inflammation activity of MeOH extract of *E. saligna*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	3.4000	0.0316	0.0707
	Test B	3.4400	0.0245	0.0548
	Test C	3.3800	0.0374	0.0837
	negative	3.5000	0.0707	0.1581
	normal	2.5200	0.0735	0.1643
	positive	3.4400	0.0510	0.1140
1 hour	Test A	3.2600	0.0400	0.0894
	Test B	3.3400	0.0510	0.1140
	Test C	3.3000	0.0632	0.1414
	negative	3.6200	0.0663	0.1483
	normal	2.5200	0.0583	0.1304
	Positive	3.1800	0.0490	0.1095
2 hour	Test A	3.0800	0.0374	0.0837
	Test B	3.1600	0.0245	0.0548
	Test C	3.1600	0.0600	0.1342
	negative	3.6400	0.0510	0.1140
	normal	2.5000	0.0548	0.1225
	Positive	3.0000	0.0316	0.0707
3 hour	Test A	2.8600	0.0510	0.1140
	Test B	2.9800	0.0374	0.0837
	Test C	3.0400	0.0510	0.1140
	negative	3.6000	0.0632	0.1414
	normal	2.5400	0.0510	0.1140
	Positive	2.7800	0.0374	0.0837
4 hour	Test A	2.7200	0.0374	0.0837
	Test B	2.8000	0.0548	0.1225
	Test C	2.8400	0.0510	0.1140
	negative	3.5200	0.0663	0.1483
	normal	2.5000	0.0447	0.1000
	Positive	2.4600	0.0510	0.1140

Appendix 16: Anti-inflammation activity of DCM extract of *E. saligna*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	3.4000	0.0548	0.1225
	Test B	3.3800	0.0490	0.1095
	Test C	3.4000	0.0707	0.1581
	negative	3.4400	0.0510	0.1140
	normal	2.4400	0.0510	0.1140
	Positive	3.240	0.103	0.230
1 hour	Test A	3.1800	0.0583	0.1304
	Test B	3.3000	0.0316	0.0707
	Test C	3.2200	0.0800	0.1789
	Negative	3.6000	0.0447	0.1000
	normal	2.4600	0.0678	0.1517
	positive	3.1800	0.0663	0.1483
2 hour	Test A	2.9600	0.0510	0.1140
	Test B	3.1600	0.0245	0.0548
	Test C	3.0600	0.0927	0.2074
	negative	3.6000	0.0548	0.1225
	normal	2.4800	0.0583	0.1304
	positive	3.360	0.147	0.329
3 hour	Test A	2.7200	0.0374	0.0837
	Test B	3.0400	0.0510	0.1140
	Test C	2.9200	0.0735	0.1643
	Negative	3.6000	0.0548	0.1225
	normal	2.4600	0.0812	0.1817
	Positive	3.1200	0.0583	0.1304
4 hour	Test A	2.7000	0.0316	0.0707
	Test B	2.8400	0.0510	0.1140
	Test C	2.8000	0.0548	0.1225
	Negative	3.5400	0.0678	0.1517
	normal	2.4600	0.0600	0.1342
	Positive	3.0200	0.0200	0.0447

Appendix 17: Antipyretic activity of MeOH extract of *P. africana*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	39.040	0.108	0.241
	Test A	38.920	0.0800	0.179
	Test A	38.940	0.129	0.288
	negative	38.880	0.0800	0.179
	normal	37.720	0.153	0.342
	positive	38.800	0.152	0.339
1 hour	Test A	38.680	0.0663	0.148
	Test B	38.620	0.0374	0.0837
	Test C	38.540	0.199	0.445
	Negative	38.960	0.0812	0.182
	normal	37.380	0.111	0.249
	positive	38.580	0.128	0.286
2 hour	Test A	38.260	0.0400	0.0894
	Test B	38.260	0.0400	0.0894
	Test C	38.240	0.175	0.391
	Negative	39.040	0.0678	0.152
	normal	37.620	0.0490	0.110
	Positive	38.260	0.108	0.241
3 hour	Test A	37.920	0.0374	0.0837
	Test B	37.980	0.0374	0.0837
	Test C	37.980	0.177	0.396
	Negative	39.000	0.0447	0.100
	normal	37.780	0.0663	0.148
	positive	37.900	0.141	0.316
4 hour	Test A	37.660	0.0600	0.134
	Test B	37.680	0.0374	0.0837
	Test C	37.680	0.116	0.259
	Negative	38.880	0.0583	0.130
	normal	37.560	0.0980	0.219
	Positive	37.460	0.133	0.297

Appendix 18: Antipyretic activity of DCM extract of *P. africana*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	38.900	0.0548	0.122
	Test B	38.960	0.121	0.270
	Test C	38.860	0.0510	0.114
	Negative	38.720	0.0735	0.164
	normal	37.340	0.0600	0.134
	Positive	38.960	0.150	0.336
1 hour	Test A	38.580	0.0490	0.110
	Test B	38.640	0.136	0.305
	Test C	38.480	0.0583	0.130
	Negative	38.840	0.0678	0.152
	normal	37.440	0.0510	0.114
	Positive	38.700	0.164	0.367
2 hour	Test A	38.260	0.0400	0.0894
	Test B	38.260	0.186	0.416
	Test C	38.160	0.0245	0.0548
	Negative	38.840	0.0872	0.195
	normal	37.400	0.0548	0.122
	Positive	38.540	0.140	0.313
3 hour	Test A	37.940	0.0400	0.0894
	Test B	37.840	0.140	0.313
	Test C	37.880	0.0374	0.0837
	Negative	38.820	0.0735	0.164
	normal	37.640	0.103	0.230
	Positive	38.340	0.154	0.344
4 hour	Test A	37.600	0.0548	0.122
	Test B	37.660	0.129	0.288
	Test C	37.660	0.0400	0.0894
	negative	38.700	0.0632	0.141
	normal	37.540	0.0872	0.195
	Positive	37.920	0.183	0.409

Appendix 19: Antipyretic activity of MeOH extract of *S. didymobotyra*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	38.960	0.0812	0.182
	Test B	38.940	0.0748	0.167
	Test C	38.820	0.0374	0.0837
	negative	38.900	0.167	0.374
	Normal	37.440	0.0510	0.114
	Positive	38.420	0.111	0.249
1 hour	Test A	38.540	0.0510	0.114
	Test B	38.560	0.0748	0.167
	Test C	38.560	0.0510	0.114
	Negative	38.940	0.154	0.344
	normal	37.180	0.0917	0.205
	Positive	38.000	0.221	0.495
2 hour	Test A	38.240	0.0510	0.114
	Test B	38.180	0.0663	0.148
	Test C	38.200	0.000000	0.000000
	negative	39.000	0.167	0.374
	normal	37.320	0.0583	0.130
	positive	37.120	0.235	0.526
3 hour	Test A	37.900	0.0707	0.158
	Test B	37.940	0.0510	0.114
	Test C	37.860	0.0245	0.0548
	Negative	39.040	0.154	0.344
	normal	37.300	0.0894	0.200
	Positive	37.200	0.118	0.265
4 hour	Test A	37.440	0.0510	0.114
	Test B	37.600	0.0316	0.0707
	Test C	37.600	0.0316	0.0707
	Negative	38.900	0.167	0.374
	normal	37.360	0.157	0.351
	Positive	36.960	0.0510	0.114

Appendix 20: Antipyretic activity of DCM extract of *S. didymobotyra*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	38.880	0.0735	0.164
	Test B	38.800	0.0447	0.100
	Test C	39.160	0.0812	0.182
	Negative	38.760	0.0400	0.0894
	normal	37.440	0.0678	0.152
	Positive	38.560	0.254	0.568
1 hour	Test A	38.600	0.0447	0.100
	Test B	38.500	0.0316	0.0707
	Test C	38.860	0.0600	0.134
	Negative	38.820	0.0374	0.0837
	normal	37.340	0.0927	0.207
	Positive	37.940	0.216	0.483
2 hour	Test A	38.240	0.0510	0.114
	Test B	38.160	0.0245	0.0548
	Test C	38.480	0.0860	0.192
	Negative	38.880	0.0374	0.0837
	normal	37.400	0.0632	0.141
	Positive	36.960	0.201	0.451
3 hour	Test A	37.920	0.0583	0.130
	Test B	37.860	0.0245	0.0548
	Test C	38.040	0.0748	0.167
	Negative	38.940	0.0510	0.114
	normal	37.360	0.103	0.230
	Positive	37.200	0.0837	0.187
4 hour	Test A	37.620	0.0735	0.164
	Test B	37.600	0.0316	0.0707
	Test C	37.580	0.0490	0.110
	Negative	38.880	0.0374	0.0837
	normal	37.300	0.134	0.300
	Positive	36.960	0.0245	0.0548

Appendix 21: Antipyretic activity of MeOH extract of *B. pilosa*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	38.940	0.0927	0.207
	Test B	38.860	0.0927	0.207
	Test C	38.940	0.108	0.241
	Negative	38.980	0.111	0.249
	normal	37.440	0.117	0.261
	Positive	38.760	0.108	0.241
1 hour	Test A	38.620	0.0490	0.110
	Test B	38.560	0.0678	0.152
	Test C	38.680	0.0860	0.192
	Negative	39.120	0.0800	0.179
	normal	37.400	0.0837	0.187
	Positive	38.520	0.132	0.295
2 hour	Test A	38.340	0.0600	0.134
	Test B	38.260	0.0400	0.0894
	Test C	38.300	0.0707	0.158
	Negative	39.220	0.0800	0.179
	normal	37.400	0.0548	0.122
	positive	38.300	0.105	0.235
3 hour	Test A	37.980	0.0663	0.148
	Test B	37.940	0.0245	0.0548
	Test C	37.920	0.0374	0.0837
	negative	39.160	0.103	0.230
	normal	37.480	0.0583	0.130
	Positive	37.920	0.124	0.277
4 hour	Test A	37.880	0.102	0.228
	Test B	37.640	0.0510	0.114
	Test C	37.860	0.112	0.251
	negative	39.060	0.103	0.230
	Normal	37.560	0.0678	0.152
	positive	38.200	0.192	0.430

Appendix 22: Antipyretic activity of DCM extract of *B. pilosa*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	38.960	0.0872	0.195
	Test B	38.800	0.0837	0.187
	Test C	38.960	0.103	0.230
	Negative	38.720	0.0583	0.130
	normal	37.540	0.144	0.321
	positive	39.020	0.116	0.259
1 hour	Test A	38.740	0.0748	0.167
	Test B	38.540	0.0510	0.114
	Test C	38.700	0.0837	0.187
	negative	38.880	0.0200	0.0447
	normal	37.540	0.125	0.279
	Positive	38.760	0.0748	0.167
2 hour	Test A	38.380	0.0583	0.130
	Test B	38.220	0.0374	0.0837
	Test C	38.420	0.0970	0.217
	negative	38.940	0.0748	0.167
	normal	37.420	0.0490	0.110
	positive	38.420	0.0800	0.179
3 hour	Test A	37.980	0.0583	0.130
	Test B	37.880	0.0374	0.0837
	Test C	38.020	0.0800	0.179
	negative	38.920	0.0800	0.179
	normal	37.440	0.0678	0.152
	positive	38.040	0.0678	0.152
4 hour	Test A	37.640	0.0678	0.152
	Test B	37.640	0.0510	0.114
	Test C	37.540	0.0510	0.114
	negative	38.840	0.0678	0.152
	normal	37.500	0.0837	0.187
	Positive	37.620	0.0663	0.148

Appendix 23: Antipyretic activity of MeOH extract of *M. indica*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	38.980	0.0970	0.217
	Test B	39.160	0.129	0.288
	Test C	39.060	0.0927	0.207
	negative	38.760	0.117	0.261
	normal	37.300	0.130	0.292
	positive	38.840	0.0927	0.207
1 hour	Test A	38.800	0.0837	0.187
	Test B	38.920	0.0970	0.217
	Test C	38.740	0.0927	0.207
	negative	38.860	0.121	0.270
	normal	37.360	0.0748	0.167
	positive	38.580	0.136	0.303
2 hour	Test A	38.500	0.0447	0.100
	Test B	38.660	0.0748	0.167
	Test C	38.400	0.0894	0.200
	negative	38.880	0.124	0.277
	normal	37.360	0.121	0.270
	Positive	38.160	0.172	0.385
3 hour	Test A	38.060	0.0812	0.182
	Test B	38.200	0.0837	0.187
	Test C	37.940	0.0400	0.0894
	negative	38.820	0.132	0.295
	normal	37.400	0.141	0.316
	Positive	37.680	0.171	0.383
4 hour	Test A	37.760	0.0510	0.114
	Test B	37.620	0.0490	0.110
	Test C	37.460	0.0748	0.167
	negative	38.680	0.111	0.249
	normal	37.420	0.128	0.286
	Positive	37.320	0.136	0.303

Appendix 24: Antipyretic activity of DCM extract of *M. indica*

Variable	C1	Mean	SE Mean	StDev
0 hour	Test A	38.860	0.0748	0.167
	Test B	38.840	0.103	0.230
	Test C	38.980	0.116	0.259
	negative	38.720	0.0800	0.179
	normal	37.120	0.116	0.259
	Positive	38.920	0.0860	0.192
1 hour	Test A	38.680	0.0663	0.148
	Test B	38.620	0.0583	0.130
	Test C	38.720	0.0970	0.217
	negative	38.660	0.0678	0.152
	normal	37.060	0.0510	0.114
	Positive	38.680	0.0663	0.148
2 hour	Test A	38.520	0.0583	0.130
	Test B	38.320	0.0374	0.0837
	Test C	38.360	0.0812	0.182
	negative	38.580	0.0583	0.130
	normal	37.180	0.0663	0.148
	Positive	38.360	0.0510	0.114
3 hour	Test A	38.280	0.0735	0.164
	Test B	37.960	0.0245	0.0548
	Test C	37.980	0.0735	0.164
	negative	38.360	0.0748	0.167
	normal	37.180	0.0583	0.130
	Positive	38.060	0.0748	0.167
4 hour	Test A	37.960	0.0678	0.152
	Test B	37.640	0.0510	0.114
	Test C	37.720	0.0374	0.0837
	negative	38.200	0.0707	0.158
	normal	37.360	0.0678	0.152
	Positive	37.740	0.0600	0.134

Appendix 25: Research authorization

NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION

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Ref No. **NACOSTI/P/16/07049/14237**

Date
16th December, 2016

Godfrey Mutuma Gitonga
Kenyatta University
P.O. Box 43844 – 00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on **"Phytochemical, anti-inflammatory, antinociceptive, antipyretic and toxicity analysis of dichloromethane and methanol extracts of five selected plants using animal model"** I am pleased to inform you that you have been authorized to undertake research in Nairobi County for the period ending **15th December, 2017.**

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

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

BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

County Commissioner
Nairobi County

The County Director of Education
Nairobi County