DETERMINATION OF DIABETIC WOUND HEALING, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF *Cyathula uncinulata* CRUDE LEAF EXTRACT IN FRUCTOSE-INDUCED DIABETIC WISTAR RATS

MUTUKU KILONZO

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DECLARATION

Declaration by the Student

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Mutuku Kilonzo

_____ **Date**_____

SSCI/CHE/M/004/22

Declaration by the Supervisors

This thesis has been submitted with our approval as university supervisors.

_____ **Date**_____

Dr. Geoffry Kiptoo
Lecturer, Department of Chemistry and Biochemistry
University of Eldoret, Kenya

_____ **Date**_____

Dr. Naomi Bisem
Lecturer, Department of Chemistry and Biochemistry
University of Eldoret, Kenya

DEDICATION

This work is especially dedicated to my mother Rael Munywoki for being my pillar of strength. I also dedicate it to my friends for being my motivators and always encouraging me.

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ABSTRACT

The treatment of a wound in a diabetic patient is difficult for clinicians and researchers since it often heals very slowly, due to lasting inflammation and is subject to prolonged oxidative stress. The current treatments have adverse side effects, are expensive, inaccessible and sometimes inefficient leading to death, infections, reduced quality of life and amputation. Therefore, there is a critical need for affordable, accessible and effective alternatives for diabetic wound management. *C. uncinulata* has been traditionally used in treatment of wounds and despite the wide spread use there is no scientific evidence to support it in the management of diabetic wounds. Therefore, this study aimed to investigate the phytochemicals, antioxidant activity and Wound healing (WH) features of *C. uncinulata* leaves extracts in fructose-induced diabetic Wistar rats. Phytochemical screening of the methanolic leaf extract showed higher presence of a high concentration of flavonoids, alkaloids and tannins, as compared to aqueous extract; methanolic extract was selected for in vivo studies. Thirty-five male rats were randomly assigned to seven groups. Two groups received topical methanolic extract, with one group treated with a low dose of 300 mg/kg and the other with a high dose of 600 mg/kg. One group was administered Floxapen (0.2 mL/day). The remaining groups served as controls, with some receiving a vehicle treatment or no treatment at all in this case diabetic control group. The rats were induced with a specific type of injury, which was then topically treated as per the experimental design. The low dose of 300 mg/kg was considered the lower dose, while 600 mg/kg represented the higher dose in the study. In vitro, the methanolic leaf extract showed significant antioxidant activity at all tested concentrations, comparable to ascorbic acid, as well as Hydrogen Peroxide (HP) scavenging, reducing power and catalase enzyme activity ($P<0.05$). Additionally, the methanolic leaf extract showed significant protection of albumin against protein denaturation at higher doses. In vivo studies, rats treated with the high dose of methanolic extract exhibited faster wound contraction, with their wounds completely healed by day 21. This outcome was similar to the group treated with the standard drug Floxapen, which also showed complete wound healing by the same time point. The Kaplan-Meier curves for the healing rate demonstrated that the treated groups healed faster than the control groups. According to this study's findings, *Cyathula uncinulata* had the potential as one of the natural therapeutic agents for the management of diabetic patient wounds due to its antioxidant, anti-inflammatory, and WH properties. These effects indicated that further studies need to be conducted to identify and describe the molecules that act in this way.

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LIST OF ABBREVIATIONS

ABS	Absorbance
AGEs	Advanced Glycation End-products
ANOVA	Analysis of Variance
BHT / BHA	Butylated Hydroxytoluene / Butylated Hydroxyanisole
BSA	Bovine Serum Albumin
CU	*Cyathula uncinulata*
DFU	Diabetic Foot Ulcer
ECM	Extracellular Matrix
HBOT	Hyperbaric Oxygen Therapy
HP	Hydrogen Peroxide
IC_{50}	50 % Inhibitory Concentration
IL-6 / IL-1β	Interleukin-6 / Interleukin-1 beta
INOS	Inducible Nitric Oxide Synthase
MΦ1 / MΦ2	Macrophage MΦ1/2
MDR	Multi-Drug Resistance
MIC	Minimum Inhibitory Concentration
MMPs	Matrix Metalloproteinases
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NSAID	Non-Steroidal Anti-Inflammatory Drug
OECD	Organisation for Economic Co-operation & Development

PAD	Peripheral Arterial Disease
PDGF	Platelet-Derived Growth Factor
PMN	Polymorphonuclear Neutrophil
RBC	Red Blood Cell
RHPDGF	Recombinant human platelet derived growth factor
ROS	Reactive Oxygen Species
RPM	Revolutions Per Minute
SC	Stratum Corneum
SEM	Standard Error of the Mean
SPSS / R	Statistical Package for the Social Sciences / R Language
TCC	Total Contact cast
TGF-β	Transforming Growth Factor-beta
TNF-α	Tumour Necrosis Factor-alpha
Tukey HSD	Tukey Honestly Significant Difference
UV-Vis	Ultraviolet–Visible Spectrophotometry
WH	Wound Healing
WHO	World Health Organization
WISTAR	Wistar Albino Rat

CHAPTER ONE

INTRODUCTION

1.1 Background

A wound exists when normal bodily tissues experience any sort of structural or functional break or interruption. The skin and its underlying tissues in the area between dermis and sub dermis are repaired by a biological sequence known as wound healing. Haemostasis, inflammation, proliferation and then maturation are the four main types of wound healing process. Both external and internal factors affect wound healing process. (Guo & DiPietro, 2010). Age, diet, sleep and also the weather conditions are also some of the factors which affect wound healing. Chronic wounds heal slowly because some processes are incomplete and disorganized. This makes them to remain in inflammation phase for long time. After an injury Platelets and coagulants begin to build up at that site. The endothelium then activates immune cells which enters into the body to begin inflammation (*Boniakowski et al.*, 2017). Neutrophils are the main cells at infiltration stage which are active in apoptosis. macrophages Phagocytosis of the cells which is performed by macrophages aids in WH process.

The MΦ2 macrophages are the one which are involved in the process of shifting towards the stage of proliferative of healing process. Diabetes condition has been deemed to suppress the transformation of MΦ1's towards MΦ2: and also, diabetic wound literature pointing towards some factors like higher oxidative stress, higher apoptotic cell number, and M macrophage preference (*Wan et al.,* 2021). During the proliferative stage of wound healing there are various physiological activities which are compromised and these activities are like angiogenesis, secretion of extracellular matrix (ECM) and wound

contraction and this is due to the failure of fibroblasts cells to differentiate into myofibroblasts. MΦ2 phenotype are the only macrophages that promotes the formation the new blood vessels and they also promote the process of the regeneration of the epithelial cells during the process of proliferation. Angiogenesis is known to be the biological process by which new blood vessels are formed from the existing ones. Angiogenesis is an important in the WH process because it aids to oxygenate hypermetabolic healing tissue and also supply it with some nutrients. When the signalling process does not occur or when myofibroblast loses their functionality, these makes keratinocytes not to migrate and promote the re-epithelialization process in the wounds. This may result to tissue remodelling delay, formation of chronic non-healing wounds and some skin barriers being compromised (Wan *et al*., 2021) The maturation or remodelling phase the last phase entails extra matrix formation (Boniakowski *et al*., 2017).

The chronic non-healing wounds affects around 1-2% of the population and the prevalence is higher among individuals aged sixty -five years and above with other associated diseases. The expenses which are due to poor diabetic wound healing are estimated to be about three billion US dollars in one year and these makes the major component of a nation's total health care expenditures as exemplified by the United States (Boniakowski *et al*., 2017). Numerous factors might slow down the process of WH by altering the tissue injury response including diabetes, being overweight, age, gender, insufficient intake of protein and energy, certain medications like steroids, Non-steroidal Anti-Inflammatory Drugs anti-rejection and immunosuppressive drugs, radiotherapy and chemotherapy, and smoking and alcohol consumption. The normal functioning of the body cells and tissues is affected by diabetes. Wounds of diabetic patients takes long period to heal than normal wounds.

Reactive oxygen species (ROS) act like signalling molecules in the WH process. They also carry out cell death of both bacterial and microbials. This occurs when they are present in right amount. High levels of ROS causes damage to the DNA, proteins and lipid peroxidation Oxidative stress and cell damage may occur due to change in environment. This is due to increase in ROS (Polaka *et al*., 2022). Also tissue and cell damage occurs when a tissue with stress is entered by phagocytic cells. This occurs during pathological inflammation process (Sherwood & Traber, 2007). Hypoxia condition may limit the function of fibroblast and epidermal cells. It also leads to increased oxidative stress and hinders the process of angiogenesis. This causes toxicity of glycosylated end products (Guo & DiPietro, 2010). Diabetes is always affected by the inflammation process and also oxidative stress, and these limits there potential to form new cells of the epithelium and building important extracellular matrix. (Oluwafemi, 2019).

It is therefore logical to deduce that speculative aggression stems from need to protect the cell from hostile forces including toxins, pathogens and/ or physical harm, with ROS serving both as a protector as well as an assailant. Free radicals and lipid peroxidation are the causes of oxidative damage and antioxidants help to remove free radicals and prevent the occurrence of lipid peroxidation which will uphold the health of the cells (Lobo *et al*., 2010). New research demonstrates the value of medicinal plants which possess bioactive compounds that function as potent antioxidants for controlling inflammation while speeding up tissue repair. Dual anti-inflammatory and antioxidant plant-based therapeutic approaches show promise as solutions for treating diabetic mellitus-linked wounds (Sherwood & Traber, 2007).

However, some natural radical scavengers are costly, and their sources are scanty or have detrimental impact on the human body; this makes plant antioxidants the best due to their efficiency, accessibility and cheapness as pointed out by Oliveira *et al*. (2025). However, steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are very useful, but they are deemed to be risky once used over time and regularly since they lead to gastrointestinal and cardiovascular issues like, high blood pressure heart failure (Ghlichloo & Gerriets, 2023). Traditional medicinal plants are used as form of medicine in many societies globally due to effectiveness, accessibility and relatively mild side effects compared to chemical products in treating different diseases (Ekor, 2014).

Diabetic ulcers, particularly Diabetic foot ulcers (DFUs), which often results in lower extremity amputations, are known as a growing health concern while inflicting at least two billion USD in healthcare service costs and loss of productivity. This is especially the case in very high mortality rates which are associated with amputations (Aaron *et al*., 2019; Burges *et al*., 2021). In a study done in the Kenyatta national hospital, it was established that the prevalence of DFU was at 4.6%. According to research, the treatment of DFUs is very costly, and expenditure in England alone is projected to exceed £1 billion per annum (Wokabi, 2021).

That is why it is important to keep searching for non-toxic diabetic wound treatment alternatives, as the conventional treatments including use of antibiotics has disadvantages like development of antibiotic resistance and adverse side effects. *Cyathula uncinulata* is a medicinal plant used traditionally in WH, antioxidant, and anti- inflammatory effects and yet it is scientifically underexplored. This research sought to investigate the preparation of water-soluble leaf extracts of *C. uncinulata* for the healing of diabetic ulcers and to evaluate

the anti-inflammatory, radical scavenging and WH ability of the said extracts using in vitro tests (Omara *et al*., 2021).

Cyathula uncinulata is an herbal medicinal plant found in most regions of Africa, which has been utilized in different traditional uses including in the fight against malaria, livestock feed and treatment of different diseases; it is a promising plant for natural WH. That is why is used in different societies around the world as medication for ailments, like wound healing it is suggested to be effective in treating diabetic ulcers and other diseases (Bisi Johnson *et al*., 2015).

Figure 1:1 Photo of A. Leaves and B. flowers of *C. uncinulata* (Source : ……)

1.2 Statement of The Problem

The prediction by WHO (2010) and Tam *et al*. (2011) shows diabetic wound treatment requirements will rise significantly based on estimated statistics from 2010 to 2030; with the annual healthcare costs from diabetic foot ulcers total $200 billion to cover the cost of slow healing, required lower limb amputations and workforce productivity losses. Major

amputations occurring from DFUs produce mortality rates exceeding those of various cancers since they reach 16.7% in the initial year and rise to 50% after five years (Yammine *et al*., 2020). Most medicines for DFUS treatment are not as effective (Ouyang *et al*., 2024). Diabetic ulcers emerge as a complex medical problem because they resist healing and maintain long-term existence. WH mechanisms in DFUs is not well known even though researchers continue to investigate the problem (Oguntibeju *et al*., 2019).

Due to the short supply of and the negative effects of synthetic antioxidants for the regulation of redox imbalances, natural antioxidants have seen a recent upsurge in interest. These are compounds obtained from plants and are ideal for their non synthetic alternatives, cheapness and ease to obtain (Oliveira *et al*., 2025)

1.3 Justification of The Study

Chronic ulcers are estimated to exist in about five (5) million persons in the US; many are adults who have one or more coexisting health conditions. Impaired wound healing results into financial burden. In one year, the total cost can be estimated to be three billion dollars. This amount to a higher figure of healthcare expenses in the United States (Palmer & Buckley, 2021). Amputation is one of the problems which is as a result of diabetic wounds. Amputation usually occurs after twelve months. Mostly 16.7% of individuals die as a result of diabetic wounds. The mortality rate is predicted to be more than 50% after 5 years period. (Yammine *et al*., 2020). Treatment of DFUS is challenging currently because the medications are becoming ineffective. (Ouyang *et al*., 2024). The antibiotics used for the treatment have also some problems like resistance, harmful side effects and they are expensive (Burges *et al*., 2021). DFUs also results to high amount being spend at the hospital. (Russell, 2004). Due to the above negative effects, there is need to explore safe

alternatives for diabetic wound treatment to be explored for long term use (Rajalakshmi *et al*., 2009)

Medicinal plants are safer, they are readily available and less costly, hence there is a growing popularity for their use. (Oliveira *et al*., 2025). Gastrointestinal and cardiovascular associated complications are some of the side effects associated with aspirin and NSAIDs intake (Ghlichloo & Gerriets, 2023) Conventional treatment of diabetic wounds is associated with some factors like high treatment failure, adverse side effects and also high treatment cost among others. Due to the above factors, there is need for exploration for safer modes of treatments. The purpose of this work was to come up with forms of treatments which are safer and also reduce global healthy burden. It also aimed to reduce the strain that DFUS causes on the economy through determination of antioxidant and anti-inflammatory activities of the methanolic extract (Emordi *et al*., 2016).

Natural products from plants helps to treat diseases mainly in the form of decoctions in both traditional herbal and traditional medicine globally. (Ahmed *et al*., 2022). However, these positive attributes of many plants and plant-derived bioactive compounds necessitate further exploration (Alam *et al*., 2022). Diabetes mellitus is a continuously emerging health threat, and an increasing number of people suffering from the chronic wound resulting from this disease require treatments with less harmful effects and lower cost. *C. uncinulata* is an economically beneficial plant used for traditional healers for treatment of various illnesses, and the scientific evidence has not been explored whether it contains some bioactive compounds contain WH, antioxidant, and anti-inflammatory activities.

1.4 Study Objectives

1.4.1 General objective

This study's overall objective was to evaluate the WH efficacy of *Cyathula uncinulata* methanolic crude leaf extract in fructose – induced diabetic Wistar rats.

1.4.2 Specific Objectives

1. To extract and perform qualitative analysis to determine the phytochemical profile of *C. uncinulata* leaves.
2. To evaluate *in vitro* the antioxidant properties of the crude leaf extract.
3. To determine *in vitro* the anti-inflammatory properties of the crude leaf extract.
4. To determine the WH efficacy of the crude leaf extract *in vivo* on fructose-induced diabetic Wistar rat.

1.5 Study Null Hypotheses

H_{1o} There are no significantly detectable levels of phytochemical compounds present in *C. uncinulata* crude leave extract.

H_{2o} There exists no significant anti-inflammatory property present in *C. uncinulata* crude leave extract.

H_{3o} There exists no significant antioxidant property present in *C. uncinulata* crude leave extract.

H_{4o} There are no significant WH properties present in *C. uncinulata* crude leave extract.

1.6 Scope of The Study

The study investigated how *C. uncinulata* showed potential for WH actions through antioxidant and anti-inflammatory mechanisms in diabetic wounds. Also, this study seeks

the opportunity to venture more into *C. uncinulata,* that has not been studied before. Despite the wide spread use of *C. uncinulata* there is no scientific evidence to support its effectiveness in management of DFUs. This model used male Wistar rats due to their availability, are cost effective, they don't have hormonal cyclicity compared to the female ones and they have been used previously in most of the researches and available as compared to other models, making them unique for diabetes research (Furman, 2015)

This work used thirty-five male Wistar rats that had diabetes which was induced through a diet containing 60% D-fructose alongside 40% standard rat feed. Plant extract preparation was initiated and the process followed by bioactive compound detection through phytochemical analysis. In vitro tests were done for antioxidant and anti-inflammatory effect of the extract. The measured extracts underwent therapeutic assessment through the tracking of WH indicators like epithelialization time and wound contraction speed.

1.7 Significance of the Study

Diabetes is a health condition that leads to slow and difficult WH. This leads to increased risks of infections and other complications related to diabetes like foot ulcers which take too long to heal. Currently, the available treatments are costly, unavailable and are not safe, hence need alternative treatment. study should provide a scientific background of the use of *C. uncinulata* leaf extract as a potential agent for WH, antioxidant, and anti-inflammatory for patients with diabetes. The results from this work will spur other studies on possible anti-diabetic, WH, antioxidant, and anti-inflammatory properties of *C. uncinulata* leaf extract.

CHAPTER TWO

LITERATURE REVIEW

2.1 Understanding Diabetic Wound Healing (WH)

Wound healing (WH) can be described as a biological response through which the body endeavours to repair injured skin tissue to regain its normal function and appearance. It is very difficult for people living diabetes to regenerate because their wound heals very slowly. They usually have challenges because their wounds are slow to heal and take a longer period of time to heal. (Villines, 2022). Carefulness should be done on the treatment of diabetic wounds since they may have some complications regardless of their sizes. Based on how difficulty they are to treat and how commonly they occur are the only type of diabetic wounds which exists. The most common type of diabetic wound is DFUs which may lead to many challenges. (Wokabi *et al*,2021). The skin sensitivity is always lowered by neuropathy and nerve damage. This decreases the ability of the person to feel pain in the feet and a person may get injury without knowing. This impairs the circulation of blood and affects the skin healing capacity. There is high risk of developing ulcer due increased mechanical stress and pressure which comes as result of wearing improper shoe or closed shoes. (Global Health Ltd., 2023).

Lack of feeling or little feeling is due to impaired underground nerve for foot wounds within diabetes where underground nerves lead to little or no feeling. One is able to get wound because of lack of protective feelings because even a minor injury can go unnoticed. Calluses, corns or blisters are the form of neuropathic injuries. If care is not provided this condition may lead to infection and may worsen injury. Constant monitoring and

prevention of diabetes related chronic wounds together with healing of ulcers are the extensive care for neuropathic ulcers (Global Health Limited, 2023).

2.2 Ischemic Wounds

Ischemic wounds in patients with diabetes are mainly because of reduced regional blood flow, widely linked to peripheral vascular disease. Peripheral arterial disease is worsened by diabetic condition. These is because they reduce the blood flow to the legs. These can result to formation of ulcers and tissue damage which are poor to heal. The most common characteristics of ischemic wounds are lack of pain, decreased blood circulation and delayed healing. Most of the high risk of serious health concern is poor blood supply. (Veronika *et al*.2022)

Diabetes foot ulcers are caused by high blood glucose level among people living with diabetes. Atherosclerotic plaques compromises healing process because it blocks the supply of blood to the wound site through blood vessels. Endothelial cells support skin function and these helps the wound to heal in the normal way. Keratinocytes and fibroblasts of the skin are being decreased by hyperglycaemia during the wound healing process while the oxidative stress increases as a result of less antioxidative enzymes (Burges *et al*., 2021).

Diabetic neuropathy slows down wound healing and these may lead to the development of foot ulcers. Autonomic neuropathy leads to anhidrosis through diabetic neuropathy. Cracking of skin due to try skin leads to openings on the body and these increases the risky to infection Tissues and even ischial gangrene are damaged by pressure caused by neuropathy at the feet. The process of wound is compromised by many factors some of which are not discussed in these chapter. Enhanced wound contraction and increased white

blood cells accumulation provided by exogenous facilitates wound healing. This occurs mostly in areas which frequently accelerates keratinocytes which are important in diabetic wound treatment. (Burges *et al*., 2021).

2.3 Microvascular Challenges in Diabetic WH

Needing lower limb amputation and slow wound healing are increased by peripheral arterial disease. Exogenous facilitates wound healing through enhanced wound contraction and increased white blood cells accumulation. The areas which accelerate keratinocytes are important in diabetic wound healing. (Burges *et al*., 2021).

It is important to administer vascular assessment and treatments within diabetic wound care since PAD is believed to be among half of DFUs, (Wokabi *et al*., 2021). Impaired circulation of oxygen among people living with diabetes makes it difficult for them to deliver oxygen in their body and these may result to tissue hypoxia forming in areas with ulcers. Endothelial cells, macrophages and the growth of new capillaries signalling are brought by change in gene expression due to low oxygen environment. Also, keratinocytes and fibroblasts modify their protein synthesis and metabolism due to very low oxygen (Yanling *et al*., 2022).

Anaemia affects many diabetic patients mostly with DFUs as a secondary condition. According to studies anaemia in patients causes severe ulcers. This is a very risky condition because it causes both loss of limb and also patient death. There should be a regular check up for people living anaemia because it is strongly connected to diabetic foot ulcer problems. These will help to regularly monitor wound care for this condition properly and these facilitates successful diabetic foot ulcer management (Burges *et al*., 2021).

2.4 Barrier disruption and infection

The skin barrier and integrity are critical for protecting the skin from moisture loss and microbial challenges. This barrier functions cooperatively with lipids, cellular connections, and specific enzymes. If it is disrupted, it causes problems like increased water loss and increased vulnerability to infections. They include reduced lipid composition in the subcutaneous layer, decreased hydration in the subcutaneous layer, the buildup of advanced glycation end products in the skin of diabetic patients, and aging skin. Such alterations in skin chemistry and response to stimuli because diabetes simulates the skin's natural aging process. This complicates the maintenance of the skin barrier role in diabetes (Mittal *et al*., 2013).

Inflammations are protective mechanisms in the human (or animal) body responding to injury, microorganisms, chemicals, and allergens. Through the principles of the process, characteristics such as heat, swellings, and potential decreased function can be observed. An inflammatory response starts through the release of chemical mediators by cells. The signals are important in the fight against infection and stimulate healing of tissues. Plant extracts contain metabolites that can specifically suppress careful anti-inflammatory targets including NF-kB, cyclooxygenases (COXs), and ROS thus provide defence against microbial encroachment (Lobo *et al*.,2010) Biochemical variation which occurs at the cellular level, inflammation leads to the free access of phospholipids which is converted into arachidonic acid and in turn transforms into leukotrienes and prostaglandins with the aid of cyclooxygenases and lipoxygenases enzymes. They facilitate a process in the biochemical steps that lead to the clinical manifestation of inflammation which is evident in the signs and symptoms mentioned above (Frum & Viljoen, 2006).

2.5 Role of Inflammatory Cells and Mediators in Tissue Repair

The body uses complex pathways of cell-interactions and immune system signalling molecules to carry out WH processes. PDGF are released by platelets when the tissue is injured. The platelets cause the blood to clot and activate cells which in turn helps to fix the injury. (Jansen *et al*., 2021). When neutrophils are essentially involved earlier on they facilitate tissue regeneration. . Immune cells accumulate on the wound site after an injury to protect the area from infections. They also clear microorganisms and help in destroying damaged tissue through help of enzymes and chemicals (Rosales, 2018).

In the later part of inflammation, the monocytes reach into the injured tissue and later they become macrophages. Macrophages are known to destroy cells and extracellular material and are known to be inflammatory substances. They oversee tissue repair in the beginning of every inflammation stage They initially perform a pro-inflammatory function by consuming both dead cells and debris that has built up. Following Pakyari *et al*. (2013), they exhibited anti-inflammatory behaviour and generated cytokines and growth factors which include TGF-β which played roles in remodelling tissues through the process of angiogenesis. Mast cells also produce histamine and other substances that make the walls of blood vessels more permeable, in effect opening the way for cells of the immunity to enter the injury point. Just like basophils, they release histamine and some other leukotrienes which enforce the immune and healing process (Pakyari *et al* .2013)

Some other cell components used during the process of repair include a few cytokines that acted as signalling molecules collectively. Interleukins, TNF- alpha, and interferons control the function of immune cells and control the process progression from inflammation to tissue repair. Together, these cells and mediators help with pathogen removal,

inflammation, and tissue rebuilding, illustrating the highly controlled nature of tissue repair (Kizhner *et al*.,2007)

2.6 Inflammation and WH

Acute inflammation is a crucial part of many healing events in the body and leads to the production of growth factors necessary for tissue repair after injury. Growth factors help in facilitation of repair and remodelling of damaged tissues (Alhakmani *et al*.,2014). When the process of inflammation lasts for a long period of time or it is abnormal at the beginning it results to slowness in the process of wound healing. As a result of chronic inflammation, the process of tissue repair and also restoration of normal function becomes very difficult for the wound to heal well and these may result to long-term complications. (Mittal etal.,2013).

2.7Antioxidants and Their Impact on WH

Free radicals which are created by some mediators like macrophages may influence the process of wound healing. The free radicals facilitate the process of neutralizing some of the pathogens that have accumulated at the wound site. (Kumaraswamy *et al*.,2008). When the free radicals build up in the body they lead to damage of DNA and lipids and these may cause stoppage of some essential enzymes from working and finally these may delay wound healing. Antioxidants play a great role since they facilitate the process of free radical neutralization and also, they prevent the cells from being damaged. According to Ghosh *et al*., 2013, it is important to increase the level of antioxidant defences since it is important as it increases the levels of antioxidants over the free radical in the body.

2.8 Mechanism of WH

WH is referred to as a complex series of biological processes that are arranged precisely to restore the damage at the dermal and epidermal levels. This is a complex mechanism that has several stages that enable the healing process of the tissue in question to be restored to its normalcy. Oguntibeju *et al*. (2019) mentioned that these stages are linear and remarkably interrelated and flexible to suggest for the complexity of wound repair. Each stage has different cellular events and biochemical changes and each show how various cells interrelate. The coordination of these events displays how the body can close it effectively ending wounds, as it can regenerate itself. The overview of the consecutive phases of the WH process indicates that the repair of injured tissue involves the highly coordinated activity of various cells (Pakyari *et al*.,2013).

2.8.1. Haemostatic Phase

Skin injury leads to the beginning of the haemostasis phase, a crucial phase of healing that strives to stop blood loss and create an initial architectural environment for subsequent phases. Following the tissue injury, platelets release substances that quickly react to type one collagen at the point of the injury. This provokes platelets to stick to the damaged area and start activating (Oguntibeju *et al*. 2019).

After getting activated, platelets release certain glycoproteins that help in summoning more platelets to that site, this is known as platelet aggregation. This process of platelet aggregation can be seen to be closely tied to the clotting cascade, particularly through the release of other factors that collectively bring about the conversion of prothrombin to thrombin (Yanling *et al*., 2022) Fibrin is a key protein involved in blood clotting and exists in two forms – soluble fibrinogen which is converted by the powerful enzyme thrombin to

insoluble fibrin strands. These fibrin strands interconnect to produce a dense net with aggregated platelets to act as a thick haemostatic plug. This plug not only acts as haemostasis point but also provides the initial architecture of the following phases of WH, describing here a well-coordinated co-concert between cellularity and biochemistry right from the onset of the healing process as described by (Oguntibeju *et al*. 2019).

2.8.2 Inflammation Phase

This is a case of a transition between the two phases of WH, where the inflammatory phase actively engages pertinent processes for vasoconstriction at the point of injury. This may take up to 2 weeks wherein the body can constrict blood vessels initially to form platelet-mediated clotting then dilate the blood vessels to enable immune cells access to the site to cause inflammation. According to Oguntibeju *et al*., (2019) this coordinated cascade starts with the formation of a clot due to vasoconstriction and platelet aggregation to stop bleeding.

The inflammation then continues, with the formation of vasodilation and increased permeability of the blood vessels (Alam *et al*.,) This enhanced permeability enables immune cells for instance macrophages to get to the wound site where they perform such functions as destruction of invading pathogens and removal of dead tissue as pointed out by Ebeling *et al*. (2014). The growth factors and prostaglandins secreted by macrophages activate fibroblast, epithelial and endothelial cells for migration and proliferation. They play key role in the subsequent proliferative phase of tissue repair and regeneration. Moreover, prostaglandins are the major endogenous substances involved in the formation of acute inflammatory response, while ROS facilitate antimicrobial effector mechanisms at the site of inflammation but are potentially toxic to the surrounding tissues in case of

overproduction. The extent of leukocyte infiltration into the wound site is a critical determinant in determining the healing process, which is why it was reported by Eming *et al*. (2007) that excessive recruitment of these cells is equally as dangerous as their absence in healing.

2.8.3 Proliferative Phase

After the inflammation is gone, the next phase continues for up to twenty-one days. In this part of healing, granulation, contraction and epithelialization are important processes for restoring tissue homeostasis. Ultimately, fibroblasts produce collagen and begin making the first granulation tissue which includes early networks of blood vessels. This matrix layer supports the structure of the next steps in the WH process by Ebeling *et al*. (2014). Following wounding, myofibroblasts help shrink the ends of the wound by lining up along the stress points. The body replaces the blood clot with healthy epithelial tissue which joins the wound and is made permanent. To allow the tissue to become strong and flexible, the type one collagen is replaced with type three during this phase (oguntibeju *et al*.,2019).

2.9 Remodelling Phase: Tissue Strengthening and Maturation

The last is the remodelling phase which may last up to 2 years from the time of the injury. This phase is dynamic in that the newly deposited collagen is continuously being reorganized into a tight bundle of fibrils which are then cross linked by enzymatic hydroxylation catalysed by vitamin C. In fact, during this phase, type one collagen synthesis and remodelling are most prominent, and tensile strength of the healing tissue is raised considerably, reaching almost the initial value (Rosales, 2018). This complex process is not only concerned with depositing new collagen fibres but also reorganizing the existing collagen fibres through enzymatic action to form an effective and strong scar

tissue. This progressive maturation of the wound matrix is required to reconstruct the regular structure of the injured site, but the aesthetic and mechanical properties of the tissue will remain impaired (Alam *et al*., 2011, Mittal *et al*, 2013)

2.10 Assessment of WH Activity: Methodologies and Models

To fully understand tissue regeneration dynamics, researchers benefit from using in vitro and in vivo methods. Research scientists commonly rely on animal models to study WH progression and duration through in vivo experiments while experimental conditions and wound type influence research findings. Surgical incisions are one of the most well-known models of WH; they involve opening small spaces on the skin and then stitches them, stapling or leaving them to heal by themselves (Abbas *et al*,.2013) Excision models, however, involves assessment of the rate of WH, which is done by observing the wound area, which is often located on the dorsum of an animal at varying intervals. The dead space model also reveals another technique where materials such as polypropylene are put in a made pocket that is implanted under the skin to allow for observation of foreign body integration and fibrosis (Ahmed and Nahor ,2012) Last , the burn wound model entails the application of hot molten wax on an animal under anaesthesia to form thermal injuries; some basic parameters recorded for assessment include wound contraction and the rate of epithelial regeneration. Collectively, these models afford a multifaceted perspective of the processes involved in WH and may identify potential therapeutic strategies with regard to treatment efficacy (Alhakmani *et al*.,2014)

2.11 Chemical Treatment for WH in Diabetes Mellitus and Challenges Faced During Treatment

With populations getting more global and the population aging and the upsurge in diabetes and its results for instance chronic, non-healing. Such chronic non healing wounds have several associated risks, including antibiotic resistant bacteria, formation of a biofilm, which shields the infection from treatment, impaired angiogenesis and blood supply to the wound site, and oxidative stress in the wound. To address these multifaceted issues, developing novel solutions such as hydrogels are considered viable applications in the medical industry. The materials are biodegradable and designed to be compatible with human tissues in a way that helps the body to heal itself by providing a structure on which cells can float and proliferate (Yuan *et al*., 2022). Thus, surface tagging and bioengineered nanoparticles can be seen as solutions to address the need for enhanced approaches in WH particularly diabetic wounds. These nanoparticles may be programmed to respond individually to the conditions of the wound and either deliver the therapeutic agents at or contribute to tissue formation at the site of the wound. Due to their small size and the possibility of post-synthesis chemical alteration, they are ideal for androgyny and movement within the heterogeneous wound milieu to target infections and promote healing while minimizing the risk of antibiotic resistance (Yasmen *et al*., 2022).

Some examples of modern polymeric dressings include daily dressing, films, foams, hydrogels, hydrocolloids and fibres which have all made the processes of wound care much easier. Wound dressings are helpful in a broad range of general wound care; as a barrier to external organisms and fluids, in control of exudates endodermal and can also deliver substances at the point of injury. Advancements in polymer base biomaterials depict that

such technologies can help in resolving clinical problems linked to wound care and, in effect, improve the patient's experience. (Palmer *et al*.,2021

2.12 Phytochemicals in WH

Phytochemicals are group of bioactive compounds that are synthesised by plant cells and are considered important for their medicinal values in the process of both treatment and control of diseases. These compounds mainly promote a healthy skin through exhibiting both antioxidant and anti-inflammatory effects, which is achieved through the process of inhibition of the NF-κB signalling pathway. (Lodhi *et al*., 2006).

Research have indicated that, when phytochemicals are used externally, they promote wound healing as they offer various functions such as antioxidant, anti-inflammatory, and antimicrobial (Ahmed *et al*., 2017). These natural compounds established themselves as cheap therapeutic substitutes and hold immense potential in dealing with the world's rising issue with antibacterial resistance. Phytochemicals give useful properties as an efficient MDR pathogens in controlling infections associated with wounds. Besides, as antioxidants, they harness curative potentials inherent in medicinal plants both in conventional and contemporary WH by carrying a diverse phytochemical content including alkaloids, tannins, flavonoids and terpenes as identified by Rajkumar *et al*. (2018)

Some disadvantages of phytochemicals in therapy include solubility and bioavailability issues and absence of clinical data. Their bioavailability low because they have very high intrinsic activity, lower absorption and their biological half life is short and these causes lower therapeutic effectiveness (Lodhi *et al*., 2006). Phytochemicals have antimicrobial, antioxidant, and WH properties because some of them, including phenolics, promotes skin

healing. This pharmacological potential is as result of its properties like having an astringent, antimicrobial and free radical scavengery (Uzma *et al*., 2020). Phytochemicals are associated with healing and their biological effects are widely known, hence more research and adequate testing are needed to be performed mostly in pharmacological fields (Walton *et al*., 2014).

2.13 Current Diabetic Wound Treatment Strategies

2.13.1 Vancomycin

This antibiotic is given to people living with diabetes mostly who have open wounds due to its efficacy against bacteria that are found on the wounds. The reason behind the use of vancomycin is because it aids in fighting against MRSA which is a bacterium which mostly leads to DFUs infections. (Peter *et al*., 2017).

Debridement is important for management of systemic wound management because it removes some of ischemic body tissue which slows down the process of wound healing. These process removes the dead tissues which interferes with the activity of the cellular cells and also ensures proper tissue granulation. (David *et al*., 2022).

Revascularization is also used the management of DFUs and PAD, but other measures should be employed, like modification of the lifestyle. Treatment of PAD involves initial medical treatment with maximal medical treatment to modifiable risk factors and revascularization. (Francesco *et al*., 2014) For effective management and wound care HBOT can be used. For proper use of HBOT it is significant to determine some indicators for identified patients and environment in and also establishing in many clinical settings the efficacy of HBOT (Gerit *et al*., 2014).

Stimulatory and inhibitory signals balances heal wounds and this makes the regrowth factors to work effective in most of chronic wounds. One of these classes of regrowth factors is recombinant human platelet derived growth factor. This is the only the recommended growth factor which is used in the treatment of treatment DFUs. In high concentration platelet growth factors, which comes from Saccharomyces cerevisiae yeast usually promotes WH closure. Growth factor therapy usually enhances WH and act as a source of hope for people living with diabetes (Francesco *et al*., 2013).

2.13.2 Plant-Based Herbal Remedies for Diabetic Chronic Wound

Flavonoids are a useful group of natural compounds possessing anti-inflammatory and antioxidant activities and promoting key healing mechanisms like angiogenesis and tissue repair. Flavonoids exert diverse skin healing by facilitating fibroblast activity and creating new collagen as they simultaneously provide antimicrobial resistance. Flavonoids exert significant effects in wound healing that facilitate good skin healing. The administration of herbal medicines and bioactive compounds in the treatment of DFUs is largely influenced by the vulnerability of DFUs to infection (Raja, 2022). These substances have naturally lowered free radicals that are harmful to tissues during WH. Flavonoids, anthraquinones, and naphthoquinones are used due to their potent in antioxidant activity. It will therefore refer to vasculogenic that involves angiogenesis, fibroblast proliferation that involves creation of new provisional extracellular matrix which plays a critical role in WH (David *et al*., 2022).

Medical science is increasingly fascinated with bioactive compounds for dual augmentation of immunity with decreased inflammation in WH conditions. The α-mangostin compound exhibits anti-inflammatory properties by its inhibitory action

towards the production of inflammatory markers TNF-α, IL-6 and INOS enzyme. Inhibition of NF-κB activity is a common action between α-mangostin and curcumin when the latter are regulatory molecules for inflammatory pathways. Bioactive molecules such as tannins and flavonoids coupled with phenolic compounds and alkaloids in medicinal plants have therapeutic impacts on diabetic conditions because their action mechanisms are predisposed towards biological processes. The research identified that phytochemicals have strong abilities to control inflammation and to allow tissue regeneration (Kooti *et al*., 2016).

2.13.3 *Cyathula uncinulata*

C. uncinulata belongs to a family of Amaranthaceae which is native to Africa, Asia, Pacific Islands, and North America. Most of these plant species are always semi erect or erect in their growth. They are around 27 species in the whole world. This plant has been used in traditional treatment of wounds in most parts of Kenya and mostly in Rift valley region. The leaves of *C. uncinulata* are always opposite phyllotaxy; they are petiolate, and their entire leaf is edged. The flowers of *C. uncinulata* are always cymose with some partial inflorescences of one to three (1-3) bisexual flowers with sometimes Plano-sterility. They have ovate, which are always membranous and they occasionally have spines which always makes this genus more morphologically distinct for these flowers. *C. uncinulata* has brut mostly to the African savanna biomes and this makes it more undesirable weed. The seeds of the species are preferably oblong or ellipsoid in shape and this increases their reproductive capacity and their abundance. Their sex organs are always well developed. (Bisi-john *et al*.2015)

The known constituents of *Cyathula uncinulata* include oleanolic acids, glycosides, 1-ethoxy-2-hydroxyl, ethyl phenol, 1,2,3-isopropylidene cyasterone and 24-hydroxycyasterone. It also performs a superb function in the physiological life in the eastern regions of the former Zaire, where it helps to enhance sexual virility among male adults. *C. uncinulata* is used as animal feed in Ethiopia and in addition it is believed that it has medicinal properties and facilitates healing of various diseases either separately or in a mixture with other herbs (Bisi-john *et al*.2015)

Research on other medicinal plant uses in South Africa revealed that *C. uncinulata* is of high antimicrobial activity due to its ethnopharmacological potential for the prophylaxis of HIV/AIDS. In Lesotho, it is applied as an emetic factor and in the treatment of urethral strictures through root decoctions. In Malagasy, it is used for treating Syphilis. In Rwandan, it is used for the treatment of snake bites and in the management of microbial infections, sterility, lactation problems, mycosis, HIV and dysentery.

In Kenya, the Kalenjin people refer to it as Kipnambwet; the Giriama community call it Munyhee; the Luhya call it kumusongolamunwa; and is used to treat snake bites and wounds in the Luo community, reflecting its importance in traditional medicine (Omara *et al*., 2021). These cross-cultural applications imply that *C. uncinulata* might have some use in some aspects of traditional and contemporary medicine and more research needs to be done into the usability and efficiency of the plant in medicine.

Figure 2:1 Photo of A. Leaves and B. flowers of C. uncinulata, (Source : Author, 2023)

2.14 Fructose as A Model for Type-2 Diabetes in Rodents

Fructose has been among the most important dietary factors in rodent models of Type 2 Diabetes Mellitus (T2DM) that have given clues to the disease pathogenesis through its metabolic derangements. Current studies reveal that chronic fructose feeding leads to insulin resistance, dyslipidemia, and β-cell dysfunction in rodents, which are comparable to major characteristics of human T2DM pathophysiology. The representations are progressively multifaceted to reduce translational divergences, such as the administration of human-like doses of fructose and concurrent dietary interventions to more closely resemble human dietary regimen and disease progression (Gunawan *et al*., 2021).

2.14.1 Mechanisms of Fructose-Induced Metabolic Dysregulation

Chronic fructose consumption disturbs glucose homeostasis through multiple mechanisms. In UCD-T2DM rats, a fructose diet of 60% enhanced diabetes onset through reducing

insulin sensitivity ($p<0.05p < 0.05p<0.05$) and depressing glucose tolerance, with elevated plasma triglycerides and markers of oxidative stress like decreased ratios of GSH/GSSG (Cummings *et al*., 2010). The effects are in line with Wistar rat studies, where 12 weeks of 20–25% supplementation with fructose raised fasting glycemia (98.28 ± 3.21 mg/dL vs. 88.14 ± 2.26 mg/dL in controls) in addition to systolic blood pressure, lowering insulin sensitivity (HOMA-IR increase) (Dupas *et al*., 2016).

2.14.2 High-Fructose Diets

Pure fructose models with 60% dietary fructose in Sprague-Dawley rats induced hyperglycaemia, dyslipidaemia, and hypertension within six to eight weeks (Gunawan *et al*., 2021). These high doses, however, surpassed typical human consumption (ten to fifteen percent of calories), so there has been a trend towards moderate protocols. Wistar rats with 20% fructose in drinking water for twelve weeks developed progressively insulin-resistant without overt obesity, quite similar to early-phase human T2DM (Dupas *et al*., 2016). This model avoids excessive dietary concentrations, favouring gradual metabolic decline.

2.14.3 Strain-Specific Responses

The rodent strain selected in fructose-induced Type 2 Diabetes (T2DM) models significantly exhibit metabolic outcomes and translational relevance. Sprague-Dawley rats become insulin resistant and develop problems with lipid levels in the blood when fed a 60% fructose diet for eight to ten weeks. The rats are appropriate for examining sudden metabolic issues (Gunawan *et al*., 2021). However, when the levels of fructose used in the rats are high compared to the amount humans eat, this approach is not yet ready for medical use because it will not mimic the type 2 diabetes in human beings. Meanwhile, the Wistar

rats took controlled amounts of fructose in their water (20–25%) for the same period of 12 weeks showed worsening metabolic syndrome, increasing insulin resistance and hypertension. The way people react to fructose in the same way as the variance demonstrates the unique differences between humans and helps explore how susceptibility to early-stage T2DM varies among individuals. Rats with a UCD-T2DM genetic influence and crossed with 60% fructose in food for four to six months show a faster development of obesity and diabetes (Cummings *et al*., 2010). In this model, rapid β-cell failure and extended increased blood sugar appear within a short time, like T2DM in humans. Even though research teams might find the level of fructose and how fast the tests are useful, these procedures do not correctly reflect the long-term impact of T2DM in people.

Such strain-specific phenotypes help to pinpoint various compromises evolved in model selection. For faster results, Sprague-Dawley rats are used, but Wistar rats help match human body metabolism and uncontrolled diabetes -T2DM rats are studied to understand late complications (Dupas *et al*., 2016). Experts are mindful that fructose dosing in laboratory animals (such as rats) is much more than what is seen in regular human diets. Copying human eating habits by adjusting the strain-specific protocol is critical to improving the utility of preclinical research.

2.15 Fructose and Oxidative Stress

Increasingly, people are blaming fructose for causing oxidative stress, a condition where there is more production of free radicals than the body can neutralize with its antioxidants. Today’s research explains how fructose increases oxidative stress and leads to different health consequences, diseases, damage to cells and metabolic issues.

Nonenzymatic glycation can lead to the AGEs accumulation at mostly due to methylglyoxal and two α-Di carbonyls are very effective when compared to fructose (Semchyshyn, 2024; Lubawy & Formanowz, 2023). Initially, it was found that when fructose is taken in large amount it reduces the antioxidant defence in the body. Taking fructose for very a longer period of time may decrease the function of main antioxidants. Liver is especially vulnerable due to its vital role in the fructose metabolism process where overconsumption of fructose leads to lipogenesis and ROS production and results in non - stem anti-inflammatory drugs and liver inflammation (Lubawy & Formanowicz, 2023; Kizhner *et al*., 2007; Midorikawa *et al* 2024).

In the molecular level, fructose-induced oxidative stress entails the activation of stress response pathways. Experiments in bacterial models like Streptococcus mutans show that fructose metabolism activates a stress response shared with that elicited by hydrogen peroxide and involves genes controlled by oxidative stress regulators. The cell pathways of stress response are mostly modulated by the breakdown of fructose in the body. This finding provides a mechanistic information on how the metabolites control oxidative stress response mostly in complex organisms including human beings although the findings were initially based on microbial models, (Walker *et al*., 2025). Mitochondrial dysfunction in mammals is always intertwined with fructose induced oxidative stress. (Lubawy & Formanowicz, 2023; Chandimali *et al*., 2025).

As a result of fructose induction, a lot of oxidative stress is always created on polyol pathway. When the levels of sugars in the body rises due to intake of a meal containing sugars it results to increased production of glucose which is later converted to sorbitol and

glucose resulting in higher fructose and reactive carbonyl species within cells. ROS build-up is prominent in unreactive tissues During oxidative stress there is built of ROS mostly in the prominent unreactive tissues which are mostly found in the nervous system and also in the kidney systems.All these are associated with increased consumption of fructose resulting into diabetic problems such as neuropathy. (Semchyshyn, 2024).

Another method preventing RCS production oxidative stress in the cells is an approach which uses enzymes. This model lowers the production of fructokinase enzyme in the body as well as boosting glyoxalase. Damage resulting from eating high fructose foods can repaired using this approach of enzyme. (Semchyshyn, 2024; Johnson *et al*., 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethical considerations

The primary goal of this research was to analyse the therapeutic properties of *Cyathula uncinulata* regarding its capacity to promote WH and reduce oxidative stress and inflammatory responses. The animal protocols complied with all standards of animal well-being in global ethical research systems. An approval from the Institutional Scientific Ethics Committee (ISEC) allowing use of rats' test subjects was obtained from the University of Eastern Africa, Baraton. The research proposal was subsequently submitted to national commission for science, technology and innovation (NACOSTI) for research licencing. The relevant approvals are provided in appendix two. The research adopted controlled procedures that ensured animal safety and minimized the possibility of discomfort and stress. This scientific study obtained *C. uncinulata* leaves material from Simat area in Uasin Gishu County in Kenya. The region is well known for its traditional use of herbs in the management of various ailments. For the identification of *C. uncinulata*, the researcher sought out the expertise of experienced local herbalists. The harvested plants were then taken to taxonomy experts at the University of Eldoret for further identification and classification and research reference.

3.2 Preparation of Aqueous Extract

C. uncinulata leaves were collected and then dried at the laboratory on the benches adjacent to the windows The extraction process was done by dissolving 500 grams of dried powdered leaves into 200milliltres of distilled water and then soaked for period of 72 hours. The mixture was filtered

using Whatman No 1 filter paper to obtain aqueous filtrate. The was then concentrated using a rotary evaporator to remove the solvent (water) yielding 1 g of concentrated crude leave extract. The crude extract was dissolved in 10 ml of distilled water to make a working solution.

3.3 Preparation of Methanolic Extract

For methanolic extract fresh leaves of *C. uncinulata* were collected and washed with clean water to remove any debris. The leaves were dried along the benches near the window at chemistry laboratory then grounded to fine powder. For maceration, about 1000 g of the leaves powder were dissolved in two litres of methanol. The mixture was macerated with methanol for seventy-two hours to ensure that the extraction process was effective and efficient. The mixture was filtered using Whatman No filter paper to obtain methanolic extract which was concentrated using a rotary evaporator with the water bath set at 50°C to evaporate the solvent. After qualitative phytochemical profiling, the methanolic extract contained more phytochemical compounds than the aqueous extract; it was therefore selected for more analysis and comparison.

3.4 Qualitative Phytochemical Screening of the Crude Extract

3.4.1 Test for Flavonoids

The crude leave extract of about 1ml was added to a concentrated sulphuric acid (1ml) and 0.5g of magnesium. A red or pink coloration that disappears within the first (3mins) indicated the presence of flavonoids.

3.4.2 Test for Phenols

To about 5mg of the crude extract 4 drops of Alcoholic Fecl3 solution was added. Formation of bluish black colour indicated the presence of phenol (*Sawant et al 2013*)

3.4.3 Test for Saponins

1ml of the crude extract was added to small amount of distilled water and shaken well then observed for the formation of froth within 20minutes of shaking (*Abbas et al 2013*).

3.4.4 Test for Alkaloids

About 1g of the crude extract was dissolved in 2ml of 0.1M hydrochloric acid and 2 drops of Mayers reagents was added. A light-yellow precipitate indicated the presence of Alkaloids

3.4.5 Test for Steroids

To 1ml of the crude extract few drops of acetic anhydride was added, boiled and cooled sulphuric acid was added from both sides of the test tube and observed for the formation of brown ring at the junction of the two layers. Green coloration of the upper layer and formation of deep red colour in the lower layer indicated a positive test for steroids (*Bhandary et al 2017*).

3.4.6 Test for Terpenoids

5ml of the crude extract was mixed with 2ml of chloroform and concentrated H2SO4 (3ml) was carefully added to form a layer. A reddish-brown colouration of the interface indicated the presence of terpenoids (*Abbas et al 201*3)

3.4.7 Test for Tannins

1ml of distilled water and 1-2 drops of ferric chloride solution was added to 0.5ml of the extracted solution. Blue colour indicated presence of Gallic tannins and green black colour indicated for catecholic tannins *(Abbas et al 2013*)

3.4.8 Test for Anthraquinones

About 0.5 mg of crude extract was shaken with 10 mL benzene, filtered and 5 mL of 10%ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonia (lower) phase indicated the presence of anthraquinones.

3.4.9 Test for Glycosides

About 0.5 g of the crude extract was treated with 2 ml of glacial acetic acid (containing 2 drops of 10% ferric chloride solution). 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a brown ring at the interface will indicates the presence of glycosides

3.5 Albumin Denaturation Inhibition Assay

The anti-inflammatory assessment of *Cyathula uncinulata* extracts was done by the albumin denaturation inhibition assay (Williams *et al*., 2008). The procedure involved 1 ml of plant extract or aspirin reference standard with a buffering agent in a small measurement container known as a cuvette. To maintain a pH of 7.8 the buffer consisting of 0.02M Tris-HCl was mixed with 1 millilitre bovine serum albumin and one millilitre of plant extract or aspirin standard. The mixture was placed in water bath at 37°C for twenty minutes, then boiled for four and allowed to cool to

room temperature. The absorbance readings of the spectrophotometer were set at 660 nm; the buffer solution without additives was applied as the reference control sample. The degree of anti-inflammatory was determined through the following formula; percent protein inhibition denaturation:

$$Inhibition\ percentage\ = \left(\frac{Absorbance\ of\ control\ -\ Absorbance\ of\ sample}{Absorbance\ of\ control}\right) \times\ 100$$

This was done to assess the capacity of the extracts to prevent protein denaturation which is a key process in inflammation.

3.6 In Vitro Antioxidant Analysis of Crude Extract

3.6.1 HP Scavenging Assay

The activity of the *Cyathula uncinulata* extracts was determined by the HP scavenging activity as described by Alam *et al*. (2013). Standard solutions with the concentrations of 20µg/mL, 40µg/mL and 60µg/mL were prepared by diluting the extract samples with the HP solution 1:1. Finally using the tubes, absorbance readings were taken after approximately 10 minutes of incubation at room temperature and the figures which were attained were related with an absorbance reading of a sample containing only the phosphate buffer without the HP. Percent HP scavenging was calculated using the formula:

$$Inhibition\ percentage\ = \left(\frac{Absorbance\ of\ control\ -\ Absorbance\ of\ sample}{Absorbance\ of\ control}\right) \times\ 100$$

This assay was employed to compare the methanolic and aqueous extracts' antioxidant activities using HP which is a form of ROS implicated in oxidative harm.

3.6.2 Catalase enzyme activity

The blood samples for the assay were drawn from the caudal vein of each rat and mixed with heparin so as to prevent clotting of the collected blood samples. The samples were also spun down to obtain plasma and red blood cell (RBC) fractions. RBCs were isolated by centrifuging at three thousand revolutions per minute for about 5 min and washed with normal saline and lysed with distilled water to develop intracellular catalase enzymes. A stock solution of lysed RBCs was prepared and then a small volume of it was added to a cuvette with the freshly prepared sodium-potassium phosphate buffer of 50mM concentration and HP at a concentration of 5mM. The overall catalase activity was calculated by taking the rate of HP decomposition and following its absorbance at 240 nm.

3.6.3 Reducing power assay

Antioxidative activity was used to check the reducing power of the extract. In this test 2.5 ml of extract was dissolved with two and a half millilitres of 0.2 millilitres phosphate buffer solution and two and a half millilitres of 10% potassium ferricyanide. After that there was incubation of the mixture in a hot water bath at 50°c for twenty minutes and then cooled to room temperature. 2.5ml of ten percent Trichloroacetic Acid solution added. Then the mixture was later centrifuged at 3000 rpm for 10min and the supernatant was collected. The mixture obtained was added to one millilitre of ferric chloride. After the reaction occurs for 10min the absorbance was measured 700nm by spectrophotometer. Then 5mg/ml ascorbic acid was used as standard. High reducing power of the plant extract was indicated by increased absorbance of the reaction mixture.

3.7 Wound Creation and Treatment Administration

The excision wound model was made under partial anaesthesia using lidocaine. Surgical incisions of 2 cm in diameter were made on the back of each rat. The wound was left open to the air and filled with normal saline to control bleeding. The dosages for each group were administered topically once a day for consecutive twenty days. Concentrations for the methanolic extracts used in the treatment was 600 mg/kg which was the high dose and 300mg/kg which was the low dose and to each dose of the extract 10 g of petroleum jelly was added and then applied topically on the wounds. The standard 0.2 ml of floxapen was applied topically to treat the rats. Wound contraction and epithelialization were assessed daily for three days and the actual wound sizes were measured through an image J software.

3.8 Experimental Design

3.8.1 Animal Procurement and Diabetes Induction

For this experiment a total of 35 adult Wistar albino rats of similar body weights ranging between 150 and 180 grams each was deemed appropriate. These were housed in metal cages that contained wooden shavings and soft grass as bedding and placed in environmental conditions of temperature 25 ± 2°C and a light/dark cycle of 12/12h. All the rats in the study were offered clean tap water ad lib and fed on a commercial pelleted diet. The Wistar rats were adapted for a week before the experiment began. The positive control was fed with a diet containing 60% fructose and 40% standard rat chow for ten consecutive weeks as described by Ross *et al*. (2009) to induce diabetes in the experimental rats. For the control group, the Wistar rats were given normal rat pellets without any supplements or test substances. The blood glucose level of the Wistar rats was monitored using

a glucometer where blood was drawn from tail of the rats and then was put on glucose strip then inserted into glucometer and the readings were taken. The rats were fed with fructose for a period of ten weeks and any rat having a mild hyperglycaemia condition of about 7 mmol/l or higher was considered as a diabetic rat and was used for the research.

3.8.2 Group Distribution

The Wistar rats were divided into 7 groups and each group consisting of five rats. The groups were as follows:

- Group 1: (Normal control) non-diabetic rats with no treatment).
- Group 2: non-diabetic rats that were injured and received *C. uncinulata* extract.
- Group 3: (Diabetic control) injured rats without any form of treatment.
- Group IV: (Experimental group) Diabetic, injured rats treated with 600 mg/kg *C. uncinulata*.
- Group 5: (Experimental group) Diabetic treated with 300 mg/kg *C. uncinulata* extract (Low dose)
- Group 6: Normal/non-diabetic rats with inflicted injuries which received Floxapen which is the standard drug.
- Group 7: (Standard treatment) diabetic injured rats treated with Floxapen.

3.8.3 Importance of Topical Application and Absorption Process

Application of *C. uncinulata* extract topically was preferred to oral route due to its ability to be directly applied at the wound site and thus increase the extract concentration at the site of action. Topical solutions ensure that bioactive compounds penetrate the skin without going through first-

pass metabolism that reduces the effectiveness of orally ingested drugs (Williams *et al*., 2020). It decreases systemic side effects and allows for deposition of higher concentrations of active compounds in the target area, thus enhancing the rate of WH. Absorption of phytochemicals in the extract happens through passive diffusion of the compounds in to dermis whereby the compounds penetrate the stratum corneum layer to reach the epidermis and other underlying tissues. This process depends on lipid solubility, molecular size, and concentration difference and enables active ingredients like flavonoids, tannins, and alkaloids to express their anti-inflammatory, antioxidant, and antimicrobial properties (Mungwari *et al*., 2024). Research has established those topical herbal formulations promote WH through the modulation of fibroblast activity, the increase in collagen content at the wound site, and the decrease in reactive oxygen species at the site (Ahmed *et al*., 2021). In this study, the methodology involved the topical application of treatments for wound healing (WH), particularly in cases of chronic diabetic vulnerability. The experiment was conducted following standard procedures to assess the effects of the treatment. Detailed analysis of previous research, such as the work by Eming *et al*. (2014), that was discussed in the literature review showed that the topical use of these herbs increases the rate of reepithelialization and angiogenesis consequently enhancing the wound contraction process. Topical application enabled *C. uncinulata* extract to reach the target site for an optimal therapeutic effect, and the findings make it a viable natural resource for the management of diabetic foot ulcer.

3.8.4 Data Analysis

The raw data was obtained was cleaned using additional SPSS before being analysed using the R statistical software. To check the statistical significance of the treatment in relation to wound contraction percentage and epithelialization time. One way ANOVA Tukey post hoc test was

conducted successively. The level of significance adopted was 0.05 probability level or 95% confidence level ($p < 0.05$).

3.9 Data Management

The raw data which was sorted and cleaned in Microsoft Excel to enhance credibility. Data analysis was conducted in R and RStudio which gave a comprehensive and conclusive analysis of the outcomes of the experiments. Basically, descriptive statistics tests were conducted alongside comparison test like the one-way ANOVA and paired T-test. This analysis framework offered a viable approach to assess the WH potential of *C. uncinulata* and its antioxidant and anti-inflammatory activity. The outcomes give informative suggestions and form the foundation for the further investigation of the plant for possible therapeutic properties.

CHAPTER FOUR

RESULTS

4.1 Phytochemical Analysis (Qualitative Data)

The heatmap figure 4.1 provides an indication of whether a specific phytochemical was present (+) or absent (-) in Methanolic Extract and Aqueous Extract flavonoids, Alkaloids, Tannins Anthraquinones, Saponins were present in both Methanolic and Aqueous Extract. Fundamentally, essential oils are famous due to their antioxidant, anti-inflammatory, and antimicrobial benefits. The aqueous extract was found to have minimal levels of bioactive compounds for instance, flavonoids, alkaloids and tannins.

Methanolic Extract was found to contain the highest number of phytochemicals. Terpenoids and Glycosides were found to be absent in the aqueous Extract, but Steroids were present. The tests found out that steroids did not dissolve in methanol but it was able to dissolve in water.

Table 4. 1: Phytochemical analysis of methanolic and aqueous leaf extracts of *C. uncinulata.*

Phytochemical	Methanolic Extract	Aqueous Extract
Flavonoids	+	+
Alkaloids	+	+
Tannins	+	+
Glycosides	+	-
Anthraquinones	+	+
Terpenoids	+	-
Steroids	-	+
Saponins	+	+

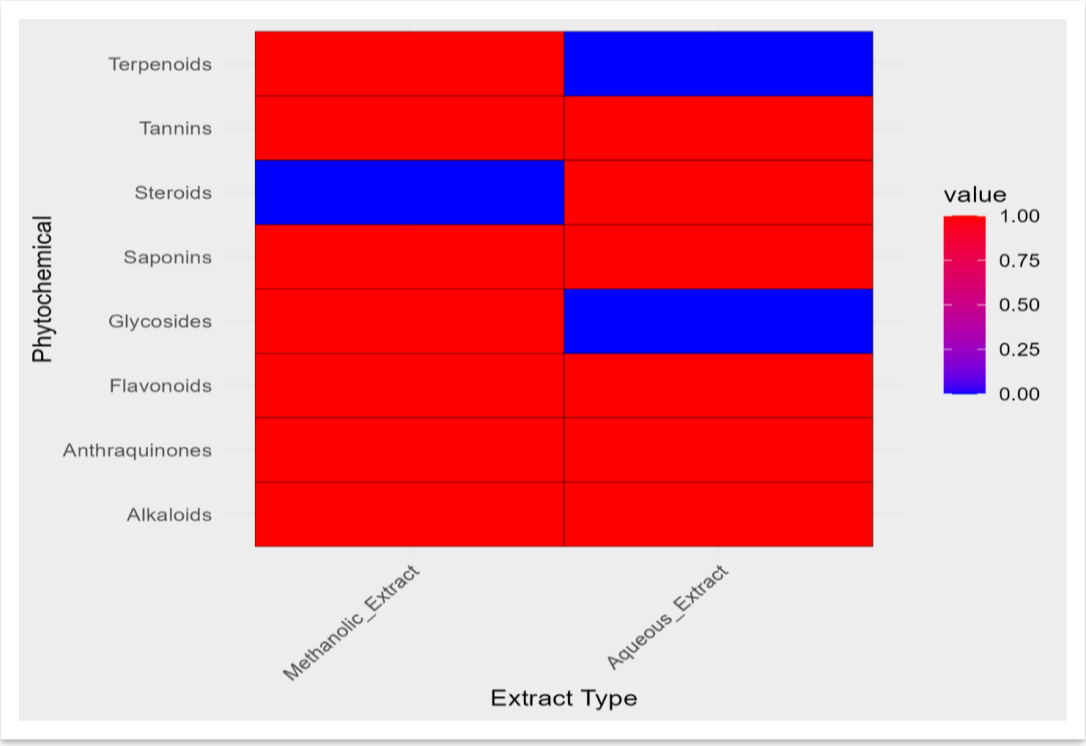

Figure 4.1:Phytochemical analysis of methanolic and aqueous leaf extract of *C. uncinulata* using heatmap

Both extracts of plant which contain flavonoids and tannins in high concentrations might be responsible for their antioxidant and anti-inflammatory effects. Flavonoids act in the process of elimination of free radicals while tannins act on the body through the prevention of oxidation and also leads to the formation of cell tissues. These compounds include alkaloids and anthraquinones suggesting that the product has antimicrobial/antifungal action and it is anti-inflammatory in nature which is good for the healthy WH.

The methanol extract of *C. uncinulata* contains more bio active compounds per unit volume and is, therefore, more active than the aqueous extract. However, the aqueous extract is effective due to its solubility and its ability to blend with the biological system used in some treatments. The phytochemical screening of the *C. uncinulata* plant showed that the plant possesses a wealth of bioactive compounds; the methanol extract contained a higher concentration of the phytochemicals than the aqueous extracts. These results offer plausibility for the pharmacological practices through the plant and traditional applications in inflammatory processes, antioxidant activity, and WH.

4.2 In vitro Antioxidant Activity

4.2.1 HP Scavenging Activity

All treatments showed a positive effect with the free radical scavenging activity improving as the concentration of the extracts increased. One-way ANOVA analysis and Tukey's post hoc test indicated existence of a significant difference between the plant extracts and the standard antioxidant at different concentrations. The percentage of scavenging activity at 20 µg/mL was found to be 60 for the methanolic extract and 45 for the aqueous extract in comparison to 85 for the standard ascorbic acid. When the concentration was increased to 40 µg/mL, the antioxidant activities were about 75% for methanolic extract, 60% for aqueous extract and close to 90 % for ascorbic acid. These variations were found to be statistically significant at the 0.05 alpha level. The highest concentration under test was 60 µg/mL at which the methanolic extract had an activity of about 85% while the aqueous extract was about 75%. Notably, there was no significant difference ($p > 0.05$) between the methanolic extract and the standard at this concentration and this was an indication that the methanolic extract was as potent as the standard substance. In every

concentration, methanolic extract gave a higher activity than aqueous extract therefore implying that methanol might be more effective in the extraction of antioxidants from *C. uncinulata*.

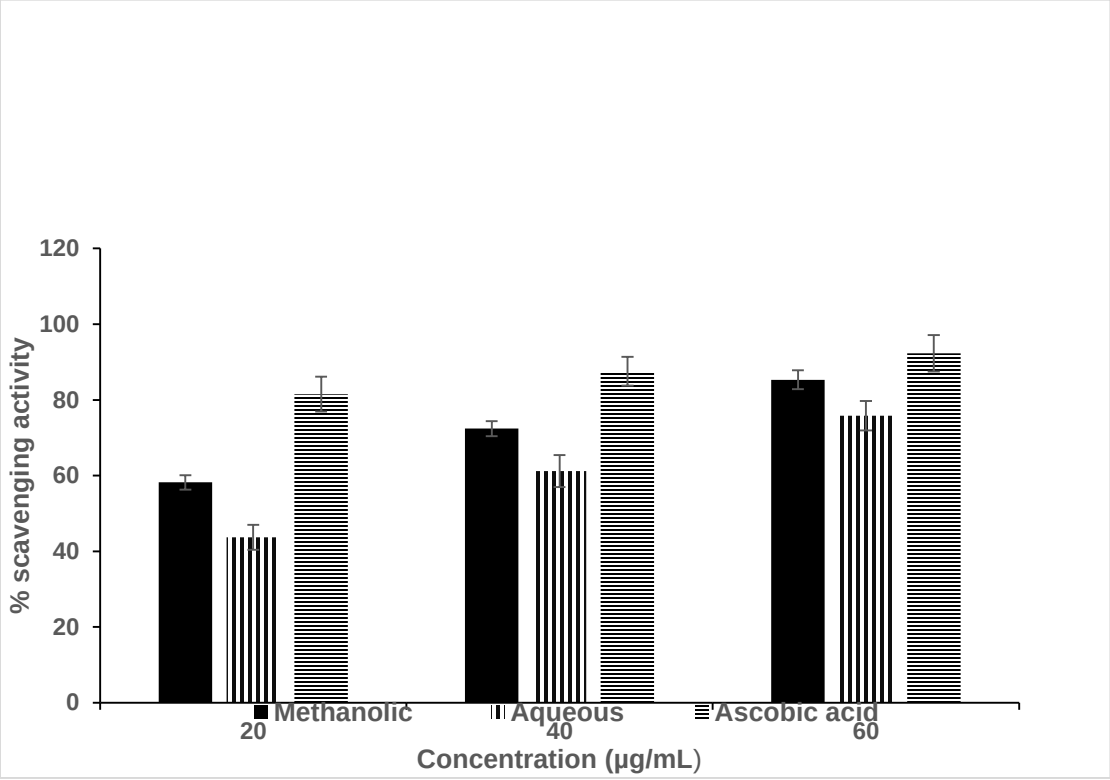

Figure 4. 2: Showing HP Scavenging Activity (%) of the methanolic and aqueous leaf extract of *C. uncinulata*

4.2.2 Reducing power assay

Table 4. 2: Antioxidant Reducing Power Assay Results

Concentration (µg/ml)	Absorbance (Reducing Power) - Methanolic Extract	Absorbance (Reducing Power) - Ascorbic Acid
100	0.429	0.67
200	0.51	1.10
300	0.53	1.80
400	1.10	2.20
500	1.50	2.52

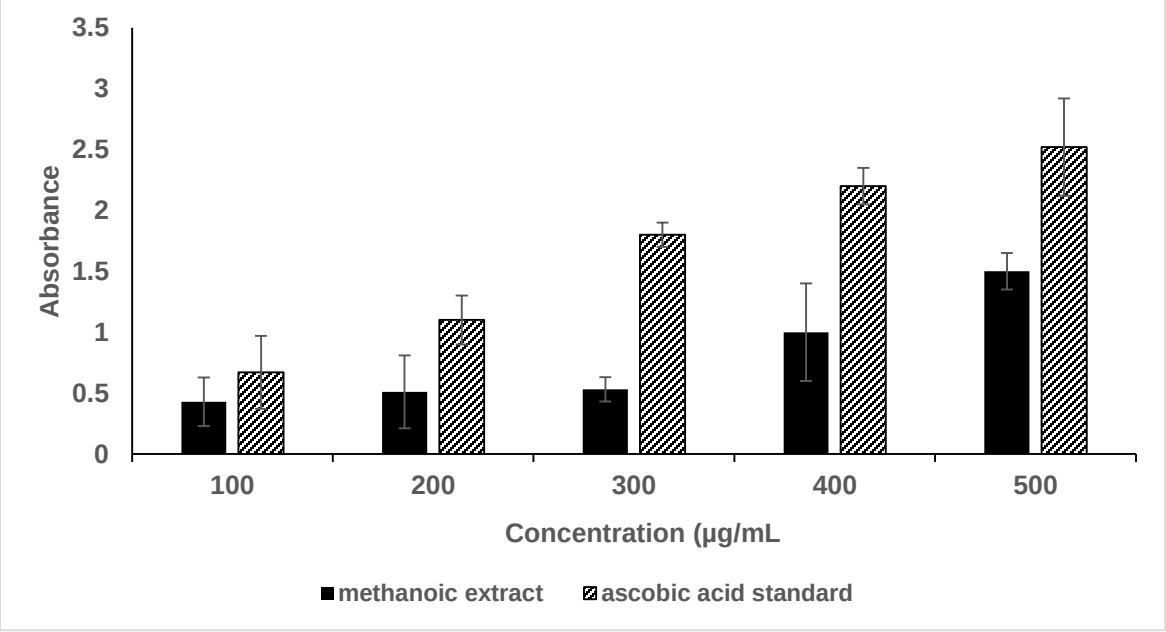

Figure 4. 3: The reducing power of *C. uncinulata* extract both methanolic leaf extract compared to standard ascorbic acid

The results revealed that the reducing power of the methanolic extract of *C. uncinulata* and ascorbic acid were enhanced with an increase of the concentrations. The results showed that at higher concentrations these samples were showing higher absorbance values and this confirm that both substances possessed higher free radical scavenging capacity as the concentration of the sample increase as shown on table 4.2. These agrees with the concept that the efficiency of antioxidants increases with the increase of concentration.

The standard antioxidant L-ascorbic acid (Vitamin C), exhibited a higher reducing power than the methanolic extract of the plant at all the concentrations applied. The findings showed that methanolic extract possessed moderate antioxidant potential which was useful in combating oxidative stress. This goes to show that *C. uncinulata* has a fairly high antioxidant content, albeit slightly ineffective than ascorbic acid. Among the phytochemicals found, tannins, flavonoids, and alkaloids present in *C. uncinulata* extract may be responsible for the reducing power. Being rich in antioxidant phytochemicals proves that the extract can be used in management of diabetic wound. This work is with agreement with previous findings that plant-based antioxidants aid in wound healing and also reduce both inflammation and oxidative stress, making the extract suitable for use in medicine.

4.2.3 Catalase enzyme activity:

Catalase is one of these enzymes that defends cells against damage by converting hydrogen peroxide which results to the release of oxygen and water. Different levels of catalase activity may be viewed in the figure 4.4 of the treatments. The control/reference sample demonstrated the most catalase enzyme activity, measuring 0.10 absorbance which shows good antioxidant protection.

High levels of oxidative stress were detected in the non-treated diabetic samples, as shown by their catalase activity in the range 0.24-0.22 absorbance. In figure 4.4 Floxapen and methanolic extract tests showed average absorbance values of 0.20–0.17 and 0.12–0.16 respectively; this is possibly because of the antioxidant activity in some part. As a result, catalase activity is highest in diabetics not receiving treatment because they have the most oxidative stress, but treatment is linked to some antioxidant capability.

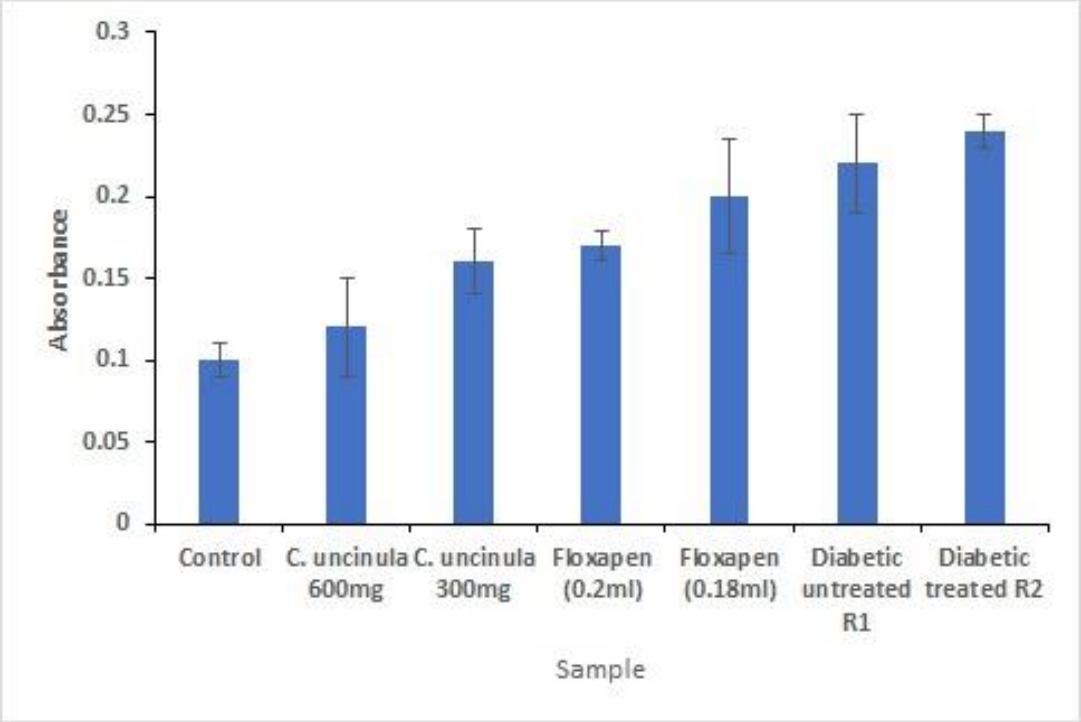

Figure 4. 4 showing catalase enzyme activity of *C. uncinulata* methanolic extract

4.3 Anti-inflammatory Activity of plant extracts

Methanolic and aqueous leaf extracts at different concentrations, which include 20, 40, and 60 µg/mL, in figure 4.5 showed a variation in anti-inflammatory activity from the standard anti-

inflammatory compound, Diclofenac sodium. The anti-inflammatory effects observed were dependent on dosage in both methanolic and aqueous extracts. The level of inhibition by the methanol extract was moderately appreciable at 20 µg/mL (45.3 ± 1.8%) while the aqueous extract was low (32.1 ± 2.2%). Diclofenac showed a significantly higher percentage of inhibition at this concentration which was 71.8 ± 3.4 percent. Increasing the concentration to the concentration 40 µg/mL, higher inhibition was obtained: 58.7 ± 2.1% for the methanolic extract and 44.5 ± 2.7% for the aqueous extract. The percentage recovery of Diclofenac remained high at this concentration (80.3 ± 2.9%) which ensure the efficacy of the method up to this level. The highest level of inhibition was recorded with the methanol extract at its peak concentration of 60 µg/mL, that showed 72.9 ± 2.4% and the standard drug showed the inhibition rate of 88.6 ± 3.1%. At the same concentration, the aqueous extract exhibited comparatively a lower inhibition rate of 56.8 ± 2.6 only. Conclusively, the methanolic extract displayed relatively higher anti-inflammatory potential than the aqueous extract for all the examined concentrations. As for specificity, a dose-dependent pattern was noticed in both extracts; however, methanolic extract exhibited a slightly lower but comparable efficacy to the standard drug that is diclofenac, and these indicated its potential as a natural alternative for the management of diabetic wound. From these findings, it can be deduced that the methanolic extract possesses anti-inflammatory ingredients that could be of potential therapeutic importance in conditions that involve inflammation.

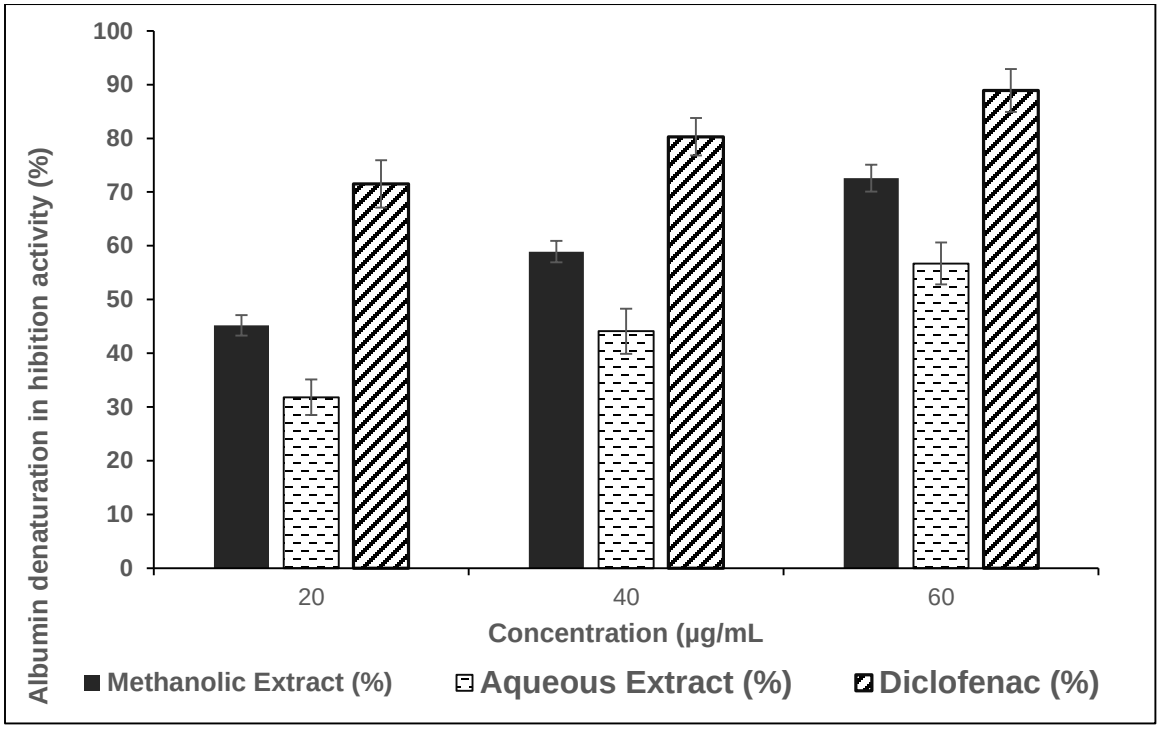

Figure 4. 5 albumin denaturation inhibition activity (%) of methanolic leaf extract of *C. uncinulata*

4.4 WH (Wound healing)

Using ANOVA test, WH outcome in the groups were compared at each of the time interval. Highly significant differences among treatment groups ($p < 0.001$). To test the hypothesis, the wound size of every rat was measured before and after the intervention; finally, the increase or decrease in the wound size was looked at to determine whether there were variations and if there were statistically significant differences of $p < 0.001$. There is variation in treatment effects by day with interaction effect being significant at $p < 0.001$ thus indicating that some treatment heals the wound faster than the others. From pairwise t-tests, both Floxapen and methanolic extracts showed a significant

effect on WH when compared to all the groups without any treatment given to them ($p<0.05$) shown in figure 4.6

In comparing of the two groups namely the diabetic and non-diabetic wounds, it is seen that overall, the diabetic group has more pronounced wound contraction than the non-diabetic group. Results indicated that diabetic groups with no treatment displayed substantially poor healing with negative values on the wound contraction showing that the wound deteriorated instead of improving. In comparison diabetic groups exhibited inferior healing compared to non-diabetic groups. For treatment effects it showed that the effect of both floxapen and high *Cyathula* is almost similar in terms of wound contraction that was noted to be almost 100%. The low *Cyathula* dosage was moderately effective but lower compared to the high dose *Cyathula* and Floxapen. Normal control groups have better healing even without going for the treatment than the diabetic control groups.

The percentage of wound contraction was established by the formula below.

$$\%\, wound\ contraction\ = \left(\frac{Initial\ wound\ area\ - Wound\ area\ on\ day}{Initial\ wound\ area}\right) \times\ 100$$

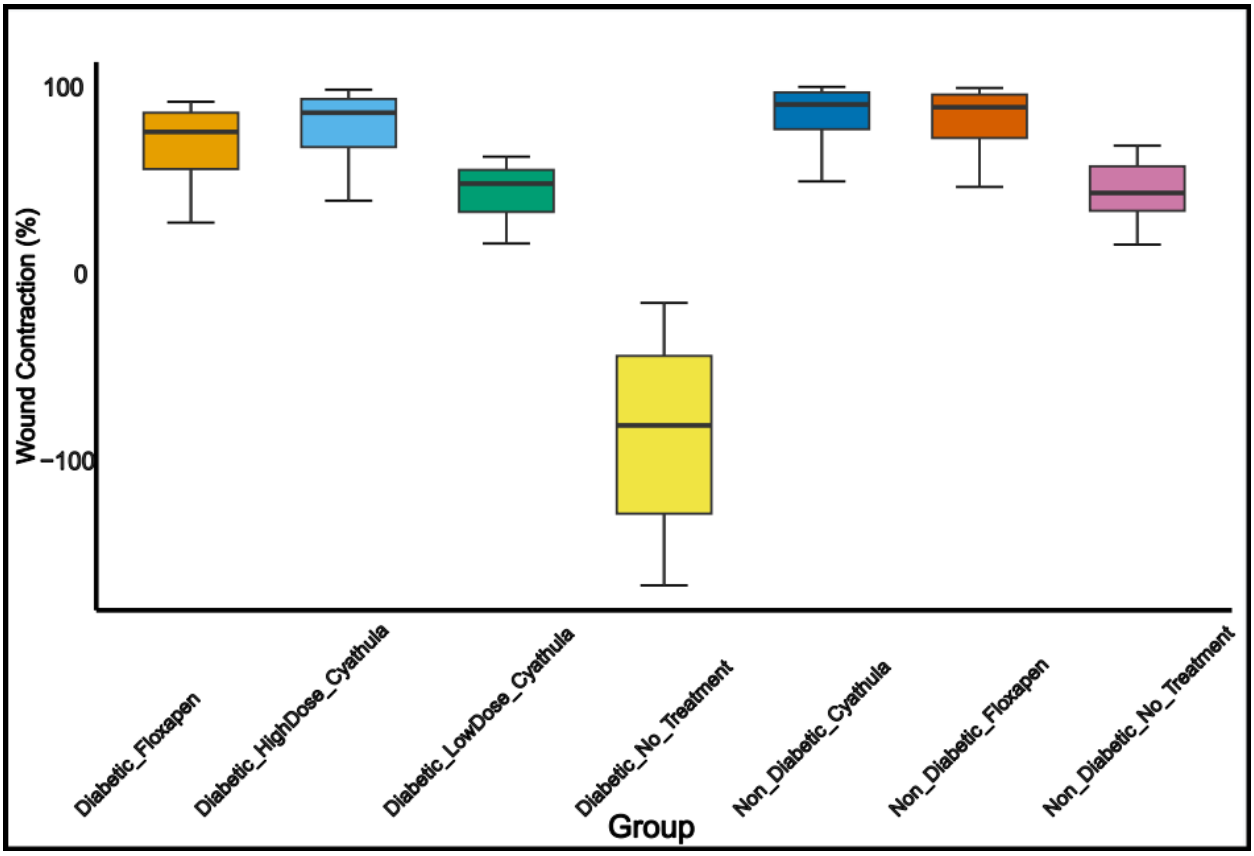

Figure 4. 6 percentage wound contraction when exposed to methanolic extract and standard floxapen drug

4.4.1 Exponential decay model of WH of different treatments of *C. uncinulata*

WH rate was estimated using exponential decay model for the different treatments. The mean score in the Diabetic no treatment group increases with time signifying that the wound deteriorates over time which is expected in diabetic ulcers. Non-diabetic and diabetic treated groups fit in an exponential decay, that suggests wound decreases quickly at the beginning and flattens later as shown on figure 4.7 below. A comparative study was conducted on *Cyathula* and Floxapen treated wounds whereby results showed that the latter healed slightly faster implying that it can aid in WH. This may be as result of the initial clearing that occurs in the initial few days characterized by clot formation and cell migration. Wound contraction reduces with time because fewer numbers of cells are required to heal the remaining tissue. The wound size gets closer to the minimum as complete healing of the wound takes time.

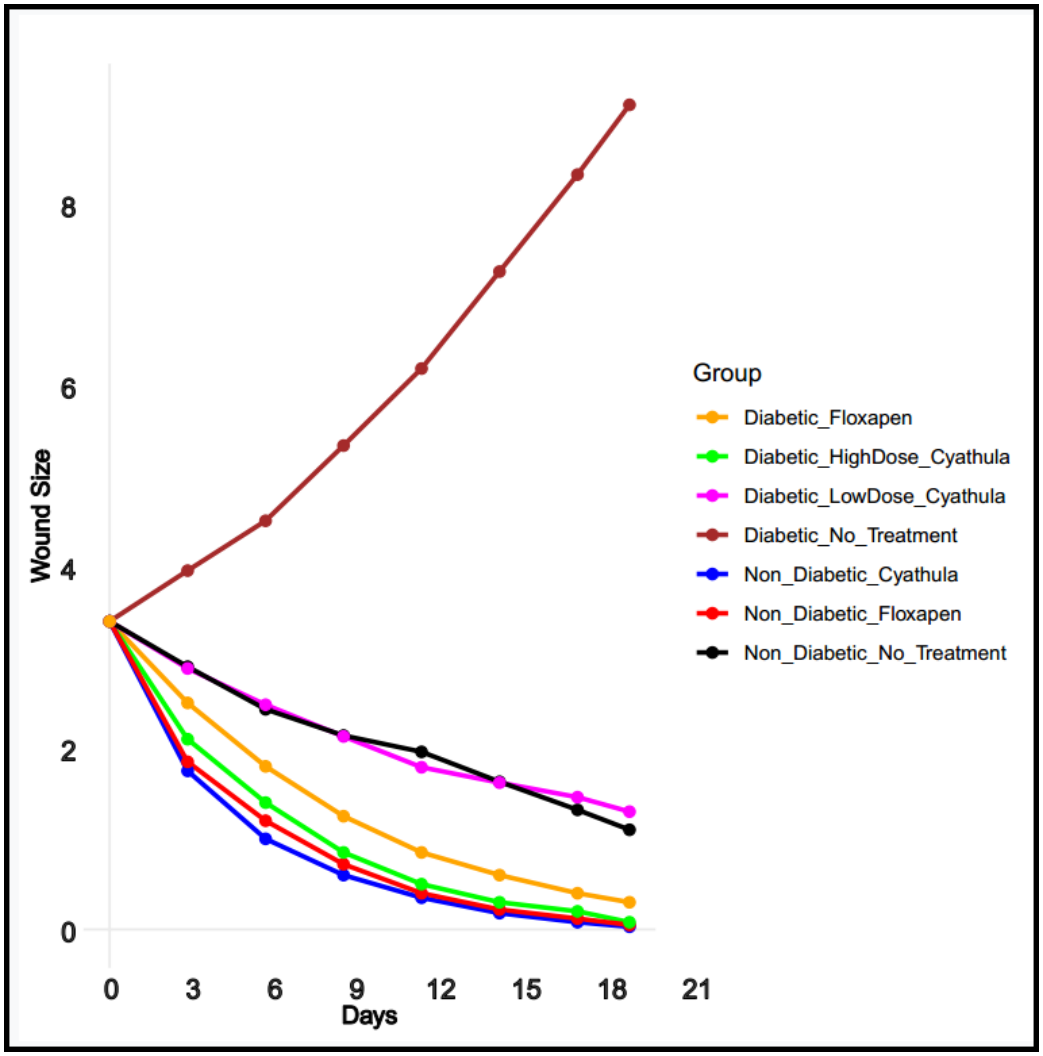

Figure 4. 7 Exponential decay model WH across different treatments of *C.uncinulata* and standard drug floxapen

4.4.2 Estimated Wound Healing Parameters

Diabetic group without treatment (+0.288) was the lowest in terms of wound size over time and this reveals that diabetes hampers the healing of wounds in the absence of treatment. From the observation, diabetic high dose *C.uncinulata* group (- 0.150) and diabetic floxapen group (- 0.149) appear to be the most efficient treatment for diabetic wounds. Nondiabetic wounds (*C. uncinulata* and Floxapen) also have healing rates as close to

diabetic ones (-0.143 to -0.146) confirming that they also facilitate wound healing in non-diabetic patients work on non-diabetic patients. They also heal faster proving its high effectivity.

Table 4. 3: Estimated WH parameters for different doses of C. uncinulata methanolic extract and standard floxapen drug

Treatment	Healing rate (k)	Comment
Diabetic_Floxapen	-0.149	Moderate WH in diabetic conditions
Diabetic_HighDose_methanol extract	-0.15	Slightly faster healing than Floxapen in diabetic wounds
Diabetic_LowDose_methanol extract	-0.101	Slower healing than high dose Cyathula and Floxapen
Diabetic_No_Treatment	0.288	Worsening wounds (wound size increases over time)
Non_Diabetic_Cyathula	-0.143	Fast healing in non-diabetic conditions
Non_Diabetic_Floxapen	-0.146	Comparable to Cyathula, slightly better healing in non-diabetics
Non_Diabetic_No_Treatment	-0.109	Healing occurred but at a slower rate

4.4.3 Kaplan Meier Survival Curve

The Kaplan-Meier survival curve displays the differences in the healing rates of the wound with the various treatment groups at different time points. In this sense, survival probability refers to the probability that a wound has not healed at a particular time. When the curve of survival probability is higher, this means that the group is taking more time to recover. The stepped pattern suggests that at least some of the individuals within the rat population healed at different rates or at different times. The p=0.2 suggests that there were no significant variations among groups but tendencies point to the possible variance in the rates of healing. The non-diabetic groups appear to have a faster rate of WH than the non-treated diabetic as shown on figure 4.8 below.

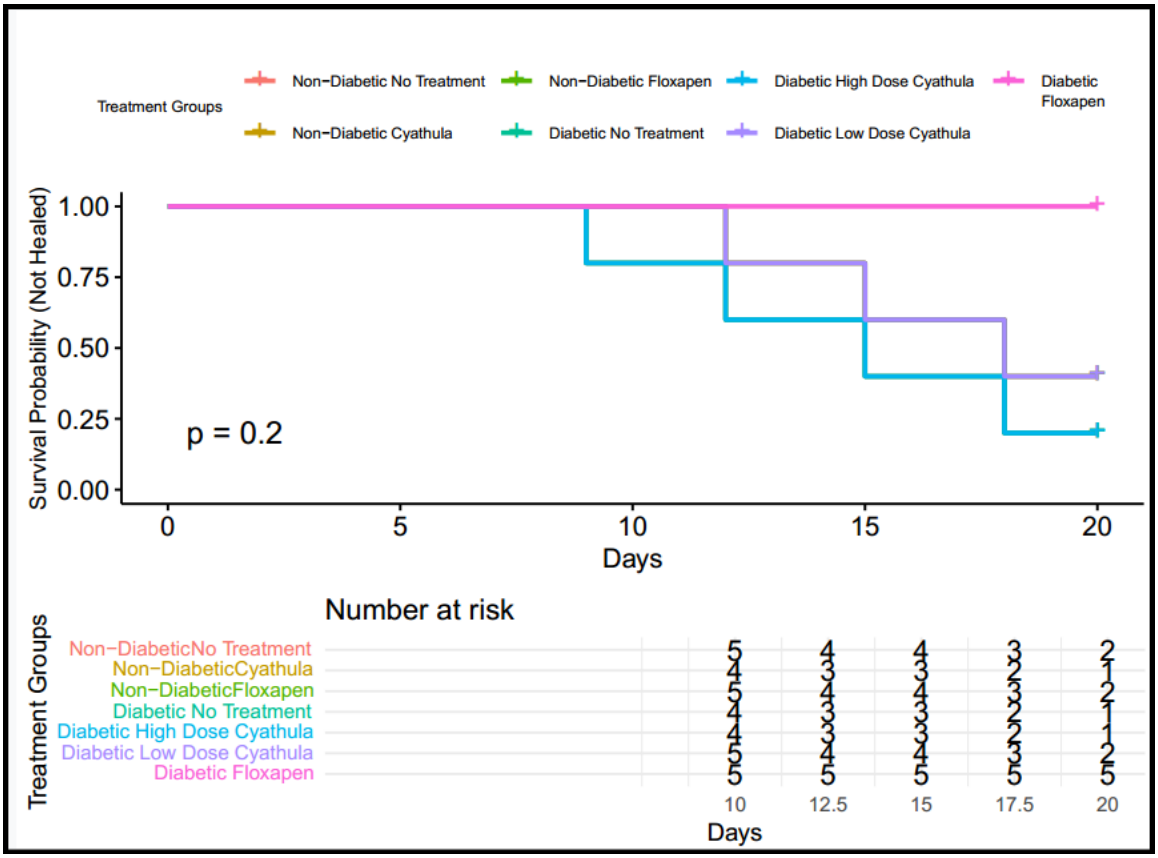

Figure 4. 8 Kaplain -Meier survival curve of diabetic and non- diabetic treatment

Non diabetic_No_Treatment group, Diabetic floxapen (pink)non-diabetic methanolic treatment (dark yellow) and Non-Diabetic Floxapen(green)diabetic no treatment group (dark blue) Diabetic Low-Dose methanol extract (dark blue) (Diabetic high dose methanolic extract (blue) and non- diabetic floxapen (green).

4.4.4 Epithelialization Time

Healing time which is the time taken for the wound to fully reepithelialise was also measured in the groups. The results depicted in the extract showed lower epithelialization time in the extract treated groups than the control group.

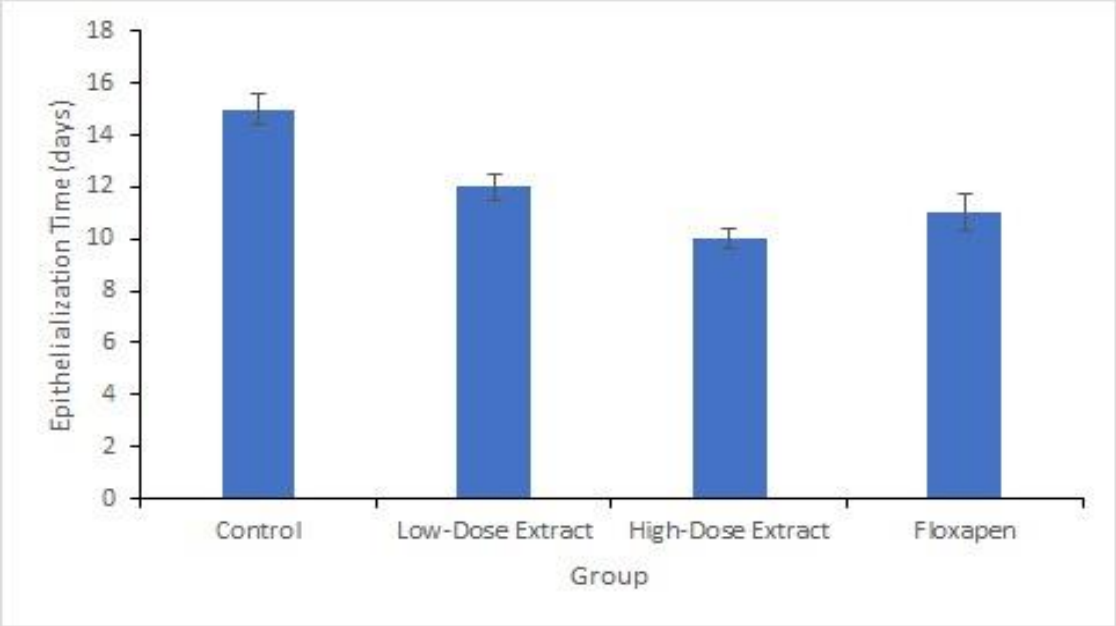

Figure 4. 9 Epithelialization time of rats treated with floxapen and methanolic extract

High-dose extract group also exhibited faster wound closure intention compared to low-dose extract intact group and Floxapen treated group at ten days as compared to twelve and eleven days respectively. Generally, epithelialization was highest in the control group for fifteen days (15 days).

4.4.5 Untreated Versus Treated Groups

Groups had variations in their capacity for angiogenesis, tissue growth and collagen production. During the study, inflammation did not improve within the control group. Instead, granulation tissue was weaker, the collagen that deposited was minimal and the number of new capillaries formed was very few. It proved that recovering from the injury would happen step by step. A review of the low-dosage extract group shows there is minor

infiltration of inflammatory cells and quick deposition of collagen, revealing mild effects of the extract on WH. In comparison to the other groups, the high dose extract produced the highest levels of regeneration, inflammation response, collagen source improvement and promotion of angiogenesis. This happens because the skin tissues repair and rebuild themselves when there's an injury. Similar levels of tissue regeneration, tissue maturity, collagen consistency and capillary density with controlled inflammation is recognized in the tissues which were treated with low doses of Floxapen as in the higher dosage group. This work clearly indicated that the WH activity of the high-dosage extract and Floxapen was much higher than that of the control and low-dosage groups.

4.5 Blood Glucose Monitoring

4.5.1 Glucose Levels Before and After Treatment

To investigate whether *Cyathula uncinulata* extracts possess hypoglycaemic properties, blood glucose was determined in the diabetic rats before and after the treatment period. There were several groups; an untreated diabetic control group, a low concentration extract diet group, a high concentration extract diet group and Floxapen group. Every rat's glucose level was measured once at the eligibility visit while an additional 4 weeks of monitoring included weekly blood glucose measurements as shown on table 4.4

Table 4. 4: Blood Glucose Levels (mg/dL) Before and After Treatment with methanol extract and standard floxapen drug

Group	Baseline (Day 0)	Z	Week 1	Week 2	Week 3	Week 4
Control	280 ± 12		275 ± 10	270 ± 15	268 ± 18	265 ± 12
Low-Dose methanol Extract	278 ± 14		230 ± 10	200 ± 9	180 ± 8	160 ± 6
High-Dose methanol Extract	282 ± 11		210 ± 12	160 ± 10	120 ± 8	98 ± 5
Floxapen	284 ± 13		215 ± 14	165 ± 9	130 ± 7	110 ± 6

The decrease was most pronounced in the group of subjects receiving the high dose of extract, whose glucose level is reduced from the initial 282 mg/dL to 98 mg/dL at week 4 ($P<0.01$). The low-dose extract group also decreased, though not to the same extent in comparison to the high-dose group. However, the Floxapen group was consistently lower but not as effective as the group receiving the high dose of extract. There were no changes in the glucose levels in the normal control group throughout the whole experiment.

4.6 Toxicity and Safety Assessment

4.6.1 Body Weight Monitoring

The rats were weighed every week to check on their health and toxicity level of *Cyathula uncinulata* extracts. The rats that received the high dose extract and Floxapen showed

significant gain in body weight over the period thus were healthier. Alternatively, the untreated diabetic control group continued to lose body weight at a constant rate shown on table 4.5. below

Table 4. 5 Body weight trends (g)

Group	Baseline (Day 0)	Week 1	Week 2	Week 3	Week 4
Control	182 ± 5	180 ± 4	176 ± 6	173 ± 7	170 ± 6
Low-Dose Extract	181 ± 6	184 ± 5	190 ± 6	195 ± 7	202 ± 5
High-Dose Extract	180 ± 5	186 ± 6	194 ± 7	204 ± 6	214 ± 5
Floxapen	182 ± 7	185 ± 5	192 ± 6	201 ± 7	210 ± 5

The metabolic status of the rats was also enhanced as shown by the increased weight gain of the rats in the high dose extract group (34 g by week 4). There were no variations among the low-dose extract group and Floxapen-treated group, both of which showed mild changes in their weight. The control group on the other hand lost 12 g during the same time, indicating that their diabetic complications were worsening.

4.6.2 Behavioural Observations

No toxic signs were noted in any rat that received the extract. The rats that received the *Cyathula uncinulata* extracts were active, grooming themselves properly and did not show any form of stress or discomfort. The everyday behavioural pattern of the Floxapen treated rats was also similar to the Controls.

4.7 Correlation Analysis

The analysis of correlation coefficient determined the relationship among phytochemical content, antioxidant, anti-inflammatory properties and WH activity. Drug-target relationships were positive in all aspects, showing a possibility of using the bioactive compounds isolated from *Cyathula uncinulata* to treat the diseases mentioned above

Table 4. 6 Correlation coefficient

Parameter	**Antioxidant Activity**	**Anti-Inflammatory Activity**	**WH Efficiency**
Phytochemical Content	0.88	0.84	0.92
Antioxidant Activity	-	0.87	0.89
Anti-Inflammatory Activity	-	-	0.91

The most significant relationship between phytochemicals and the re-esterification process was obtained, r = 0.92 for overall WH efficiency which further supports the premise that these compounds are vital in the WH process. *C. uncinulata* exhibited significant healing properties and significant correlations between antioxidant and anti-inflammatory indices with values of r = 0.89 and r = 0.91 respectively.

CHAPTER FIVE

DISCUSSION

5.1 Effects of topical application of methanolic extract on wound healing

The research provided a strong positive support for the application of *C uncinulata* extracts on WH, antioxidant profile and anti-inflammatory activities in diabetic rats. This research intended to establishing the viability of *C uncinulata* for treatment especially in a diabetic ulcer that takes long time to heal due to impacts like hyperglycaemia, poor blood circulation, neuropathy and weakened immune system the disease. The outcome of this work indicated that *C. uncinulata* possesses active phytochemical constituents that could possibly be responsible for the therapeutic effect that may warrant the plant to be considered as having the potentiality to treat diabetic ulcer. *Cyathula uncinulata* extract was applied locally to the wound site to enhance the bioavailability and localized therapeutic impact of the extract as compared to oral administration. Topical treatments draw the bioactive compounds directly into the skin and skip through first-pass metabolism that decreases the efficiency of orally administered drugs (Williams *et al*., 2020). It decreases the side effects to the entire body and ensures that more of the active ingredients get to the target area, healing the wound faster. Phytochemicals within the extract are absorbed passively through the dermis into the outer layer of skin known as the stratum corneum, epidermis and the subcutaneous tissues. This process depends on lipid solubility, molecular weight, and concentration gradient to enable active compounds like flavonoids, tannins, and alkaloids to have anti-inflammatory, antioxidant, and antimicrobial effects, as stated by Mungwari *et al*. (2024). Research has indicated that topical formulations prepared from herbs improve WH through the regulation of fibroblast behaviour, accrual of collagen, and diminished oxidative stress at the point of injury (Ahmed *et al*., 2021). In fact, earlier

related research on WH and chronic diabetic ulcers has established topical application to bear significant benefits. For example, Eming *et al*. (2014) explained that topical application of herbs increases the formation of epithelial cells and angiogenesis, thus enhancing wound contraction. This topical application method which was used in this work allowed the extract to provide maximum healing benefits and this made it a potential natural treatment for DFUs. The excision model was being chosen because it was able to determine different wound healing parameters like width contraction (Pandey *et al*., 2019).

Unlike other models of wound healing like the incision and burn models, this model mimics the typical chronic wounds which are found in diabetic patients because they are slow to heal (Ahmed & Abbaspour 2021). Use of the excision wound model allows one to apply dosage on the wound site and this aids in maximum absorption of the extract on the wound site. This is in agreement with other work on WH. For instance, Eming *et al*. (2014) employed it in aggravating the fact that the wounds in diabetic models heal slower. It is also used to assess the effectiveness of the existing tam in wound management. Because this model was repeatable, the methods accurate, and pertinent to the applied goals of the study, the excision model was deemed more effective in determining the WH properties of *Cyathula uncinulata*.

5.2 WH Efficacy of *Cyathula uncinulata* Extract

Compared to the control group, diabetics treated with *C. uncinulata* extract showed a much-improved rate of wound healing. There was a significant reduction in the wound contraction percentage in the high-dose extract where the time taken to achieve complete re-epithelialization was lower than diabetes control. From the results *C. uncinulata* contains some bioactive compounds that are responsible for wound healing. This is because

the extract had ability to promote the growth of granular tissue, collagen deposition and formation of new blood vessels. All these processes are important in different phases of wound healing. This is in supports by work which showed that phytochemicals contained in the plant extract aids in WH (Bin *et al*., 2021).

Therefore, the rate of inflammation in treated rats was less compared to the control group thus the rate of healing is faster within the short time frame. This is another crucial aspect which is inflammation regulation. It also delays the time to heal the wound and increases the rate at which the wound is infected. There was increase in collagen which was deposited and wound contraction rate in extract-treated groups which was useful in tissue repair and WH. This might be attributed to the flavonoids and alkaloids that are present in *C. uncinulata* since they were found to possess anti-inflammatory properties (Wan *et al*., 2021).

5.3 Antioxidant Properties and Their Role in WH

The increased levels of ROS impair the normal functioning of the cell by altering the antioxidant defence mechanism hence worsening the WH especially in diabetic patients. This educative study also substantiates the fact that *C. uncinulata* has the ability to scavenge free radicals whereby the methanolic extract possesses a higher antioxidant activity as compared to the aqueous extract. This indicates that the extract has phytochemical compounds that prevent the formation of free radicals and reduce the amount of oxidative stress on the injured tissue (Lobo *et al*., 2010). These results are in agreement with other related work on medicinal plants which was done within the family of *Cyathula uncinulata* by mungwari which showed antioxidant effects in both in vivo and in vitro model (Mungwari *et al*., 2024). Increased activity of enzyme catalase was

observed in treated diabetic rats compared to diabetic untreated control rats. Catalase is an enzyme which breaks down hydrogen peroxide to water and oxygen hence resembling oxidative stress. The treated rats indicated higher levels of catalase activity compared to untreated rats which was evident by decreased absorbance and these implied a maintenance of antioxidant defence system altered by fructose induced diabetes.

The HP scavenging activity and the reducing power assay led to an enhanced antioxidant activity and these suggests that *C. uncinulata* can combat oxidative stress on diabetic ulcer. Flavonoids, tannins and phenolics, pigments which come from other medicinal plant as indicated in other studies, promotes the growth of new cells and the repair function of tissues by virtue of their antioxidant nature or as protectants against destructive actions on cells of the skin, specifically, the keratinocytes and the fibroblasts (Polaka *et al*., 2022). This is further confirmation of our hypothesis that the healing benefit of the *C. uncinulata* can be attributed to a decrease in oxidative stress linked to diabetic conditions.

5.4 Anti-Inflammatory Activity of *Cyathula uncinulata* Extract

Inflammation is a critical component of ulcer healing; nevertheless, extended and delayed inflammation in diabetic ulcers slows healing and increases complications. In summary, the findings of the albumin denaturation inhibition tests conducted suggests that *C. uncinulata* has anti-inflammatory properties which is an important process during early period of WH. A measure that is known to be consistent with anti-inflammatory activity is the capacity to minimize albumin denaturation or the capacity to stabilize the protein molecules that may be affected by inflammation (Sherwood & Traber, 2007).

This implies that there is a somewhat related trend in the inhibition of heat induced albumin denaturation whereby the potency of the extract rises with concentration and that the methanol extract is more potent than the aqueous extract. This is in consonance with other work by Emordi that have indicated that methanolic extracts contain high levels of bioactive anti-inflammatory agents (Emordi *et al*., 2016). The anti-inflammatory activity of *C. uncinulata* may be due to presence of phytochemicals like phenols, saponins, alkaloids and flavonoids in which earlier work has indicated to contain phytochemicals. The decrease in inflammation facilitated to improved wound contraction, re-epithelialization and tissue regeneration as observed in treated groups. These is in agreement with previous work by Tellechea on medicinal plants with dual antioxidant and anti-inflammatory properties which facilitates wound healing in diabetes (Tellechea *et al*.,2020). The higher dose of the extract indicated higher noticeable effects with limited side effects and this emphasized its potential as safer alternative and also showed comparable efficacy when compared to Diclofenac (standard drug).

Another factor considered concerning the level of inflammation of a wound in diabetic ulcers is the balance between the pro-inflammatory cytokines and the anti-inflammatory cytokines. A diabetic wound has increased levels of TNF-α and IL-6 that prolong the inflammation phase of WH. The reduced numbers of neutrophils accompanied with more active macrophages at the point of injury facilitates the healing process of the wound, after transitioning from the inflammatory stage into the proliferative stage. This is important in diabetic WH because, if the inflammation phase fails to heal, the wound becomes a chronic one.

5.5 Study Strengths and Weaknesses

A major strength of this work is the technique employed in evaluating the WH, antioxidant, and anti-inflammatory properties of *Cyathula uncinulata*. The in vitro and in vivo approaches offered the complementary assessment of the effect of the plant on bioactive agents. The components of the aqueous extracts in relation to the phytochemistry and bioactivities as deduced from the in vitro tests were complemented with an in vivo WH model. This dual approach helps to increase the reliability of the study and contributes to the further progress in using *C. uncinulata* for clinical purposes.

There is a clear specification of the experimental design with suitable control groups. The animals used in this experiment were diabetic and non-diabetic to compare the responses of WH with and without the complication of diabetes. The effect of *C. uncinulata* extracts could be compared with the control groups which were performed using Floxapen, a well-known antibiotic. This comparative approach supports the evidence and also demonstrates the efficacy of plant extract in treating diabetic ulcer or even in conjunction with other treatments.

In applying one-way ANOVA and Tukey post hoc test, the observed differences were statistically significant. This ruled out interference of random variance results. The results showed that the phytochemicals present in the *C. uncinulata* and its antioxidant and anti-inflammatory properties, and WH potential which might have facilitated the effectiveness of the wound treatment.

One weakness of the study is that the researchers only used less than 200 rats and this may restrict the generalization of their results to the population. Although the experimental

groups were back then well defined, it might have been beneficial if more rats were used in an effort to add more strength to this work and possibly differentiate the outcomes better. A further limitation was the use of a single animal model. An additional weakness is that while Wistar rats are used in experimental studies, there might be differences between species including metabolism and immune response. However, since the study was short-term, long-term effects of *C. uncinulata* on diabetic WH and related issues such as chronic toxicity were not evaluated. Almost all the studies were focused on the extent of WH factors such as contraction rate and time for the formation of epithelial, and other general effects, immune status, cytokines and metabolic alterations were also underexplored. Other researchers should explore other parameters to get a better picture of the plant's healing powers.

5.6 Clinical Impact

This work had a lot of implications, particularly in the treatment of DFUs that end up in health care facilities. DFUs are among the most severe diabetic comorbidities and result in numerous hospitalizations, infections, and amputations. Due to high prevalence of DFUs, increasing rate of resistance to antimicrobials, and the need to find other ways of treating diabetic wound. There is need to find natural sources of phytochemicals. This work therefore aimed at establishing that *C. uncinulata* is a natural and cheap remedy for treating DFUs. The extract enhanced the wound contraction rate, epithelialization and collagen accumulation in the repairing tissues comparable to that of Floxapen in clinical use. Findings suggest that *C. uncinulata* can be used together with the conventional medicine to treat wounds since there are no traditional anti-wound care drugs in developing countries.

Furthermore, the antioxidant and anti-inflammatory property of the plant extract suggest other potential utilization in clinical medicine in apart from WH. It might also ameliorate diabetic arthritis because of reduced oxidative stress, and the inflammatory properties study shows that *C. uncinulata* may have an effect on chronic inflammation in diabetes and other metabolic disorders. It is used in other skin disorders including skin inflammations, skin injuries including burn injuries, and post-surgery rehabilitation. Because antibiotic resistance is on the rise, there is a concern of identifying methods of WH that are effective and safe and are likely not to increase the level of antibiotic resistance. The extract shows antibacterial activity which might be utilized as a natural source of an antibiotic in treating bacterial infections in diabetic ulcers.

However, the findings have to be further investigated in clinical, placebo-controlled trials to ensure that *C. uncinulata* poses no harm and is effective at combating diabetic wounds. Perhaps, additional studies of this extract preparation can also require standardization of the extract into a concentrated form that can be diluted into various wound dressings or topical gels so that correct therapeutic amounts can be administered frequently. Therefore, more work is required to understand the side effects and any interaction that such drugs might have with other anti- diabetic medications. Therefore, the findings might be viewed as valuable for the further application of *C. uncinulata* in the treatment of diabetic ulcers. With further clinical evaluation, this plant extract could be another cheap, natural, and effective treatment modality for WH and other complications of diabetes in human use.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This work intended to evaluate and determine the WH, antioxidant, and anti-inflammatory potential of a methanolic extract of *Cyathula uncinulata* in Wistar albino rats following diabetic induction. Thus, the plant extract could heal the wound, reduce oxidative stress, and inflammation. These findings substantiated the fact that *C. uncinulata* can be used as natural remedy in in treating DFUs patients in an effort to discourage the use of synthetic drugs and their side effects and this should be done after clinical trial have been done.

Other findings of this research also include determination of the possible capacity of the plant extract in enhancing the rate of WH. The results demonstrated that the Wound treated with *C. uncinulata*, promoted the wound contraction and deposition of collagen and re-epithelialization comparing to the normal contralateral wound in rats. The findings are in harmony with the previous studies by Amoolya *et al*, (2014) where size of wound diameter was considerably exponential in the diabetic experimental animals up on topical application of *M. oleifera* and *Michelia champaca* as compared to normal and diabetic control. The results also showed that the rate of wound contraction was higher in high dose extract group. This can be attributed to how the extract helps in the healing of the tissue. The finding also revealed that *C. uncinulata* possesses actual concentration of antioxidant that is important for the healing of wound. Other related anti-oxidants have been indicated to lead to inflammation and even hamper the healing of wounds in diabetic patients because of high ROS. The outcome of the HP scavenging and reducing power assay indicated that *C. uncinulata* extract has the capacity to scavenge ROS that induce oxidative stress. It is

important to note here that such oxidation inhibition has some effect on the plant's capacity to meet this need through the creation of healing wounds, within cells suffering from oxidation stress while simultaneously repairing them.

Another notable property of *C. uncinulata* was the anti-inflammatory effect, in addition to the antioxidant effect. There are several characteristics of chronic wounds in diabetic patients including inflammation which affects the normal healing process and its phases. The results of the tests that we conducted such as the albumin denaturation inhibition assay revealed that *C. uncinulata* extract possess the property to prevent protein coagulation and reduce inflammation. These results demonstrated that the extract of *C. uncinulata* has slightly significant efficacy WH and anti-inflammatory properties as the Floxapen antibiotic used in wound treatment. This is crucial in the current world where antibiotic resistant strains are a cause of worry to patients because *C. uncinulata* has the potential to be a natural antibiotic. The prospective claim of the extract as an antimicrobial further enhances the overall therapeutic capacity of the extract and can be incorporated into the overall management of diabetic wound care. The findings also indicated that the extract contained key phytochemical compounds which included saponins, flavonoids, tannins and alkaloids. Saponins facilitates wound healing process through promoting the synthesis of collagen and formation of new blood vessels. Flavonoids act by scavenging free radicals and these protects cells from oxidative stress which may delay the wound healing process. Tannis facilitates wound contraction since it has astringent properties and also prevents microbial invasion. Alkaloids have antimicrobial and analgesic effects and they work by reducing microbial load.

6.2 Recommendations

- More research work is required to optimize this extract for clinical trials and to validate the findings reported in this study.
- The long-term effects and potential toxicity of the plant extract should be carried on to determine its safety since the research was done for short period of time.
- Also, other different models should be studied since the study used one model and confirm it efficacy. *Cyathula uncinulata* should be integrated in diabetic wound treatment since it indicated potential healing outcomes.
- Other research work should focus on characterizing the bioactive compounds of the extracts since they have anti-inflammatory, antioxidant and WH properties in this research.
- Standardization of extraction methods as well as formulation into usable forms such as ointment or wound dressing forms will also be important. Future work should focus on mapping the molecular signalling affected such as antioxidant defence (NrF2) and inflammation (NFkB and MAPk pathways) and more research should be carried out to determine the significance of the drug in the management of other complications of diabetes. Thus, *Cyathula uncinulata* can be a good source of natural medicine for treating the diabetic wound for its antioxidant, anti-inflammatory and WH activity profile. Wound contraction and stimulation of collagen formation and the reduction in ROS levels*, C. uncinulata* should be deemed as an option or even a supplement to the traditional methods of WH and all these should be done after clinical trials. Thus, with furthering the research and obtaining more clinical data, *C. uncinulata* could further the interventions for

diabetic ulcers by providing an accessible and affordable solution to a medical issue that affects millions of people in the world people in the wor

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APPENDICES

Appendix I: NACOSTI Research Permit

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Date of Issue: 11/January/2025

RESEARCH LICENSE

This is to Certify that Mr.. Fidelis Kilonzo Mutuku of University of Eldoret, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Uasin-Gishu on the topic: DETERMINATION OF DIABETIC WOUND HEALING,ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF CYATHULA UNCINULATA IN DIABETIC INDUCED WISTAR RATS for the period ending : 11/January/2026.

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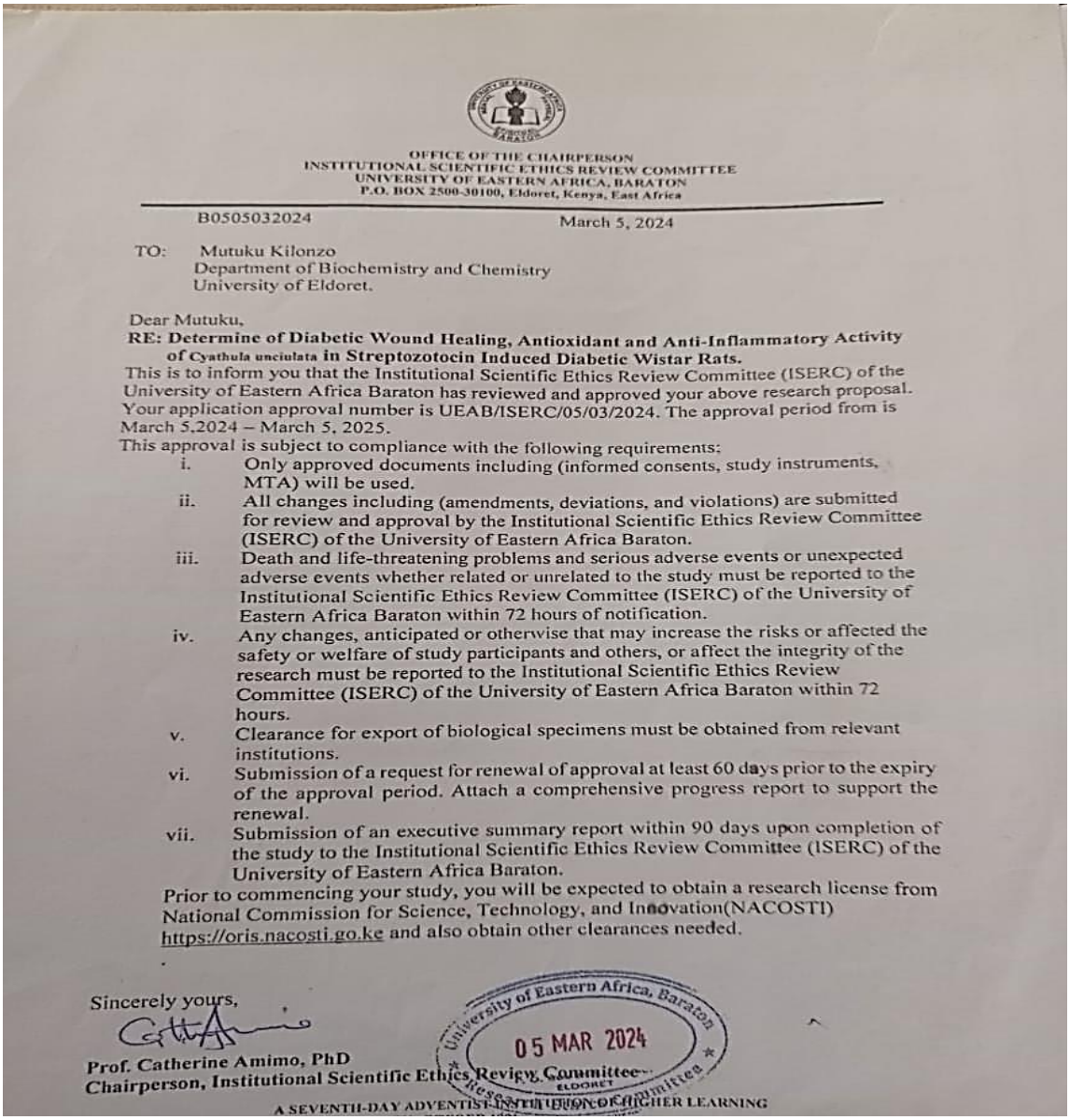
See overleaf for conditions

Appendix II: Wound Healing Progression

Appendix III: Ethical Clearance Letter



OFFICE OF THE CHAIRPERSON
INSTITUTIONAL SCIENTIFIC ETHICS REVIEW COMMITTEE
UNIVERSITY OF EASTERN AFRICA, BARATON
P.O. BOX 2500-30100, Eldoret, Kenya, East Africa

B0505032024 March 5, 2024

TO: Mutuku Kilonzo
Department of Biochemistry and Chemistry
University of Eldoret.

Dear Mutuku,

RE: Determine of Diabetic Wound Healing, Antioxidant and Anti-Inflammatory Activity of *Cyathula unciulata* in Streptozotocin Induced Diabetic Wistar Rats.

This is to inform you that the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton has reviewed and approved your above research proposal. Your application approval number is UEAB/ISERC/05/03/2024. The approval period from is March 5,2024 – March 5, 2025.

This approval is subject to compliance with the following requirements:

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton.
iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected the safety or welfare of study participants and others, or affect the integrity of the research must be reported to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to the expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology, and Innovation(NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Sincerely yours,

Prof. Catherine Amimo, PhD
Chairperson, Institutional Scientific Ethics Review Committee

University of Eastern Africa, Baraton
05 MAR 2024
ELDORET
Research Committee

A SEVENTH-DAY ADVENTIST INSTITUTION OF HIGHER LEARNING

Appendix IV: Similarity Report

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