

**UNVEILING BIOACTIVE POTENTIAL OF METHANOL AND
DICHLOROMETHANE EXTRACTS OF *Ficus sycomorus* L: LINKING
PHYTOCHEMICAL COMPOSITION TO ANTI-INFLAMMATORY ACTIVITY**

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DECLARATION

I, Muthee Eunice Wothaya, duly declare that this thesis is my original work and has not been presented for a degree in any other university or for any other award.

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DEDICATION

I dedicate this thesis to my parents Joseph Muriuki and Grace Muthoni for laying a solid academic foundation in my life. Special dedication also goes to my husband Benjamin Mwangi for his sacrifice and financial support.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
bw	Bodyweight
COX	Cyclooxygenase
CRP	C-reactive protein
GC-MS	Gas Chromatography-Mass Spectrometry
IFN-γ	Interferon-gamma
IL	Interleukins
IκBα	Inhibitor of nuclear factor kappa B
LC-MS	Liquid Chromatography-Mass Spectrometry
MAPK	Mitogen-activator protein kinase
NF-κB	Nuclear factor-kappa B
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
PGE2	Prostaglandin E2
ROS	Reactive oxygen species
TNF-α	Tumor Necrosis Factor-alpha
WHO	World Health Organization

ABSTRACT

Inflammation is the mechanism by which the immune system initiates the healing process. Inflammation is treated using synthetic drugs linked to severe effects and resistance necessitating the need for safer alternatives. Herbal remedies have active phytochemicals that are highly effective in treating inflammation. They are arguably affordable and with fewer severe effects, which makes them suitable alternative agents. *Ficus sycomorus* is used by Mbeere community to treat inflammation but there lacks the science-based data to support the claim. This study aimed at determining anti-edema properties of DCM and MeOH stem bark and leaf extracts of *F. sycomorus* in mice, as well as quantitative phytochemical analysis. The medicinal plant samples were collected from Embu County, Kenya. The plant was identified by a taxonomist at the National Museums of Kenya. Dichloromethane and methanol were used in the extraction. Liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) techniques were used for phytochemical analysis. Swiss albino mice aged from seven to eight weeks and weighing between 20 and 22 g. The animals were randomly classified into six groups (n=5): normal control, negative control, positive control, and three experimental groups. The normal control mice received normal saline (0.1 ml). Carrageenan (0.1 ml) was used to induce edema. One hour after edema induction, the negative and positive control mice intraperitoneally received 0.1 ml of normal saline and diclofenac (0.1 ml-15 mg/kg bw), respectively. The three experimental groups intraperitoneally received the extracts at dosages of 50, 100, and 200 mg/kg bw. The study employed one factorial analysis of variance to compute for significant variations across the groups under investigation. In case of statistical variations, Tukey's post hoc was employed. The significance threshold was inferred at $p < 0.05$. Results revealed the extracts' anti-inflammatory effects ranging from 2.67 to 16.54%. The Me OH extracts showed the best effects (16.54%) at dose of 200 mg/kg bw compared with DCM extracts. The LC-MS analysis identified ferulic acid – phenolic (14.27 %) as the highest abundant phytochemical followed by catechin - flavonoid (16.87%). The most abundant compound identified by GC-MS was zierone – terpenoid (40.68%). Some of these phytochemicals are linked to anti-inflammatory effects. Conclusions drawn from the study is that the extracts can be used in the development of alternative therapeutic agents with anti-inflammatory effect. The study recommends assessment of safety profiles and isolation of fractions of the studied extracts.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The term "inflammation" (phlogosis) is defined as the body's immunological reaction to tissue damage caused by exposure to infections, radiation, and noxious chemicals, as well as trauma (Chen *et al.*, 2017). It is marked by pathophysiological responses such as accumulation of blood cells and plasmatic fluid in the area of injury (Hassanzadeh *et al.*, 2021). Acute and chronic inflammation are the two recognized types of inflammation. The former is a short-term reaction with localized effect and responds where a problem exists, whereas chronic inflammation has a long-term and whole-body effects and produces mild inflammation in the entire body, which contributes to progression of ailments, including rheumatoid arthritis, multiple sclerosis, chronic asthma, colitis and psoriasis, bronchitis, and dermatitis (Chiurchiù *et al.*, 2018; Elsayed and Norredin, 2019; Pahwa *et al.*, 2023).

Globally, the leading causes of significant mortality are chronic inflammatory diseases (Barcelos *et al.*, 2019). The WHO (World Health Organization) recognizes chronic illnesses as a public health concern. Over 50% of all deaths worldwide are attributable to chronic inflammatory diseases, including neurodegenerative ailments, chronic kidney disease, autoimmune disorders, stroke, liver disease, diabetes mellitus, cancer, and ischemic heart disease (Furman *et al.*, 2019).

Although inflammation is important to the well-being of the body, it subjects individuals to discomfort and distress. Essential inflammation may be irritating, while excessive

inflammatory reaction may present deleterious effects on an individual (Furman *et al.*, 2019). The treatment of inflammation often involves the use of non-steroidal anti-inflammatory drugs. Among these medications are Ibuprofen, Etoricoxib, Celecoxib, Naproxen, Indomethacin, and Diclofenac (Wongrakpanich *et al.*, 2018). Nonetheless, these conventional medicines are known to possess numerous side effects, including gastrointestinal, kidney, liver, and heart toxicities, among others (Bindu *et al.*, 2020).

Medicinal plants are also used to manage inflammation (Oguntibeju, 2018). According to Khan and Ahmad (2019), the WHO estimates that 80% of people in underdeveloped nations treat illnesses and disorders using medicinal plants. The demand for herbal medicines has risen as a result of consumers realizing that natural products have fewer adverse effects, are accessible, and are more affordable (Jiang *et al.*, 2022; Khumalo *et al.*, 2022). Many communities residing in rural areas use herbal formulations periodically owing to their cultural beliefs (James *et al.*, 2018). Herbal medicines have become increasingly popular and widely used in the past ten years (Zhang *et al.*, 2018).

Herbal formulations are crucial sources for development of novel therapeutic agents (Atanasov, 2021). Under stressful condition, medicinal plants often synthesize secondary metabolites (Hussein *et al.*, 2019). These metabolites possess various pharmacological effects against various ailments and disorders. The potency of herbal medicines is attributed to a number of phytochemicals, including steroids, saponins, terpenoids, tannins, flavonoids, alkaloids and phenolic acids (Batiha *et al.*, 2020). These phytochemicals are known to alleviate inflammation (Shingala *et al.*, 2021).

Research investigations are currently being conducted to assess whether the traditional medicinal uses of medicinal herbs are supported by empirical scientific data. *Ficus sycomorus L.*, commonly known as mulberry fig, is used in treatment of inflammation among populations living in Embu County, Kenya. Nonetheless, the scientific evidence supporting the acclaimed medicinal use of *F. sycomorus* was lacking. The current investigation, therefore, aimed to determine the *in vivo* anti-edema potential and quantitative phytochemical profiles of methanol and dichloromethane extracts of *Ficus sycomorus L.*

1.2 Statement of the Problem

Inflammation reduces the victims' productivity and results in pain, discomfort and distress. Synthetic drugs used to treat inflammation include Glucocorticoids (Budesonide, Beclomethasone, Betamethasone, Dexamethasone, and Hydrocortisone) (Berardicurti *et al.*, 2020; Yasir *et al.*, 2020) and NSAIDs (such as Naproxen Celecoxib Ibuprofen, and Diclofenac) (Bindu *et al.*, 2020). Nevertheless, these drugs are known to possess severe effects, including gastrointestinal (gastrointestinal bleeding and peptic ulcers), liver and kidney toxicities, as well as cardiovascular diseases (Gokul *et al.*, 2023) and development of resistance. There is therefore, the necessity for alternative anti-inflammatory drugs.

Ficus sycomorus has widely been used by communities living in Embu County to treat inflammation. Despite its extensive traditional use in Embu County, no published pharmacological studies have validated its anti-inflammatory effects or identified the specific bio active phytochemicals responsible.

1.3 Justification of the Study

Medicinal plants are important bio-resource for providing human health needs (Dar *et al.*, 2017). The use herbal remedies considered natural with minimal side effects (Boy *et al.*, 2018; Goel and Kulshrestha, 2021). Medicinal plants are endowed with phytochemicals with anti-edema properties and therefore may be crucial in developing novel anti-inflammatory agents (Akhtar, 2022). The continued use of herbal formulations with anti-inflammatory effects have necessitated the demand for evidence-based efficacy on herbal formulations. The current study aims to bridge this gap by conducting an in depth evaluation of the anti-inflammatory effect of methanol and dichloromethane extracts of *F. sycomorus* L using an *in vivo* murine model. Additionally, it will provide comprehensive phytochemical profiles of the extracts identifying the key active compounds with potential anti-inflammatory activity.

1.4 Research Questions

- i. Do methanol and dichloromethane leaf and stem bark extracts of *Ficus sycomorus* exhibit anti-inflammatory activity in mice?
- ii. What is the quantitative phytochemical composition of methanol and dichloromethane leaf and stem bark extracts of *Ficus sycomorus*?

1.5 Study Objectives

1.5.1 Main Objective

To evaluate the anti-inflammatory activity and determine the quantitative phytochemical composition of methanol and dichloromethane extracts from leaves and stem bark of *Ficus sycomorus*.

1.5.2 Specific Objectives

- i. To evaluate the anti-inflammatory effects of methanol and dichloromethane leaf and stem bark extracts of *Ficus sycomorus* in mice.
- ii. To determine the quantitative phytochemical composition of methanol and dichloromethane leaf and stem bark extracts of *Ficus sycomorus*.

1.6 Significance of Study

Ficus sycomorus is traditionally used in African medicine for treating inflammation-related disorders (wounds, arthritis, and gastrointestinal issues). However, scientific validation of its anti-inflammatory effect was limited. This study provides evidence-based data on its efficacy. Identification of bioactive compounds in methanol and dichloromethane extracts can reveal new lead molecules for drug development. Comparing methanol (polar) and dichloromethane (non-polar) extracts identifies which solvents yield the most potent anti-inflammatory compounds. This study filled the gap of knowledge on the anti-inflammatory effect of methanol and dichloromethane extracts of *Ficus sycomorus*. The information provide in this study may contribute in the development of new anti-inflammatory drugs with minimal side effects.

1.7 Scope and Limitations of Study

The scope of this study included *in vivo* validation of anti-inflammatory effect of methanol and dichloromethane extracts of *Ficus sycomorus* on carrageenan-induced paw edema. Also, the study identified phytochemicals of methanol and dichloromethane extracts of *Ficus sycomorus* using liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry, respectively. The study also compared the efficacies of methanol (polar) and dichloromethane (non-polar) extracts.

This study on the anti-inflammatory potential and phytochemical analysis of *Ficus sycomorus* extracts has several key limitations. The research is confined to methanol and dichloromethane extracts, potentially missing bioactive compounds soluble in other solvents. The phytochemical profiling identified major compounds but cannot fully capture potential synergistic effects between components.

The study lacks investigation into the extracts molecular mechanisms of action and did not assess long-term toxicity or pharmacokinetics. Additionally, variations in plant composition due to seasonal or environmental factors were not accounted for, which may affect reproducibility. Most importantly, the absence of clinical trials limits the direct applicability of findings to human therapeutics. These constraints suggest the need for more comprehensive follow-up studies to validate and expand upon these preliminary findings.

CHAPTER TWO

LITERATURE REVIEW

2.1 Inflammation

Inflammation talks about body immune reaction to tissue damage or harm caused by burns, insect's bites and stings, bacterial and fungal infections or chemicals and radiations exposure (Chen *et al.*, 2017). The damaged cells discharge chemical substances, including bradykinin, histamine, and prostaglandins. These chemicals induce blood vessels to leak fluid into the area where tissue damage has occurred, causing swelling (Bunman *et al.*, 2017).

2.1.1 Biochemistry of Inflammation

The inflammatory process is essential for immune monitoring, tissue regeneration, and tissue repair following injury (Choi *et al.*, 2023). Inflammation can result from many factors including infectious agents (such as *mycobacterium tuberculosis*, protozoa and fungi), exposure to irritant, trauma, autoimmune disorders, as well as inflammatory and biochemical inducers such as reactive oxygen species (Chen *et al.*, 2017). Proinflammatory mediators such interleukins-(1 β , 6, and 8), nitric oxide, reactive oxygen species (ROS), and prostaglandins can be produced during infections by bacteria (Kany *et al.*, 2019).

Periodic episodes of intense inflammation led to chronic inflammation, which causes blood vessels to dilate, plasma flow to rise, blood vessels to become more permeable, and

neutrophils to enter wounded tissue through the capillary wall. The composition of white blood cells also shifts when macrophages and lymphocytes begin to take over the transient neutrophils (Abdulkhaleq *et al.*, 2018). When entering the tissue location, the primary inflammatory cells, like macrophages, and lymphocytes, release growth factors, enzymes and pro-inflammatory cytokines. This accelerates tissue damage, secondary curative therapy, the development of granulomas, and fibrosis (Stone *et al.*, 2021).

In response to internal or external stimuli, tissue-susceptible cells release cytokines, specifically IL-1 β and TNF- α . These cells include dendritic cells and macrophages. As a result, the injury-location endothelium cells become unbound by integrins and selectins, which promote chemotaxis of the circulating leukocytes. Additionally, dendritic cells, neutrophils and monocytes recruitment stimulates cytokines synthesis, aids in the phagocytosis of the antigen, and serves as a cell that presents antigen to lymphocytes (Abdulkhaleq *et al.*, 2018).

Once the leukocytes have reached the site of local damage, the numerous cytokines and chemokines discharged by the macrophages and dendritic cells trigger the leukocytes to flow, which in turn discharge more inflammatory cytokines, and COX-2 (cyclooxygenase-2) and prostaglandin E2 (PGE2) (Stone *et al.*, 2021).

Neutrophils are the main cells in acute inflammation; they produce granules containing lysozyme, myeloperoxidase, and matrix metalloproteinases (Herrero-Cervera *et al.*, 2022). When they come into contact with an antigen, they cause its destruction (Aroca-Crevillén

et al., 2024). Additionally, neutrophils eliminate antigens by phagocytosing them, releasing ROS (reactive oxygen species), and synthesizing TNF- α , and IL-(6 and -1 β) (Paclet *et al.*, 2022). Lymphocytes such as B- and T-lymphocytes, make up the 2nd line of defense. The synthesis of antibodies and immunological complexes, the release of cytokines, lymphocyte activation, and other intricate processes are only a few of the complicated ways that lymphocytes contribute to inflammation (Abdulkhaleq *et al.*, 2018). Three processes by which circulating platelets may contribute to inflammation are platelet aggregation, and thrombus formation (Stone *et al.*, 2021).

2.1.2 Signs and Symptoms of Inflammation

It is characterized by swelling that develops when the fluids build up in the tissues, redness which happens when there is a proliferation in the blood stream to the capillaries in the affected area, heat that occurs as a result of increased blood flow in the affected area. Elevated capillary absorbency results in excess fibrinogen and other proteins leaking into the interstitial spaces, causing fluid to clot in the interstitial space, and substantial granulocytes and monocytes to migrate to the injured tissue (Stone *et al.*, 2021). Destruction to the structure, role, and consistency of nucleic acids, lipids and proteins (Zhang *et al.*, 2019).

2.1.3 Classifications of Inflammation

Two distinct types of inflammation exist: acute and chronic. The key indications of acute toxicity are pain, redness, loss of function heat and swelling (Chen *et al.*, 2017). This inflammation can be induced by tissue injury due to shock, microbial invasion, toxic compounds or touch of a hot substance. It starts fast, becomes severe in a short term and

response in minutes and hours, depending on the nature of the injury (Soliman and Barreda, 2022). Essentially, acute inflammation is categorized by exudation of plasma protein and fluid and migration of leukocytes mainly neutrophils and later macrophages resulting in edema. Some causes and infections that can lead to acute inflammation, including sore throat, acute bronchitis, trauma, wound, among others (Chen *et al.*, 2017).

An extended period of inflammation, ranging from many months to years, is known as chronic inflammation. Chronic inflammation develops due to persistent acute inflammation, autoinflammatory diseases and exposure to irritants (Chen *et al.*, 2017). Pro-inflammatory cytokines, ROS, metalloproteinases, inflammatory enzymes (cyclooxygenase-2), chemokines, together with activated macrophages, neutrophils, and T lymphocyte infiltrates are the hallmarks of chronic inflammation (Pahwa *et al.*, 2023).

The pro-inflammatory markers that are in high levels in chronic inflammation include IL-1 β , IL-8, IL-13, C-reactive protein and IFN- γ (interferon-gamma) (Ruparelia *et al.*, 2017). The increased pro-inflammatory molecules also stimulate the production of nitric acid, ROS, and platelets by immune cells like mast cells eosinophils, monocytes, and platelets, which in turns causes destruction to the structure, purpose, and integrity of nucleic acids, proteins, and lipids (Zhang *et al.*, 2019).

Chronic inflammation manifests as fever, skin rashes, repeated infections, depressive symptoms, and physical pain (Furman *et al.*, 2019; Pahwa *et al.*, 2023). Numerous illnesses, such as inflammatory asthma, psoriasis, idiopathic pulmonary fibrosis,

rheumatoid arthritis, bowel disease, and chronic obstructive lung disease, are mostly brought on by chronic inflammation (Pahwa *et al.*, 2023).

2.1.4 Treatment of Inflammation

2.1.4.1 Conventional Treatment

2.1.4.1.1 Non-steroidal Anti-inflammatory Drugs (NSAIDs)

Celecoxib, Etoricoxib, Ketoprofen, Naproxen, Indomethacin, Ibuprofen and Diclofenac are some examples of NSAIDs (Thakur *et al.*, 2018; Braun *et al.*, 2020). These drugs act by suppressing cyclooxygenase (COX) enzyme that contributes to inflammation and are also used to relieve pain. The enzyme COX converts arachidonic acid into prostaglandins, thromboxanes, and prostacyclins. It has been proposed that the therapeutic benefits of NSAIDs are due to the absence of these eicosanoids. Specifically, prostaglandin E2 causes vasodilation, aids in nociception and pyrexia, whereas thromboxanes are implicated in platelet adhesion (Hang *et al.*, 2018; Ruslin *et al.*, 2020).

Diclofenac is a NSAID that is derived from phenylacetic acid (Wojcieszynska *et al.*, 2023). NSAIDs block the enzymes that produce prostaglandins (PGs), cyclooxygenase (COX)-1 and -2. PGs contribute to inflammation and pain signalling (Fokunang *et al.*, 2018). The typical drug design that produced diclofenac was based on the structures of indomethacin, mefenamic acid, and phenylbutazone (Wojcieszynska *et al.*, 2023). After a long-term use, diclofenac is linked with the incidence of gastro-intestinal severe effects, cardiovascular, hepatic and renal toxicity. Diclofenac has been found to exert prothrombotic effects by

changing levels of PG1-2. It may also raise the dangers for heart outbreaks and myocardial infarction (Hafeez *et al.*, 2019).

COX comprises two isoenzymes: COX-1 and COX-2. The gastrointestinal tract lining, platelet aggregation, and renal function are all dependent on the constitutive production of COX-1 by the body. COX-2 is not constitutively expressed by the organism; rather, it is expressed in response to inflammation. With the exception of celecoxib, which only targets COX-2, most of NSAIDs are nonselective to COX. Notably, COX-2 selective NSAIDs offer anti-inflammatory therapy without endangering the integrity of the gastric mucosa. The NSAIDs have been related to severe effects, including gastrointestinal, renal and hepatic toxicities (Hang *et al.*, 2018; Ruslin *et al.*, 2020).

2.1.4.1.2 Glucocorticoids

Glucocorticoids are frequently utilized to treat autoimmune and inflammatory diseases. They include drugs such as hydrocortisone, methylprednisolone, prednisolone, beclomethasone, betamethasone, and dexamethasone. However, these drugs are allied with side effects for instance Cushing syndrome, ulceration and gastritis, immune suppression, diabetes, weight gain, depression, among others (Berardicurti *et al.*, 2020; Yasir *et al.*, 2020).

2.1.4.2 Alternative and Complementary Treatment of Inflammation

2.1.4.2.1 Physical Exercise and Diet

Exercise improves physical function and quality of life and lowers inflammatory mediators such as ROS and pro-inflammatory cytokines (Sirico *et al.*, 2018). Numerous dietary and lifestyle adjustments may be beneficial in lowering inflammation mediators. Polyphenolic compounds are found in vegetables and fruits may act as potent anti-inflammatory agents (Philippou *et al.*, 2021). Anthocyanins, which are pigments found in fruits, are known to have strong anti-edema and antioxidant activities. They also reduce lipid peroxidation and COX-2, an inflammatory mediator (Rahman *et al.*, 2022).

2.1.4.2.2 Herbal Management

The utilization of therapeutic plants as a source of therapies for treating various human ailments has been practiced throughout history since ancient times. More than 400 medicinal plant species have been identified in Kenya, where traditional medicine is widely practiced and accepted by the culture (Gakuya *et al.*, 2020; Mbuni *et al.*, 2020). In Kenya, one of the disorders treated using herbal formulations is inflammatory (Kigen *et al.*, 2017).

Medicinal plants have gained substantial interest due to their ethno pharmacological uses in treatment and management of medical ailments including inflammatory diseases (Zhang *et al.*, 2018). Plant-derived formulations are used to treat inflammation (Hussein and El-Anssary, 2019). Plant exhibits a broad spectrum of anti-edema and immunomodulatory activities with through their secondary metabolites with relatively few safety concerns

(Shingala *et al.*, 2021). Extensive research evidence that phytochemicals could be helpful in treating inflammation (Zhang *et al.*, 2019).

Plant extracts have shown to manage levels of different inflammatory mediators including interleukins (1, 6 and 10) and TNF- α . The presence of phytochemical defense systems in plants including secondary metabolites, has elicited the attention of researchers with regard to bioactive phytochemicals (Zhang *et al.*, 2019).

2.2. *Ficus sycomorus* L.

2.2.1 Plant Description

Ficus sycomorus L. is commonly referred to as mulberry fig (English), Mukuyu (Mbeere) Mkuyu (Swahili), Olam (Luo). It belongs to Moraceae family. It is a tree with a height of around 20m (Bussmann *et al.*, 2022). Its branches emerge from the lower stem, making it have an umbrellas-like shapes. Leaves are heart-shaped, dark green with yellow veins, spirally arranged to a maximum of 14 cm long. It produces flowers and fruits (2 to 3 cm in diameter) throughout the year with a pick season in between July and December. The fruit is green while unripe but turns yellow or red when ripe. The ripe fruits harbor several hundreds to thousands of seeds. The ripe fruits are also highly nutritive and flavored (Orwa *et al.*, 2022). Its natural habitat includes the tropics of African countries, Oman, Laban and Arabian Peninsula. The plant has been domesticated in Israel, Cyprus, Madagascar and Egypt. The global climate has led to the plant growth in some rainfall areas (Erhirhie *et al.*, 2018).



Figure 2.1: Photo of *Ficus sycomorus* L.

2.2.2 Medicinal Uses

Africans and South America topically apply white latex of the plant burns, warts and ulcers to inhibit infections and aid in healing. Water extract of powdered stem bark is administered three times in a day to treat of epilepsy, pains and diarrhea. The roots have

laxative and anthelmintic properties. Boiled bark extracts of this plant are also used in the management of chest infections and sore throat. Leaf is used in treating jaundice and snake bites. In Nigeria and plateau state stem bark is used to treat animal diarrhea, and infertility and low sperm count in humans (Erhirhie *et al.*, 2018).

Despite the wide use of *Ficus sycomorus* in traditional medicine, its anti-inflammatory activities and phytochemical profile remain under explored. This study aims to bridge this gap by analyzing the methanol and dichloromethane extracts of the plant for their potential bioactive compounds. Although various studies have reported ethnomedicinal uses of *Ficus sycomorus*, limited research has systematically evaluated its anti-inflammatory efficacy using methanol and dichloromethane extracts. Furthermore, most existing studies do not provide a detailed phytochemical analysis linking specific compounds to anti-inflammatory.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Medicinal Samples Collection

Selection and collection of *Ficus sycomorus* medicinal samples (leaves and stem bark) was based on ethnobotanical data obtained from a traditional medical practitioner in Gitiburi village, Embu County, and Kenya. Plant materials were collected with acceptable bio-conservation methods. They were later transported to the National Museums of Kenya in the Department of Botany, herbarium section, where botanical authentication was done by an acknowledged taxonomist. The plant sample was assigned a voucher number (EWM-001) and then archived at the National Museums of Kenya. Using a handheld Global Positioning System device (model type Garmin Etrex H), the coordinates where the medicinal plant samples were collected were obtained and recorded as “South West 68 0 36’33” S 37 3 7’15” E”.

3.2 Preparation of Plant Materials

The medicinal samples underwent a thorough sorting, cleaning, cutting into small pieces, and two weeks of shade drying. The dried plant materials were ground using electric mill and stored in paper bags ready for extraction.

3.3 Extraction

A conical flask with a clearly label was filled with three hundred grams (300g) of powdered *F. sycomorus* leaves and stembarks that had been separately weighed. Each conical flask was filled with one litre of dichloromethane and methanol, sealed, and left to stand for

twenty-four hours while being constantly rotated every six hours. The mixtures were decanted and then filtered through Whitman filter paper number 1. A rotary evaporator was utilized to concentrate the MeOH and DCM filtrates under reduced pressure and at temperatures of 64°C and 45°C, respectively.

3.4 Research Authorization and Approval

The proposal for this study was duly approved by the Kenyatta University Graduate School. (Appendix I). In addition, authorization to undertake the research was granted by the National Commission for Science, technology and Innovation (Appendix II). The Kenyatta University's Ethics Committee for the Care and Use of Laboratory Animals granted ethical approval for the use research animals (Approval number PKUA/007/007).

3.5 Assessment of Anti-inflammatory Effects of the Extracts

3.5.1 Laboratory Animal Husbandry

In this investigation utilized female Swiss albino mice weighing 20-22 grams, and aged 7-8 weeks. They were selected due to their genetic uniformity, well-documented physiological response in inflammation models and widespread use in pharmacological research. The mice were purchased and raised in the Animal Breeding and Research Facility at Kenyatta University.

The mice were acclimatized for 48 hours before experimentation. They were maintained in standard propylene cages under ambient room temperature 25°C with 12 hours' daylight. The mice were supplied with water *ad libitum* and nourished with the standard mice pellet

(Unga Holding Limited, Kenya). The animals were handled ethically following *ARRIVE* guidelines for reporting animal model research. Pain and distress were minimized, where necessary and euthanasia was conducted in accordance with Kenyatta University's Guidelines for Care and Use of Laboratory Animals. After the experiment any the animals were sacrificed using chloroform and disposed according to the guidelines (Vogel *et al.*, 2002).

3.5.2 Induction of Inflammation

Inflammation was induced in mice through injection of Carrageenan (0.1ml, 1% commercial grade type 1) solution into left hind paw sub-plantar tissue. Inflammation was confirmed by measuring paw diameter before and after induction using digital Vernier calipers.

3.5.3 Study Design

This investigation used a completely random controlled study design was selected to ensure unbiased allocation of treatment groups, minimizing systematic variations and allowing for robust comparative analysis of anti-inflammatory effects. This approach enhances internal validity by reducing confounders.

3.5.4 Experimental Design

Randomly, mice were assigned 6 groups (n = 5): negative control (edema control), normal control (non-edema control), diclofenac control (positive control) and 3 extract-treated mice. A sample size of five animals per group was determined based on previous studies

Moriasi *et al.* (2020) and Mworira *et al.* (2021) demonstrating that the number provide sufficient statistical power to detect meaningful differences in anti-inflammatory response. The normal control mice received normal saline (vehicle) containing 5% dimethyl sulphoxide (DMSO).

After an hour of edema induction, the edema control mice were administered the vehicle. An hour after the diclofenac control mice were induced with inflammation, they were administered diclofenac sodium (15mg/kg bw (body weight)). The three extract-treated mice were induced with edema one hour before administration of the *F. sycomorus* extracts at 50, 100 and 200 mg/kg bw (Table 3.1).

All the treatments were intraperitoneal administered. The independent variables in this study included the different doses of the extracts (50, 100 and 200 mg/kg bw), the vehicle control, and the diclofenac control. The dependent variable was the inhibition of edema, measured using digital Vanier calipers at different times intervals (1st, 2nd, 3rd and 4th hours post treatment).

Table 3.1: Protocol for Anti-inflammatory Assay

Status	Treatment
Group I	Carrageenan + Normal saline + 5% DMSO
Group II	Normal saline + 5% DMSO
Group III	Carrageenan + Diclofenac (15mg/kg bw)
Extract low dose	Carrageenan + <i>F. sycomorus</i> extract (50 mg/kg bw)
Extract medium dose	Carrageenan + <i>F. sycomorus</i> extract (100 mg/kg bw)
Extract maximal dose	Carrageenan + <i>F. sycomorus</i> extract (200 mg/kg bw)

The paw diameter was measured thirty minutes before injecting carrageenan and then in the 1st, 2nd, 3rd and 4 hours after treatment. The formula by Umamageswari *et al.* (2015) was utilized to compute the % inhibition of edema;

$$\text{Percentage inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where;

V_c = Edema volume in the control group

V_t = Edema diameter in the treated group

3.6 Determination of Phytochemical Profile

3.6.1 Gas Chromatography-Mass Spectrometry (GC-MS)

One gram of DCM leaf and stem bark extract of *F. sycomorus* were each dissolved in 1mL dichloromethane (Sigma Aldrich grade). A 1.5 mL Eppendorf tube was used to weigh the samples, and each weight was recorded in milligrams. Each sample was dissolved in one milliliter of dichloromethane, vortexed for thirty seconds, sonicated for fifteen minutes in an ultra-bath, and then centrifuged 10,000 rpm for 5 minutes. Subsequently, the samples were placed in 2 ml auto sampler vials and then subjected to GC-MS analysis. Each extract was injected at a volume of 1 μ .

The plant extracts were analyzed using the following parameters using an Agilent Gas Chromatograph (7683 Agilent Technology, Inc., Beijing, China) connected to a 5975 c inert XL EI/CI Mass Spectrometer in full scan mode gas chromatography Column HP_5MS is a low bleed capillary with 0.25 mm diameter, 0.25 μ m film thickness, and 30 m length that contain 5 % phenyl methyl siloxane. Before analyzing the extracts, the

temperature of the oven, the flow rate of Helium and the electron gun of the gas chromatography and mass spectrometry were programmed. An electron ionization apparatus with an ionization energy of 70 Ev was used during analysis.

At a steady flow rate of 7.2 milliliters per minute, helium (99.99 %) was utilized as the carrier gas. The injector and mass transfer line temperatures were adjusted to 250°C and 200°C, respectively. The oven was programmed to operate at 50°C/minute for fifty minutes, reaching 285°C in nine minutes, with a fifty-minute run time. The oven's original operating temperature was 35°C for five minutes, followed by a 10-degree Celsius rise every minute to 280°C for ten minutes. The mass spectrometry was operated with the following parameters: 250 °C for the interface temperature, 1666 μ sec for scan speed, 40-550M/Z for scan range, 70 eV for ionization energy, and 50-70 minutes for total retention period.

The concentration of each component in the sample was calculated using beta-sitosterol linear equation $y = 186096x + 1000000$. The spectrum of the known components kept in the National Institute of Standard and Technology computer library was used to identify unknown phytochemicals.

3.6.2 Liquid Chromatography-Mass Spectrometry (LC-MS)

Samples (1g) were each dissolved in 1mL (90:10 methanol, deionized distilled H₂O), vortexed for ten seconds, sonicated for thirty minutes, centrifuged at 14000rpm for ten minutes at 4°C and then the supernatant (0.1μL) analyzed utilizing ultra-performance

liquid chromatography-mass spectrometry (UPLC-MS). The ACQUITY UPLC I-class apparatus from Waters Corporation in Milford, Massachusetts was utilized to perform the chromatographic separation. It was outfitted with an ACQUITY UPLC BEH C18 column (Waters Corporation, Wexford, Ireland) (2.1mm x 150mm, 1.7 μ m particle size; oven temperature 45°C). A steady flow rate of 0.2mL per minute was maintained.

Two solvents, water and MeOH, each acidified with formic acid (0.01%), formed the mobile phase. The gradient system that was utilized was 0-2 minutes, 5% B, 2-4 minutes, 40% B, 4-7 minutes, 40% B, 7-8.5 minutes, 60% B, 8.5-10 minutes, 60% B, 10-15 minutes, 80% B, 15-19 minutes, 80% B, 19-20.5 minutes, 100% B, 20.5-23 minutes, 100% B, 23-24 minutes and 95% B, 24-26 minutes, 95% B. The auto sampler tray was cooled to 5°C.

The ultra-performance liquid chromatography was interfaced with an ESI (electrospray ionization) in positive ionization mode. The settings that were used were the mass-to-charge ratio (m/z) range of 40-2,000, the sampling cone voltage of 30V, the capillary voltage of 0.5 kV, the source temperature of 150°C, and the desolvation temperature of 120°C. The flow rate for nitrogen desolvation was 800 L/h.

Mass Lynx version 4.1 SCN 712 software was utilized to collect the data. Adducts, common segments, literature online databases (METLIN, ChemSpider), and, when accessible, co-injections to validate with genuine samples were used to generate molecular ion peaks. After the mass spectra for each peak was generated, possible phytochemicals

were established. The linear equation of the oleic acid standard ($y = 6181.1x + 21034$) was used to calculate the concentration of the components.

3.7 Statistical Data Analysis

A Microsoft Excel Spreadsheet was used to record raw data. The data was cleaned and then organized for analysis utilizing Minitab statistical software version 21.0. Means \pm SEM (standard error of the mean) were computed to generate descriptive statistics. To ascertain the statistical variations between the various test groups, inferential statistic one factorial ANOVA (analysis of variance) was employed. In case of statistical variations, pairwise mean comparisons were performed using Tukey's multiple comparisons. An independent/unpaired t-test was employed to compare the effects of the two extracts. A $p < 0.05$ was used to define a significance level. Tables and figures were employed to illustrate the analyzed data.

CHAPTER FOUR

RESULTS

4.1 Extract Yields

Semi-solid extracts were obtained, allowed to dry, weighed using precision weighing balance and then refrigerated at 4°C awaiting phytochemical analysis and bioassays. Percentage extract yields were computed using a formula used by Moriasi *et al.* (2020);

$$\% \text{ Extract Yield} = \frac{\text{Mass of the obtained extract}}{\text{Mass of the powdered sample}} \times 100$$

The yield of the methanol leaf and stem bark extracts was 17.5% and 9.5%, respectively. The yield of the DCM leaf and stem bark extracts was 5.2% and 4.6%, respectively. In general, relative higher yields were obtained for the methanolic extract of *Ficus sycomorus* compared with the DCM counterpart.

4.2 *In Vivo* Anti-inflammatory Activities of Dichloromethane and Methanol Stem Bark Extracts of *Ficus sycomorus*

The administration of *Ficus sycomorus* DCM stem bark of *Ficus sycomorus* extracts reduced the inflamed paw diameter of Swiss albino mice in the treatment period as indicated in table 4.1. However, the percentage change in paw edema in the edema control (negative control) mice was substantially higher relative to mice that received diclofenac and DCM stem bark extract at 50, 100, and 200 mg/kg bw from 1st hour of the treatment onward (p<0.05). The extract showed a response that was dosage dependent in the 1st, 3rd, and 4th hours (Table 4.1).

The inflamed paw diameter was reduced by 4.02, 6.51, and 6.68%, in the 1st hour by the *Ficus sycomorus* DCM stem bark extract at 50, 100, and 200 mg/kg bw, respectively. The anti-edema effects of DCM stem bark extract varied significantly between the three doses ($p < 0.05$). Conversely, the anti-edema effect of DCM extract did not vary significantly at ($p > 0.05$) 100, and 200 mg/kg bw. Similarly, the effect of diclofenac (positive control) did not differ significantly in comparison to the effect of the extract ($p > 0.05$) at 50 mg/kg bw as depicted in table 4.1.

The paw diameter was decreased to 92.41, 91.09, and 91.31% in the 2nd hour by the *Ficus sycomorus* DCM stem bark extract dosages of 50, 100, and 200 mg/kg bw, respectively. At all three doses, the extract's anti-edema was statistically equivalent ($p > 0.05$) to that of diclofenac. The anti-edema effect of the extract noted a dose-independent response as shown in table 4.1.

The DCM extract at 50, 100, and 200 mg/kg bw attenuated the paw edema by 10.36, 11.74, and 12.2 %, respectively in the 3rd hour. The anti-edema effect of *Ficus sycomorus* DCM stem bark extract at 100, and 200 mg/kg bw did not vary statistically ($p > 0.05$). Similarly, the anti-edema effect of diclofenac did not vary considerably compared to the effect of 50 mg/kg bw of the extract as depicted in table 4.1 ($p > 0.05$).

The inflamed paw diameter declined to 87.52, 86.31, and 85.53%, in the 4th hour when *Ficus sycomorus* DCM stem bark extract was administered at 50, 100, and 200 mg/kg bw, respectively. The anti-edema effect of the extract at the three studied doses differed

substantially in mice ($p < 0.05$). Nevertheless, the effect of diclofenac did not differ substantially from that of the extract at the three doses as illustrated in table 4.1 ($p > 0.05$)

Table 4.1: *In Vivo* Anti-edema Effect of DCM Stem Bark Extract of *Ficus sycomorus*

Percentage change in paw diameter				
Group	1hr	2hr	3hr	4hr
Normal control	0.00±0.00 ^d (0.00)	0.00±0.00 ^c (0.00)	0.00±0.00 ^e (0.00)	0.00±0.00 ^e (0.00)
Negative control	101.76±0.39 ^a (-1.76)	103.70±0.42 ^a (-3.70)	106.00±0.16 ^a (-6.00)	107.15±0.15 ^a (-7.15)
Positive control	95.17±0.42 ^b (4.83)	92.09±0.20 ^b (7.91)	89.23±0.17 ^{bc} (10.77)	86.37±0.45 ^{bc} (13.63)
50mg/kg bw	95.98±0.39 ^b (4.02)	92.41±0.55 ^b (7.59)	89.64±0.23 ^b (10.36)	87.52±0.42 ^b (12.48)
100mg/kg bw	93.49±0.46 ^c (6.51)	91.09±0.40 ^b (8.91)	88.26±0.52 ^{cd} (11.74)	86.31±0.49 ^{bc} (13.69)
200mg/kg bw	93.32±0.35 ^c (6.68)	91.31±0.43 ^b (8.69)	87.75±0.34 ^d (12.25)	85.53±0.28 ^c (14.47)

Descriptive statistics are represented as mean plus or minus SEM. The percentage of inflammation inhibition is presented by the values in parenthesis. There is no statistically significant variation between descriptive statistics with the same letter along the column ($p > 0.05$), using one factorial ANOVA and Tukey's multiple comparisons.

The *Ficus sycomorus* methanol stem bark extract also noted anti-edema effect following induction of paw edema using carrageenan (Table 4.2). This was noted by decline in paw diameter after the animals received the extract at the three doses. However, from the 1st hour onward, the percentage change in paw diameter of edema control mice was significant higher in contrast to those of mice that were administered with diclofenac and extract ($p < 0.05$) at the three tested doses. From the first hour onward, the methanol extract ameliorated paw edema dose-dependently (Table 4.2).

The paw inflammation was reduced to 94.88, 94.07, 90.33, and 94% in the 1st hour by using methanol stem bark extract of *Ficus sycomorus* at 50, 100, and 200mg/kg bw, and

diclofenac, respectively. The anti-edema effect of the methanol extract dose of 50, and 100mg/kg bw, and diclofenac did not differ significantly in mice as shown in table 4.2 ($p>0.05$).

The paw inflammation was ameliorated by 8.46, 10.80, and 12.68%, in the 2nd hour by the *Ficus sycomorus* extract at 50, 100, and 200 mg/kg bw, respectively. There was no considerable variation in the extract's anti-edema activity across the three doses ($p>0.05$) in this hour. At 50 mg/kg bw, the anti-edema effect of the extract did not differ significantly to the effect of the reference drug as illustrated in table 4.2 ($p>0.05$).

The paw inflammation was lowered to 88.99, 87.08, 85.17, and 88.44 % in the third hour by *Ficus sycomorus* extract at 50, 100, and 200 mg/kg bw, including diclofenac, respectively. The three extract doses showed significantly different anti-edema effects in mice ($p<0.05$). Nonetheless, the anti-edema effect at 50 mg/kg bw of the extract did not vary considerably from the ($p>0.05$) effect of diclofenac (Table 4.2).

In the 4th hour, the mice that received methanol extract dosages of 50, 100, and 200mg/kg bw noted a decline in paw inflammation by 12.79, 14.61, and 16.54%, respectively. The anti-edema effect of the methanol extract at the three doses differed substantially ($p<0.05$). The anti-edema activity of diclofenac statistically matched the extract's activity at 50mg/kg bw as detailed in table 4.2 ($p>0.05$).

Table 4.2: *In Vivo* Anti-edema Effect of Methanol Stem Bark Extract of *Ficus sycomorus*

Group	Percentage change in paw diameter			
	1hr	2hr	3hr	4hr
Normal control	0.00±0.00 ^d (0.00)	0.00±0.00 ^e (0.00)	0.00±0.00 ^e (0.00)	0.00±0.00 ^e (0.00)
Negative control	101.35±0.38 ^a (-1.35)	103.49±0.40 ^a (-3.49)	104.46±0.43 ^a (-4.46)	106.59±0.27 ^a (-6.59)
Positive control	94.00±0.43 ^b (6.00)	91.43±0.36 ^b (8.57)	88.44±0.40 ^b (11.56)	86.73±0.21 ^b (13.27)
50mg/kg bw	94.88±0.17 ^b (5.12)	91.54±0.33 ^b (8.46)	88.99±0.26 ^b (11.01)	87.21±0.22 ^b (12.79)
100mg/kg bw	94.07±0.25 ^b (5.93)	89.20±0.15 ^c (10.80)	87.08±0.15 ^c (12.92)	85.39±0.21 ^c (14.61)
200mg/kg bw	90.33±0.18 ^c (9.67)	87.32±0.19 ^d (12.68)	85.17±0.17 ^d (14.83)	83.46±0.26 ^d (16.54)

Descriptive statistics are represented as mean plus or minus SEM. The percentage of inflammation inhibition is presented by the values in parenthesis. There is no statistically significant variation between descriptive statistics with the same letter along the column (($p > 0.05$), using one factorial ANOVA and Tukey's multiple comparisons.

In comparison, the ant-edema activities of DCM and methanol stem bark extracts at 50mg/kg bw did not vary statistically in the 2nd, 3rd and 4th hours as illustrated in figure 4.1 ($p > 0.05$). Conversely, the paw diameters of that received methanol stem bark extract were substantially higher relative to paw diameter of mice that received DCM stem bark extract in the 1st hour as detailed in figure 4.1 ($p < 0.05$).

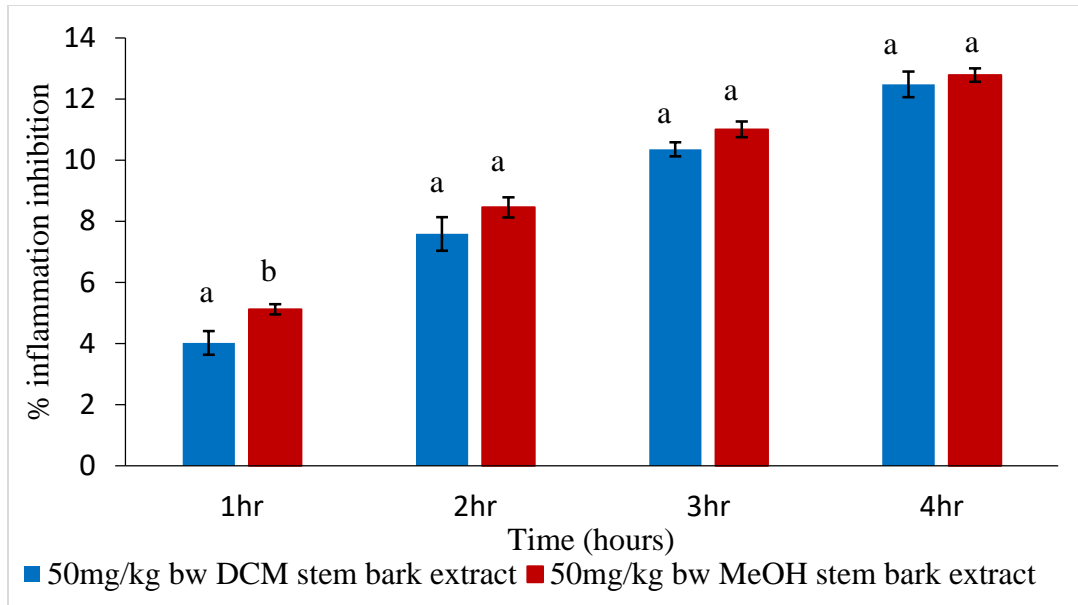


Figure 4.1: Comparison of *In Vivo* Anti-edema Effects of DCM and MeOH Stem Bark Extracts of *Ficus sycomorus* at 50mg/kg bw.

Using an independent t-test, bars with the same letter within the same hour do not differ significantly ($p > 0.05$).

At 100mg/kg bw, the paw diameters of mice that received DCM stem bark extract were significantly lower than those of mice that received methanol stembark in the 2nd hour ($p < 0.05$). Nevertheless, at the 1st, 3rd and 4th hours, the effect of the two extracts did not vary considerably as shown in figure 4.2 ($p > 0.05$).

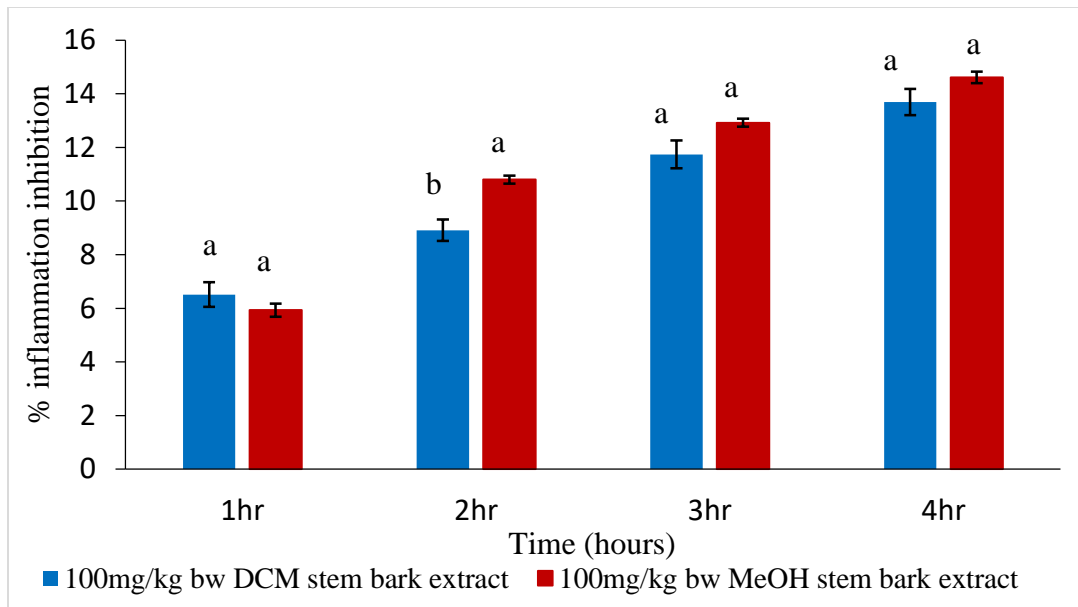


Figure 4.2: Comparison of *In Vivo* Anti-edema Effects of DCM and MeOH Stem Bark Extracts of *Ficus sycomorus* at 100mg/kg bw.

Using an independent t-test, bars with the same letter within the same hour do not differ significantly ($p > 0.05$).

At 200mg/kg bw, the paw diameters of mice treated with DCM extract were considerably lower relative to the paw diameter of methanol extract in the entire treatment period as detailed in figure 4.3 ($p < 0.05$).

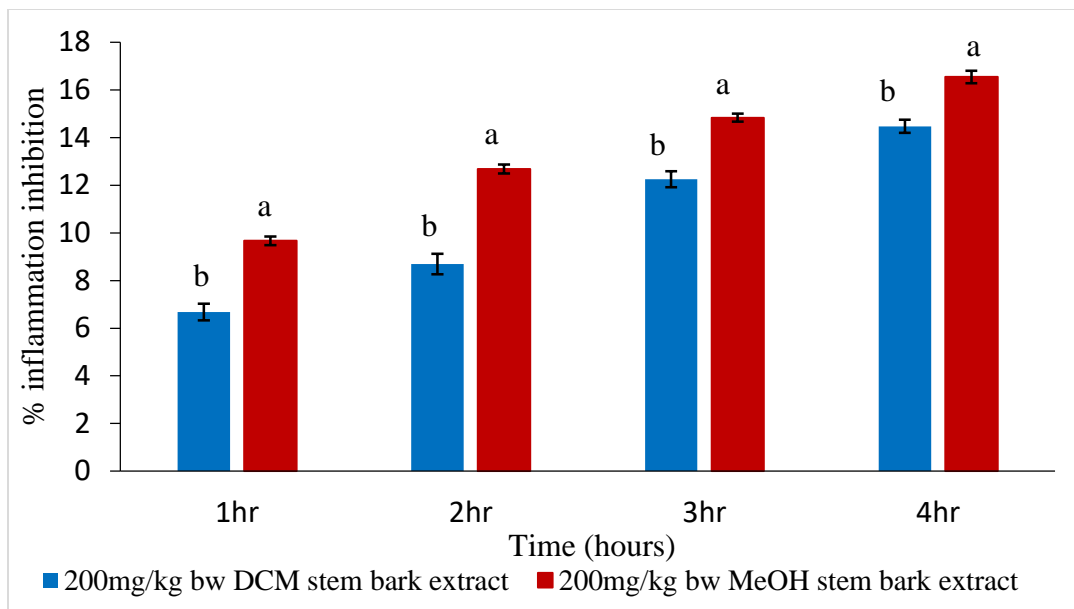


Figure 4.3: Comparison of *In Vivo* Anti-edema Effects of DCM and MeOH Stem Bark Extracts of *Ficus sycomorus* at 200mg/kg bw.

Using an independent t-test, bars with the same letter within the same hour do not differ significantly ($p > 0.05$).

4.3 *In-Vivo* Anti-inflammatory Activities of Methanol and Dichloromethane Leaf Extracts of *Ficus sycomorus*

In general, the dichloromethane leaf extract of *Ficus sycomorus* at 50, 100 and 200mg/kg bw noted anti-edema effect in mice that were induced with inflammation. This effect was revealed by a decline in paw inflammation following therapy with the extract (Table 4.3). The percentage change in paw diameter in edema control mice was substantially greater relative to paw diameter of mice administered with diclofenac and leaf extract at all dose levels from hour 2 onward ($p < 0.05$). In the entire treatment period, the anti-edema effect of DCM leaf extract of *Ficus sycomorus* reduced the paw edema dose-dependently apart from the 1st hour which was dose independent (Table 4.3).

The DCM extract of *Ficus sycomorus* at 50, 100, and 200mg/kg bw and diclofenac ameliorated the inflamed paw by 4.50, 4.37, 4.72, and 5.29%, in the 1st hour, respectively.

The anti-edema effect of the extract at the three doses did not vary considerably relative to the activity ($p>0.05$) of diclofenac as shown in table 4.3.

The DCM extract at 50, 100, and 200mg/kg bw and diclofenac alleviated the diameter of the inflamed paw by 7.63, 8.15, 8.19, and 8.24%, respectively, in the second hour. The anti-edema activity at 50, 100, and 200mg/kg bw of *Ficus sycomorus* extract did not differ considerably and was comparable ($p>0.05$) to the effect of diclofenac as depicted in table 4.3.

The *Ficus sycomorus* extract at 50, 100, and 200mg/kg bw ameliorated the inflamed paw by 9.20, 9.93, and 11.48%, in the third hour, respectively (Table 4.3). The extract's anti-edema effect at 50 and 100 mg/kg bw did not differ substantially in mice ($p>0.05$). The anti-edema effect of diclofenac did not vary statistically compared to the effect of *Ficus sycomorus* extract at 200mg/kg bw as detailed in table 4.3 ($p>0.05$).

The inflamed paw diameter was reduced by 10.95, 12.29, 13.73, and 12.91% in the fourth hour by *Ficus sycomorus* extract at 50, 100, and 200mg/kg bw, and diclofenac, respectively. The anti-edema activity of DCM of *Ficus sycomorus* extract at 50, 100, and 200mg/kg bw differed considerably ($p<0.05$). The effect of diclofenac did not vary considerably compared from the effect of 100 and 200mg/kg bw of *Ficus sycomorus* extract as detailed in table 4.3 ($p>0.05$).

Table 4.3: *In Vivo* Anti-edema Effect of DCM Leaf Extract of *Ficus sycomorus*

Group	Percentage change in paw diameter			
	1hr	2hr	3hr	4hr
Normal control	0.00±0.00 ^c (0.00)	0.00±0.00 ^c (0.00)	0.00±0.00 ^d (0.00)	0.00±0.00 ^e (0.00)
Negative control	101.98±0.64 ^a (-1.98)	103.72±0.22 ^a (-3.72)	104.50±0.25 ^a (-4.50)	106.44±0.37 ^a (-6.44)
Positive control	94.71±0.35 ^b (5.29)	91.76±0.37 ^b (8.24)	88.36±0.40 ^c (11.64)	87.09±0.27 ^{cd} (12.91)
50mg/kg bw	95.50±0.25 ^b (4.50)	92.37±0.46 ^b (7.63)	90.80±0.42 ^b (9.20)	89.05±0.41 ^b (10.95)
100mg/kg bw	95.63±0.28 ^b (4.37)	91.85±0.46 ^b (8.15)	90.07±0.14 ^b (9.93)	87.71±0.25 ^c (12.29)
200mg/kg bw	95.28±0.28 ^b (4.72)	91.81±0.26 ^b (8.19)	88.52±0.19 ^c (11.48)	86.27±0.23 ^d (13.73)

Descriptive statistics are represented as mean plus or minus SEM. The percentage of inflammation inhibition is presented by the values in parenthesis. There is no statistically significant variation between descriptive statistics with the same letter along the column (($p > 0.05$), using one factorial ANOVA and Tukey's multiple comparisons.

Moreover, the methanol leaf extract of *Ficus sycomorus* at 50, 100 and 200mg/kg bw ameliorated paw edema as shown in Table 4.4. The percentage change in paw diameter of edema control mice was considerably higher relative percentage change of mice ($p < 0.05$) in the other studied groups. In the entire treatment period, the extract reduced paw edema in a dose-dependently (Table 4.4).

The inflamed paw diameter was reduced by 2.67, 3.42, 3.94, and 3.91% in the first hour by the *Ficus sycomorus* extract at 50, 100, and 200mg/kg bw and by diclofenac, respectively. The effect of 50, 100, and 200mg/kg bw of *Ficus sycomorus* extract did not vary substantially and statistically matched the effect of the reference drug as shown in table 4.4 ($p > 0.05$).

The *Ficus sycomorus* extract at 50, 100, and 200mg/kg bw in the 2nd hour alleviated paw edema to 94.48, 93.77, and 93.11%, respectively. The anti-edema activity of *Ficus sycomorus* extract at 50, and 100mg/kg bw did not vary considerably in mice ($p>0.05$). Additionally, the anti-edema effect at 100, and 200mg/kg bw of *Ficus sycomorus* extract also noted statistical similarities ($p>0.05$). Further, the effect of diclofenac statistically matched that of leaf methanol extract at 200mg/kg bw ($p>0.05$) in mice as presented in table 4.4.

The paw edema was reduced by 8.75, 10.45, 11.83, and 10.70% in the 3rd hour by *Ficus sycomorus* extract at 50, 100, and 200mg/kg bw and diclofenac, respectively. The anti-edema effect of *Ficus sycomorus* extract at 100, and 200mg/kg bw did not vary considerably and was comparable ($p>0.05$) from that of diclofenac (Table 4.4).

The paw diameter was reduced to 88.97, 87.93, 85.23, and 86.86% in the 4th hour by *Ficus sycomorus* extract at 50, 100, and 200mg/kg bw and diclofenac, respectively. The anti-edema effect of the extract at three studied doses varied significantly in mice ($p<0.05$). The effect of diclofenac was substantially lower relative to the effect of the extract ($p>0.05$) at 200mg/kg bw in mice as depicted in table 4.4.

Table 4.4: *In Vivo* Anti-edema Effect of Methanol Leaf Extract of *Ficus sycomorus*

Group	Percentage change in paw diameter			
	1hr	2hr	3hr	4hr
Normal control	0.00±0.00 ^c (0.00)	0.00±0.00 ^e (0.00)	0.00±0.00 ^d (0.00)	0.00±0.00 ^f (0.00)
Negative control	101.57±0.25 ^a (-1.57)	103.52±0.25 ^a (-3.52)	104.51±0.54 ^a (-4.51)	106.64±0.19 ^a (-6.64)
Positive control	96.09±0.23 ^b (3.91)	92.59±0.46 ^d (7.41)	89.30±0.54 ^c (10.70)	86.86±0.17 ^d (13.14)
50mg/kg bw	97.33±0.38 ^b (2.67)	94.48±0.22 ^b (5.52)	91.25±0.18 ^b (8.75)	88.97±0.26 ^b (11.03)
100mg/kg bw	96.58±0.41 ^b (3.42)	93.77±0.33 ^{bc} (6.23)	89.55±0.30 ^c (10.45)	87.93±0.24 ^c (12.07)
200mg/kg bw	96.06±0.32 ^b (3.94)	93.11±0.08 ^{cd} (6.89)	88.17±0.42 ^c (11.83)	85.23±0.34 ^e (14.77)

Descriptive statistics are represented as mean plus or minus SEM. The percentage of inflammation inhibition is presented by the values in parenthesis. There is no statistically significant variation between descriptive statistics with the same letter along the column (($p > 0.05$), using one factorial ANOVA and Tukey's multiple comparisons.

In comparison, the anti-edema effect of MeOH leaf extract of *Ficus sycomorus* at 50mg/kg bw significantly reduced paw edema compared to effect of DCM extract at the corresponding dosage in the 1st and 2nd hours ($p < 0.05$). Nonetheless, there was no significance variations in the anti-edema effect of DCM and methanol leaf extracts at 50mg/kg bw in mice in the 3rd and 4th hours as illustrated in figure 4.4 ($p > 0.05$).

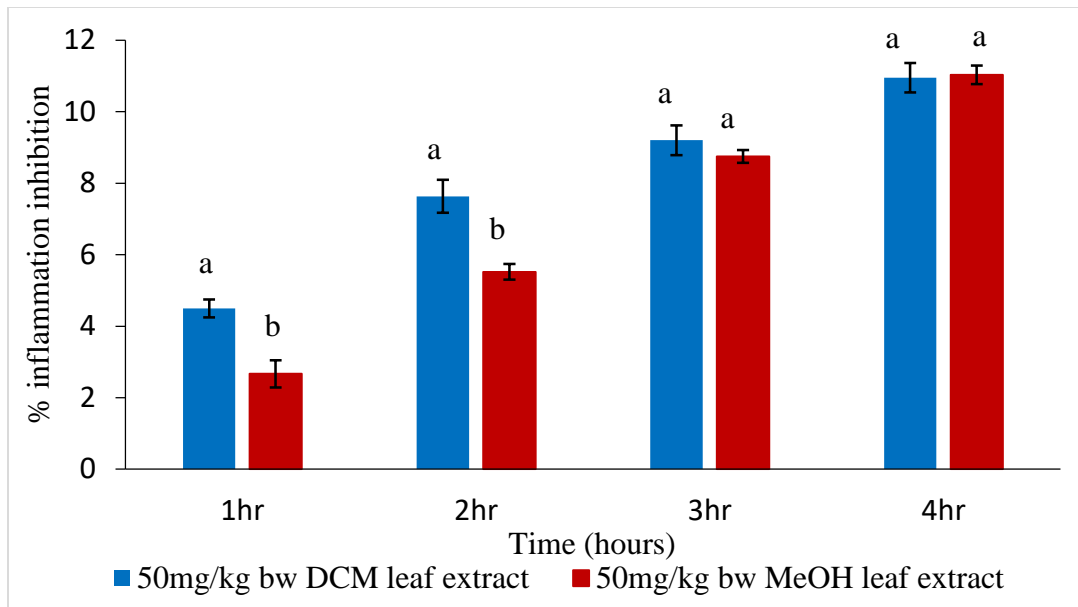


Figure 4.4: Comparison of *In Vivo* Anti-edema Effects of DCM and Methanol Leaf Extracts of *Ficus sycomorus* at 50mg/kg bw.

Using an independent t-test, bars with the same letter within the same hour do not differ significantly ($p > 0.05$).

In the 1st, 3rd and 4th hours, the anti-edema effects of DCM and methanol leaf extracts at 100mg/kg body weight did not vary considerably in mice ($p > 0.05$). Nonetheless, the anti-edema effect of DCM leaf extract of *Ficus sycomorus* at 100mg/kg bw was considerably greater relative to that of methanol leaf extract at the same dosage in the second hour as shown in figure 4.5 ($p < 0.05$).

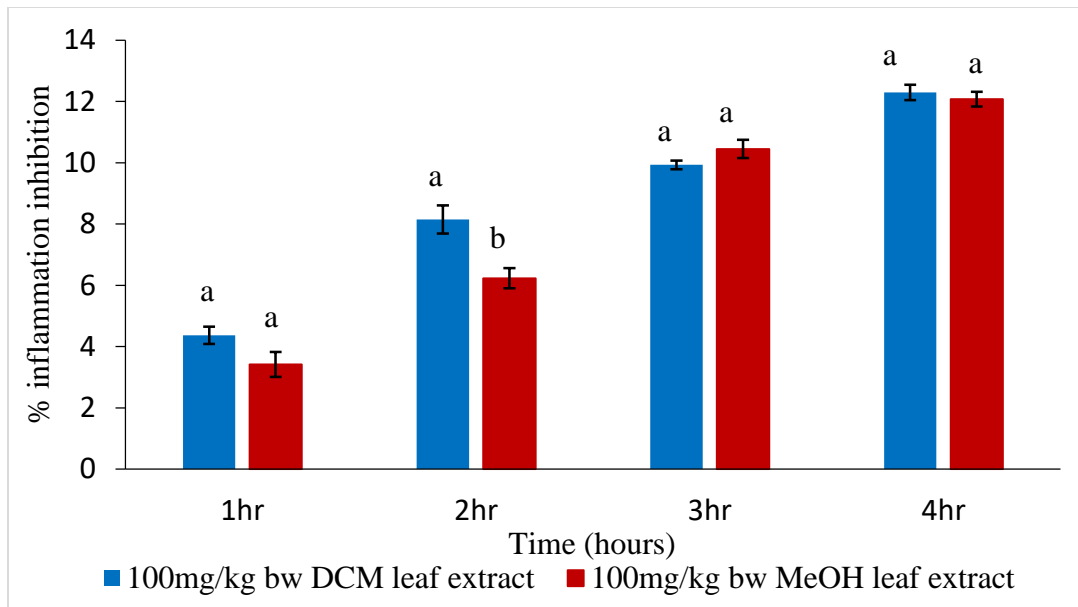


Figure 4.5: Comparison of *In Vivo* Anti-edema Effects of DCM and Methanol Leaf Extracts of *Ficus sycomorus* at 100mg/kg bw.

Using an independent t-test, bars with the same letter within the same hour do not differ significantly ($p > 0.05$).

In the first and third hours, the anti-edema activities of the two leaf extracts at 200mg/kg bw did not vary considerably in mice ($p > 0.05$). Nevertheless, the anti-edema of the DCM leaf extract at 200mg/kg bw varied significantly in contrast to that methanol leaf extract ($p < 0.05$) in the 2nd and 4th hours as shown in figure 4.6.

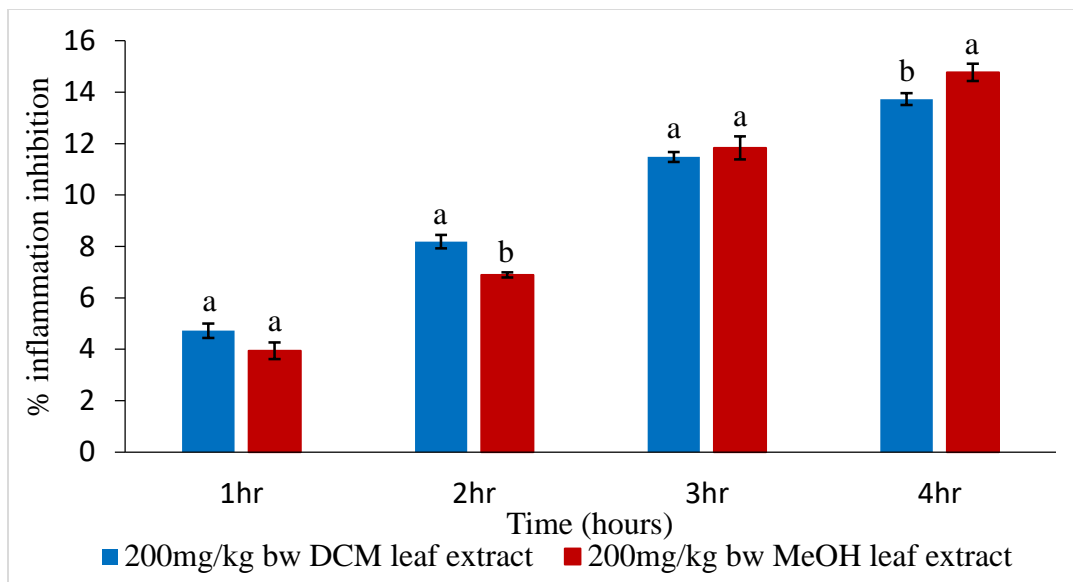


Figure 4.6: Comparison of *In Vivo* Anti-edema Effects of DCM and Methanol Leaf Extracts of *Ficus sycomorus* at 200mg/kg bw.

Using an independent t-test, bars with the same letter within the same hour do not differ significantly ($p > 0.05$).

4.4 LC-MS Analysis of Methanol Extracts of *Ficus Sycomorus*

The LC-MS analysis of MeOH stem bark extract of *Ficus sycomorus* detected 20 phytochemical compounds belonging to 10 classes (Table 4.5; Figure 4.7). These included tannins (1), phenolics (2), phenols (2), flavonoids (6), fatty acid (2), flavonols (2), ester (1), sterol (2), phytosterol (1) and isoflavones (1). Catechin had the most abundance of 16.87% followed by Chlorogenic acid with an abundance of 14.02%. The least percentage abundance of 0.09% was reported in stigma sterol as shown in table 4.5.

Table 4.5: LC-MS Analysis of Methanol Stem Bark Extract of *Ficus sycomorus*

Rt (min)	Compound name	Molecular formula	Chemical class	Relative Abundance %
1.31	Ferulic	C ₁₀ H ₁₀ O ₄	Phenolic acid	10.5
1.46	Catechin	C ₁₅ H ₁₄ O ₆	Tannins	16.87
1.59	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	Ester	14.02
1.64	Oleic acid	C ₁₈ H ₃₄ O ₂	Fatty Acid	8.38
1.69	Vanillic acid	C ₈ H ₈ O ₄	Phenols	7.77
2.95	P-coumaric	C ₉ H ₈ O ₃	Phenols	0.29
3.28	Procyanidin A1	C ₃₀ H ₂₄ O ₁₂	Flavonols	0.51
4.88	Procyanidin B2	C ₃₀ H ₂₆ O ₁₂	Flavonols	1.78
5.08	Rutin	C ₂₇ H ₃₀ O ₁₆	Phenolic	0.29
6.07	Arachidic acid	C ₂₀ H ₄₀ O ₂	Fatty Acid	2.82
8.37	Genistein	C ₁₅ H ₁₀ O ₅	Isoflavones	11.32
8.88	Eriodictyol	C ₁₅ H ₁₀ O ₆	Flavonoid	8.66
8.99	Kaempferol	C ₁₅ H ₁₀ O ₆	Flavonoid	4.47
9.04	Biochanin A	C ₁₆ H ₁₂ O ₅	Flavonoid	2.68
9.13	Luteolin	C ₁₅ H ₁₀ O ₆	Flavonoid	0.63
9.21	Apigenin	C ₁₅ H ₁₀ O ₅	Flavonoid	0.45
9.62	Quercetin	C ₁₅ H ₁₀ O ₇	Flavonoid	4.8
10.77	Campesterol	C ₂₈ H ₄₈ O	Sterol	0.35
10.83	Stigmasterol	C ₂₉ H ₄₈ O	Phytosterol	0.09
10.94	Beta-Sitosterol	C ₂₉ H ₅₀ O	Sterol	3.3

RT = retention time

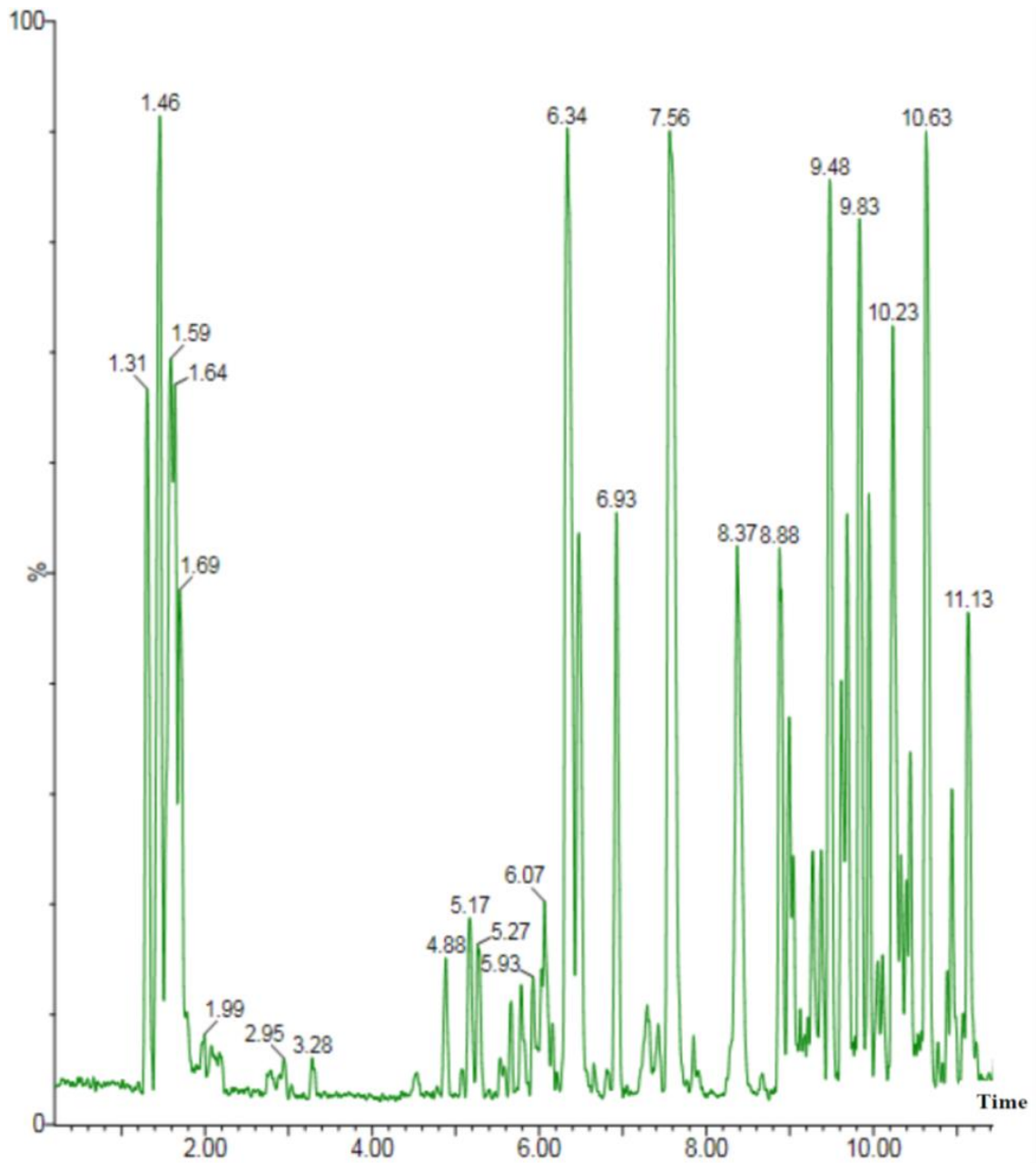


Figure 4.7: Chromatogram of LC-MS Analysis of *Ficus sycomorus* Stem Bark Methanol Extract.

The LC-MS analysis of MeOH leaf extract of *F. sycomorus* detected 18 phytochemical compounds belonging to 7 classes (Table 4.6; Figure 4.8). These included phenolics (3), flavonoids (7), fatty acid (2), flavonols (2), ester (1), sterol (2), and isoflavones (1). Ferulic acid had the highest abundance of 14.27% followed by genistein with an abundance of 10.69%. The least percentage abundance of 1.86% was reported in procyanidin B2 (Table 4.6).

Table 4.6: LC-MS Analysis of Methanol Leaf Extract of *Ficus sycomorus*

Rt (min)	Compound Name	Molecular formula	Chemical class	Relative abundance %
1.32	Ferulic acid	C ₁₀ H ₁₀ O ₄	Phenolic	14.27
1.46	Catechin	C ₁₅ H ₁₄ O ₆	Flavonoids	8.67
1.51	4-hydroxycinnamic acid	C ₉ H ₈ O ₃	Phenols	4.65
1.54	Oleic acid	C ₁₈ H ₃₄ O ₂	Fatty acid	2.74
1.58	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	Ester	5.24
1.62	Oleic acid	C ₁₈ H ₃₄ O ₂	Fatty acid	1.97
4.60	Procyanidin A1	C ₃₀ H ₂₄ O ₁₂	Flavonols	3.89
4.77	Procyanidin B2	C ₃₀ H ₂₆ O ₁₂	Flavonols	1.86
4.87	Rutin	C ₂₇ H ₃₀ O ₁₆	Phenolic	7.2
8.45	Genistein	C ₁₅ H ₁₀ O ₅	Isoflavones	10.69
8.90	Eriodictyol	C ₁₅ H ₁₂ O ₆	Flavonoids	4.17
8.94	Kaempferol	C ₁₅ H ₁₀ O ₆	Flavonoids	5.62
9.00	Biochanin A	C ₁₆ H ₁₂ O ₅	Flavonoids	7.85
9.06	Luteolin	C ₁₅ H ₁₀ O ₆	Flavonoids	5.91
9.20	Apigenin	C ₁₅ H ₁₀ O ₅	Flavonoids	2.59
9.57	Quercetin	C ₁₅ H ₁₀ O ₇	Flavonoids	5.31
10.77	Campesterol	C ₂₈ H ₄₈ O	Sterol	4.87
10.93	Beta-Sitosterol	C ₂₉ H ₅₀ O	Sterol	2.49

RT = retention time

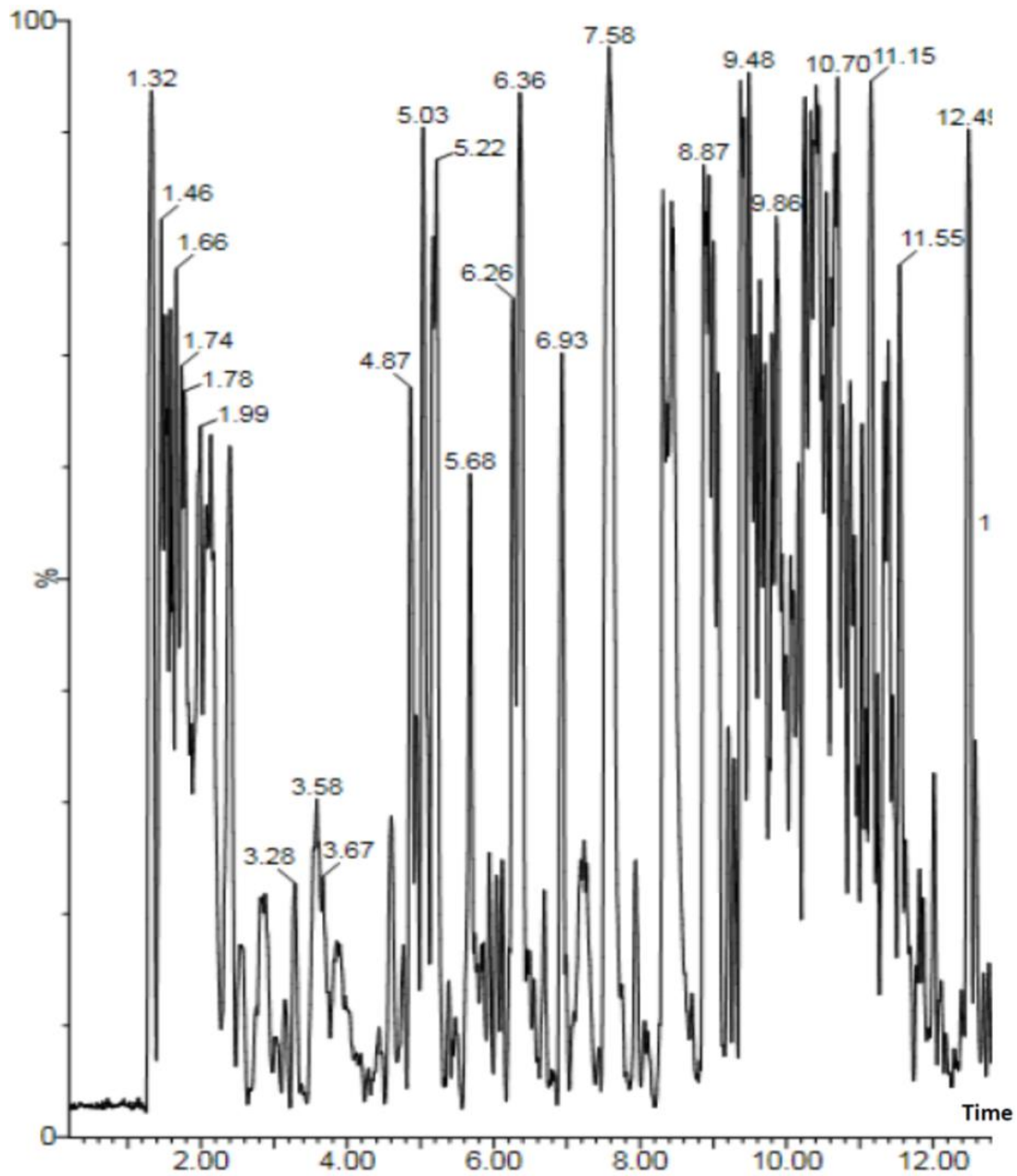


Figure 4.8: Chromatogram of LC-MS Analysis of *Ficus sycomorus* Leaf Methanol Extract.

4.5 GC-MS Analysis of DCM Stem bark and Leaf Extracts of *Ficus sycomorus*

The GC-MS analysis of DCM stem bark extract identified twenty phytochemicals with different retention times (Table 4.7; Figure 4.9). The phytochemicals were classified into different classes including alkaloid, alkene, pyrazine, alcohol, cyclic amine, furan, fatty

acid methyl ester, sesquiterpenoids, monoterpenoid, fatty acid and phytosterol as detailed in table 4.7. The most abundant phytochemical was an alcohol, A-neogammacer-22(29)-en-3-ol, acetate, (3.beta) (82.60%), followed by sesquiterpene, zierone (14.93%) (Table 4.7).

Table 4.7: GC-MS Analysis of DCM Stem Bark Extract of *Ficus sycomorus*

RT (min)	Compound Name	Molecular Formula	Chemical class	Relative Abundance %
6.69	Furan,2,5-dihydro-	C ₄ H ₆ O	Furan	0.01
9.47	3,6,6-Trimethyl-cyclohex-2-enol	C ₉ H ₁₆ O	Alcohol	0.15
9.69	Pyrrolidine	C ₄ H ₉ N	cyclic amine	0.01
11.15	Morphinan,7,8-didehydro-3-methoxy-17-methyl-6	C ₁₉ H ₂₃ NO	Alkaloid	0.01
13.50	P-Cymene	C ₁₀ H ₁₄	<i>Monoterpene</i>	0.13
13.79	1-Phenyl-1-butene	C ₁₀ H ₁₂	Alkene	0.01
14.03	Cyclopentene<3,5-dimethylene-1,4,4-trimethyl->	C ₁₀ H ₁₄	Alkene	0.12
14.75	Benzene,2,4-dimethyl-1-(1-methylethyl)-	C ₁₁ H ₁₆	monoterpene	0.10
16.01	6-Methyl-4-indanol	C ₁₀ H ₁₂ O	Alcohol	0.01
23.51	Methyl palmitate	C ₁₇ H ₃₄ O ₂	Fatty acid methyl ester	0.08
24.38	Isopropyl Palmitate	C ₁₉ H ₃₈ O ₂	Fatty acid	0.08
26.49	3,6-Dimethylpiperazine-2,5-dione	C ₆ H ₁₀ N ₂ O ₂	Pyrazine	0.06
27.90	E-8-Methyl-9-tetradecen-1-ol acetate	C ₁₇ H ₃₂ O ₂	Alcohol	0.15
31.80	1-Methyl-2-phenylbenzimidazole	C ₁₄ H ₁₂ N ₂	Benzimidazole	0.25
31.12	2,3,6-trimethylhept-5-en-1-ol	C ₁₀ H ₂₀ O	Alcohol	0.36
38.98	Beta -sitosterol	C ₂₉ H ₅₀ O	Phytosterol	0.17
41.31	13,27-cyclours-11-en-3-ol, acetate	C ₃₂ H ₅₀ O ₂	Alcohol	0.35
42.19	Zierone	C ₁₅ H ₂₂ O	Sesquiterpenoid	14.93
43.58	A-Neogammacer-22(29)-en-3-ol, acetate, (3.beta)	C ₃₂ H ₅₂ O ₂	Alcohol	82.60
46.22	Olean-18-en-28-oic acid,3-oxo-, methyl ester	C ₃₁ H ₄₈ O ₃	Fatty acid methyl ester	0.42

RT = retention time

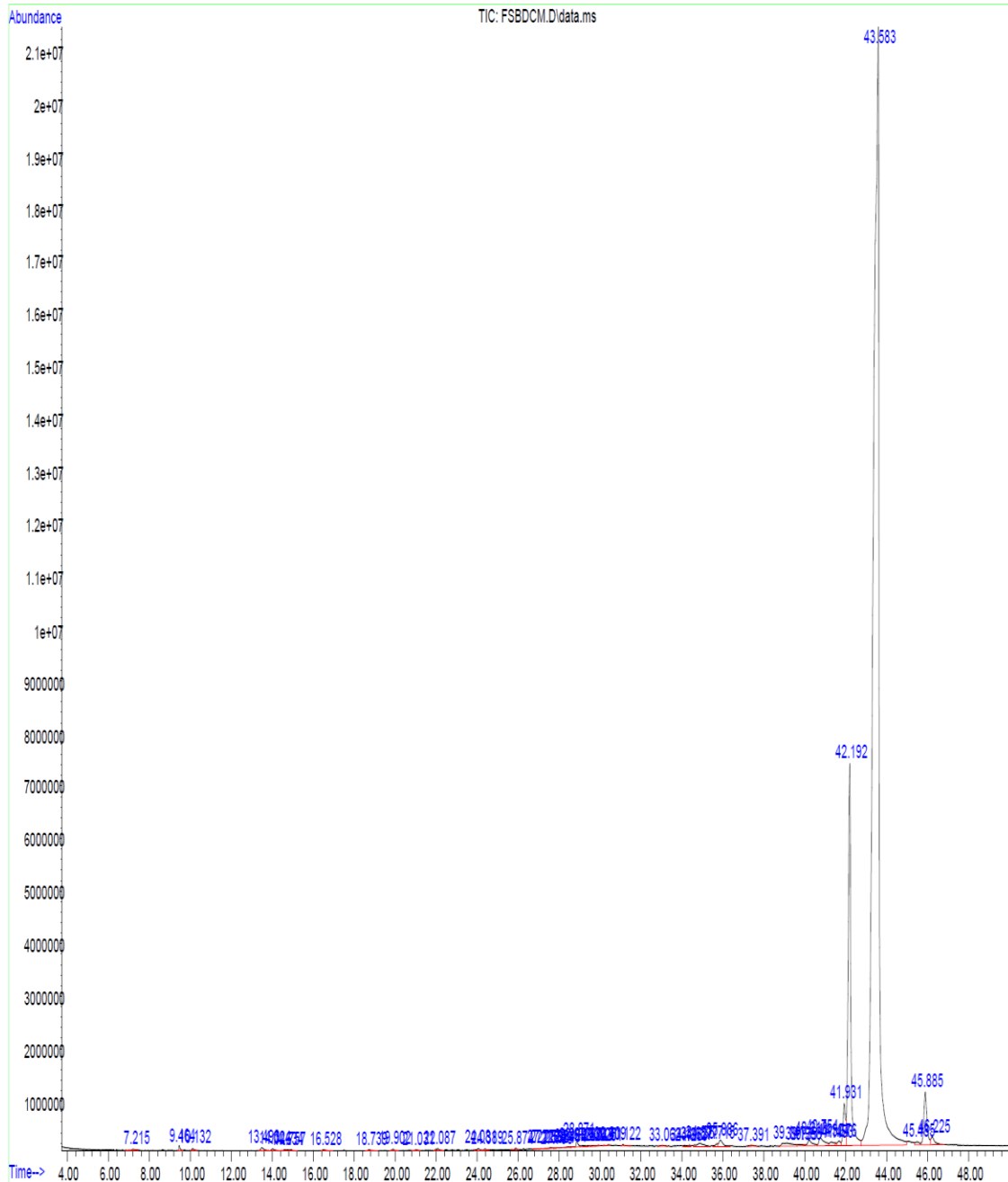


Figure 4.9: Chromatogram of GC-MS Analysis of *Ficus sycomorus* DCM Stem Bark Extract.

About 25 phytochemicals with different retention time and belonging to different phytochemical classes were detected using GC-MS analysis (Table 4.8; Figure 4.10). The phytochemicals were belonging to classes of alkenes, dicarboxylic acid, aldehyde,

monoterpene, fatty acid, acrylic diterpenoids, esters, fatty alcohol, carboxylic esters, fatty acid methyl ester, diterpene, Vitamin, phytosterol, triterpenes, sesquiterpene and alcohol as illustrated in table 4.8. The most abundant phytochemical was zierone (sesquiterpene) at 40.68% (Table 4.8).

Table 4.8: GC-MS Analysis of DCM Leaf Extract of *Ficus sycomorus*

RT (min)	Compound Name	Molecular Formula	Chemical Class	Relative Abundance %
6.71	2-Hexene,2,5,5-trimethyl	C ₉ H ₁₈	Alkenes	0.13
7.47	Oxalic acid	C ₁₄ H ₂₂ O ₄	dicarboxylic acid	0.34
9.47	2-Butenal,3-methyl-	C ₅ H ₈ O	Aldehyde	1.85
13.74	P-Cymene	C ₁₀ H ₁₄	Monoterpene	0.33
18.92	Methyl p-tert-butyl phenyl acetate	C ₁₃ H ₁₈ O ₂	Fatty acid	0.73
22.52	Phytol	C ₂₂ H ₄₂ O ₂	Diterpenoid	1.27
25.17	Acetamide,2-cyano-	C ₃ H ₄ N ₂ O	Esters	0.37
26.67	Benzaldehyde,2-nitro-,diaminomethylidenedrazone	C ₈ H ₉ N ₅ O ₂	Aldehyde	0.52
25.39	2,2,3,4-tetramethyl-3-ol,Isobutylpentyl ether	C ₉ H ₂₀ O	Fatty alcohol	0.55
22.79	Citronellyl valerate	C ₁₅ H ₂₈ O ₂	Carboxylic esters	0.65
23.51	Methyl palmitate	C ₁₇ H ₃₄ O ₂	Fatty acid methyl ester	0.77
24.05	Octadecagon<n->	C ₁₈ H ₃₈ O	Fatty alcohol	0.76
31.12	Pseudo phytol<6E,10Z->	C ₂₀ H ₃₆ O	Diterpene	10.15
39.52	Benzo h quinolone,2,4-dimethyl-	C ₁₅ H ₁₃ N	Aldehyde	0.93
33.54	Z-11(13-Methyl)tetradecen-1-ol acetate	C ₁₇ H ₃₂ O ₂	Hydrocarbon	3.75
35.15	Vitamin E	C ₂₉ H ₅₀ O	Vitamin E	6.61
37.32	Beta and Gamma Tocopherol	C ₂₈ H ₄₈ O ₂	Vitamin E	1.32
38.98	Gamma-Sitosterol	C ₂₉ H ₅₀ O ₂	Phytosterol	3.72
39.14	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b	C ₃₀ H ₄₈ O	Triterpenes	3.17
39.72	Cyperotundone	C ₁₅ H ₂₂ O	Sesquiterpene	5.97
40.19	13,27-Cyclours-11-en-3-ol, acetate	C ₃₂ H ₅₀ O ₂	Alcohol	6.17
40.78	18, 19-Secolupan-3-ol, (3.beta. 17.)	C ₃₀ H ₅₄ O	Alcohol	2.48
42.12	Zierone	C ₁₅ H ₂₂ O	sesquiterpene	40.68
45.86	Olean-18-en-28-oic acid,3-oxo-,methyl ester	C ₃₁ H ₄₈ O ₃	Triterpenes	6.72
49.58	2-Ethylacridine	C ₁₅ H ₁₃ N	Fatty acid	0.06

RT = retention time

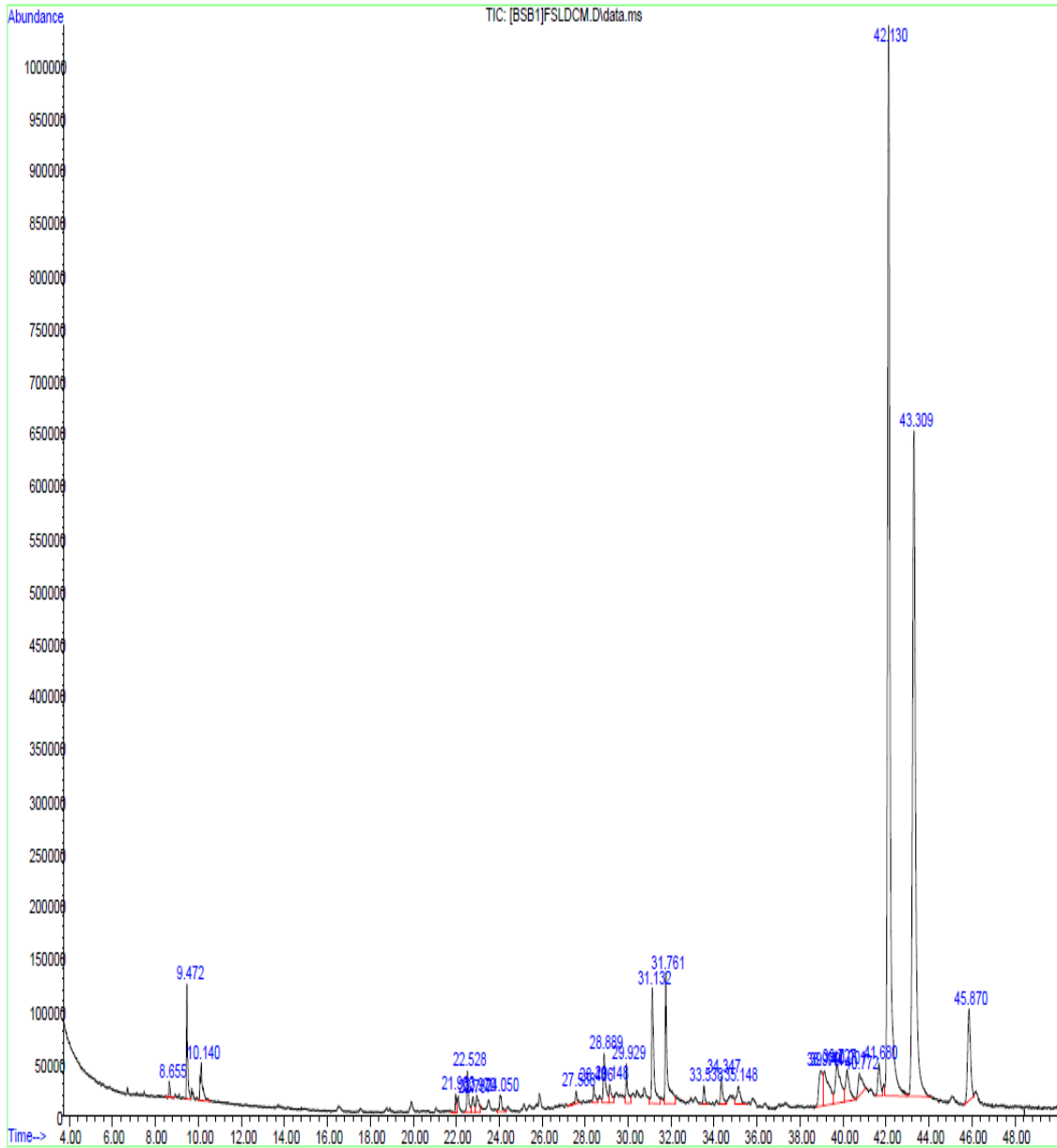


Figure 4.10: Chromatogram of GC-MS Analysis of *Ficus sycororus* DCM Leaf Extract.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Inflammation causes suffering and minimizes victims' productivity (Arnold *et al.*, 2021; Liberale *et al.*, 2022). The NSAIDs and corticosteroid drugs are often prescribed to treat inflammation. Nevertheless, these drugs are associated with hepatic, renal and gastrointestinal toxicities (Tung *et al.*, 2023). This necessitates the need of alternative therapeutic agents. Medicinal plants are largely used to manage diseases and disorders, particularly in developing nations (Oguntibeju, 2018). *Ficus sycomorus* is used by Kenyan communities to manage inflammation and pain, among other uses. However, the scientific data to validate these claims was lacking. This investigation aimed to determine the *in vivo* anti-edema potential and quantitative phytochemical profiles of DCM and methanol extracts of *Ficus sycomorus*.

In the current study, the *Ficus sycomorus* MeOH and DCM stem bark and leaf extracts revealed anti-edema effects following induction of paw edema using carrageenan in mice. This was demonstrated by an attenuation of paw edema following administration of the extracts at 50, 100, and 200mg/kg bw in mice. Generally, MeOH extracts have more polar, non-volatile and high molecular weight compounds than DCM, which extracts mostly non-polar, volatile and low molecular weight compounds (Wrona *et al.*, 2019). This suggested that both polar and non-polar compounds had anti-inflammatory potential.

The findings of current investigation corroborate with that of Alemu *et al.* (2018) who demonstrated the anti-edema effect of *Leonotis ocymifolia* (Burm. F.) MeOH extract following induction of paw edema using carrageenan in mice. Besides, Asefa *et al.* (2022) documented that MeOH extract of *Verbascum sinaiticum* Benth had an anti-edema effect following induction of paw edema using carrageenan in mice. Further, Mworira *et al.* (2021) demonstrated that *Senna didymobotrya* (Fresenius) and *Eucalyptus globulus* (Labill) DCM leaf extracts had anti-edema effects following induction of paw edema using carrageenan in mice. Moreover, Veronica *et al.* (2017) found that *Acacia mellifera* DCM stem bark extract had an anti-edema effect following induction of paw edema using carrageenan in mice.

The most widely used standard model for acute inflammatory research to assess the anti-edematous effects of medicinal plants is carrageenan-induced paw inflammation (Zhang *et al.*, 2022). This model produces reproducible results and hence the choice in this study. Edema caused by carrageenan is classified into two phases. The 1st phase occurs after injection of carrageenan and produces pro-inflammatory mediators, including bradykinins, histamines, serotoninins, and reactive oxygen species (Patil *et al.*, 2019). These pro-inflammatory mediators promote instant onset of edema, hyperalgesia, and erythema (Zhang *et al.*, 2022). Increased vascular permeability and venous blockage are the primary causes of peripheral edema (Kim *et al.*, 2020). The second phase typically appears 3 hours after injection of carrageenan. Prostaglandins are the primary players in this phase, including IL-(1 β , and -6) and TNF- α (Patil *et al.*, 2019).

In this study, the changes in paw edema thickness were utilized to establish the anti-edema potential of the extracts. Carrageenan caused an increased paw edema one hour after the injection and then edema subsided after 4 hours. The DCM stem bark extract produce a dose-dependent response in the 1st, 3rd and 4th hours of the study. The MeOH and DCM stem bark extracts had dose-dependent response throughout the experiment. The higher doses of 200mg/kg bw had better anti-inflammatory effects than the lower doses of 50mg/kg bw. This may be ascribed to rapid metabolism and clearance, as well as deactivation of bioactive substances at lower than higher dosages (Veronica *et al.*, 2017). It was also noted that the four extracts were more efficacious in the 3rd and 4th hours of the study. This could be ascribed to the gradual passive diffusion of anti-inflammatory components in the peritoneal cavity through the cell membrane (Mworia *et al.*, 2021).

In the current study, diclofenac, a nonsteroidal anti-inflammatory drug was chosen as the reference drug. Diclofenac exerts an anti-edema effect by blocking COX-2 activity. This inhibits the synthesis of PGE₂, one of the essential components of inflammatory mediators. The four extracts in this study are believed to have suppressed the synthesis of PGE₂, including other inflammatory mediators such as histamines, bradykinins, serotoninins, pro-inflammatory cytokines and reactive oxygen species. This could be attributed to phytochemicals that were identified using LC-MS and GC-MS screening.

The MeOH and DCM extracts usually extract a higher proportion of polar and non-polar compounds, respectively (Wrona *et al.*, 2019). In quantitative analysis of phytochemicals, LC-MS and GC-MS techniques are utilized to detected non-volatile and volatile

phytocompounds, respectively. The two techniques usually separate compounds using separation column and then quantify them using mass spectrum according to their mass-to-charge ratio (Gonfa *et al.*, 2023). The LC-MS and GC-MS analyses were, therefore, utilized to quantify phytocompounds that were extracted using methanol and DCM, respectively.

The LC-MS analysis of *Ficus sycomorus* methanol extracts revealed various secondary plant metabolites that possess anti-inflammatory effect. These secondary metabolites included phenolic acids, terpenoids, alkaloids, phytosterols and flavonoids (Nisar *et al.*, 2023). The classes of phytochemicals that were detected in this study included flavonoids, phenolic acids, tannins, fatty acids and phytosterols. Flavonoids are polyphenols that possess a benzopyrone ring with polyphenolic or phenolic groups at distinct positions (Ullah *et al.*, 2020). Flavonoids are categorized into multiple sub-classes such as flavonols, flavones, flavanones, flavans, isoflavones, neoflavonoid, flavonolignans, flavonoid glycosides, anthocyanidins and chalcones (Ekalu and Habila, 2020). The structure of flavonoids comprises $C_6C_3C_6$ with a heterocyclic oxygenated benzopyran ring and two aromatic rings (Abou Baker, 2022). There are several health benefits associated with flavonoids, including antidiabetic, antiviral, anti-edema, anticancer, and antioxidant activities (Ekalu and Habila, 2020; Safe *et al.*, 2021).

A number of mechanisms are involved in the production of anti-inflammatory effects by flavonoids, including enhanced immune mechanisms and ROS scavenging, inhibition of lipoxygenase activity, suppression of leukotriene synthesis, inhibition of synthesis of TNF-

α , IL-1 β , and -6), and inhibition of PGE2 and COX-2 synthesis (Ekalu and Habila, 2020; Abou Baker, 2022). The anti-edema activities of flavonoids are ascribed to their structure. For instance, position and number of OH groups, ring unsaturation, -C=O groups, glycosides with high lipophilicity, and methylation (Sharma *et al.*, 2018; Abou Baker, 2022). The enhanced anti-edema effect of flavonoids may be explained by increased number of OH groups in the ring that inhibit lipoxygenase activity. The OCH₃ group also raises the lipophilicity and bioavailability of flavonoids, which in turn increases the suppression of lipoxygenase activity (Abou Baker, 2022).

The flavonoids that were identified in this study included catechins, genistein, luteolin, biochanin, eriodictyol, quercetin and kaempferol. Catechins (flavanol) exert their anti-edema effect by regulating the deactivation of inflammation signaling pathways such as and MAPKs (mitogen-activated protein kinases) and NF- κ B pathways (Kim and Heo, 2022). According to reports, genistein (isoflavone) possesses anti-edema properties by scavenging of ROS, and suppression in production of PGE2, TNF- α , and IL-1 β (Goh *et al.*, 2022). Rutin (Phenolic) exerts its anti-edema effects by upregulating the expression of inhibitor of κ B α (nuclear factor kappa B) and downregulating IL-6 and TNF- α levels (Lan *et al.*, 2022).

The strong anti-edema actions of luteolin (flavone) are achieved by inhibiting NF- κ B and STAT3 (signal transducer activator of transcription-3) signaling pathways (Aziz *et al.*, 2018). By modifying the MAPKs and NF- κ B signaling pathways, biochanin (isoflavone) prevents the generation of inflammatory cytokines (Sarfranz *et al.*, 2020). Eriodictyol

(flavanones) possesses anti-inflammatory properties through suppressing the MAPKs and NF- κ B signaling pathways (Deng *et al.*, 2020). It has been shown that quercetin (flavonol) reduces inflammation by suppressing the NF- κ B and MAPKs pathways, including downregulating expression of TNF- α , and IL-(1 β and -6) (Li *et al.*, 2019; Al-Khayri *et al.*, 2022). Kaempferol, also known as flavonol, inhibits the NF- κ B pathway and reduces the levels of TNF- α and IL-1 β expression.

The phenolic acids were also identified in this study. Phenolic acids are polyphenols that have methoxyl groups, one or more hydroxyl and a carboxylic acid group (Chen *et al.*, 2020). Hydroxycinnamic and hydroxybenzoic acids are the two main classes of phenolic acids. Chlorogenic, ferulic acids, and p-coumaric are examples of hydroxycinnamic acids, while hydroxybenzoic acids include syringic, salicylic, protocatechuic, vanillic, ellagic, gallic, and gentisic acids (Kumar and Goel, 2019). Naturally occurring antioxidants, phenolic acids have a number of medicinal benefits, like hepatoprotective, anti-allergic, anti-cancer, antibacterial, and antiviral properties. They also have anti-edema properties (Sehrawat *et al.*, 2022).

Using LC-MS analysis, phenolic acids including chlorogenic, ferulic acids, p-coumaric and vanillic were found in the four extracts. Chlorogenic acid exerts its anti-edema effects by suppressing levels of PGE₂, NF- κ B, IL-(1 β , and -6), and TNF- α . Ferulic acid reduces inflammation by blocking NF- κ B and MAPKs signaling pathways, and by downregulating TNF- α , and IL-(1 β , and -6) levels (Liu *et al.*, 2022). p-Coumaric acid inhibits TNF- α and

IL-6 to induce anti-edema action (Zhu *et al.*, 2018). Vanillic acid inhibits the NF- κ B pathway and downregulate TNF- α and IL-1 β levels (Ziادلou *et al.*, 2020; Hu *et al.*, 2022).

The LC-MS analysis also identified phytosterols. The steroid skeleton that makes up phytosterols is defined by a saturated bond between C-5 and C-6 sterol moieties. Numerous studies have documented the impressive pharmacological properties of phytosterols, which include antioxidant, chemo preventive, anti-edema, antidiabetic, and anti -atherosclerotic activities (Salehi *et al.*, 2021). This study identified phytosterols such as β -sitosterol and stigmasterol. Beta-sitosterol exerts its anti-inflammatory effect via scavenging of ROS and inhibition of TNF- α , and IL-(1 β and -6) (Choi *et al.*, 2012). Stigmasterol has shown its anti-edema effects by lowering TNF- α levels and blocking activity of COX-2 enzyme (Bakrim *et al.*, 2022).

Fatty acids were also identified using LC-MS analysis. Carboxylic acids that have lengthy aliphatic chains and can be either unsaturated or saturated are known as fatty acids. They characterized with a methyl group (-CH₃) and a carboxyl group (-COOH) (Guimarães and Venâncio, 2022). Oleic acid, a mono-unsaturated omega-9 fatty acid, was identified using LC-MS in this study. Studies have shown that oleic acid reduces edema by suppressing COX-2 activity and inhibiting the MAPK and NF- κ B pathways (Hong *et al.*, 2022).

The GC-MS analysis also detected compounds that have been associated with anti-inflammatory effects, including terpenoids, terpenes, alkaloids, fatty acids, vitamins, phytosterol, and fatty acid methyl ester. Terpenoids are a modified class of terpenes that

have distinct functional groups and methyl groups that have undergone oxidation at various positions. Terpenoids are hydrocarbons containing oxygen (Masyita *et al.*, 2022). Based on their carbon units, terpenes, also known as terpenoids, are categorized as follows: hemiterpenes, monoterpenes, diterpenes, sesterterpenes, sesquiterpenes, triterpenes, and carotenoids/tetraterpenes with 5, 10, 15, 20, 25, 30 and 40 carbon atoms, respectively (Adefegha *et al.*, 2022).

The terpenes that were identified in this study included p-cymene (monoterpene), benzene,2,4-dimethyl-1-(1-methylethyl)- (monoterpene), pseudo phytol<6E,10Z-> (diterpene), and olean-18-en-28-oic acid,3-oxo-,methyl ester (triterpene), while terpenoids included phytol acetate<E-> (diterpenoid), cyperotundone (sesquiterpenoids) and zierone (sesquiterpenoid). Numerous studies have demonstrated that naturally occurring terpenoids and their derivatives have anti-edema properties. Terpenes and terpenoids have been found to downregulate the levels of TNF- α (Gallily *et al.*, 2018).

Alkaloids were also detected using the GC-MS analysis. Alkaloids are naturally occurring organic compounds with nitrogen atoms (Dey *et al.*, 2020). Morphinan, 7,8-didehydro-3-methoxy-17-methyl-6 was an alkaloid that was identified in this study. Alkaloids suppress production of histamine, as well as expression of COX-2, IL-1 β , and TNF- α , to achieve their anti-inflammatory actions (Wang *et al.*, 2018; Souza *et al.*, 2020).

Fatty acids were also detected using GC-MS analysis. They included methyl p-tert-butyl phenyl acetate and isopropyl hexadecanoate. Fatty acids are documented to possess anti-

edema effect (Tortosa-Caparrós *et al.*, 2017). Using GC-MS analysis, fatty acid methyl esters were also detected in this investigation. These are esters of fatty acids. They are derived through the transesterification of fats with methanol. It has been documented that they suppress the expression of TNF- α , NF- κ B and IL-6 (Saeed *et al.*, 2012). Methyl palmitate, and olean-18-en-28-oic acid, 3-oxo-, methyl ester were some of the fatty acid methyl esters that were identified in this study.

Vitamins were also detected using GC-MS analysis in this study. Vitamins exert their anti-edema effects through the scavenging of ROS (Pisoschi *et al.*, 2022). Vitamin E and beta and gamma tocopherol were some of the vitamins that were identified in this study. Other phytochemicals that were detected using GC-MS were phytosterol. As was earlier discussed, phytosterols have demonstrated to possess anti-edema properties. Gamma-sitosterol is one of the phytosterols that was detected using GC-MS in this study.

According to this investigation, at the higher dose of 200mg/kg bw, the MeOH stem bark extract exhibited superior activity in comparison to the other three extracts. The better anti-edema effect of MeOH stem bark extract could be attributed to the polar phytochemicals. It is also possible that phytochemicals that had better anti-inflammatory effects were highly concentrated in the stem bark.

5.2 Conclusions

The study concluded that the four studied extracts of *Ficus sycomorus*;

- i. Ameliorated carrageenan-induced inflammation in mice which suggests their potential as natural anti-inflammatory agents.
- ii. Have phytochemicals associated with anti-inflammatory effects which indicate that the extracts may contribute to the development of plant based therapeutic options for managing inflammation.

5.3 Recommendations

5.3.1 Recommendation from the Study

This study recommends that MeOH extracts of *F. sycomorus* at the dose level of 200 mg/kg bw should be used as a potential alternative anti-inflammatory agent.

5.3.2 Recommendations for Further Studies

- i. Assessing the safety profiles of the studied extracts in animal model to determine their potential toxicity and therapeutic window.
- ii. Investigating the anti-edema properties of isolated extract fractions to identify the most bioactive compounds responsible for the observed effects.

REFERENCE

- Abdulkhaleq, L.A., Assi, M.A., Abdullah, R., Zamri-Saad, M., Taufiq-Yap, Y.H., and Hezme, M.N.M. (2018).** The crucial roles of inflammatory mediators in inflammation: A review. *Veterinary World*, 11(5), 627.
- Abou Baker, D.H. (2022).** An ethno pharmacological review on the therapeutical properties of flavonoids and their mechanisms of actions: A comprehensive review based on up-to-date knowledge. *Toxicology Reports*, 9, 445-469.
- Adefegha, S.A., Oboh, G., and Oluokun, O.O. (2022).** Food bioactives: the food image behind the curtain of health promotion and prevention against several degenerative diseases. *Studies in Natural Products Chemistry*, 72, 391-421.
- Akhtar, M.A. (2022).** Anti-Inflammatory Medicinal Plants of Bangladesh-A Pharmacological Evaluation. *Frontiers in Pharmacology*, 13, 809324.
- Alemu, A., Tamiru, W., Nedi, T., and Shibeshi, W. (2018).** Analgesic and Anti-Inflammatory Effects of 80 % Methanol Extract of *Leonotis ocymifolia* (Burm. f.) Iwarsson Leaves in Rodent Models. *Evidence-Based Complementary and Alternative Medicine*, (1), 1614793.
- Al-Khayri, J.M., Sahana, G.R., Nagella, P., Joseph, B.V., Alessa, F.M., and Al-Mssallem, M.Q. (2022).** Flavonoids as potential anti-inflammatory molecules: A review. *Molecules*, 27(9), 2901.
- Arnold, N., Lechner, K., Waldeyer, C., Shapiro, M.D., and Koenig, W. (2021).** Inflammation and Cardiovascular Disease: The Future. *European Cardiology*, 16, e20.
- Aroca-Crevillén, A., Vicanolo, T., Ovadia, S., and Hidalgo, A. (2024).** Neutrophils in physiology and pathology. *Annual Review of Pathology: Mechanisms of Disease*, 19(1), 227-259.
- Asefa, M., Teshome, N., and Degu, A. (2022).** Anti-Inflammatory and Analgesic Activity of Methanolic Root Extract of *Verbascum sinaiticum* Benth. *Journal of Inflammation Research*, 15, 6381-6392.
- Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., and Supuran, C.T. (2021).** Natural products in drug discovery: Advances and opportunities. *Nature Reviews Drug Discovery*, 20(3), 200-216.
- Aziz, N., Kim, M.Y., and Cho, J.Y. (2018).** Anti-inflammatory effects of luteolin: A review of *in vitro*, *in vivo*, and *in silico* studies. *Journal of Ethno Pharmacology*, 225, 342-358.

- Bakrim, S., Benkhaira, N., Bourais, I., Benali, T., Lee, L.H., El Omari, N., and Bouyahya, A. (2022).** Health benefits and pharmacological properties of stigmasterol. *Antioxidants*, 11(10), 1912.
- Barcelos, I.P., Troxell, R.M., and Graves, J.S. (2019).** Mitochondrial dysfunction and multiple sclerosis. *Biology*, 8(2), 37.
- Batiha, G.E.S., Beshbishy, A.M., El-Mleeh, A., Abdel-Daim, M.M., and Devkota, H.P. (2020).** Traditional uses, bioactive chemical constituents, and pharmacological and toxicological activities of *Glycyrrhiza glabra* L. (Fabaceae). *Biomolecules*, 10(3), 352.
- Berardicurti, O., Ruscitti, P., Pavlych, V., Conforti, A., Giacomelli, R., and Cipriani, P. (2020).** Glucocorticoids in rheumatoid arthritis: the silent companion in the therapeutic strategy. *Expert Review of Clinical Pharmacology*, 13(6), 593-604.
- Bindu, S., Mazumder, S., Bandyopadhyay, U. (2020).** Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochemical Pharmacology*, 180, 114147.
- Boy, H.I. A., Rutilla, A.J.H., Santos, K.A., Ty, A.M.T., Alicia, I.Y., Mahboob, T., and Nissapatorn, V. (2018).** Recommended medicinal plants as source of natural products: a review. *Digital Chinese Medicine*, 1(2), 131-142.
- Bunman, S., Dumavibhat, N., Chatthanawaree, W., Intalapaporn, S., Thuwachosuan, T., and Thongchuan, C. (2017).** Burn Wound Healing: Pathophysiology and Current Management of Burn Injury. *The Bangkok Medical Journal*, 13(2), 91-91.
- Chen, J., Yang, J., Ma, L., Li, J., Shahzad, N., and Kim, C.K. (2020).** Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. *Scientific Reports*, 10(1), 2611.
- Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., and Zhao, L. (2017).** Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, 9(6), 7204-7218.
- Chiurchiù, V., Leuti, A., and Maccarrone, M. (2018).** Bioactive lipids and chronic inflammation: Managing the fire within. *Frontiers in Immunology*, 9, 38.
- Choi, B., Lee, C., and Yu, J.W. (2023).** Distinctive role of inflammation in tissue repair and regeneration. *Archives of Pharmacal Research*, 46(2), 78-89.
- Choi, J.N., Choi, Y.H., Lee, J.M., Noh, I.C., Park, J.W., Choi, W.S., and Choi, J.H. (2012).** Anti-inflammatory effects of β -sitosterol- β -D-glucoside from *Trachelospermum jasminoides* (Apocynaceae) in lipopolysaccharide-stimulated RAW 264.7 murine macrophages. *Natural Product Research*, 26(24), 2340-2343.

- Dar, R.A., Shahnawaz, M., and Qazi, P.H. (2017).** General overview of medicinal plants: A review. *The Journal of Phytopharmacology*, 6(6), 349-351.
- Deng, Z., Hassan, S., Rafiq, M., Li, H., He, Y., Cai, Y., and Yan, T. (2020).** Pharmacological activity of eriodictyol: The major natural polyphenolic flavanone. *Evidence-based Complementary and Alternative Medicine*, (1), 6681352.
- Dey, P., Kundu, A., Kumar, A., Gupta, M., Lee, B.M., Bhakta, T., and Kim, H.S. (2020).** Analysis of alkaloids (indole alkaloids, isoquinoline alkaloids, tropane alkaloids). In *Recent advances in Natural Products Analysis* Elsevier pp. 505-567
- Ekalu, A., and Habila, J.D. (2020).** Flavonoids: isolation, characterization, and health benefits. *Beni-Suef University Journal of Basic and Applied Sciences*, 9(1), 1-14.
- Elsayed, Y., and Nakashima, J.M. (2019).** Chronic inflammation in the etiology of disease across the life span. *Nature Medicine*, 25(12), 1822-1832.
- Erhirhie, E.O., Iiodigwe, E.E., and Ihekwereme, C.P. (2018).** *F. sycomorus L* (Moraceae): A review on its phytopharmacology and toxicity profiles. *Discovery Phytomedicine*, 5(4), 64-71.
- Fokunang, C., Fokunang, E.T., Frederick, K., Ngameni, B., and Ngadjui, B. (2018).** Overview of non-steroidal anti-inflammatory drugs (NSAIDS) in resource limited countries. *Journal Medical Toxicology*, 4(1), 5-13.
- Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., and Slavich, G.M. (2019).** Chronic inflammation in the etiology of disease across the life span. *Nature Medicine*, 25(12), 1822-1832.
- Gakuya, D.W., Okumu, M.O., Kiama, S.G., Mbaria, J M., Gathumbi, P.K., Mathiu, P.M., and Nguta, J.M. (2020).** Traditional medicine in Kenya: past and current status, challenges, and the way forward. *Scientific African*, 6(4), e00360.
- Gallily, R., Yekhtin, Z., and Hanuš, L.O. (2018).** The anti-inflammatory properties of terpenoids from cannabis. *Cannabis and Cannabinoid Research*, 3(1), 282-290.
- Goel, A., and Kulshrestha, S. (2021).** Review on Anti-Rheumatoid Arthritis Potential of Medicinal Plants. *International Journal of Current Research and Review*, 13(03), 16-32.
- Goh, Y.X., Jalil, J., Lam, K.W., Husain, K., and Premakumar, C.M. (2022).** Genistein: a review on its anti-inflammatory properties. *Frontiers in Pharmacology*, 13, 820969.

- Gokul, A.J., Chandur, V.K., and Shabaraya, A.R. (2023).** Non-Selective COX Inhibitor NSAIDs Induced Nephropathy a Systematic Review. *International Journal of Pharmaceutical Sciences*, 1(1), 433-440.
- Gonfa, Y.H., Tessema, F.B., Bachheti, A., Rai, N., Tadesse, M.G., Singab, A.N., and Bachheti, R.K. (2023).** Anti-inflammatory activity of phytochemicals from medicinal plants and their nanoparticles: A review. *Current Research in Biotechnology*, 6, 100152
- Guimarães, A., and Venâncio, A. (2022).** The potential of fatty acids and their derivatives as antifungal agents: A Review. *Toxins*, 14(3), 188.
- Hafeez, F., Zahoor, A.F., Ahmad, S., Ahmad, M., Faiz, S. (2019).** Recent progress in the synthesis of diclofenac based NSAIDs analogs/derivatives. *Synthetic Communications*, 49(3), 325-350.
- Hang, Y., Qin, X., Ren, T., Cao, J. (2018).** Baicalin reduces blood lipids and inflammation in patients with coronary artery disease and rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. *Lipids in Health and Disease*, 17(1), 1-6.
- Hassanzadeh, F., Jafari, E., Khayambashi, N., Hajhashemi, V. (2021).** Synthesis and anti-inflammatory effects evaluation of 1, 3 substituted isatin derivatives. *Thai Journal of Pharmaceutical Sciences*, 45(4), 248-252.
- Herrero-Cervera, A., Soehnlein, O., and Kenne, E. (2022).** Neutrophils in chronic inflammatory diseases. *Cellular and Molecular Immunology*, 19(2), 177-191.
- Hong, J.H., and Lee, Y.C. (2022).** Anti-Inflammatory Effects of Cicadidae Periostracum Extract and Oleic Acid through Inhibiting Inflammatory Chemokines Using PCR Arrays in LPS-Induced Lung inflammation *In Vitro. Life*, 12(6), 857.
- Hu, R., Wu, S., Li, B., Tan, J., Yan, J., Wang, Y., and He, J. (2022).** Dietary ferulic acid and vanillic acid on inflammation, gut barrier function and growth performance in lipopolysaccharide-challenged piglets. *Animal Nutrition*, 8, 144-152.
- Hussein, R.A., and El-Anssary, A.A. (2019).** Plant secondary metabolites as anti-inflammatory agents. In *Herbal Medicine* (pp. 11-30). IntechOpen.
- James, P.B., Wardle, J., Steel, A., and Adams, J. (2018).** Traditional, complementary and alternative medicine use in Sub-Saharan Africa: a systematic review. *British Medical Journal Global Health*, 3(5), e000895.
- Jiang, W., Chen, X., Guo, M., Yu, J., Yang, M., and Pang, X. (2022).** Analysis of fungal microbiomes in edible medicinal *Morinda officinalis* Radix and *Alpiniae oxyphyllae* Fructus using DNA metabarcoding. *Foods*, 11(12), 1748.

- Kany, S., Vollrath, J.T., Relja, B. (2019).** Cytokines in Inflammatory Disease. *International Journal of Molecular Sciences*, 20(23), 6008
- Khan, M.S.A., and Ahmad, I. (2019).** Herbal medicine: current trends and future prospects. In *New look to Phytomedicine* Academic Press.pp. 3-13.
- Khumalo, G.P., Van Wyk, B E., Feng, Y., and Cock, I.E. (2022).** A review of the traditional use of Southern African medicinal plants for the treatment of inflammation and inflammatory pain. *Journal of Ethnopharmacology*, 283, 114436.
- Kigen, G., Kipkore, W., Wanjohi, B., Haruki, B., and Kemboi, J. (2017).** Medicinal plants used by traditional healers in Sangurur, Elgeyo Marakwet County, Kenya. *Pharmacognosy Research*, 9(4), 333.
- Kim, J.M., and Heo, H.J. (2022).** The roles of catechins in regulation of systemic inflammation. *Food Science and Biotechnology*, 31(8), 957-970.
- Kim, K.H., Im, H.W., Karmacharya, M.B., Kim, S., Min, B.H., Park, S.R., and Choi, B.H. (2020).** Low-intensity ultrasound attenuates paw edema formation and decreases vascular permeability induced by carrageenan injection in rats. *Journal of Inflammation*, 17, 1-8.
- Kumar, N. and Goel, N. (2019).** Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, 24, e00370.
- Lan, Z., Wang, H., Wang, S., Zhu, T., Ma, S., Song, Y., and Tian, C. (2022).** Rutin protects against cyclophosphamide induced immunological stress by inhibiting TLR4-NF- κ B-mediated inflammation and activating the Nrf2-mediated antioxidant responses. *Pharmacological Research-Modern Chinese Medicine*, 4, 100135.
- Li, T., Li, F., Liu, X., Liu, J., and Li, D. (2019).** Synergistic anti-inflammatory effects of quercetin and catechin via inhibiting activation of TLR4–MyD88-mediated NF- κ B and MAPK signaling pathways. *Phytotherapy Research*, 33(3), 756-767.
- Liberale, L., Badimon, L., Montecucco, F., Lüscher, T.F., Libby, P., and Camici, G.G. (2022).** Inflammation, aging, and cardiovascular disease: JACC review topic of the week. *Journal of the American College of Cardiology*, 79(8), 837-847.
- Liu, Y., Shi, L., Qiu, W., and Shi, Y. (2022).** Ferulic acid exhibits anti-inflammatory effects by inducing autophagy and blocking NLRP3 inflammasome activation. *Molecular and Cellular Toxicology*, 18(4), 509-519.
- Masyita, A., Sari, R.M., Astuti, A.D., Yasir, B., Rumata, N.R., Emran, T.B., and Simal-Gandara, J. (2022).** Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chemistry*, 13, 100217.

- Mbuni, Y.M., Wang, S., Mwangi, B.N., Mbari, N. J., Musili, P.M., Walter, N.O., and Wang, Q. (2020).** Medicinal plants and their traditional uses in local communities around Cherangani Hills, Western Kenya. *Plants*, 9(3), 331.
- Mworia, J.K., Kibiti, C.M., Ngeranwa, J.J., and Ngugi, M.P. (2021).** Anti-inflammatory potential of dichloromethane leaf extracts of *Eucalyptus globulus* (Labill) and *Senna didymobotrya* (Fresenius) in mice. *African Health Sciences*, 21(1), 397-409.
- Moriasi, G., Ileri, A., and Ngugi, M. (2020).** Cognitive-Enhancing, *Ex Vivo* Antilipid Peroxidation and Qualitative Phytochemical Evaluation of the Aqueous and Methanolic Stem Bark Extracts of *Lonchocarpus eriocalyx* (Harms.). *Biochemistry Research International*, (1), 8819045.
- Nisar, A., Jagtap, S., Vyavahare, S., Deshpande, M., Harsulkar, A., Ranjekar, P., and Prakash, O. (2023).** Phytochemicals in the treatment of inflammation-associated diseases: the journey from preclinical trials to clinical practice. *Frontiers in Pharmacology*, 14, 1177050.
- Oguntibeju, O. O. (2018).** Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. *Journal of Inflammation Research*, 11, 307-317.
- Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., and Anthony, S. (2022).** *Ficus sycomorus*. In Agroforestry Database: A tree reference and selection guide version 4.0. *World Agroforestry*. <https://www.worldagroforestry.org/treedb2/species-profile.php?Spid=704>. Retrieved 03 July 2025.
- Paclet, M.H., Laurans, S., and Dupré-Crochet, S. (2022).** Regulation of neutrophil NADPH oxidase, NOX2: a crucial effector in neutrophil phenotype and function. *Frontiers in Cell and Developmental Biology*, 10, 945749.
- Pahwa, R., Goyal, A., and Jialal, I. (2023).** Chronic Inflammation. In *StatPearls [Internet]*. StatPearls Publishing.
- Patil, K. R., Mahajan, U. B., Unger, B. S., Goyal, S. N., Belemkar, S., Surana, S. J., and Patil, C.R. (2019).** Animal models of inflammation for screening of anti-inflammatory drugs: implications for the discovery and development of phytopharmaceuticals. *International Journal of Molecular Sciences*, 20(18), 4367.
- Philippou, E., Petersson, S.D., Rodomar, C., and Nikiphorou, E. (2021).** Rheumatoid arthritis and dietary interventions: systematic review of clinical trials. *Nutrition Reviews*, 79(4), 410-428.
- Pisoschi, A.M., Pop, A., Iordache, F., Stanca, L., Geicu, O.I., Bilteanu, L., and Serban, A.I. (2022).** Antioxidant, anti-inflammatory and immunomodulatory roles of

vitamins in COVID-19 therapy. *European Journal of Medicinal Chemistry*, 232, 114175.

- Rahman, M.M., Rahaman, M.S., Islam, M.R., Hossain, M.E., Mithi, F.M., Ahmed, M., and Uddin, M.S. (2022).** Multifunctional roles of anthocyanins in inflammation and oxidative stress. *Biomedicine and Pharmacotherapy*, 153, 113526.
- Ruparelia, N., Chai, J.T., Fisher, E.A and Choudhury, R.P. (2017).** Inflammatory processes in cardiovascular disease: a route to targeted therapies. *Nature Reviews Cardiology*, 14(3), 133-144.
- Ruslin, R., Bruggemann, R., and Visser, T. (2020).** Thromboxane A2 modulation in cardiovascular disease: A balancing act. *European Journal of Pharmacology*, 886, 173442.
- Saeed, N.M., El-Demerdash, E., Abdel-Rahman, H.M., Algandaby, M., Al-Abbasi, F.A., and Abdel-Naim, A.B. (2012).** Anti-inflammatory activity of methyl palmitate and ethyl palmitate in different experimental rat models. *Toxicology and Applied Pharmacology*, 264(1), 84-93.
- Safe, S., Jayaraman, A., Chapkin, R.S., Howard, M., Mohankumar, K., and Shrestha, R. (2021).** Flavonoids: structure–function and mechanisms of action and opportunities for drug development. *Toxicological Research*, 37, 147-162.
- Salehi, B., Quispe, C., Sharifi-Rad, J., Cruz-Martins, N., Nigam, M., Mishra, A.P., and Koch, W. (2021).** Phytosterols: From preclinical evidence to potential clinical applications. *Frontiers in Pharmacology*, 11, 1819.
- Sarfraz, A., Javeed, M., Shah, M.A., Hussain, G., Shafiq, N., Sarfraz, I., and Rasul, A. (2020).** Biochanin A: A novel bioactive multifunctional compound from nature. *Science of the Total Environment*, 722, 137907.
- Sehrawat, R., Rathee, P., Akkol, E.K., Khatkar, S., Lather, A., Redhu, N., and Khatkar, A. (2022).** Phenolic acids-versatile natural moiety with numerous biological applications. *Current Topics in Medicinal Chemistry*, 22(18), 1472-1484.
- Sharma, A., Sharma, P., Tuli, H.S., and Sharma, A.K. (2018).** Phytochemical and pharmacological properties of flavonols. *Encyclopedia of Life Sciences John Wiley and Sons*, 1-12.
- Shingala, Z., Chauhan, B., and Baraiya, J. (2021).** A review on medicinal plants as a source of anti-inflammatory agents. *Journal of Pharmacognosy and Phytochemistry*, 10(6), 364-371.
- Sirico, F., Bianco, A., D'Alicandro, G., Castaldo, C., Montagnani, S., Spera, R., and Nurzynska, D. (2018).** Effects of physical exercise on adiponectin, leptin, and

inflammatory markers in childhood obesity: *Systematic Review and Meta-Analysis*. *Childhood Obesity*, 14(4), 207-217.

- Soliman, A.M., and Barreda, D.R. (2022).** Acute inflammation in tissue healing. *International Journal of Molecular Sciences*, 24(1), 641.
- Souza, C.R., Bezerra, W.P., and Souto, J.T. (2020).** Marine alkaloids with anti-inflammatory activity: Current knowledge and future perspectives. *Marine Drugs*, 18(3), 147.
- Stone, W.L., Basit, H., and Burns, B. (2021).** Pathology, inflammation. In *StatPearls [Internet]*. StatPearls Publishing.
- Thakur, S., Riyaz, B., Patil, A., Kaur, A., Kapoor, B., and Mishra, V. (2018).** Novel drug delivery systems for NSAIDs in management of rheumatoid arthritis: *An overview*. *Biomedicine and Pharmacotherapy*, 106, 1011-1023.
- Tortosa-Caparrós, E., Navas-Carrillo, D., Marín, F., and Orenes-Piñero, E. (2017).** Anti-inflammatory effects of omega 3 and omega 6 polyunsaturated fatty acids in cardiovascular disease and metabolic syndrome. *Critical Reviews in Food Science and Nutrition*, 57(16), 3421-3429.
- Tung, B.T., Linh, T.V., Thao, T.P., and Thuan, N.D. (2023).** Anti-inflammatory Agents from Medicinal Plants. *Phytochemical Drug Discovery for Central Nervous System Disorders: Biochemistry and Therapeutic Effects*, 9, 219-250.
- Ullah, A., Munir, S., Badshah, S.L., Khan, N., Ghani, L., Poulson, B.G., and Jaremko, M. (2020).** Important flavonoids and their role as a therapeutic agent. *Molecules*, 25(22), 5243.
- Umamageswari, M.S., and Maniyar, Y.A. (2015).** Evaluation of anti-inflammatory activity of aqueous extract of leaves of *Solanum melongena* linn. in experimental animals. *Journal of Clinical and Diagnostic Research*, 9(1), 1-3.
- Veronica, S.A., Cheruiyot, K.S., Bosibori, M.J., Munene, I.M., Murugi, N.J., and Piero, N.M. (2017).** Anti-inflammatory, analgesic and antipyretic effects of dichloromethane stem bark extract of *Acacia mellifera*. *The Journal of Phytopharmacology*, 6(4), 239-246.
- Wang, C.Y., Jang, H.J., Han, Y.K., Su, X.D., Lee, S.W., Rho, M.C., and Kim, Y.H. (2018).** Alkaloids from *Tetrastigma hemsleyanum* and their anti-inflammatory effects on LPS-induced RAW264. 7 cells. *Molecules*, 23(6), 1445.
- Wojcieszynska, D., Łagoda, K., and Guzik, U. (2023).** Diclofenac Biodegradation by Microorganisms and with Immobilized Systems—A Review. *Catalysts*, 13(2), 412.

- Wongrakpanich, S., Wongrakpanich, A., Melhado, K., and Rangaswami, J. (2018).** A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. *Aging and Disease*, 9(1), 143-150.
- Wrona, O., Rafińska, K., Walczak-Skierska, J., Możeński, C, and Buszewski, B. (2019).** Extraction and determination of polar bioactive compounds from alfalfa (*Medicago sativa L.*) using supercritical techniques. *Molecules*, 24(24), 4608.
- Yasir, M., Goyal, A., Bansal, P., and Sonthalia, S. (2020).** Corticosteroid Adverse Effects. In *StatPearls [Internet]*. StatPearls Publishing.
- Zhang, L., Dou, X.W., Zhang, C., Logrieco, A.F., and Yang, M.H. (2018).** A review of current methods for analysis of mycotoxins in herbal medicines. *Toxins*, 10(2), 65.
- Zhang, L., Virgous, C., and Si, H. (2019).** Synergistic anti-inflammatory effects and mechanisms of combined phytochemicals. *The Journal of Nutritional Biochemistry*, 69, 19-30.
- Zhang, X., Retyunskiy, V., Qiao, S., Zhao, Y., and Tzeng, C.M. (2022).** Alloferon-1 ameliorates acute inflammatory responses in λ -carrageenan-induced paw edema in mice. *Scientific Reports*, 12(1), 16689.
- Zhu, H., Liang, Q.H., Xiong, X.G., Wang, Y., Zhang, Z.H., Sun, M.J., and Wu, D. (2018).** Anti-inflammatory effects of p-coumaric acid, a natural compound of *Oldenlandia diffusa*, on arthritis model rats. *Evidence-Based Complementary and Alternative Medicine*, 2018(1), 5198594.
- Ziadlou, R., Barbero, A., Martin, I., Wang, X., Qin, L., Alini, M., and Grad, S. (2020).** Anti-inflammatory and chondroprotective effects of vanillic acid and epimedin C in human osteoarthritic chondrocytes. *Biomolecules*, 10(6), 932.

APPENDICES
Appendix I: Research Approval



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Website: www.ku.ac.ke

Internal Memo

FROM: Dean, Graduate School

DATE: 9th March, 2022

TO: Ms. Muthee Eunice Wothaya
C/o Biochemistry, Microbiology &
Biotechnology Department

REF: I56/38031/2017

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

=====

This is to inform you that Graduate School Board, at its meeting on 2nd March, 2022, approved your Research Proposal for the M.Sc. Degree entitled, "Antiinflammatory Activities of Methanolic and Dichloromethane Extracts of *Ficus sycomorus* L. in Swiss Albino Mice."

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

DR. EDWIN OBUNGU

FOR: DEAN, GRADUATE SCHOOL



CC. Chairman, Department of Biochemistry, Microbiology & Biotechnology
Supervisors:

1. Dr. Mathew Piero Ngugi
C/o Biochemistry, Microbiology & Biotechnology Dept.
Kenyatta University
2. Prof. Alex K. Machocho
C/o Department of Chemistry
Kenyatta University



REPUBLIC OF KENYA



NATIONAL COMMISSION FOR
SCIENCE, TECHNOLOGY & INNOVATION

Ref No: 112841

Date of Issue: 22/March/2022

RESEARCH LICENSE



This is to Certify that Mr.. Eunice Wothaya Muthee of Kenyatta University, has been licensed to conduct research in Nairobi on the topic: Anti-inflammatory activities of methanolic and dichloromethane extracts of *Ficus sycomorus* L. in Swiss albino mice for the period ending : 22/March/2023.

License No: NACOSTI/P/22/16460

112841

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR
SCIENCE, TECHNOLOGY &
INNOVATION

Verification QR Code



NOTE: This is a computer generated License. To verify the authenticity of this document,
Scan the QR Code using QR scanner application.



**KENYATTA UNIVERSITY
ANIMAL CARE AND USE COMMITTEE**

Fax: 8711242/8711575
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Nairobi, 00100

Website: www.ku.ac.ke

Tel: 8710901/12

Our Ref: **KU/ACUC/APPROVAL/VOL.1/02**

Date: 30th August, 2022

Eunice Muthee
P.O BOX 43844-00100
Nairobi.

Dear Eunice,

**RE: ANTI-INFLAMMATORY ACTIVITIES OF METHANOLIC AND
DICHLOROMETHANE EXTRACTS OF FICUS SYCOMORUS L. IN SWISS ALBINO
MICE**

This is to inform you that **KENYATTA UNIVERSITY ANIMAL CARE AND USE COMMITTEE** has approved the study protocol together with the attached SOPs dated 22nd August 2022. Your application approval number is **PKUA/007/007**. The approval period is from **30th August 2022 to 30th August 2023**.

This approval is subject to compliance with the following requirements;

- i. Only approved documents including SOPs will be used in the study
- ii. All amendments, deviations are to be submitted using relevant forms for review and approval by the Animal Care and Use Committee
- iii. Death and life threatening problems of the study animals and handlers and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to the Animal Care and Use Committee within 72 hours of notification.
- iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study animals and handlers or affect the integrity of the research should be reported to the Animal Care and Use Committee.
- v. Clearance for export of biological specimens must be obtained from relevant institutions.

Appendix IV: Structures of Phytochemicals

