Wound Healing and Anti-Inflammatory Activities of Leaf Gel of *Aloe trigonantha* L.C. Leach in Rats

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This is to certify that the thesis prepared by Haile Tazeze, entitled "Wound healing and anti-inflammatory activities of crude leaf gel of *Aloe trigonantha* in rats" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abstract

Wound healing and anti-inflammatory activities of leaf gel of *Aloe trigonantha* L.C. Leach in rats

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Aloe trigonantha L.C. Leach, which locally called 'Eret' (Amharic), is among endemic *Aloe* species of Ethiopia. Traditionally its leaf is used for the treatment of different ailments including wound and infectious as well as inflammatory diseases. However, there was no *in vivo* studies which prove its claimed use for wound healing and anti-inflammatory activity. Therefore, the present study was aimed at evaluating the *in-vivo* wound healing and anti-inflammatory effects of the leaf gel of the plant in rats. The leaf gel powder of the plant was prepared after drying the gel in lyophilizer. It was studied for wound healing activity topically by incorporating in simple ointment base in concentration of 5% (w/w) and 10% (w/w). Excision and incision models were used for wound healing activity in rats. Parameters including wound contraction and period of epithelialization were studied in case of the excision wound model, while wound tensile strength was evaluated using incision wound model. Xylene induced ear edema model and cotton pellet induced granuloma model were used for anti-inflammatory study. The leaf gel powder of *A. trigonantha* was given orally at dose of 100, 200 and 400 mg/kg in both models of anti-inflammatory studies. An anti-inflammatory effect was measured by reduction of ear edema weight and reduction of wet exudate and dry granuloma weight in both of xylene induced ear edema and cotton pellet induced granuloma models respectively. Treatment of wound with ointment containing 5% and 10% (w/w) of the gel exhibited significantly increased wound contraction rate, shorter epithelialization time, higher skin breaking strength ($p < 0.05$) compared to control. *A. trigonantha* leaf gel powder also produced dose-dependent significant reductions ($p < 0.05$) of inflammation compared to control in both models. Therefore, results of the current study demonstrated that *A. trigonantha* is a potential wound-healing and anti-inflammatory agent in rat models of wound and inflammation which provides evidence for the traditional claim

Key words: *Aloe trigonantha*, wound healing, anti-inflammatory, granuloma model, ear edema model

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List of acronyms/abbreviations

A. Trigonantha	*Aloe trigonantha*
PDGF	Platelet Derived Growth Factor
EGF	Epidermal Growth Factor
PG	Prostaglandin
VEGF	Vascular Endothelial Growth Factor
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
ROS	Reactive Oxygen Species
COX	Cyclooxygenase
TGF	Transforming Growth Factor
EMC	Extracellular Matrix
TNF	Tumor Necrosis Factor
MMP	Matrix Metalloproteinase
IL	Interleukin
IBD	Inflammatory Bowel Disease
OECD	Organization for Economic Cooperation and Development
WHO	World Health Organization
EPHI	Ethiopian Public Health Institute
SEM	Standard Error of the Mean

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1 Introduction

1.1 Overview and classification of wound and inflammation

Skin forms an important and effective barrier against insults such as xenobiotics, bacteria and dehydration (1). On top of its function as protector, skin also serves as regulator of circulation and temperature through its pores and extensive vascular plexuses; as well as sensor for pressure, touch and pain (2). Its structure is composed of the epidermis and the dermis layer (3).

Wound is abnormality in the structural, cellular, and functional epithelial integrity of skin which is commonly caused by physical, chemical, thermal, microbial, or immunological factors (4). Based on physical appearance of the wound, it could be grouped into different groups such as; open wound where skin is torn, cut, or punctured; closed wound where blunt force trauma causes a contusion; and burn wound caused by fire, heat, radiation, chemicals, electricity, or sunlight (5). Infectious wound is common in developing countries due to poor hygienic condition (6).

Healing of wound is a sophisticated, strictly controlled process that plays a great role in maintaining the protective function of skin (7). It is a complex process accomplished by coordinated interactions between cells in the dermis and epidermis (4). It is also accomplished by an interaction of blood cells, proteins, proteases, growth factors, and extracellular matrix (ECM) components (8). Although wound healing is a normal process which takes place on its own, there are prolonged wounds by different reasons grouped into internal/systemic factors (e.g. dehydration, abnormal nutritional state, concurrent disease, vascular insufficiency, age etc.) and external/local factors (pressure, temperature, bacterial burden, wound size, foreign bodies etc.) (9,10).

Peripheral blood mononuclear cells, resident skin cells, cytokines, chemokines, and regulatory molecules also participates in the wound healing process (3). Following cutaneous wounding, circulating monocytes migrate to the site of injury and differentiate into macrophages, which have a role in all phases of wound healing, and depletion of these macrophages results in delayed wound repair, reduced granulation tissue formation, and delayed re-epithelialization and scar deposition (11).

Inflammation is a complex natural protective response of the body from an external noxious stimuli like radiation, chemical, physical, infectious and immunological stimuli (12). The response to this stimulus is known as an inflammatory response, which is the first defense of the organism to tissue damage manifested by: flushing, heat, edema, swelling, pain, and functional impairment (13–15). The aim of this response is to avoid the stimulus and initiate local tissue healing (15).

Uncontrolled inflammation is taken as one of the pathophysiologic cause of most chronic diseases such as, chronic inflammatory diseases, diabetes, cancer and cardiovascular diseases. Scientific reports shows that various reactive nitrogen species and reactive oxygen species (ROS), as well as pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α), interleukin-1 β (IL- 1β) and IL-6 have a great role in inflammation (16). The neutrophils and macrophages which are main innate immunity cells reach the site of injury for phagocytic activity. If infection is not controlled by these cells, lymphocytes are stimulated and incorporate the adaptation and memory functions, initiating the immune system to produce specific responses (15).

Wound location, etiology, presenting symptoms or type of injury, its depth and tissue loss as well as clinical appearance of the wound are some of the classification methods of wound (17). According to these classification methods the followings are some types of wound: bites, burns, surgical wound, laceration and acute inflammatory phase followed by synthesis of collagen and extracellular molecules , and also it can be classified as acute and chronic based on its duration and nature of healing process (18). The causal factors, the type of injury, pre-existing conditions, and signaling inhibitors are some of the factors that cumulatively determines whether wound healing process is acute or chronic (19). Wound can also be classified based on the degree of contamination into three groups as follows: aseptic wounds, contaminated wounds, and septic wounds (20).

In diverse disease processes, wound healing cascade can be affected resulting in chronic, non-healing wounds that affects the patient quality of life and economy of the patient as well as the medical system (7). Chronic wound could occur in the form of vascular ulcers, diabetic ulcers, and pressure ulcers. These wounds have some common shared features and results in failure of wound to heal include a prolonged or excessive inflammatory phase, persistent infections,

formation of drug-resistant microbial biofilms, and the inability of dermal and/or epidermal cells to respond to reparative stimuli (21).

1.1.1 Acute wound

Healing of acute wound normally progresses via a regulated healing process to restore anatomic and functional integrity which is caused mainly by cuts or surgical incisions (22). The predictable and expected time frame of healing for acute wound is usually within 2-3 months depending on the size, depth and the extent of damage in the skin (23). Platelets, keratinocytes, immune surveillance cells, microvascular cells, and fibroblasts are factors that plays key roles in the well organized and predictable restoration of tissue integrity (21).

1.1.2 Chronic wound

Chronic wounds are those wound that fail to progress through the normal stages of healing which was described under acute wound and these wounds cannot be repaired in an orderly and timely manner, because of disturbance in one or more phases of normal wound healing (20). It is caused by local infection, hypoxia, trauma, foreign bodies and also by systemic problems such as diabetes mellitus, malnutrition, immunodeficiency or medications, which unable to progress normal healing process and enter a state of pathologic inflammation (22). Mostly, the majority of chronic wounds begin as minor traumatic injuries in patients with underlying pathologies, such as diabetes-induced and nondiabetic neuropathies (21).

1.1.3 Acute inflammation

Acute inflammation has been considered as a part of true innate immune reaction of an organism to remove the injurious stimuli and to initiate the healing process by reversing tissue homeostasis towards normality (24,25). It keeps the integrity of organisms through activation of immune cells, but if it persists it may lead to complications, like rheumatoid arthritis, inflammatory bowel diseases, asthma, allergy, atherosclerosis, immune-inflammatory diseases and even neoplastic transformation (25,26). An acute inflammation involves events like exudation of fluids including plasma proteins, dilatation of arterioles, venules, and capillaries with increased vascular permeability, and leukocyte migration into the inflammatory sites (27).

1.1.4 Chronic inflammation

Chronic inflammation is a sustained inflammatory process that leads to a progressive shift in the type of cells present at the area of inflammation and recognized by concurrent destruction and healing of the tissue (25,28). The noxious stimuli affect tissue for long duration and the body recognizes most of the stimuli as foreign antigens by the host immune response and may add to the tissue destruction (25). Chronic inflammation involves infiltration and activation of many inflammatory and immune cells, that releases different inflammatory mediators to interact and activate structural cells at the site of inflammation (29).

The major damages caused during chronic inflammation are mediated by the host inflammatory response, not by the foreign invaders. It results in non-healing of wounds and causes pain, ulceration, scarring, and fibrosis (28). It can also be detrimental by; preventing wound remodeling and matrix synthesis leading to delay in wound closure and increase in wound pain (22). Chronic inflammation thus triggers the generation and progression of pro-inflammatory cytokines, transcription factors and oncogenes (30).

1.2 Phases of wound healing and inflammation

Wound healing involves; inflammation, re-epithelialization, granulation tissue formation, neovascularization, wound contraction, and remodeling of ECM and this process is regulated by a sophisticated signaling mechanism that involves various growth factors, inflammatory cells, chemokines, cytokines, matrix molecules, and nutrients (7,19). During these phases of healing, various types of growth factors such as; transforming growth factor alpha & beta (TGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), and keratinocyte growth factor) are released by cells to promote angiogenesis, fibroblast stimulation, epithelialization, and eventual wound closing (31). Synthesis, remodeling, and deposition of structural ECM molecules, are essential for initiating repair and progression into the healing state. Cellular responses to trauma involve cell–cell, cell–matrix interactions, and crosstalk between different cell populations by soluble mediators (32). Generally, wound healing can be broadly grouped under three phases; inflammatory phase (consisting of hemostasis and inflammation), proliferative phase (consisting granulation tissue formation, contraction and re-epithelialization) and finally the maturation phase which determines the strength and appearance of the healed tissue (33).

1.2.1 Coagulation/inflammatory phase

This phase is the first step in repair of wound manifested by adhesion of platelets to damaged blood vessels for initiating the extrinsic and intrinsic coagulation pathways to begin a hemostatic reaction, and forms a scaffold made up of fibrin clot for the migration and proliferation of cells involved in wound healing (1,21). Fibrin clot in addition to its primary role in hemostasis; it forms a barrier against the invasion of microorganisms, organizes the matrix for cell migration, and maintains the skin's integrity. Here, cellular response is characterized by the influx of leukocytes into the site of injury (34). Collagen and other ECM in contact with blood components and platelets, forms blood clot composed of fibronectin, fibrin, and thrombospondin (20). Collagen also activates the clotting cascade to initiating the inflammatory phase (2).

This phase is marked by vasoconstriction and platelet aggregation which mostly occurs during the coagulation, followed by vasodilation, leukocyte emigration and phagocytosis to produce inflammation at the wound site (4). Neutrophil migration into the wound site results in phagocytosis of bacteria and foreign debris, and release of proteolytic enzymes. After three days of injury, activated macrophages replace neutrophils as the dominant cell type in the wound site to activate the nuclear-factor κB (NF- κB) mediated release of proinflammatory cytokines and matrix metalloproteinases (MMPs). MMPs involves in phagocytosis of damaged cells and release of growth factors from the ECM (35). Simultaneously, blood monocytes also infiltrate the injury site and differentiate into macrophages, releasing proteases to debride the wound (36). The resident macrophages degrades all the invading microbes, eliminate tissue debris, and activate the proliferation of fibroblasts and the epithelial cells at the wound site (37).

Macrophages are the principal players in phase transition of wound healing and it has an M1 and M2 phenotype. The M1-M2 transition is crucial to prevent the occurrence of chronic non-healing wounds. The activated M1 macrophages involves in the production of pro-inflammatory mediators whereas the alternative M2 subset, involves in the synthesis of anti-inflammatory mediators and the production of ECM, in the initiation of fibroblast proliferation as well as in angiogenic processes (20,34,37). The cannabinoid 2 receptor (CB2R) revealed an anti-inflammatory effect by inhibiting pro-inflammatory M1 macrophage emission rather than activating anti -inflammatory M2 macrophages (38).

1.2.2 Proliferative phase

Proliferation phase involves angiogenesis, granulation tissue formation, re-epithelialization, collagen deposition, wound contraction, formation of different proteins, and lasts up to 3 weeks (4,8,39). The aim of this phase is to decrease the injured tissue area by contraction and reinforcing the injured tissues (fibroplasia), forming a possible epithelial barrier to activate keratinocytes (34). Keratinocyte participates in re-epithelialization, (migration, proliferation and differentiation of cells), re-formation of a functional epidermis, closing the lesion and protecting underlying tissues from further trauma (36). This phase is characterized initially by the formation of granulation tissue within the wound bed and finally by replication and migration of epithelial cells across the skin wound edges (2). Following migration of these epithelial cells across the wound bed, the wound is contracted by myofibroblasts, which clutch the wound edges and undergo contraction (38). The epithelial cells starts to migrate from the wound edges within a few hours of wounding and the migration stops as soon as the advancing epithelial cells meet, and then the basement membrane begins to form (20). During this phase keratinocytes and fibroblasts interact and increase cell proliferation rate and wound contraction strongly. They are activated by macrophages to release the factors for increasing angiogenesis, collagen production, and epithelialization (40).

Fibroplasia begins with the formation of granulation tissue and manifested by the proliferation of fibroblasts (34). Fibroblast is the connective tissue cell responsible for collagen deposition needed to repair the tissue injury, whereas collagen is protein that act as a base for the extracellular matrix formation within the wound (20), and required to repair tissues by giving strength, integrity and structure to the injured tissues for restoration of the anatomic structure and function (41).

1.2.3 Matrix remodeling and scar formation phase

Maximum tensile strength of wound through reorganization, degradation, and resynthesizing of the ECM is restored during remodeling stage of wound healing (34). This phase lasts from 3 weeks to 2 years, a new collagen is synthesized and tissue tensile strength increased due to intermolecular cross-linking of collagen (4). Fibroblasts synthesize ECM to form granulation tissue perfused with newly formed blood vessels and results in wound contraction and matrix

remodeling (21). It collaborates with myofibroblasts to produce collagen and matrix proteins in addition to ECM, to modify the immature collagen matrix into mature scar tissue (42).

The degradation of type III collagen produced and deposited by fibroblast during the proliferative phase is replaced by type I collagen in a slow process by MMP enzymes (2). The formation of mature type I collagen with gradual degradation of abundant ECM and the immature type III collagen is critical in this final phase of wound healing (43). Slowly, the activity of tissue inhibitors of MMPs increases to end with a drop in activity of MMP enzymes, thereby promoting new matrix accumulation. Unwounded dermis contains 80% type 1 and 25% type 3 collagen, whereas wound granulation tissue expresses 40% type 3 collagen (20).

Restoration of structure and function of the skin could be achieved with wound contraction, remodeling and resolution of the granulation tissue ECM by proteolysis and endocytosis, with minimal scar formation (11). The tensile strength of a healing scar improves slowly, at the beginning of maturation phase wounds have only about 20% to 30% of the strength of normal skin, and finally it reaches only 70% to 80% of the normal strength at the end of the remodeling phase. The abnormality of collagen deposition or synthesis affects the scar tissue tensile strength, so synthesis or deposition of collagen has to be balanced to produce a normal scar (2).

1.2.4 Phases of inflammation

Based on the duration and mechanisms of response inflammation has three different phases: An acute transient phase characterized by local vasodilation and increased capillary permeability, sub-acute phase, characterized by intrusion of leukocytes and phagocytic cells and proliferative phase of tissue degeneration and fibrosis (13). Inflammatory reaction, a sensory response perceived as pain, and a repair process are the serious events of inflammation. The inflammatory reaction also in turn has the following successive phases such as; a silent phase, where pro-inflammatory mediators are released by the resident cells of the damaged tissue, a vascular phase of vasodilation and increased vascular permeability, and a cellular phase, which is a phase of leukocytes infiltration to the site of injury (44).

1.3 Pathophysiology of wound and inflammation

Local factors such as edema, infection, tissue hypoxia, ischemia, necrosis and growth factor imbalance, and systemic causes like metabolic disease, nutritional status and general perfusion

disturbances or pre-existing illness are among many pathophysiological and metabolic factors that affect wound healing (20). During tissue injury the motile white blood cells move into the wound to begin engulfing cellular debris; whereas the myofibroblasts of the wound produce the wound contraction and cover the debris (43). Excessive neutrophil infiltration is a biological marker of chronic wounds, this abundance of neutrophils results in over-production of ROS, causing direct damage to ECM, cell membrane and thereby it causes premature cell senescence (1).

Complications of chronic wounds of human are broad, and patients are at risk of severe pain, septicaemia, hospitalization, and amputations. Wounding of the skin barrier causes microorganisms to colonize the damaged area and successive formation of biofilm. *Staphylococcus aureus* (SA), coagulase-negative staphylococci, *Enterococcus faecalis*, *Proteus species*, anaerobic bacteria, and *Pseudomonas aeruginosa* (PA) are some of the most common bacteriological findings of chronic wounds (45).

Pain and tissue destruction are the initial effect of the inflammatory pathway which stimulated during injury by the production of inflammatory mediators. Disruption in any of the healing stages can result in abnormal repair, with lack of restoration of integrity (25). Edema formation, leukocyte infiltration and granuloma formation are among the different components those contributes to inflammatory reaction and associated symptoms (46,47). Infiltrating leukocytes are the principal cellular components of the inflammatory response which combats invading pathogens as well as involved in tissue degradation and tissue formation (32). As risk factor exposure prolongs, chronic systemic inflammation generates pro-inflammatory mediators from the site of injury and travels to distant sites to attack cells and results in a new inflammation sites and inflammatory disease (25).

Inflammation involves a mediator release, enzyme activation, cell migration, extravasation of fluid, tissue breakdown and repair (13). Inflammatory mediators such as nitric oxide, prostaglandin(PG), and TNF-α can be produced by activation of different signaling molecules like, macrophages, monocytes, and neutrophils due to bacterial and viral infections (48). TNF-α secreted by monocytes/macrophages has an important role in the pathophysiology of inflammation by initiating other pro-inflammatory cytokines (such as IL-1β, IL-6 and IFN-ϒ). So, agents that block TNF-α action during chronic inflammatory condition reveal an anti-

inflammatory activity (30). PGs and leukotrienes are the main mediators of inflammation which act by attracting Polymorphonuclear leucocytes to the site of inflammation, and causes damage to tissue by releasing free radicals (13). PGs E1 and E2 are considered to be the final mediators of acute inflammation, and released in the site of inflammation to play a homeostatic role for white blood cells and fibroblasts (43).

1.4 Prevalence of wound and inflammation

Wound affect physical and mental health of millions of patients and impose significant cost on patients by causing physical disabilities (49). About 1-2% of individuals in all populations worldwide acquires a chronic wound during their life-time (36). Non healing wound due to burn and chronic skin ulcer affects a total of 9 million people (8), and its clinical management accounts for loss of USD 25 billion in USA which represents a great burden in public health expenditure (18). Similarly in Scandinavian countries, 2-4% of the total healthcare expenses are lost for the clinical management of chronic wounds (18). Because of increment in number of population older than 65 years in 2030 and the high risk of developing diabetes for children in United States (US), the anticipated risks of diabetes and age-associated non-healing chronic wounds continue to increase dramatically (19). So, non-healing wounds are significant problems for healthcare systems all over the world.

A point study conducted in Nigeria indicated that about 31.55% of the total patients attended the hospital were being managed for wound (50). According to a study conducted in Black Lion Hospital the overall prevalence of bacterial isolates from infected postoperative patients showed that 75.6% of them were had a bacterial infected wound, this implies that unless wound is properly managed it may has a secondary complication that can cost a lot (51). Another study done in some private and public hospitals of Bahir Dar city, indicated that the overall prevalence of surgical site infection following surgical procedures was reached up to 9.9% of patients those undergo surgical procedure and chronic wound was the major causes of this infection (52).

Higher rates of inflammatory bowel diseases (IBD) are seen in western countries. In the US, specifically about 30,000 new IBD cases are reported annually. In Africa, prevalence of allergic diseases exceeds the commonly reported diseases like tuberculosis and HIV/AIDS (25).

1.5 Management of wound and inflammation

A careful wound bed preparation, minimizing bacterial burden, thorough debridement of nonviable tissue, control of edema, optimizing the vascular status to allow for optimal nutrient supply, prevent additional trauma and reduction of mechanical stress are fundamental prerequisites for effective wound healing (53). Wound closure management is categorized into different methods of wound closure intention such as; primary intention which is done when surgical wounds can be closed immediately with sutures and tend to heal rapidly with less scar area. If a wound is contaminated and left open to prevent infection and wound closure is performed after a few days, it is called as delayed primary healing. The other is secondary intention closure which performed when the tissue loss has been more extensive, the wound must be left open due to sepsis, the reparative process is prolonged and has wide area of scar, as the defect must fill with extensive granulation tissue (54).

1.5.1 Conventional drugs of wound and inflammation

Currently wound is treated by debridement, irrigation, antibiotics, tissue grafts, and proteolytic enzymes methods, which have undesirable side effects (55). Because of possibility of chronic wound to harbor microbes, topical preparation of the following antibiotics (amikacin, bacitracin, chloramphenicol, clindamycin, gentamicin, nitrofurazone ointment 0.2% and polymyxin B) are chosen based on their ability to inhibit the growth of pathogenic organisms (56). Topical growth factor application and incisional priming with PDGF or IL-1 can optimize wound healing time by modifying inflammation and accelerating the proliferative phase. Electrical field stimulation may also improve the remodeling phase by stimulating more efficient fibroblast recruitment and collagen deposition (20). Silicone gel sheeting is the only remedy with high evidence which has been used widely for hypertrophic scar treatment. Some of its method for scar reduction include improved hydration, increased temperature and change in scar mechanical tension (53).

Inflammation and wound healing are governed by collection of growth factors, cytokines, and the inflammatory cells that produce them. Polypeptide growth factors, which promote and control cell proliferation at the site of wound is the main constituents of many present day wound-healing medicines (57). A study shows that topical VEGF has significant repair effects of wound in the treatment of diabetic complications characterized by impaired neovascularization. It enhances early angiogenesis and tensile strength in the ischemic wound in a rat. Currently, one

of the few available commercial products of cytokines and growth factors proven to be efficacious in randomized, double-blind studies is PDGF (8). However, majority of the present day pharmacologic cytokines and growth factors have a limited role in clinical practice though it remains still under investigation.

The enzyme-based therapeutics is widely used in conventional medicines because of their selectivity and efficiency. The first enzyme based therapy (intravenous trypsin) was began in 1950 to treat inflammation due to rheumatoid arthritis, ulcerative colitis, and atypical viral pneumonia as well as post-surgical swelling and bruises caused by sports injuries. The other enzyme based therapeutics serratiopeptidase also started in Japan in 1957, and currently it is broadly used in Japan and Europe as the anti-inflammatory and pain treatment of choice. The use of empirically encapsulated enzyme like; trypsin, chymotrypsin, and bromelain of oral route administration is growing because of good achievement of enzyme based therapeutics (58).

Anti-inflammatory agents such as non-steroidal anti-inflammatory drugs (NSAIDs) are successfully used in treating chronic inflammation associated disease; however, the long-term use of these drugs may result in damage to the gastric intestinal mucosa and heart, thereby limiting their usage (30). The long-term use of NSAIDs causes adverse effects, mainly due to inhibition of COX-1 leading to reduced prostaglandin production, so selective COX-2 inhibitors are beneficial to minimize the gastro intestinal side effects of NSAIDs though these also cause cardiovascular side effects (59). Recent studies have revealed an increased cardiovascular risk with nonselective NSAIDs with high COX-2 inhibition, whereas nonselective NSAIDs with high COX-1 inhibition seem to have higher gastrointestinal risk (60). But, the most common side effect of NSAIDs prolonged use is gastric bleeding (58).

Other drugs used for inflammation are corticosteroids, their predominant effect is to control multiple inflammatory genes that have been activated during the chronic inflammatory process as well as they have additional effects on the synthesis of anti-inflammatory proteins and postgenomic effects when given in high concentrations (29).The autoimmune disorders like rheumatoid arthritis require an effective dose of steroids such as prednisolone in an advanced stage but, long-term use of these drugs also affects immune system severely. These existing

complications of chemical based anti-inflammatory drugs led to the discovery of new drugs to replace conventional drugs (58).

1.5.2 Herbal medicines in wound and inflammation

Wound treatment involves a number of pharmacological and nonpharmacological measures to promote wound healing in short duration, additionally herbal remedies have high potential for the management and treatment of wounds and burns in many countries (5). The healing effects of medicinal plant depends on their secondary metabolites such as alkaloids, flavonoids, saponins, tannins, essential oils, terpenoids and phenolic compounds (5,61). According to World Health Organization (WHO) traditional medicine has been promoted as a source of less expensive, comprehensive medical care, especially in developing countries. Majority of the traditional medicines are used for the treatment of wounds and skin disorders, in contrary to a minimum number of modern drugs for these health problem (62). More than 70% of some developed countries and up to 95% of developing countries population use traditional medicine as primary healthcare to address their healthcare needs and concerns (63). According to Dan *et al.*, a study conducted in Colombia people those used folk medicine for the treatment of common infections have a reduced risk of getting infectious diseases from resistant organism than people of urban areas treated with antibiotics (6). Several studies reported that therapeutic plant extracts has been utilized in the management of wound healing due to their minor toxicity and easy accessibility (42).

The genus *Aloe* comprises about 600 species. It is anticipated to be used for medicinal purposes starting from 2000BC. But, only a few species of *Aloe* have been reported to contain many bioactive ingredients (64). It has a long history of use as a topical therapeutic for the treatment of burns, wounds and a variety of other skin conditions by applying the leaf gel as either freshly or after its extraction (65). *Aloe* can increase cell proliferation, wound closure rate, transforming growth factor beta at the wound site, blood vessel count, and collagen fiber density while decreasing wound secretions, inflammation, and scar formation (31).

Aloe vera gel acts by enhancing keratinocyte multiplication and migration, expression of proliferation related factors, and epidermis formation (43). *Aloe vera* contains more than 100 active components possessing wound healing, antibacterial, anti-inflammatory and other different medicinal value (6). It has been used effectively in burn wound management and it

significantly increases the collagen synthesis and remodels collagen composition (type III) to promote wound healing, contraction and breaking strength resulting in scar (40). Experimentally *Aloe vera* gel showed a significant reduction of wound thickness and altered the neutrophil, macrophage and fibroblast cells. It also caused an apparent wound contraction. Many of the medicinal effects of *Aloe vera* is because of the polysaccharide found in the inner gel. Different study outcomes from *in vitro,* animal and human studies revealed that topical *Aloe* gel has promising effect on immunomodulatory properties that may improve wound healing and skin inflammation (66). The other *Aloe* species called *Aloe littoralis* also has effects on wound, burn and inflammation. Gel formulations of *Aloe littoralis* and its raw mucilaginous gel were evaluated in the incision wound model and showed significant wound healing activity (67).

1.6 The experimental plant

Aloe trigonantha (A. trigonantha) L.C. Leach, which locally called as 'Eret' (Amharic), is among the endemic *Aloe* species of Ethiopia, which was first described in 1971 and found in an area between Bahir Dar and Gondar in Amhara region of Ethiopia (68). *A. trigonantha* grows on dry stony ground near roads and along field margin at altitude between 1900 and 2100m (Fig. 1B). It has secondary branching up to 50 racemes or more, its leaves are stemless at young age and in a dense rosette, 25– 40× 5–8 cm with uniformly green color (Fig. 1A). This species has three-angled perianth with a truncate base which helps to distinguish the species from the other related group. The main flowering period is from September to January (69). Among the total 32 number of endemic *Aloe* species, 26 are endemic to Ethiopia with 81.25% of endemism, out of which *A. trigonantha* is one species (70).

Like many other *Aloe* species traditionally employed, the local people of Bahir Dar and Gondar area extensively use the leaves of *A. trigonantha* for treatment of malaria, eye infection, wound (68), and inflammatory diseases (71). The leaves latex of *A. trigonantha* has significant effect against a broad range of bacteria particularly gram-negative pathogens (71). Its latex also revealed a significant antimalarial and antifungal effects according to thesis research conducted at Addis Ababa University (68).

A B

Figure 1. Pictures of *Aloe trigonantha* L.C. Leach; young leaf (A) and flower of the plant (B)

1.7 Rationale of the study

Although chronic wound is prevalent worldwide affecting over 40 million people globally, its significance is superseded by their causes, its costs are poorly documented, and appropriate care and education is not completely available (1). Because of different factors on conventional drug such as bacterial resistance, adverse effects, costs, environmental degradation, and pollution coupled with improper usage of these medicines, the use of medicinal plants as effective and safer alternatives in the management of various diseases such as wound has been increased (4,55,72). A clinical study showed plant derived medicine prevented 85% of legs from limb amputation in diabetic foot ulcer patients provoking more studies to be done on plant medicines (4). The limitations of current wound healing treatments also call for an urgent development of a safe, effective, and economical formulation for use in wound healing (8). Since conventional drugs result in adverse effects; development of safe, effective and affordable, wound healing and

anti-inflammatory products from plant materials is still needed (73). Though the experimental plant, *A. trigonantha* used for wound healing, there are no *in vivo* studies which prove its claimed use for wound healing and anti-inflammatory action. Therefore, the present study was aimed to evaluate the *in-vivo* wound healing and anti-inflammatory effect of the leaf gel of *A. trigonantha*.

2 Objective

2.1 General objective

To evaluate the wound healing and anti-inflammatory activity of the leaf gel of *A. trigonantha* in rats.

2.2 Specific objectives

- To conduct acute oral toxicity test of the leaf gel of *A. trigonantha* in rats
- To conduct acute dermal toxicity test of the leaf gel of *A. trigonantha* in rats
- To evaluate wound healing activity of the leaf gel of *A. trigonantha* in rats
- To evaluate anti-inflammatory activity of the leaf gel of *A. trigonantha* in rats
- To conduct phytochemical screening of the leaf gel *A. trigonantha*

3 Materials and Methods

3.1 Materials

3.1.1 Chemicals, solvents and instruments

Chemicals, solvents and instruments used in the present study include: ketamine hydrochloride (NEON Laboratories limited, India), xylene (Farmitalia Carlo Erba, Italy), indomethacin 25mg capsule (Cadila Pharmaceuticals PLC, Ethiopia), nitrofurazone (2mg) 0.2% ointment (Galentic pharma (India) Pvt Ltd.), mercuric chloride (Blulux Analytical Reagent Pvt Ltd, India), potassium iodide (Calibre Engineering Chemistry, India). sulfuric acid (Loba chemical, India), chloroform (Indenta Chemical Pvt Ltd. India), ferric chloride ($FeCL_3$) (Sisco Research Laboratories Pvt. Ltd Maharashtra. India), acetic anhydride (Lot A13/45/67/A), distilled water (Ethiopian Pharmaceutical manufacturing), electronic balance (KERN-ANG 220-4, Germany), lyophilizer (OPR-FDU-5012, Korea), deep freezer (AFTRON AFF 545, Denmark), tissue drying oven (Laboratory Oven Lab-Tech, India).

3.1.2 Plant material

The plant leaf gel used in this study was collected from Amhara regional state in northwest part of Ethiopia, Kutkuatema small village; 568 KMs away from Addis Ababa on the way to Bahir Dar, where the leaf gel is used traditionally for various conditions. The fresh leaves were transported using plastic bag. It was then authenticated by Dr. Getachew Addis, a botanist at Ethiopian Public Health Institute (EPHI), and sample specimen was deposited at the herbarium of EPHI with a voucher number (HGDB/002/18) for future reference.

3.1.3 Animals

Healthy Wistar albino rats of either sex, were used for the experiment. The animals were kept in an air-conditioned area at room temperature, under 12hrs dark and 12hrs light cycle. Animals were allowed to acclimatize for one week before commencement of the study and provided with a commercial food pellets and water *ad libitum*. All procedures and techniques used in this study were conducted in accordance with the national research council guidelines for the care and use of laboratory animals (74).

3.2 Methods

3.2.1 Preparation of plant material

Preparation of the plant leaf gel was carried as per the method described previously by Rahman *et al.,* (40) and Farzadinia *et al.,* (75). Briefly the whole leaf was washed with distilled water. Then spikes and margins were avoided before slicing the leaf, and the cortex was carefully separated from the parenchyma using a knife. Fresh *A. trigonantha* filets were cut into smaller pieces and blended with electrical blender to homogenize with little amount of water. The homogenate was kept in deep freezer to minimize enzymatic degradation, air oxidation and loss of active ingredients. Then it was dried in a lyophilizer under cold vacuum conditions. The dried sample of the plant was pulverised into fine powder using mortar and pestle. Then the pounded product was kept in refrigerator in dark closed bottle container until its use.

3.2.2 Ointment formulation

The formula of simple ointment formulation was used from British Pharmacopoeia (BP, 1988) in order to prepare simple ointment base for leaf gel ointment formulation (76).

Table 1. Master formula and reduced formula for simple ointment formulation

Ingredients	Master formula (gm)	Reduced formula (gm)
Wool fat	50	15
Hard paraffin	50	15
White soft paraffin	850	255
Cetostearyl alcohol	50	15
QS	1000	300

QS: Quantity Sufficient

A total of 300 g of the simple ointment base was prepared using the reduced formula according to method described by Gebremeskel *et al.,* (77) with slight modification on the ratio of ingredient taken. Out of this 100 g was used as a control and the rest 200g was used to prepare 100 g of each 5% and 10% ointments of the leaf gel powder. First, all the ingredients of the simple ointment base were mixed, heated gently with stirring until homogenous and stirred until cool. Then, to prepare the 5% and 10% ointment preparation, 5 g and 10 g of the of the plant

powder were measured and added to 95 g and 90 g of the base respectively, mixing with the simple ointment (base) by levigation on the surface of the ointment slab to make ointment of uniform consistency and smooth texture (78).

3.2.3 Acute oral toxicity study

The limit test was done as per OECD guidelines-425 in five female rats (age, 11-12 weeks; weight, 200g -250g). The rats were nulliparous and non-pregnant, and a dose of 2000mg/Kg of the test substance in distilled water was first given for one female rat. This rat was followed for 24 hours with giving special attention on the first four hours to observe survival and any sign of acute behavioral and anatomical change. After confirming the survival of this rat, the rest four rats were administered with the same concentration of the gel powder sequentially to test toxicity of the plant on all rats. Food was given four hours after administration of the test substance. The rats were then observed every day for 14 days for any signs of toxicity and/or mortality as well as to follow if any gross behavioral and autonomic profiles changed (79).

3.2.4 Acute dermal toxicity study

Acute dermal toxicity was determined according to OECD 404 (80), in three female nulliparous and non-pregnant rats. Approximately 24 hours before the test, fur was removed by closely clipping the dorsal area of the trunk on both flanks using a suitable depilatory material. Special attention was given not to abrade the skin. Skin was washed with sterile water a day before toxicity test procedure. A limit test dose of 2000mg/kg of the 10% (w/w) leaf gel ointment was applied on the shaved back of the rats within a range that was approximately 10% of the body surface area and held in contact to the skin with a gauze patch to facilitate good contact and uniform distribution of the test chemical on the skin for the duration of the exposure period (24 hours) (81). The test was first conducted in a single rat for initial testing, and then after observing the response of the first rat for 24 hours a confirmatory test was done on another two rats simultaneously. At the end of the exposure period, the ointment was removed and the animals were observed for 14 days for development of any adverse skin reactions like inflammation, irritation or redness. Behavior, general condition, posture and reflexes, attitude towards food, and water were also evaluated.

3.2.5 Grouping and dosing of animals

The fasting body weight of each animal was measured initially after overnight fasting thereafter animals were randomly selected and housed individually in a cage for the wound healing and dermal toxicity tests, so that wound conditions would not be aggravated by biting and the bedding materials. In excision model, randomly four groups of rats consisting of six animals in each group were employed. Each groups were treated with the following test and control ointments such as; 5% (w/w) and 10% (w/w) crude gel ointments of test, simple ointment (negative control), and 0.2% (w/v) nitrofurazone (positive control). The same procedures were also used for incision wound model except, addition of fifth group, i.e., the untreated group.

For assessment of anti-inflammatory activity, in both xylene induced ear edema and cotton pellets induced model, five groups of rats, six animals per group were used in each models. Distilled water was used as a control, indomethacin (10mg/kg) as a standard drug and three different doses of the dried leaf gel powder at (100mg/kg, 200mg/kg, and 400mg/kg) was assigned to group of I-V, respectively. The dose calculation was dependent on the safe dose of the plant product obtained after limit test at 2000mg/kg. The whole administrations were given orally, with a maximum volume of 10 ml/kg. Both the dried gel powder and the standard drug were dissolved in distilled water to get an oral suspension.

3.2.6 Excision wound model

The excision wound was done according to techniques described by Subalakshmi *et al.,* (82). On the day of inducing wound, animals were anesthetized by intra peritoneal injection of ketamine in 1ml/kg and back of the animals were shaved with scissors. Then, circular area was marked on the shaved area and the full thickness of the marked area was carefully excised using razor blade and about 450mm^2 of wound area was formed. The wound was hemostasis with cotton swab soaked in normal saline. After 24hr of wound creation, both controls and plant product which was prepared in the form of ointments were applied gently once a day to cover the wounded area for 21 days. Then, wound contraction was monitored and measured as percent of contraction every 3 day.

Wound contraction was evaluated by measuring wound size and calculated as

$$\% \text{ Wound contraction} = \frac{\text{Initial wound size- specific day wound size}}{\text{Intial wound size}} \text{x}100$$

The period of epithelialization was also calculated as the number of days required for falling off the dead tissue remnants without any residual raw wound (55).

3.2.7 Incision wound model

The measure of tensile strength was performed according to techniques used Mulisa *et al.,* (83). Animals were anesthetized in the same manner described for excision wound model before wounding them. Then, 3cm long longitudinal paravertebral incision was made through the skin and subcutaneous tissue using razor blade, after the dorsal fur of each rat had shaved with scissor. The parted skin was sutured 1cm apart using catgut and curved needle. The continuous thread (polyglycolic acid 2/0) catgut on both wound edges was tightened for good closure of the wounds. After 24 h of wound creation (on 1st day), rats were treated topically with the test substance and references drugs for the consecutive nine days. The sutures were removed on the 8^{th} day and tensile strength was measured on the 10^{th} day using continuous water flow method.

The anesthetized rats were then laid on table and forceps were applied on the back of the rats one centimetre away from the sutured skin as shown in Fig 3. The forceps on one side of the rat was tied firmly to a stable metal stands fixed to the operation table, whereas the other side forceps was connected to an empty polythene bag by string running over a pulley and the bag was connected to water reservoir. Wound tensile strength was measured by constant and continuous water flow technique which was released from the reservoir into the plastic bag. The gradual increased weight was acted as a pulling force to disrupt the wound. Then the rubber tube was clamped as soon as the stitched wound began to open and the polythene container was weighed. So, the weight of the liquid was recorded as weight required to break the suture and used to measure the tensile strength.

The tensile strength was then calculated as follow:

$$\%\ \text{Tensile strength of leaf gel} = \frac{\text{TS leaf gel- TSso}}{\text{TSso}} \text{x}100$$

$$\%\ \text{Tensile strength of Reference} = \frac{\text{TSreference- TSso}}{\text{TSso}} \text{x}100$$

$$\%\ \text{Tensile strength of SO} = \frac{\text{TSSo- TSLU}}{\text{TSLU}} \text{x}100$$

Where, 'TS' is tensile strength, 'SO' is simple ointment (vehicle) and 'LU' is left untreated (negative control).

3.2.8 Xylene induced ear edema model

Acute anti-inflammatory activity of *A. trigonantha* leaf gel was evaluated by xylene induced ear edema in rats. An established method described by Kumer A *et al.,* (73) was used with slightly modification; thirty rats were randomly divided into five groups. The grouping was made as follows, group I treated with distilled water (10mL/kg body weight) as negative control. Group II treated with 10mg/kg indomethacin and considered positive control. Test groups (group III, IV and V) were orally administered with crude leaf gel at doses of 100, 200 and 400mg/kg body weight.

Acute ear edema was induced by topical application of 30μL of xylene on the anterior and posterior surfaces of the right ear lobe of each animal, one hour after respective treatment. The left ear was untreated with xylene and served as control. The experimental animals were sacrificed (2 hours after xylene application) and 10 mm diameter of circular sections of both ears was punched out. Finally, the cut portion of each ear was weighed accurately. The weight of ear edema was calculated from the difference between the weight of right ear and weight left ear. Then percentage inhibition of ear edema was determined by the following formula.

$$\text{Inhibition}\ (\%) = 1 - \frac{\text{weight of edema leaf gel or standard (mg)}}{\text{weight of edema (negative control)(mg)}}\ \text{X}\ 100$$

3.2.9 Cotton pellet induced granuloma model

The method described by Mengi and Bakshi (2009) (84) was followed to assess the exudate formation and proliferative (granulomatous) components of chronic inflammation. Male Wistar albino rats (240–280 g; 11–12weeks) were fasted over night with free access to water. The rats were randomly divided into five groups, six rats per group. Each groups were treated in the same manner as that of xylene induced ear edema model and treatments were given daily for seven days orally.

Sterile cotton pellet was prepared by rolling of a cotton piece of 20 ± 1 mg weight and sterilized by autoclaving for two hours at 120∘C. Thirty minutes after treatment with the reference drug and test agent, the rats were anesthetized with ketamine at a dose of 1ml/kg, and subcutaneous tunnel was made aseptically using blunted forceps and surgical blade on both sides of groin. Two sterilized cotton pellets weighing 20 ± 1 mg each were then implanted bilaterally in the subcutaneous tunnel and sutured with chromic catgut (polyglycolic acid 2/0). On the 8^{th} day, the rats were sacrificed after anesthetized with ether. The pellets surrounded by granuloma tissue were freed from extraneous tissue after it carefully dissected out. Then, the wet weight was taken immediately after removal before it dried at 60∘C for 24hrs and the net dry weight was determined. The exudate amount (mg), granulation tissue formation (mg), and percent inhibition of exudate and granuloma tissue formation were calculated according to the formula given below following methods used by Aishwarya *et al.,* (13):

$$Exudates\ inhibition\ (\%)\ = 1 - \frac{Exudates\ in\ treated\ group}{Exudates\ in\ control\ group}\ X\ 100$$

$$Granuloma\ inhibition\ (\%)\ = \ 1\ - \frac{Granuloma\ in\ Treated\ group}{Granuloma\ in\ control\ group}\ X\ 100$$

Where;

$$measure\ of\ exudates(mg) = immediate\ wet\ weight\ of\ pellet - constant\ dry\ weight$$

$$measure\ of\ granuloma\ (mg) = constant\ dry\ weight\ of\ cotton\ pellet - initial\ weight\ of\ pellet$$

3.2.10 Preliminary phytochemical screening of crude leaf gel

The qualitative screening of secondary metabolites were carried out following standard procedures to identify the presence of alkaloids, flavonoids, glycosides, terpenoids, saponins, tannin, and steroids.

Test for alkaloids

Ten mg of plant dried gel sample was dissolved with 5ml of distilled water, two drops of Mayer's reagent was added in a test tube. Appearance of white creamy precipitate was considered as the indication of the presence of alkaloids (85).

Test for Flavonoids

Ten mg of the gel was dissolved with 5ml of water, a few drops of 2 % lead acetate solution was added. The configuration of yellow colour was taken as evidence for the presence of the flavonoids in the plant leaf gel (86).

Test for glycoside

About 20mg of the gel powder was treated with 2ml of glacial acetic acid solution which was contained in 1 drop of 2% ferric chloride solution and 1ml of concentrated H_2SO4. A reddish brown colour formed at the junction of the two layers was taken as an indication of the presence of glycoside in the leaf gel (86).

Test for terpenoids

Fifty mg of the gel powder was dissolved in 5ml of distilled water and evaporated on the water path after mixed with 2 ml of chloroform then boiled with 2 ml of H2SO4 concentrated. A grey color produced indicated the presence of terpenoids (87).

Test for saponins

Five milliliter of distilled water was added to 50mg of crude plant gel product in a test tube and it was shaken vigorously. The foam formation used as an indication for the presence of saponins (87).

Test for tannins

Few amounts of the gel was dissolved in 2 ml of water, then 4 drops of diluted ferric chloride solution was added. A green precipitation was indicated the presence of tannins compound in the plant (86).

Test for phenols and tannins

Two milliliter of 2% solution of FeCl3 was added to crude leaf gel. Black or blue-green color was used as an indication for the presence of tannins and phenols (87).

Test for steroids

Two milliliter of each chloroform and concentrated H2SO4 were mixed with fifty mg of the crude powder of the leaves gel. Presence of red color in the lower chloroform layer was used as an indication of steroids availability (87).

3.2.11 Data analysis

All statistical analyses were performed using international business machine of statistical package for the social Sciences, (IBM SPSS), version 25 for windows (SPSS inc, Chicago, Illinois, USA). Statistical differences between groups was analyzed by one-way analysis of variance (ANOVA) followed by Tukey post hoc test for multiple comparisons to compare results among groups. The experimental results were expressed as mean ± standard error of the mean (S.E.M) and $P < 0.05$ was considered as statically significant for each parameter.

4 Results

4.1 Acute oral toxicity effect of *Aloe trigonantha*

Acute oral toxicity study results showed that the plant leaf gel was safe at least up to the tested dose. Physical and behavioral observations of the experimental animals revealed no visible overt signs of acute toxicity like lethargy, tremor, fatigue, paralysis, autonomic and behavioral changes. Therefore, the LD50 of the gel powder was greater than 2000 mg/kg. None of the rats died, and also there was no observed sign of toxicity till the end of the 14th day.

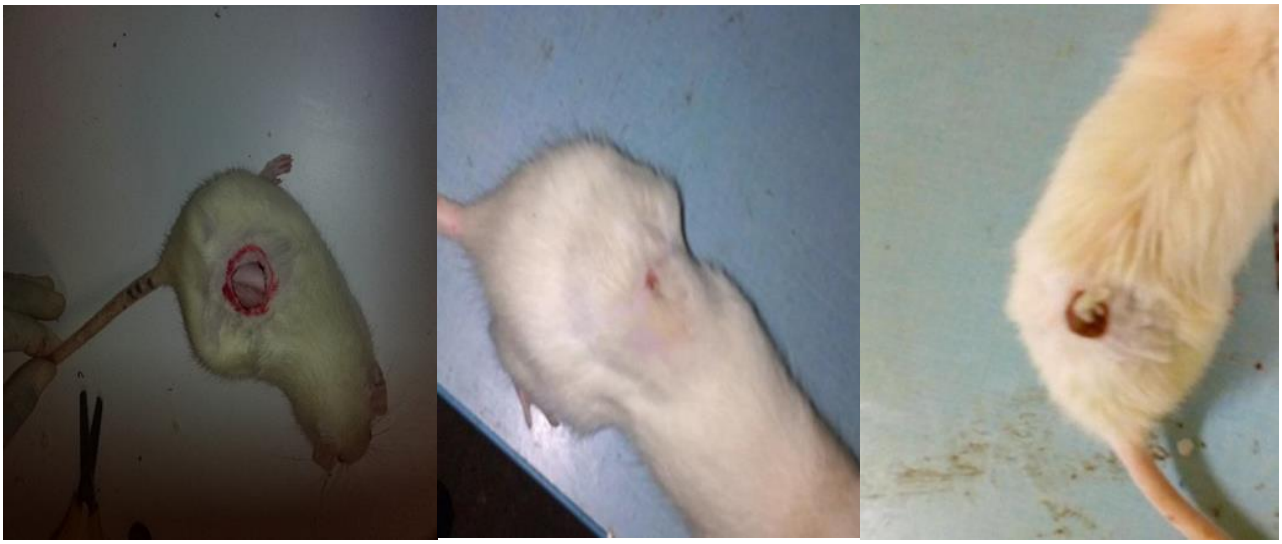
4.2 Acute dermal toxicity effect of *Aloe trigonantha*

After application of 10% ointment of the gel, there was no sign of inflammation, irritation or redness at the site of application and no changes were observed in fur, eyes, and behavior of treated animals. There was also no overt signs and symptoms observed when the animals were monitored for 48 h. Moreover, no signs of toxicity, no edema and erythema as well as no mortality were noted during the 14 days cage side observation. This indicates that the plant is nonirritant and safe to the animal in dermatological applications at least up to the applied concentration.

4.3 Effect of *Aloe trigonantha* on wound healing

4.3.1 Excision wound model

The dried leaf gel powder formulated in ointment form was found to be highly active on excision wounds. This was shown by the percent of wound contraction and epithelialization period that shows the rate at which wound healing progresses. The test substance treated animal showed a fast contraction of wound compared to the simple ointment treated rat (Fig. 2). The results obtained from the excision wound experiment are summarized in Table 2.



A B C

Figure 2. Pictures of excision wound at day 0 of wounding (A), day 18 of 10% *A. trigonantha* gel treated rat (B) and day 18 of simple ointment treated rat (C)

The rats treated with the plant leaf gel ointment were found to have good wound contraction and fast epithelialization period compared to ointment base treated group animals (Table 2). Animals treated with 10% (w/w) gel ointment and 0.2% nitrofurazone ointments showed significant decrease (17.1% and 18%, respectively, $p < 0.05$) in epithelialization period as evidenced by shorter period for fall of the dead tissue remnants. Likewise, 5% (w/w) ointment treated group also exhibited significant decrease (12.6%, $p < 0.05$) in epithelialization period compared to control group with slightly less percent of period/ prolonged number of days required for epithelialization than 10% gel ointment. Similarly, the wound contraction of the gel ointment treated groups between each other showed that the 10% ointment was statistically significant at day 15 and day 18 compared to the 5% of the gel ointment.

Table 2. Effect of *Aloe trigonantha* leaf gel ointment on percent of wound contraction and period of epithelialization by excision wound model in rats

Groups	Percent of contraction							Period of epithelialization (in days)
	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	
I	14.25 ± 1.64	31.3 ± 2.13	50.25 ± 3.57	66.93 ± 3.67	81.88 ± 1.08	88.72 ± 1.02	95.16 ± 0.91	18.5 ± 0.43
II	18.71 ± 1.15	41.68 ± 3.29	70.4± 1.32 a*	87.79 ± 0.94 a*	93.67 ± 0.54 a*b*	97.81 ± 0.27 a*b*	99.21 ± 0.18 a*	15.17 ± 0.31 a*
III	16.72 ± 2.81	34.58 ± 3.34	57.75 ± 3.27	79.77 ± 2.53 a*	88.43 ± 1.96 a*	94.42 ± 1.12 a*	97.42 ± 0.43 a*	16.17 ± 0.48 a*
IV	18.08 ± 1.25	41.22 ± 2.74	70.42 ± 1.2 a*	86.69 ± 1.63 a*	93.61 ± 0.77 a*b*	97.77 ± 0.45 a*b*	99.06 ± 0.21 a*	15.33 ± 0.49 a*

All values are expressed as Mean ± SEM (n=6); Analysis was performed by one way ANOVA; a: compared against simple ointment; b: compared against 5% gel; statistical significance is denoted by *p < 0.05; I: simple ointment; II: nitrofurazone ointment; III: 5% leaf gel ointment; IV: 10% leaf gel ointment

The wound area of all groups were measured on the 3rd, 6th, 9th, 12th,15th, 18th and 21st days for excision wound model and percentages of wound contraction was calculated on these days. Simple ointment treated rats showed less percentage of wound contraction. Increased percentage of wound contraction was observed in animals treated with 10% ointment (Fig. 2), and nitrofurazone starting from the 9th day of wounding (P < 0.05). Treatment with 5% of the gel

ointment revealed significantly higher wound contraction than simple ointment control group starting from the 12th day ($p<0.05$) (Table 2).

4.3.2 Incision wound model

The tensile strength effect of *A. trigonantha* on sutured wound by incision wound model was done by water flow technique as presented in Fig. 3 and also demonstrated wound healing (stitch stability) effect of the gel ointment. The mean tensile strength in the group treated with simple ointment tended to increase by about 4.4 % compared to untreated controls, which failed to reach statistical significance. However, tensile strength was significantly increased by about 26.5 % ($p < 0.05$), 37.6 % ($p < 0.05$), and 38.3 % ($p < 0.05$) with 5 % gel ointment, 10 % gel ointment and nitrofurazone 0.2% ointment respectively, compared to controls treated with the ointment base. The 10% gel ointment and nitrofurazone also gave a significant tensile strength effect compared to the 5% gel ointment ($P<0.05$) (Table 3). Generally, the 10% leaf gel ointment gave a significant wound breaking strength than the 5% gel ointment and simple ointment treated group and comparable to nitrofurazone 0.2% ointment treated group.

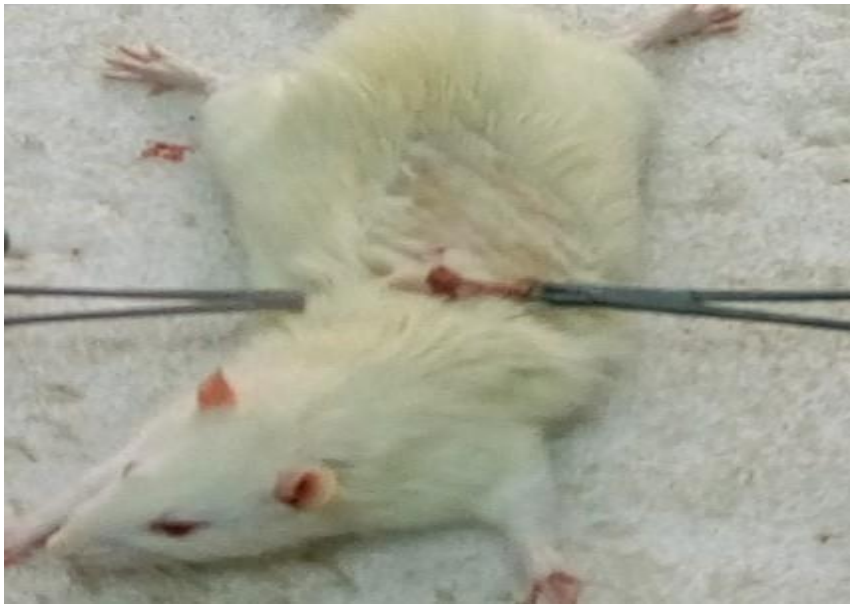


Figure 3. Water flow technique to measure tensile strength of skin wound

Table 3. Effect of *Aloe trigonantha* on tensile strength in incision model using continuous water flow technique rats

Group	Tensile strength in gram (mean ± SEM)	% Tensile strength
I	342.33 ± 5.302	
II	357.33 ± 6.3810	4.4%
III	494.00 ± 6.787 a*b*c*	38.3%
IV	452.17 ± 6.483 a*b*	26.5%
V	491.67 ± 7.032 a*b*c*	37.6%

All values are expressed as Mean ± SEM (n=6); Analysis was performed by one way ANOVA; a: compared against untreated group, b: compared against simple ointment; c: compared against 5% gel ointment; statistical significance is denoted by *p < 0.05; I: left untreated; II: simple ointment; III: nitrofurazone ointment; IV: 5% leaf gel ointment; V: 10% leaf gel ointment

4.4 Anti-inflammatory activity of *Aloe trigonantha* gel

4.4.1 Xylene induced ear edema model

In acute inflammation study of xylene induced ear edema model, the plant material showed concentration based inhibition of inflammation. The highest dose (41.5%) showed comparable inhibition of inflammation with indomethacin (42.6%) and revealed significant effect compared to the negative control group ($p< 0.05$). The 200mg/kg dose also significantly inhibited inflammation by inhibiting ear edema ($p<0.05$) compared to negative control group (Table 4).

Table 4. Acute inflammation inhibition effects of *Aloe trigonantha* using xylene induced ear edema model in rats

Substance given	Edema weight (mg) (mean ± SEM)	% inhibition of edema
I	10.83 ± 0.49	
II	6.22 ± 0.64 *a	42.61%
III	7.92 ± 1.22	26.6%
IV	7.25 ± 1.01 *a	33.07%
V	6.33 ± 0.70 *a	41.54%

All values are expressed as Mean ± SEM (n=6); Analysis was performed by one way ANOVA; a: compared against distilled water treated group; statistical significance is denoted by *p < 0.05; I: distilled water (control); II: indomethacin 10mg/kg (standard); III: 100mg/Kg leaf gel powder; IV: 200mg/Kg leaf gel powder; V: 400mg/Kg leaf gel powder

4.4.2 Cotton pellet induced granuloma model

The cotton pellet was removed on the eighth days as shown in Fig. 4A, and measured its wet weight then, it was dried in a tissue drying oven for twenty four hours (Fig. 4B). The *A. trigonantha* leaf gel powder was found to reduce the weight of cotton pellet exudate and granuloma in a dose dependent manner (Table 5). It was significantly ($p < 0.05$) prevented the formation of inflammatory exudates and granuloma mass at all tested doses compared to the control. Intergroup comparisons revealed that the 400mg/kg is significantly superior to the 200 and 100mg/kg in both exudate and granuloma inhibition, similarly the 200mg/kg was also significantly higher than 100mg/kg at ($p < 0.05$). It was also shown that 400mg/kg gave relatively comparable effect to indomethacin in both exudate and granuloma inhibition at ($p < 0.05$). Furthermore, the anti-inflammatory effect of the *A. trigonantha* was in dose dependent fashion with ($R2 = 0.942$). Generally, this plant showed 23.4%, 29%, and 34.3% inhibition of inflammatory exudate and 22.1%, 30.9%, and 38.6% reduction of granuloma at 100mg/kg, 200mg/kg, and 400mg/kg doses respectively, while indomethacin showed 34.8% and 38.8% inhibition of exudates and granuloma respectively at ($p < 0.05$).

A B

Figure 4. Photographs of a freshly removed cotton pellet after seven days of treatment (A) and dried cotton pellet in tissue drying oven after 24 hours (B)

Table 5. Exudate and granuloma inhibition effects of *Aloe trigonantha* in cotton pellet induced granuloma model in rats

Groups	Measure of exudate formation (mg) (mean ± SEM)	% inhibition	Measure of granuloma formation (mg) (mean ± SEM)	% inhibition
I	294.500 ± 1.7654		77.67 ± 1.4298	
II	193.333 ± 1.0541*abc	34.8%	47.5 ± 1.3844*abc	38.8%
III	225.500 ± 1.5438*a	23.4%	60.5 ± 1.118*a	22.1%
IV	209.167 ± 0.6009*ab	29%	53.667 ± 0.6667*ab	30.9%
V	193.500 ± 0.4282*abc	34.3%	47.667 ± 1.022*abc	38.6%

All values are expressed as Mean ± SEM (n=6); Analysis was performed by one way ANOVA; a: compared against distilled water treated group; b: compared to 100mg/kg; C: compared to 200mg/kg; statistical significance is denoted by *$p < 0.05$; I: distilled water (control); II: indomethacin 10mg/kg (standard); III: 100mg/Kg leaf gel powder; IV: 200mg/Kg leaf gel powder; V: 400mg/Kg leaf gel powder

4.5 Preliminary phytochemical screening of *Aloe trigonantha* gel

Preliminary phytochemical screening of the leaf gel of *A. trigonantha* indicated the presence of four classes of secondary metabolites (Table 6).

Table 6. Preliminary phytochemical screening results of *Aloe trigonantha* leaf gel

Secondary metabolites	Availability results
Alkaloids	-
Flavonoids	+
Glycosides	+
Terpenoids	-
Saponins	-
Tannins	+
Steroids	-
Phenols and tannins	+

The phytochemical screening test had revealed that the *A. trigonantha* gel has secondary metabolites like flavonoids, glycosides phenols and tannins. However, saponins, alkaloids, terpenoids and steroids were not detected.

5 Discussion

In the present study, the wound healing and anti-inflammatory activity of the crude leaf gel of *A. trigonantha* has been evaluated using excision and incision model for the wound healing and xylene induced ear edema and cotton pellet granuloma method for the anti-inflammatory activity test. Both excision and incision *in vivo* methods were used in this study because of complexity of wound healing cascade and difficulty of evaluating the effects of an agent in any *in vitro* model (77). The dried gel for topical administration was prepared in the form of ointment to facilitate a sustained drug release at the application sites. This ointment base has also an advantage of acting as a barrier for moisture by hard and white soft paraffin whereas the wool fat and cetostearyl alcohol are thickeners and because of this characteristics they are used for stabilization of ointment (63).

The main processes that are involved in wound healing are epithelialization, contraction, and connective tissue deposition and governed by the biosynthesis and deposition of new collagens at the site of the wound. It also involves migration of endothelial cells, leading to the neovascularization of connective tissues to synthesize ECM (17). The variable of measurement in excision wound were the rate of wound contraction and the period of epithelialization. So, visible appearances and measurements of wound contraction become an essential parameters in macroscopic evaluation for wound healing (88). This study showed that *A. trigonantha* significantly accelerated the contraction of wounds at ($P<0.05$). The 10% (w/w) gel ointment treated group revealed significant wound area contraction starting from day 9 of wounding, whereas the 5% (w/w) ointment treated group showed statistically significant wound area contraction starting from the 12^{th} day on wards. The outcomes of topical formulation of *Aloe* gel at 100% of *Aloe* being considered most effective, and other with less than 50% showing less effect (65), this evidence supports result of the present study in which the 10% is more effective than 5% gel ointment in both models of wound. This implies that as leaf gel concentration increases the wound contraction rate also increases. The wound contraction could be mediated by specialized myofibroblasts found in the granulation tissue. So, the increase in wound contraction is probably due to the ability of the gel to enhance activity of fibroblasts (88).

Re-epithelialization is mandatory for restoration of the integrity of the skin and makes it less vulnerable to infection. The leaf gel ointment treated rats showed decreased time to epithelialization compared with the control group (Table 2). The period of epithelialization was significantly reduced from 18.5 days (negative control) to 15, 16, and 15 days for nitrofurazone, 5% and 10% gel ointment treated groups, respectively. Shortening of epithelialization period could be due to proliferation of the epithelial cells and increasing in their viability by the crude gel ointment of the plant (83). The anti-inflammatory effect or a probable induction of macrophage cell proliferation, stimulation of fibroblast migration, and proliferation and production of collagen could be the possible mechanisms of the gel ointment for its wound healing potential (63).

The incision wound treated with leaf gel ointment showed a significant tensile strength in both the 5% and 10% gel ointment treated groups (Table 3). This tensile strength effect could be associated with the increment of collagen synthesis, angiogenesis, fibroblast growth and stabilization of fibers with crosslinking of the protein (55). Therefore, the overall effect improves circulation for oxygen and nutrients supply that are vital for wound healing cascade (77).

Inflammation could occurs in three distinct phases, such as an acute transient phase characterized by local vasodilation and increased capillary permeability, sub-acute phase, characterized by infiltration of leukocytes and phagocytic cells and chronic proliferative phase, in which tissue degeneration and fibrosis occurs. The acute vascular process results from vasodilation and capillary permeability leading to hyperemia and edema formations at the inflammatory site. These processes are all accompanied by pro-inflammatory cytokine, and activations of complement factors (13). In acute inflammation testing, xylene was used for induction of inflammation due to a vasodilatation. It is also known to cause local increases in capillary permeability, inflammatory cell infiltration, and ear acute exudative inflammatory edema. During the process of inflammation, vasodilatation brings about plasma extravasation and inflammatory mediators release, which trigger the acute inflammation response (89).

Acute inflammation induced in xylene induced ear edema model is frequently used to validate the *in vivo* evaluation of anti-inflammatory topical steroids and nonsteroidal anti-inflammatory agents, mainly those inhibiting phospholipase A2 (14,90,91). It is also partially associated with substance-P and the release of inflammatory mediators such as histamine, kinin, and fibrinolysin

(43). Kinin is said to be the main mediator of granuloma, as it both causes vasodilation and increase vascular permeability in the early stages of inflammation (46). The significant inhibition of inflammation in xylene-induced ear edema in rats ($P< 0.05$) treated with all doses of leaf gel powder indicated that the gel may have an active ingredient that could either antagonize the effect or reduce release of substance-P or any of the above pro-inflammatory mediators.

In the present study, the gel significantly inhibited the xylene induced increases in ear edema weight in a dose related manner. This inhibition capacity of the gel can be regarded as the evidence of anti-inflammatory efficacy through reducing vasodilation and improving edematous condition. There was significant reduction of the ear edema formation at the dose of 400mg/kg showed an activity comparable to standard drug (Table 4). So, the inhibition of ear edema indicated that *A. trigonantha* leaf gel could have attenuated vasodilatations and plasma extravasation of neurogenic inflammation, which were common in the early stage of acute inflammation. Additionally, it may have a membrane-stabilizing effect that reduces capillary permeability and/or has inhibitory effects on mediators (92).

Excessive proliferation of macrophages, neutrophils and fibroblast are basic sources of granuloma formation in inflammation (46). Cotton pellet induced granuloma model also assess the ability of agents to reduce leukocyte infiltration and granuloma formation in the area of inflammation (93), and the significant effects of this model ($P< 0.05$) indicates that the gel may has strong ability of reduction in leukocytes migration during the process of inflammation. The gel effectively and significantly reduced cotton pellet-induced exudate and granuloma, thereby suggesting its activity in both the exudative and proliferative phase of the inflammation. This reduction in transudate and granuloma formation by administration of the gel may be correlated with reduction of the number of fibroblasts and the synthesis of collagen and mucopolysaccharides that are involved in the formation of granuloma tissue (92).

The process of granuloma formation takes place due to release of proinflammatory mediators, lysosomal enzymes and reactive oxygen species (26). The effect of the gel in this model showed significant effects of minimizing both the wet and dry weight ($P < 0.05$) compared to the distilled water treated group (Table 5). The exudate and granuloma inhibition effect could be due to the increased levels of anti-inflammatory cytokines and decreased production of proinflammatory

mediators like myeloperoxidase, nitric oxide and some interleukins, or by either inhibiting the lysosomal enzymes or stabilizing the membrane (47). Although we have not measured the levels of different anti-inflammatory cytokines or proinflammatory mediators, they may contribute in wound healing and anti-inflammatory properties of the plant gel.

The preliminary phytochemical screening of the leaf gel of *A. trigonantha* showed the presence of flavonoid, glycoside, phenols and tannins (Table 6). The wound healing and anti-inflammation effects of the gel might be due to the presence of any one or more of the above phytochemical constituents. Studies have shown that secondary metabolites like flavonoids are known to have therapeutic values such as antimicrobial, astringent, anti-inflammatory, antioxidant and wound healing effects (81). It also decreases lipid peroxidation by avoiding or slowing the onset of cell necrosis and improving vascularity. The inhibition of lipid peroxidation is believed to increase the viability of collagen (94).

According to Cheng *et al.*, the secondary constituents such as flavonoids, tannins, glycosides and other phenolic compounds have a good anti-inflammatory effects (48,95). Tannins and flavonoids are also known by their anti-inflammatory properties via inhibiting enzymes involved in inflammation, especially enzymes involved in arachidonic acid metabolic pathway, and synthesis of prostaglandins. Tannins could also affects the inflammatory response via free radical scavenging properties (43), and inhibition of inductive nitric oxide species (iNOS) in macrophages (96). Flavonoids play a role in inhibition of lipoxygenase and cyclooxygenase, an enzyme which highly involves in induction of inflammation (95), and blockage of molecules like cytokines, and matrix metalloproteinase. It has a significant activity in both proliferative and exudative phases of inflammation as well as in the inhibition of the development of the induced granuloma (96).

Phenolic compounds work in a similar way as NSAIDs do; in addition to COX some of phenolic compounds also inhibit other pro-inflammatory mediators by inhibiting their activity or gene expression. According to reports of Ambriz-Perez *et al.*, some dietary flavonoids have shown an anti-inflammatory effects by modulation of the inflammation mediators such as IL-6 (59). Pandey *et al.*, also reports that flavonoid- rich product gives a significant ($P < 0.05$) anti-inflammatory effects by inhibiting expression of inflammatory mediators like: IFN-ϒ, and TNF-α, so these evidence supports that the effects of *A. trigonantha* gel on inflammation probably

depends on its flavonoid content by inhibiting the above pro-inflammatory factors (30). Therefore, the presence of flavonoids, tannins and phenols may be responsible for the wound healing and anti-inflammatory effects of *A. trigonantha* Leaf gel.

6 Conclusion

The leaf gel of *A. trigonantha* possesses a potential wound healing and anti-inflammatory activities which rationalize the folklore therapeutic claim that the plant has been used for. Topical application of the gel ointment on excision as well as incision models revealed comparable wound healing effect to that of the standard (0.2% nitrofurazone) ointment. Oral administration of the dried gel also demonstrated dose dependent anti-inflammatory effect. The anti-inflammatory activity may be attributed for the wound healing effect of the gel. From the present results, *A. trigonantha* could be considered a future possible wound healing and anti-inflammatory agent.

7 Recommendations

Further studies should be done on the following aspects;

- Subacute and chronic toxicity of the gel of *Aloe trigonantha*
- Fractionation, isolation, identification and purification of the crude leaf gel to find the active constituents that contributed for reduction of inflammation and wound healing duration
- Establishing the mechanism/s for wound healing and anti-inflammatory activity of the leaf gel

References

1. Zhao R, Liang H, Clarke E, Jackson C, Xue M. Inflammation in chronic wounds. *International Journal of Molecular Sciences*. 2016;17(12):1–14.

2. Prasetyono TOH. General concept of wound healing , revisited. *Medical Journal of Indonesia*. 2009;18(3):208–16.

3. Cañedo-Dorantes L, Cañedo-Ayala M. Skin acute wound healing: A comprehensive review. *International Journal of Inflammation*. 2019;2(7):1-15.

4. Oguntibeju OO. Medicinal plants and their effects on diabetic wound healing. *Veterinary World*. 2019;12(5):653–63.

5. Thakur R, Jain N, Pathak R, Sandhu SS. Practices in wound healing studies of plants. *Evidence-based Complementary and Alternative Medicine*. 2011;24(2):1-17.

6. Dan MM, Sarmah P, Rao Vana D, Dattatreya A. Wound Healing: Concepts and Updates in Herbal Medicine. *International Journal of Medical Research & Health Sciences*. 2018;7(1):170–81.

7. Han G, Ceilley R. Chronic Wound Healing: A Review of Current Management and Treatments. *Advances in Therapy*. 2017;34(3):599–610.

8. Sinno H, Prakash S. Complements and the Wound Healing Cascade: An Updated Review. *Plastic Surgery International*. 2013;2013:1–7.

9. Europe wound management. Guidelines for the assessment & management of wounds. *Northamptonshire Healthcare*. 2015;1–37.

10. Namunana S, Lutoti S, Nyamaizi G, Agaba G, Apun I, Ssebunnya C, *et al*. Formulation, Development and Validation of a Wound Healing Herbal Ointment from Extracts of *Bidens pilosa* and *Aloe barbadensis. Journal of Pharmacy and Pharmacology Research*. 2018;02(02):32–8.

11. Nissinen LM, Kähäri VM. Collagen Turnover in Wound Repair - A Macrophage Connection. *Journal of Investigative Dermatology*. 2015;135(10):2350–2.

12. Mondal A, Maity TK, Bishayee A. Analgesic and Anti-Inflammatory Activities of

Quercetin-3-methoxy-4?-glucosyl-7-glucoside Isolated from Indian Medicinal Plant *Melothria heterophylla*. *Journal of Medicine*. 2019;59(6):1–11.

13. Aishwarya M.N.L, Mounika P, Mounika J, Shankar M, G. Mallikarjuna G, Niranjan Babu M NN. Anti-Inflammatory Activity of *Murraya exotica* Linn against Cotton Pellet induced Granuloma in Wistar Rats. *International Journal of Pharmaceutical Sciences Review and Research.* 2018;49(22):111–6.

14. Chen H, Pu J, Liu D, Yu W, Shao Y, Yang G, *et al*. Anti-inflammatory and antinociceptive properties of flavonoids from the fruits of black mulberry (*Morus nigra* L). *PLoS One*. 2016;11(4):1–14.

15. Serra MB, Barroso WA, Silva NN Da, Silva SDN, Borges ACR, Abreu IC, *et al*. From Inflammation to Current and Alternative Therapies Involved in Wound Healing. *International Journal of Inflammation*. 2017;6(6):1-17.

16. El-Shitany NA, Shaala LA, Abbas AT, Abdel-dayem UA, Azhar EI, Ali SS *et al*. Evaluation of the anti-inflammatory, antioxidant and immunomodulatory effects of the organic extract of the red sea marine sponge *Xestospongia testudinaria* against carrageenan induced rat paw inflammation. *PLoS One*. 2015;10(9):1–16.

17. Elzayat EM, Auda SH, Alanazi FK, Al-Agamy MH. Evaluation of wound healing activity of henna, pomegranate and myrrh herbal ointment blend. *Saudi Pharmaceutical Journal*. 2018;26(5):733–8.

18. Haillu Abdissa. Evaluation of anti-inflammatory and wound healing activities of the 80% methanol fruit extracts of *Dovyalis abyssinica* A. Rich. in mice, MSc Thesis. Addis Ababa University; 2011.

19. Lordani TVA DLC, Ferreira FBP, Monich MDST, Da Silva CM, Lordani CRF, Bueno FG TJ. Therapeutic effects of medicinal plants on cutaneous wound healing in humans: a systematic review. *Mediators of Inflammation*. 2018;2018(4):1-12.

20. Velnar T, Bailey T, Smrkolj V. The wound healing process: An overview of the cellular and molecular mechanisms. *Journal of International Medical Research*. 2009;37(5):1528–42.

21. Demidova-Rice TNTN, Hamblin MRMR, Herman I. IM. Acute and Impaired Wound Healing: Pathophysiology and Current Methods for Drug Delivery, Part 1: Normal and Chronic Wounds: Biology, Causes, and Approaches to Care. *Advance Skin and wound care.* 2012;25(8):304–14.

22. Arun M, Satish S, Anima P. Herbal boon for wounds. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2013;5(2):1-12.

23. Selvaraj Dhivya, Viswanadha Vijaya Padma ES. Wound dressings – a review. *Biomedicine (ISSN 2211-8039).* 2015;5(4):24–8.

24. Ahmed AU. An overview of inflammation: mechanism and consequences. *Front Biol*. 2011;6(4):274–281.

25. Leyikun T. Evaluation of the analgesic and anti-inflammatory activities of 80 % methanol leaf extract of *Stephania abyssinica* (Menispermaceae) in mice, MSc Thesis. Addis Ababa University; 2015.

26. Patil KR, Patil CR. Anti-inflammatory activity of bartogenic acid containing fraction of fruits of *Barringtonia racemosa* Roxb. in acute and chronic animal models of inflammation. *Journal of Traditional and Complementary Medicine.* 2017;7(1):86–93.

27. Helen Geremew. Evaluation of Analgesic and Anti -inflammatory activity of *Moringa stenopetala* Bak . (Moringaceae) in mice, MSc Thesis. Addis Ababa University; 2014.

28. Hadagali MD, Chua LS. The anti-inflammatory and wound healing properties of honey. *European Food Research and Technology*. 2014;239(6):1003–14.

29. Barnes PJ. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. *British Journal of Pharmacology*. 2006;148(3):245–54.

30. Pandey S, Cabot PJ, Shaw PN, Amitha K. Anti-inflammatory and immunomodulatory properties of *Carica papaya. Journal of Immunotoxicology.* 2016;13(4):590–602.

31. Singh Parhar N, Shah K, St John G, A Shah S. Plant-based wound healing cream for the treatment of a topical wound. *Pharmacology, Drug Development & Therapeutics*. 2016;1(1):4–5.

32. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: Molecular and cellular mechanisms. J*ournal of Investigative Dermatology*. 2007;127(3):514–25.

33. Hajhashemi V, Ghannadi A, Heidari AH. Anti-inflammatory and wound healing activities of *Aloe littoralis* in rats. *Research in Pharmaceutical Sciences.* 2012;7(5):73–8.

34. Gonzalez ACDO, Andrade ZDA, Costa TF, Medrado ARAP. Wound healing - A literature review. *Anais Brasileiros de Dermatologia.*. 2016;91(5):614–20.

35. Shah A, Amini-nik S. The Role of Phytochemicals in the Inflammatory Phase of Wound Healing. *International Journal of Molecular Sciences Review*. 2017;18(5):1-17.

36. Shedoeva A, Leavesley D, Upton Z, Fan C. Wound Healing and the Use of Medicinal Plants. *Evidence-Based Complementary and Alternative Medicine*. 2019;1(10):1-30.

37. Nguyen V, Truong C, Cao B, Nguyen Q, Vo V, Dao T, *et al*. Anti-inflammatory and wound healing activities of calophyllolide isolated from *Calophyllum inophyllum* Linn. *PLoS One*. 2017;12(10):1–16.

38. Du Y, Ren P, Wang Q, Jiang S, Zhang M, Li J, *et al*. Cannabinoid 2 receptor attenuates inflammation during skin wound healing by inhibiting M1 macrophages rather than activating M2 macrophages. *Journal of Inflammation*. 2018;15(25):1–12.

39. Porter S. The role of the fibroblast in wound contraction and healing. *Wounds UK*. 2007;3(1):33–40.

40. Rahman S, Islam R, Rana M, Spitzhorn L, Rahman MS, Adjaye J, *et al*. Characterization of burn wound healing gel prepared from human amniotic membrane and *Aloe vera* extract. *BMC Complementary and Alternative Medicine.* 2019;5(19):1–15.

41. Dons T, Soosairaj S. Evaluation of wound healing effect of herbal lotion in albino rats and its antibacterial activities. *Clinical Phytoscienc*e. 2018;4(6):1–7.

42. Samira Negahdari, Hamid Galehdari, Mahnaz Kesmati , Anahita Rezaie GS. Wound Healing Activity of Extracts and Formulations of *Aloe vera*, Henna, *Adiantum capillus-veneris*, and Myrrh on Mouse Dermal Fibroblast Cells. *International Journal of Preventive Medicine.* 2019;8(1):1-18.

43. Pawar RS, Toppo FA. Plants that heal wounds. A review. *Kerba Polonica*. 012;58(1):47–65.

44. Vergnolle N. The inflammatory response. *Drug Development Research*. 2003;59(4):375–81.

45. Trøstrup H, Bjarnsholt T, Kirketerp-Møller K, Høiby N, Moser C. What Is New in the Understanding of Non Healing Wounds Epidemiology, Pathophysiology, and Therapies. *Hindawi Publishing Corporation Ulcers*. 2013;1(4):1–8.

46. Afsar SK, Rajesh Kumar K, Venu Gopal J, Raveesha P. Assessment of anti-inflammatory activity of Artemisia vulgaris leaves by cotton pellet granuloma method in Wistar albino rats. *Journal of Pharmacy Research*. 2013;7(6):463–7.

47. Paschapur MS, Patil MB, Kumar R, Patil SR. Influence of ethanolic extract of *Borassus flabellifer* L. male flowers (inflorescences) on chemically induced acute-inflammation and poly arthritis in rats. *International Journal of PharmTech Research.* 2009;1(3):551–6.

48. Cheng J, Ma T, Liu W, Wang H, Jiang J, Wei Y, *et al*. *In vivo* evaluation of the anti-inflammatory and analgesic activities of compound Muniziqi granule in experimental animal models. *BMC Complementary and Alternative Medicine.* 2016;16(1):1–10

49. Barku VYA, Boye A, Ayaba S. Phytochemical screening and assessment of wound healing activity of the leaves of *Anogeissus leiocarpus. European Journal of Experimental Biology.* 2013;3(4):18–25

50. Adigun IA, Rahman GA, Yusuf IF, Ofoegbu CKF. The Point Prevalence and Cost of Wound Management in a Nigerian Teaching Hospital. *Journal of the Nigeria Medical Association*. 2010;51(6): 23-23.

51. Asres GS, Legese MH, Woldearegay GM. Prevalence of Multidrug Resistant Bacteria in Postoperative Wound Infections at Tikur Anbessa Specialized Hospital , Addis Ababa , Ethiopia. *iMedPub Journals*. 2017;1–9.

52. Fisha K, Azage M, Mulat G, Tamirat KS. The prevalence and root causes of surgical site infections in public versus private hospitals in Ethiopia : a retrospective observational cohort study. *Patient Safety in Surgery*. 2019;13(26):1–9.

53. Sorg H, Tilkorn DJ, Hager S, Hauser J, Mirastschijski U. Skin Wound Healing: An Update on the Current Knowledge and Concepts. *European Surgical Research*. 2017;58(1):81–94.

54. Singh S, Young A, McNaught CE. The physiology of wound healing. *Surgery (United Kingdom)*. 2017;35(9):473–7

55. Liu H, Lin S, Xiao D, Zheng X, Gu Y, Guo S. Evaluation of the wound healing potential of *Resina Draconis (Dracaena cochinchinensis)* in animal models. *Evidence-Based Complementary and Alternative Medicine.* 2013;1(4):1-10.

56. Beshir K. Evaluation of Wound Healing Activity of 70% Ethanol Leaf Extract of *Becium grandiflorum* Lam. (Lamiaceae) in Mice, MSc Thesis. Addis Ababa University; 2017.

57. Juneja K, Mishra R, Chauhan S, Gupta S, Roy P, Sircar D. Metabolite profiling and wound-healing activity of *Boerhavia diffusa* leaf extracts using *in vitro* and *in vivo* models. *Journal of Traditional and Complementary Medicine.* 2019;1(2):1-8.

58. Tiwari M. The role of serratiopeptidase in the resolution of inflammation. *Asian Journal of Pharmaceutical Sciences.* 2017;12(3):209–15.

59. Ambriz-pérez DL, Leyva-lópez N, Gutierrez-grijalva EP, Heredia JB. Phenolic compounds : Natural alternative in inflammation treatment (A Review). *Cogent Food & Agriculture*. 2016;41(1):1–14.

60. Al-Saeed A. Gastrointestinal and cardiovascular risk of nonsteroidal anti-inflammatory drugs. *Oman Medical Journal*. 2011;26(6):385–91.

61. Laut M, Ndaong NA, Utami T. Cutaneous wound healing activity of herbal ointment containing the leaf extract of *Acalypha indica* L. on mice. *Journal of Physics: Conference Series.* 2019;1146(1):1-6.

62. Nagesh H, Basavanna P, Kishore M. Evaluation of wound healing activity of ethanolic extract of *Azadirachta indica* leaves on incision and excision wound models in Wister albino rats. *International Journal of Basic and Clinical Pharmacology*. 2015;4(6):1178–82.

63. Demilew W, Adinew GM, Asrade S. Evaluation of the Wound Healing Activity of the Crude Extract of Leaves of *Acanthus polystachyus* Delile (Acanthaceae). *Evidence-based Complementary and Alternative Medicine.* 2018;1(5)8:1-9.

64. Moriyama M, Moriyama H, Uda J, Kubo H, Nakajima Y. Beneficial Effects of the Genus Aloe on Wound Healing , Cell Proliferation , and Differentiation of Epidermal Keratinocytes. *PLoS One.* 2016;11(10):1–15.

65. Quave CL. Wound Healing with Botanicals: a Review and Future Perspectives. *Current Dermatology Reports.* 2018;7(4):287–95.

66. Akev N, Can A, Sutlupinar N, Candoken E, Ozsoy N, Ozden, TY, Yanardag R UE. Twenty years of research on *Aloe vera. Journal of Pharmacy of Istanbul University.* 2015;45(2):191–215.

67. Firdous SM, Sautya D. Medicinal plants with wound healing potential. Vol. 13, *Bangladesh Journal of Pharmacology*. 2018; 13(1):41-52.

68. Megersa M. Antimalarial and Antimicrobial Activities of Anthrones and Chromone from the Leaf Latex of *Aloe trigonantha*, MSc Thesis. Addis Ababa University; 2013.

69. Demissew S, Nordal I. *Aloes* and Lilies of Ethiopia and Eritrea. 2nd ed. Addis Ababa, Ethiopia.: Shama Books; 2010. 1–351 p.

70. Alemayehu Mekuria. Chromosome study on seven endemic *Aloe* species (ALOACEAE) of Ethiopia, MSc Thesis. Addis Ababa University; 2007.

71. Megeressa M, Bisrat D, Mazumder A, Asres K. Structural elucidation of some antimicrobial constituents from the leaf latex of *Aloe trigonantha* L.C. Leach. *BMC Complementary and Alternative Medicine*. 2015;15(1):1–7.

72. Lodhi S, Jain AP, Rai G, Yadav AK. Preliminary investigation for wound healing and anti-inflammatory effects of *Bambusa vulgaris* leaves in rats. *Journal of Ayurveda and Integrative Medicine.* 2016;7(1):14–22

73. Kumer A, Hashem AK, Biswas K, Aa SS. Antithrombotic and anti-inflammatory activities of leaf methanolic extract of *Euphorbia hirta* Lin. *International Journal of*

Complementary & Alternative Medicine. 2019;12(4):154–62.

74. Garber JC, Barbee RW, Bielitzki JT, Clayton LA, Donovan JC, Hendriksen CFM, *et al*. National Institute of Health: Guide for The Care and Use of Laboratory Animals. 2010. 1–218 p.

75. Farzadinia P, Khatamsaz S, Movahed A, Mohammadi M. Anti-inflammatory and Wound Healing Activities of *Aloe vera* , Honey and Milk Ointment on Second-Degree Burns in Rats. *The International Journal of Lower Extremity Wounds*. 2016;1(7):1–7.

76. British Pharmacopoeia 2009. Monographs for medicinal substances and formulated preparations. London: The Stationery Office; 2009.

77. Gebremeskel L, Bhoumik D, Sibhat GG, Tuem KB. *In vivo* wound healing and anti-inflammatory activities of leaf latex of *Aloe megalacantha* baker (Xanthorrhoeaceae). *Evidence-based Complementary and Alternative Medicine.* 2018;2018(7):1-7.

78. Allen L V, Popovich NG, Ansel HC. Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems ninth edition. 1985.

79. OECD/OCDE. Acute Oral Toxicity – Up-and-Down-Procedure (UDP). Oecd Guidel Test Chem. 2008;425(October):1–27.

80. OECD 404. Guidelines for the Testing of Chemicals. Acute Dermal Irritation/Corrosion. OECD Guidel Test Chem Sect 4 Heal Eff. 2015;(July 28th):1–8.

81. Kokane DD, More RY, Kale MB, Nehete MN, Mehendale PC, Gadgoli CH. Evaluation of wound healing activity of root of*Mimosa pudica. Journal of Ethnopharmacology.* 2009;124(2):311–5.

82. M. Subalakshmi, A. Saranya, M. Uma Maheswari, A. Jarina, S. Kavimani RM. Aa overview of the current methodologies used for the evaluation of drugs having wound healing activity. *International Journal of Experimental Pharmacology.* 2014;4(2):127–31.

83. Mulisa E, Asres K, Engidawork E. Evaluation of wound healing and anti- inflammatory activity of the rhizomes of *Rumex abyssinicus* J . (Polygonaceae) in mice. *BMC Complementary and Alternative Medicine.* 2015;15:1–10.

84. Mengi SA, Bakshi VJ. Evaluation of the aqueous extract of *Rotula aquatica* roots in acute and chronic inflammatory conditions in rats. *Pharmaceutical Biology*. 2009;47(6):491–5.

85. Banu KS, Cathrine L. General Techniques Involved in Phytochemical Analysis. *International Journal of Advanced Research in Chemical Science*. 2015;2(4):25–32.

86. Kamal T, Muzammil A, Akintunde R, Rahma MS, Omar MN. Preliminary phytochemical screening test of *Garcinia griffithii* Plant. *Innova Ciencia.* 2012;4(4): 68-74.

87. Jaradat N, Hussen F, Ali A Al. Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata* decne. *Journal of Materials and Environmental Science.* 2015;6(6):1771–8.

88. ISABELA K.R. AGRA, LUANA L.S. PIRES PSMC, EURÍPEDES A. SILVA-FILHO SS and EB. Evaluation of wound healing and antimicrobial properties of aqueous extract from *Bowdichia virgilioides* stem barks in mice. *Annals of the Brazilian Academy of Sciences.* 2013;85(3):945–54.

89. Li C, Wu X, Zhao X, Su Z, Chen H, Wang X, *et al*. Anti-Inflammatory Property of the Ethanol Extract of the Root and Rhizome of *Pogostemon cablin* (Blanco) Benth. *The Scientific World Journal.* 2013;1(10):1-12.

90. Tank Chintankumar J, Borkhataria Chetan H, Baria Ashok H, Patel Rakesh P, Tamizharasi S, Sureja DK, *et al*. Formulation and evaluation of aceclofenac loaded maltodextrin based proniosome. *International Journal of ChemTech Research*. 2009;1(3):567–73.

91. Bagad AS, Joseph JA, Bhaskaran N AA. Comparative Evaluation of Anti-Inflammatory Activity of Curcuminoids , Turmerones , and Aqueous Extract of *Curcuma longa*. *Advances in Pharmacological Sciences.* 2013;1(11):1-7.

92. Hosseinzadeh H, Haddadkhodaparast MH, Arash AR. Antinociceptive , Antiinflammatory and Acute toxicity Effects of *Salvia leriifolia* Benth. *Phytotherapy Research.* 2003;425:422–5.

93. Owoyele B V., Nafiu AB, Oyewole IA, Oyewole LA, Soladoye AO. Studies on the analgesic, anti-inflammatory and antipyretic effects of *Parquetina nigrescens* leaf extract.

Journal of Ethnopharmacology. 2009;122(1):86–90.

94. Ambiga S, Narayanan R, Gowri D, Sukumar D, Madhavan S. Evaluation of wound healing activity of flavonoids from *Ipomoea carnea* Jacq. *Ancient science of life.* 2007;26(3):45–51.

95. Lotanna AD, Emeka IE, Jegbefume OM, Onyeka OC, Adaobi OM, Obiora IS. Mechanisms of anti-inflammatory activity of the leaf extract and fractions of *Millettia aboensis. International Research Journal of Pharmacy.* 2013;4(9):54–9.

96. Alemu A, Tamiru W, Nedi T, Shibeshi W. Analgesic and Anti-Inflammatory Effects of 80 % Methanol Extract of *Leonotis ocymifolia* (Burm . f .) Iwarsson Leaves in Rodent Models. *Evidence-Based Complementary and Alternative Medicine*. 2018;1(11):1-8.