



**Anti-inflammatory, anti-oxidant and wound-healing  
properties of selected South African medicinal plants**

**Submitted in fulfillment for the Degree of Master of Applied Sciences in  
Biotechnology in the Department of Biotechnology and Food Technology,  
Durban University of Technology, Durban, South Africa**

**Nonkululeko Betty Mzindle**

**SUPERVISOR:**

**PROFESSOR B. ODHAV**

## REFERENCE DECLARATION

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I, Miss NB Mzindle– 21346190 and Prof Bharti Odhav do hereby declare that in respect of the following dissertation: **Anti-inflammatory, anti-oxidant and wound-healing properties of selected South African medicinal plants**

1. As far as we ascertain:
  - a) no other similar dissertation exists;
  
2. All references as detailed in the dissertation are complete in terms of all personal communication engaged in and published works consulted.

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Date

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Signature of supervisor

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Date

## AUTHOR'S DECLARATION

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This study presents original work by the author. It has not been submitted in any form to another academic institution. Where use was made of the work of others, it has been duly acknowledged in the text. The research described in this dissertation was carried out in the Department of Biotechnology and Food Technology, Faculty of Applied Sciences, Durban University of Technology, South Africa, under the supervision of **Professor Bharti Odhav**.

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**Student's signature**

## DEDICATION

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This work is entirely dedicated to my daughter **Anelisa Mzindle** for being my pillar of strength, for making me laugh everyday when I get home, for being my motivator and for loving me.

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## ABBREVIATIONS

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<b>AIDS</b>	: Acquired Immune Deficiency Syndrome
<b>CaCl<sub>2</sub>·2H<sub>2</sub>O</b>	: Calcium chloride dihydrate
<b>COX</b>	: Cyclooxygenase
<b>DMEM</b>	: Dulbecco's Modified Eagle Medium
<b>DMSO</b>	: Dimethyl sulfoxide
<b>DNA</b>	: Deoxyribonucleic acid
<b>DPPH</b>	: 1, 1-diphenyl-2-picrylhydrazyl
<b>EDTA</b>	: Ethylene diamine tetra acetic acid
<b>ELISA</b>	: Enzyme-linked immunosorbent assay
<b>ENA</b>	: Extract not available
<b>FBS</b>	: Fetal bovine serum
<b>IGF</b>	: Insulin-Growth Factor
<b>IKB</b>	: Inhibitor kB kinase
<b>IL</b>	: Interleukin
<b>KCL</b>	: Potassium chloride
<b>LOX</b>	: Lipoxygenase
<b>MgCl<sub>2</sub>·6H<sub>2</sub>O</b>	: Magnesium chloride hexahydrate
<b>MTT</b>	: 3-(4, 5-dimethylthiazolyl)-2, 5-diphenyltetrazolium bromide
<b>NaCl</b>	: Sodium chloride
<b>NADPH</b>	: Nicotinamide adenine dinucleotide
<b>Na<sub>2</sub>SO<sub>4</sub></b>	: Sodium sulphate
<b>NDGA</b>	: Nordihydroguaiaretic acid
<b>NFKB</b>	: Nuclear factor kappa beta
<b>NO</b>	: Nitric oxide
<b>NSAIDS</b>	: Non-steroidal anti-inflammatory drugs
<b>PAF</b>	: Platelet activation factor
<b>PBS</b>	: Phosphate buffered saline
<b>PDGF</b>	: Platelet derived growth factor
<b>PGD<sub>2</sub></b>	: Prostaglandin D <sub>2</sub>

<b>PGE2</b>	: Prostaglandin E2
<b>PGF2<math>\alpha</math></b>	: Prostaglandin F2 alpha
<b>PGs</b>	: Prostaglandins
<b>ROS</b>	: Reactive oxygen species
<b>SD</b>	: Standard deviation
<b>TGF-<math>\beta</math></b>	: Tumor growth factor beta
<b>TNF-<math>\alpha</math></b>	: Tumor necrosis factor alpha

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## ABSTRACT

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South Africa has a wide range of medicinal plants that are used traditionally by black Zulu South Africans for the treatment of a range of illnesses, including inflammatory ailments; disease conditions caused by oxidative stress and wound healing. It has been indicated that bioactive compounds isolated from plants contribute to their anti-inflammatory, antioxidant and wound healing properties; hence, herbal remedies have been widely used traditionally in many countries in the management and treatment of wounds.

Inflammation is the main condition that relates to a variety of diseases affecting most of the world's population. It is the body's immune response to infection and injury and is induced by the release of pro-inflammatory mediators —prostaglandins and leukotrienes—following wound occurrence. Wounds result in disruption of living tissue caused by oxidative stress.

Anti-inflammatory agents, antioxidants, and antimicrobials play an important role in the wound healing process and they prevent aggravated wound conditions. Controlling inflammation during wound repair is important to minimize any additional complications that may result; hence, chemical agents such as non-steroidal anti-inflammatory drugs (NSAIDs), synthetic antioxidants and steroids are frequently used. These drugs block the enzymes that are responsible for prostaglandin synthesis in inflammation, react with free radicals thereby interfering with oxidation process as a result affect one or more phases of wound healing. The use of these drugs, however, has been limited as they can cause detrimental side effects when used over long periods of time. There is, consequently, a need to find alternative natural therapeutic drugs. Studies on medicinal plants confirmed that herbal drugs exhibit fewer side effects in comparison with chemical agents and are more cost-effective. Thus the aim of this study was to investigate South African medicinal plants, for anti-inflammatory, antioxidant and wound healing properties.

Dissolved extracts of thirty-eight medicinal plants were evaluated for their anti-inflammatory activity using the 5-lipoxygenase assay as well as free radical scavenging activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Their safety was evaluated using brine shrimp lethality assay. Proliferation and viability of fibroblast cells was determined by the 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay furthermore a scratch wound assay was used to study the properties of wound healing *in vitro* and to confirm the

anti-inflammatory activities of the dissolved extracts. Migration rate was evaluated quantitatively by an image analyzer. Methanol was chosen for extraction because it completely dissolves extracts. Anova was used for statistical analysis.

Almost all aqueous extracts were found to be effective in inhibiting lipoxygenase enzyme when compared to nordihydroguaiaretic acid (NDGA). Aqueous extracts exhibited remarkably high percentage inhibition of lipoxygenase with most above 100% when compared to methanolic extracts. *Amaranthus dubius* and *Portulaca oleracea* were found to have good biological activities in the inhibition of 5-lipoxygenase enzymes when compared to the other plants. However, *Galinsoga parviflora* and *Syzygium cordatum* were least effective in inhibiting enzyme activity with percentages as low as -2% and 34% respectively. Percentage inhibitions for methanolic extracts were lower than that of aqueous extracts. *Amaranthus spinosus* had the highest percentage inhibition among all the methanolic extracts and *Galinsoga parviflora* had the lowest. The methanolic plant extracts were found to be more effective in scavenging DPPH free radicals than the corresponding aqueous extracts. All the methanolic extracts exhibited free radical scavenging ability in the range of 60%–104%. *Asystasia gangetica*, *Ficus sur*, *Heteropyxis natalensis*, *Hibiscus sabdariffa*, *Pelargonium* sp. showed notably higher scavenging abilities, ranging from 101%–104% compared to Rutin. Methanolic extracts of *Heteropyxis natalensis* and *Hibiscus sabdariffa* exhibited scavenging ability even at the lowest concentration of 10µg/ml. Furthermore, aqueous extracts displayed remarkably lower activities than methanolic extracts with thirty-one extracts having a scavenging capacity ranging from 22%–59%.

None of the extracts were found to be detrimental to brine shrimp. Almost all the extracts were shown to stimulate the growth of fibroblast cells except the methanolic extract of *Solanum nodiflorum*, which was shown to be killing the cells at high concentrations with a percentage viability of 46%. As the concentration decreased, however, the viability of cells with this extract increased to 143%. An increase in the number of fibroblast cells was observed in the scratched area of the treated cells and a significant migration rate was also noted with some of the extracts. Aqueous extracts of *Sonchus oleraceus* (86%), *Justicia flava* (85%) and *Dichrostachys cinerea* (85%) and methanolic extracts of *Senna occidentalis* and *Hibiscus sabdariffa* were found to have the highest migration rate compared to untreated

cells that served as a control. No cell migration was observed with methanolic extract of *Solanum nodiflorum*. Instead, the extract was found to be toxic to the cells.

Some of the plants evaluated in this study have been studied for either anti-inflammatory, antioxidant and wound healing properties *in vivo*, however, no work has been conducted to demonstrate a correlation between anti-inflammatory, antioxidant and wound healing properties of plant species *in vitro*. The current study was, therefore, conducted to review medicinal herbs considered as anti-inflammatory, antioxidants and wound healing agents as well as collecting evidence for their effectiveness and pharmacological mechanisms in modern science. In the plant species investigated *Amaranthus dubius*, *Asystasia gangetica*, *Bidens pilosa*, *Buddleja saligna*, *Carpobrotus dimidiatus*, *Chenopodium album*, *Dichrostachys cinerea*, *Emex australis*, *Ficus sur*, *Guilleminea densa*, *Hibiscus sabdariffa*, *Physalis viscosa*, *Syzygium cordatum*, *Taraxacum officinale* and *Tulbaghia violacea* demonstrated good anti-inflammatory and wound healing properties. In conclusion the results from this study demonstrated promising anti-inflammatory and antioxidant activities as well as wound healing properties, furthermore it was also shown that the plant extracts were not toxic to the cells hence this suggested that the plants investigated, can be used as substitutes or to formulate wound healing agents that are safe to use in primary healthcare.

# 1. INTRODUCTION

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South Africa has a wide range of medicinal plants that have been and are still being used traditionally by people to treat a variety of diseases. About 80% of black South Africans still rely on using plants for medicinal purposes due to the fact that they are cheap, safe, easily accessible, culturally acceptable and better tolerated by patients (Shaikh, 2011). Statistics show that about 25% of the world's plants are found in South Africa and about 3000 medicinal plants are often used for traditional medicine in South Africa with 2942 used for human administration (Van Wyk, 2008). Most Zulu medicinal plants are found in KwaZulu-Natal making it the main province in South Africa where healing using traditional Zulu medicine is practiced (Jager and Van Staden, 2005); Lin *et al.*, 1999). Most of these plants are collected from the wild and some are found in areas that are too dry where weedy species are reported to be more predominant (Lewu and Afolayan, 2009).

Medicinal plants have demonstrated potent healing properties and can be used to treat a variety of conditions such as swelling, burns, and wounds as well as diseases such as malaria. It's for these reasons that medicinal plants have primarily been used by traditional healers over the years to treat health issues and has subsequently attracted scientists to study the active constituents in the plants that possess these healing properties ultimately leading to the development of novel drugs. It is through these studies, that scientists have identified the key mechanisms by which these medicinal plants are able to promote healing, inhibit oxidation process and either inhibit or affect enzymes or processes or pathways responsible for the development of various ailments (Lin *et al.*, 1999).

Inflammation is caused when a body is attacked by hostile agents such as pathogenic microorganisms, chemical substances and parasites or the body is exposed to tissue damage (Agnihotri *et al.*, 2010). Heat, redness, swelling and pain are some of the clinical symptoms of inflammation (Anilkumar, 2010). When a cell is injured inflammatory mediators such as cytokines, tumor necrosis factor, interleukin-1 from leukocytes, monocytes and macrophages are released as a response to pain, thereby alerting the body of any vascular alterations in the area of injury. When this happens the production of cyclooxygenase (COX) and 5-lipoxygenase (5-LO) is increased and prostaglandins and leukotrienes are synthesized (Anilkumar, 2010; Iwalewa *et al.*, 2007). This process can lead

to inflammatory-related diseases such as rheumatic fever, rheumatoid arthritis, ankylosing spondylitis, polyarthritis nodosa, systemic lupus erythematosus and osteoarthritis all of which require treatment (Anilkumar, 2010). There are agents that are used to control pain and inflammatory disorders by blocking the enzymes COX that is needed for prostaglandin synthesis. There are three groups of anti-inflammatory agents that have been used to treat inflammatory-related disorders. These include non-steroidal anti-inflammatory drugs (NSAIDs) (i.e. aspirin), indomethacin and glucosaticoid drugs (Okem, 2011). These drugs, however, have shown to cause adverse side effects such as water and salt retention when used over long periods of time, , making them unsafe to use as inflammatory agents. There is, therefore, a greater need for the development of effective and non-toxic anti-inflammatory drugs (Fawole *et al.*, 2009).

In addition during the inflammatory process, excessive production of reactive oxygen species (ROS) may be seen which is also produced as part of the body's defence system, they are produced during attack to eliminate invading microorganisms. These free radicals however may have damaging effects to the tissues if not removed or controlled, hence antioxidants play a major role in preventing degradation of essential cellular components caused by oxidation (Amponsah et al, 2013). Antioxidants are therefore molecules that are capable of slowing down or preventing oxidation of other molecules; they scavenge free radicals and inhibit lipid peroxidation thereby preventing reactions that result in cell damage to commence (Partap and Pandey, 2012). Studies have shown that many plants possess secondary metabolites that are able to scavenge free radicals thereby preventing various disease conditions including inflammatory diseases. Whole grain, fruits and vegetables are some of the primary sources of naturally occurring antioxidants that are currently used worldwide (Kumaraswamy and Satish, 2008), they are safe and cost-effective. Hence, as research of using plants as natural antioxidants increases, it is important to study plants for their antioxidant property to understand their mode of action and help provide information of how they can aid in prevention or treatment of a variety of diseases (Molan et al, 2012).

Wound healing is a cellular response to injury that results in the reduction and closure of the wound. It is a complex process involving cellular and biochemical interactions that leads to the functional barrier being restored. There are various phases involved in the

process of wound healing, namely inflammation, proliferation and migration of different cell types such as keratinocytes, fibroblasts, and endothelial cells. The proliferation of fibroblast cells is very important in tissue repair since these cells are involved in migration, contraction and collagen production (Balekar *et al.*, 2012). On this basis, an extract that stimulates the growth of fibroblast can be useful in helping wounds to heal. Pain, reddening, and edema are some of the symptoms that are observed in the surrounding tissue when wounding occurs. These are also symptoms of inflammation caused by the release of prostaglandins and leukotrienes (Houghton *et al.*, 2005). Synthetic steroids are chemical substances used to treat wounds. They also work in combination with anti-inflammatory drugs. These chemical substances, however, have been found to have detrimental side effects such as decreasing the production of tumor growth factor beta (TGF- $\beta$ ) and Insulin-Growth Factor-1 (IGF-1) as well as decreasing disposition of collagen that is essential during the wound healing process, hence, the need to find alternative therapeutic drugs (Juniarti *et al.*, 2012). When it comes to management and treatment of wounds, herbal remedies have been widely used traditionally in many countries (Balekar *et al.*, 2013). Multiple mechanisms of the natural agents are involved in inducing healing and regeneration of the damaged tissue. The belief is that these natural agents affect one or more phases during the wound healing process (Demirci *et al.*, 2014). They prevent fresh wounds from bleeding, inhibit microbial growth and, consequently, speed up wound healing (Barku *et al.*, 2013).

Many plants contain well known biologically active compounds such as alkaloids, curcumin, phenols, tannins, glycosides, terpenoids, saponins, flavonoids, and steroids. These secondary metabolites are able to inhibit cyclooxygenase (COX) and lipoxygenase (LOX) enzymes involved during inflammatory phase which thereby reduces (Fawole *et al.*, 2009). They have also been found to affect one or more phases of the wound healing process (Demirci *et al.*, 2014). From studies performed so far, it has been noted that flavonoids are the major inflammatory agents found in plants. Under various conditions, these flavonoids have been shown to inhibit secretion of pro-inflammatory cytokines such as interleukin-6 (IL-6), increase interleukin-10 (IL-10) secretion (Thring *et al.*, 2011) and some act as phospholipase inhibitors, which inhibit cyclooxygenase and lipoxygenase pathways (Agnihotri *et al.*, 2010). As inflammation is one of the stages of the

wound healing process, using plants that have an ability to reduce inflammation should be able to promote wound healing.

The aim of this study was to investigate local medicinal plants for anti-inflammatory properties and to evaluate their wound healing potential *in vitro* using mouse fibroblast cells.

The objectives of the study were:

1. To screen aqueous and methanolic extracts for anti-inflammatory activity using lipoxygenase inhibitor screening assay kit
2. To screen aqueous and methanolic extracts for antioxidant activity by using the DPPH radical scavenging assay
3. To evaluate safety and toxicity of the plant extracts by using the brine shrimp lethality assay and cytotoxicity MTT assay
4. To evaluate the *in vitro* wound-healing property of the plant extracts using a scratch wound assay and healing time in fibroblast cells
5. To correlate the wound closure time to the anti-inflammatory activity of the plants

The plants used here were selected based on the fact that they are used traditionally by South African people in KwaZulu-Natal for medicinal purposes. Their ability to heal wounds was based on their antioxidant activity as scientific research has shown that plants with antioxidant activity prevent tissue damage, thereby which facilitating the wound healing process (Barku *et al.*, 2013).

## 2. LITERATURE REVIEW

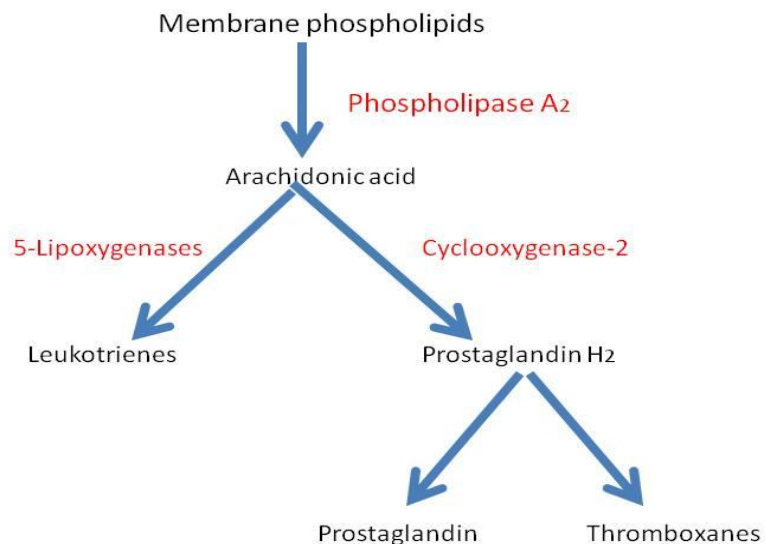
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### 2.1 Inflammation Overview

Inflammation is a reaction of cells or tissues to injury, infections, chemical irritants or allergies usually characterized by heat, swelling, redness and loss of function. It is an indication that the body is under attack by a disease or disorder and, therefore, the body prepares itself to repair the damage caused to the cell or tissue. Inflammation occurs when chemical mediators (prostaglandins, nitric oxide, leukotrienes, vasoactive amines, and cytokines) are released by cells such as mast cells, platelets, neutrophils and monocytes/macrophages due to an injury, infection, chemical irritation or allergies. These mediators are called pro-inflammatory signaling molecules and they determine the severity of inflammation depending on the duration of injury. Once released, these mediators target specific cell receptors and, thereby, increase vascular permeability, promote neutrophil chemotaxis, increase direct enzymatic activity, which leads to induced pain and stimulates muscle contraction (Iwalewa *et al.*, 2007).

Inflammation can occur in three phases, acute transient phase, delayed subacute phase and chronic proliferate phase. Vascular permeability, due to the inflammatory exudate in the first phase (i.e. acute transient phase) leads to edema. This is followed by migration of the leukocytes and phagocytes from blood to vascular tissue, which results in the killing of bacteria, viruses, or parasites and leads to wound repair (i.e. delayed subacute phase). In the third phase, fibrosis occurs after the tissue is degraded (Anilkumar, 2010) and, if inflammation persists, it may promote the development of diseases such as rheumatoid arthritis, heart disease, Alzheimer's, asthma, Acquired Immune Deficiency Syndrome(AIDS), cancer, diabetes and many more inflammatory-related disorders. This is due to the overexpression of proinflammatorychemical mediators. Migration of leukocytes, nitric oxide (NO), Arachidonic acid metabolism, reactive oxygen species (ROS), nuclear factor kappa B( NF-Kappa B) and pro-inflammatory cytokines are the main causes of inflammation (Iwalewa *et al.*, 2007). Many plant chemicals inhibit NFKB, NO, COX and ROS generation, and they also inhibit enzyme-tyrosine, which prevents the entry of microorganisms and they have immunoprotective/immunomodulatory properties.

Cyclooxygenase enzymes are involved in the synthesis of prostaglandins and leukotrienes, the end products of arachidonic acid metabolism (Figure 1). When cell injury occurs, phospholipids are liberated and arachidonic acid is formed, catalyzed by the enzyme phospholipase A<sub>2</sub>. Arachidonic acid is then converted to leukotrienes and prostaglandins under the action of cyclooxygenases and lipoxygenases, resulting in inflammation (Frum, 2006).



**Figure 1: Formation of leukotriene and prostaglandin pro-inflammatory mediators by the inflammatory cells released following an injury (Frum, 2006)**

As outlined in Figure 1, the enzyme phospholipase A<sub>2</sub> is directly responsible for the formation of prostaglandins and leukotrienes, the inflammatory mediators, via the formation of arachidonic acid. Polymorphonuclear leucocytes are, subsequently, attracted to the site of inflammation which leads to tissue damage most likely as free radicals are released as a response to inflammation. Arachidonic acid is highly reactive and is rapidly metabolized by cyclooxygenase to prostaglandins, which are major components that induce pain and inflammation (Kumar *et al.*, 2011). PGE<sub>2</sub>, PGD<sub>2</sub>, and PGF<sub>2</sub>α are the various types of prostaglandins that exist in our bodies. They are responsible for a number of physiological functions such as pain and temperature control, smooth muscle relaxation, vasodilation, cytoprotection and platelet aggregation (Frum, 2006).

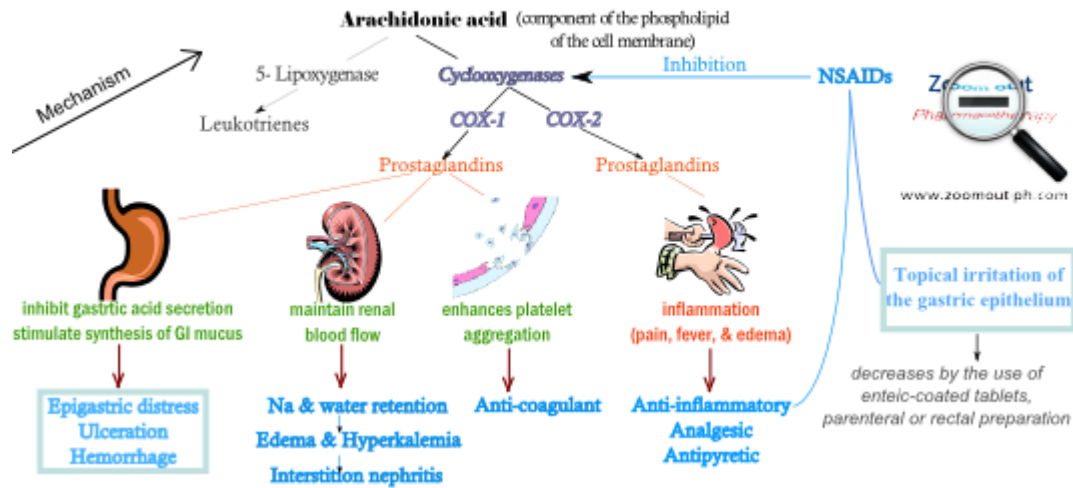
Some medicinal plants have been shown to inhibit the actions of prostaglandins. These plants contain flavonoids, which have been shown to inhibit the enzymes responsible for the production of leukotrienes and prostaglandins, therefore, inhibiting these pro-inflammatory mediators (Frum, 2006). Anti-inflammatory activity of any given compound can be evaluated using a cyclooxygenase assay or 5-lipoxygenase assay where cyclooxygenase 1 or 2 and 5-lipoxygenase enzymes are inhibited by a chemical compound extracted from a plant material (Fawole *et al.*, 2009).

### **2.1.1 Causes and Treatment**

Inflammation is a consequence of the body's response to injury caused by trauma, ultraviolet, burns, excessive cooling, corrosive chemicals, microbial infections or allergic reactions. Microbial infections include bacteria, viruses, protozoa, fungi and parasites (Iwalewa *et al.*, 2007). The death of tissues due to lack of oxygen or nutrients may also be the cause of inflammation. Apart from injury caused to cells other factors may contribute to an inflammatory response. These include improperly healed joints, muscles and broken bones. Also, if the immune system is compromised, due to chronic health conditions, inflammation may result. Another factor is not balancing the Omega 3 and 6 fatty acids, required for the prevention of a disease (Amatangelo, 2007).

Non-steroidal anti-inflammatory drugs are used by almost the entire population around the world to fight against inflammatory-related conditions. These drugs suppress the release of chemical mediators, such as prostaglandins, associated with stimulating an inflammatory response. They achieve this by inhibiting or blocking the enzymes—cyclooxygenases and lipoxygenases—responsible for their production (see figure 2). In spite of this, the majority of these drugs result in various side effects. One particular anti-inflammatory drug corticosteroids result in adverse side effects, including suppressing the immune system and preventing the body from fighting against pathogenic microorganisms, all of which leads to impaired healing (Wassung, 2012).

## Non-steroidal Anti-inflammatory Drugs - Mechanism of Action



**Figure 2: Inhibition of cyclooxygenases and lipoxygenases required for the conversion of Arachidonic acid to prostaglandins responsible for inflammation by non-steroidal anti-inflammatory drugs**

<http://www.zoomout-ph.com/2012/02/rheumatoid-arthritis-concept-map.html>

### 2.1.2 Inflammatory cells and mediators involved in tissue repair

Platelets, neutrophils, macrophages, monocytes, mast cells and basophils are cells of the immune system that are involved in inflammatory disorders and tissue repair. Wound healing involves a number of tissues and cells working together. The manner in which the wound heals depends on the amount of infiltrating leukocytes in the wound area (Eming *et al.*, 2007).

#### 2.1.2.1 Platelets

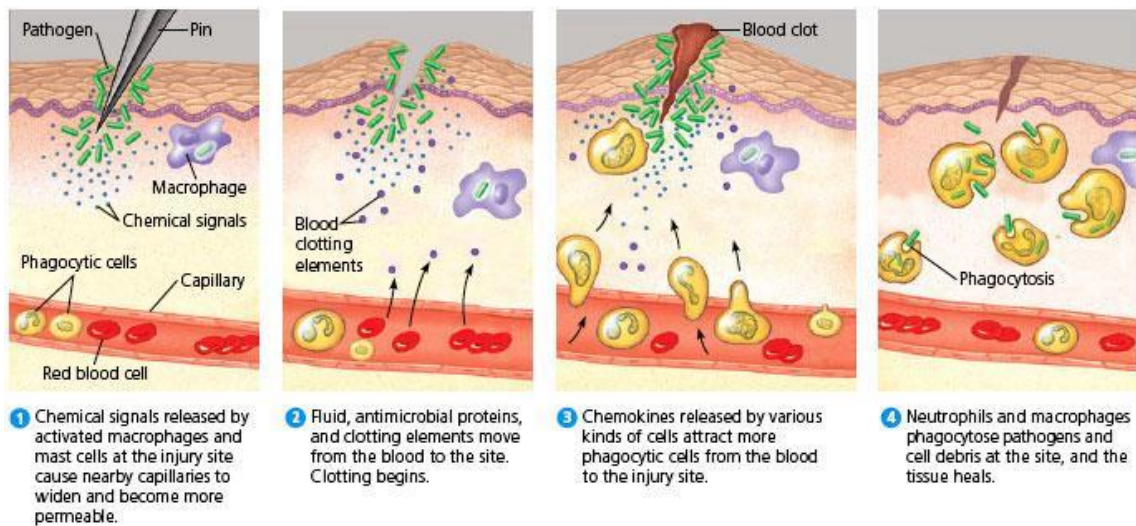
Platelets are implicated as inflammatory cellular elements because they release pro-inflammatory mediators which are thromboxane A<sub>2</sub>, serotonin, tumor growth factor beta (TGF- $\beta$ ), platelet-derived growth factor (PDGF) and lipo-oxygenase (LOX). Due to the activity of phospholipase A<sub>2</sub> and acetyltransferase in mast cells, basophils, eosinophils and endothelial cells, pro-inflammatory mediator known as platelet activation factor (PAF) results from membrane phospholipids (Iwalewa *et al.*, 2007). During wound healing platelets release factors that increase aggregation response and instruct inflammatory cells to initiate coagulation cascade (Eming *et al.*, 2007).

### 2.1.2.2 Neutrophils and Macrophages

Within few hours of injury, pro-inflammatory cytokines activate neutrophils to migrate across the endothelial cell wall of blood capillaries at the wound site leading to the formation of different classes of adhesion molecules required for attachment of leukocytes. Mostly chemokines and their receptors are usually the important mediators for neutrophil recruitment during tissue repair (Eming *et al.*, 2007). Neutrophils and other phagocytes release oxygen free radicals and non-radical reactive oxygen intermediates that have been implicated in inflammatory disorders. This may be accomplished by interference with NADPH oxidase, which is a powerful oxidant-producing enzyme localized on the surface membrane of neutrophils (Iwalewa *et al.*, 2007). Neutrophils are usually the first cells deployed to the sites of the tissue injury to deal with the causes of inflammation. They release antimicrobial substances and proteases to destroy pathogens and cell debris by phagocytosis (Okem, 2011) but if the wound is not contaminated, migration of neutrophils stops (Reddy *et al.*, 2012).

Macrophages are found at the wound site after two days of injury. They work in the late inflammatory phase and are responsible for changing from wound inflammation to wound repair. Most of them are recruited from the blood. Chemotactic factors including the growth factors, pro-inflammatory cytokines, and chemokines macrophage inflammatory proteins control the penetration of macrophages at the wound site, therefore enabling these cells to successfully perform their role in wound healing (Eming *et al.*, 2007) by stimulating migration and proliferation of cells to form a tissue matrix.

They also digest and kill pathogens and destroy the remaining neutrophils (Reddy *et al.*, 2012) as outlined in figure 3.



**Figure 3: Neutrophils and macrophages involved in first line of defense, they ingest pathogens and cell debris at the wound site**

<https://www.studyblue.com/notes/n/peri-inflammatory-response/deck/5830391>

### 2.1.2.3 Mast Cells and Basophils

Mast cells play an important role in acute and chronic inflammatory diseases such as asthma, inflammatory bowel disease and rheumatoid arthritis (Iwalewa *et al.*, 2007). They are responsible for recruiting neutrophils and monocytes to the wound area during the haemostatic phase (Tsala *et al.*, 2013). Inflammation and vascular changes during tissue repair are promoted by the pro-inflammatory mediators and cytokines found within the mast cells. They also participate in sterile inflammatory conditions exacerbated by stress, such as atopic dermatitis, interstitial, cystitis etc. After an injury visibility of mast cells may be unclear due to degranulation however after 48 hours they return to normal and their amount increases as tissue repair proceeds (Eming *et al.*, 2007). Basophils and mast cells produce histamine a chemical that causes many symptoms of allergies (Iwalewa *et al.*, 2007).

### 2.1.2.4 Cytokines

The expression of many proteins including all pro-inflammatory cytokines and enzymes of the arachidonic acid cascade are regulated by the transcription factor —nuclear factor kappa beta (NF $\kappa$ B). This is a complex factor that binds to deoxyribonucleic acid(DNA) and activates gene transcription. In normal cells, this factor inhibits protein I kappa B (I $\kappa$ B). A number of inflammatory stimuli activate NF $\kappa$ B which is then translocated into the nucleus and binds to DNA and activates pro-inflammatory cytokines, chemokines, tumor necrosis

factor alpha (TNF $\alpha$ ), interleukin 1(IL1), phospholipaseA<sub>2</sub>, lipoxygenase, cyclooxygenase 2(COX-2). Asthma and other inflammatory diseases are due to the abnormal activation of the NF $\kappa$ B pathway (Okem, 2011). Lymphocytes produce interferon gamma (IFN- $\gamma$ ) which inhibits migration of fibroblasts and synthesis of collagen (Tsala *et al.*, 2013).

#### **2.1.2.5 Prostaglandins**

These are a group of long-chain fatty acids that are synthesized by all cells except red blood cells. They have physiological roles which involve inflammatory reaction, pain, and fever. They can also activate the enzymatic pathway or they can cause contraction of smooth muscle (Okem, 2011).

## **2.2 Oxidants and Antioxidants**

Normally there is a balance between oxidants and antioxidants in a cell, these levels are controlled by body's antioxidative defense system. Nevertheless, failure of the body's antioxidative defense system to appropriately function or overproduction of reactive oxygen species may lead to increased production of free radicals therefore creating an imbalance in the cell (Sagnia *et al.*, 2014). Free radicals are any chemical species that contain unpaired electrons; they can be from a normal metabolism or external source such as exposure to an organic chemical in the environment (Lai *et al.*, 2011). Free radicals include singlet oxygen, superoxide anion, hydroxyl radical and hydrogen peroxide (Narayanaswamy and Balakrishnan, 2011); they are produced during an oxidation reaction where electrons are transferred from a substance to an oxidizing agent. Free radicals are highly reactive substances with hydroxyl radical being the most reactive (causes severe damage to biomolecules) and when they react with inhaled oxygen in the body, formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) occurs (Pandey *et al.*, 2012) which may attack lipids, proteins, enzymes, DNA as well as RNA (Muchuweti *et al.*, 2006). Oxidation reactions are both beneficial and harmful to life, if not balanced they may lead to oxidative stress resulting in tissue damage. Oxidative stress is caused by increased levels of free radicals in the body due to an imbalance between oxidants and antioxidants; it is the cause of many disease conditions (Sagnia *et al.*, 2014).

During an inflammatory response, ROS stimulate the release of inflammatory cytokines and chemokine mediators. Consumption of oxygen is increased which results in formation of superoxide anion that quickly gets converted to hydrogen peroxide by an enzyme called superoxide dismutase. In turn hydrogen peroxide gets reduced by transition metal to form hydroxyl radical which is one of the strongest oxidizing agents. Furthermore hydroxyl radical produces peroxyl radical after it has reacted with polyunsaturated fatty acids. In addition during the inflammatory process reactive nitrogen species may be produced, reaction of these species with superoxide anion may result in a very toxic compound that may oxidize some biomolecules resulting in cell damage. Nevertheless these reactive species have important roles in host defense mechanism as they are mainly produced to neutralize invading microorganisms (Miguel, 2010).

Human body can to some extent fight the effects of free radicals (oxidants) on its own, there are four sources of antioxidants in a biological system these include enzymic antioxidants, large molecules, small molecules and some hormones. And because there are multiple free radicals that are not similar these antioxidants may react differently to each radical (Prior *et al*, 2005). Superoxide dismutase is one of the antioxidant enzyme that scavenge and detoxify the effects of ROS in the cell, there are however other several enzymes that work with each other to efficiently destruct the action of ROS (Nahak *et al*, 2013). Nevertheless in the event of overproduction of free radicals, where the antioxidative defense system in the cell fails to function properly or a decrease in antioxidant level is observed, external sources of antioxidants are required to fight against the damaging effects of reactive species.

Antioxidants protect cells from being damaged by slowing down or preventing oxidation of biomolecules to occur, they inhibit initiation of oxidation chain reactions and therefore turn ROS into stable and harmless molecules (Nahak *et al*, 2013). They do this in two ways, they prevent access and generation of ROS to important biological sites, in addition they trap, absorb electrons and bind, inactivate metal ions thereby scavenging and destroying reactive oxygen species. There are primary, secondary and co-antioxidants, their classification is based on their mode of action. Primary antioxidants form a more stable radical by donating a hydrogen atom that will react with a lipid radical. Secondary

antioxidants react with initiating enzymes thereby preventing their action resulting in reduced oxygen levels (Miguel, 2010).

Use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has been decreased over the years as they present with many side effects, their use have strong restrictions and they are expensive (Patel *et al*, 2010). This has created a need to find antioxidants from natural sources. Antioxidants from tea, wine, fruits, vegetables and spices are already used as additives or antioxidant supplements as research has shown that they are high in antioxidant concentration, there is however still a great need to further study medicinal plant for their antioxidant properties. Research has shown that many antioxidant properties of plants are due to the presence of flavones, flavonoids, anthocyanins and phenolic compounds (Choi *et al*, 2002; Khalaf *et al*, 2008). These phytochemicals directly react with ROS and decompose peroxide, inhibit enzymes and quench oxygen molecules in this way preventing oxidative stress (Choi *et al*, 2002). Phenolic compounds are known to scavenge lipid radicals (Ravipati *et al*, 2012), terminate the action of free radicals and they have strong antioxidant activities (Narayanaswamy and Balakrishnan, 2011). As hydroxyl radical is the most reactive species, their number and arrangement somehow correlates to the antioxidant activity (Muchuweti *et al*, 2006). Some antioxidants dissolve in water, they react with oxidants in the cytoplasm and blood plasma whereas some dissolve in lipids, they protect cell membrane from lipid peroxidation (Partap and Pandey, 2012). During an inflammatory response antioxidants may scavenge lipidoxyl formed during enzymic peroxidation thereby inhibiting formation of lipid hydroperoxide necessary for catalytic cycle of LOX hence preventing inflammation to occur (Kumaraswamy and Satish, 2008). Some antioxidants quench superoxide, some directly scavenge singlet oxygen thereby helping in detoxifying many inhaled oxidizing air pollutants (Partap and Pandey, 2012).

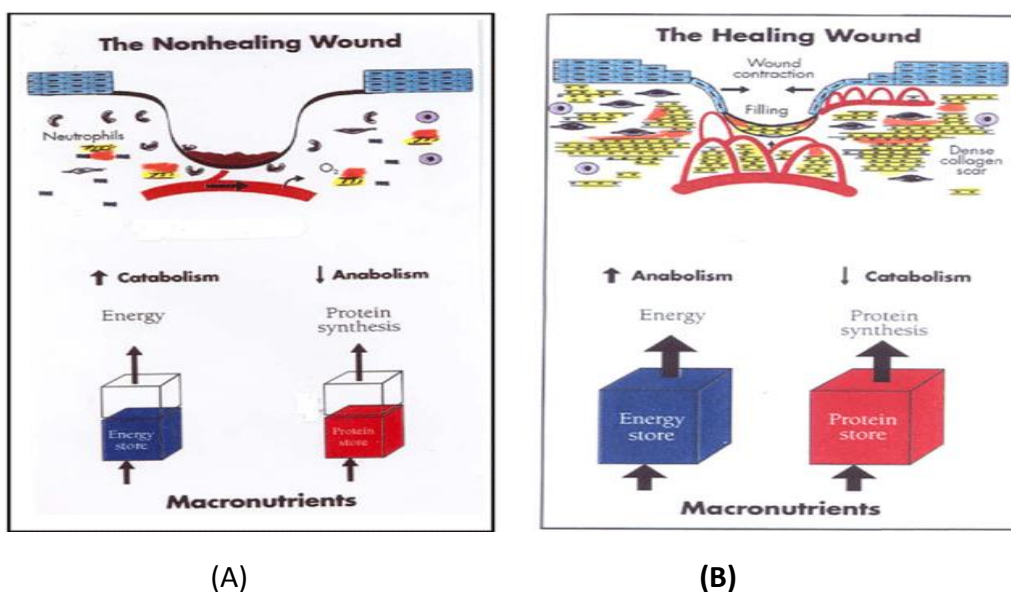
### **2.3 Wound healing**

Wound healing is a process whereby the body repairs the tissue damaged due to physical, chemical and microbiological processes. The process is made up of different phases that interconnect and overlap; these include haemostasis and inflammation, neovascularization, granulation, re-epithelialization and remodeling. Plant constituents

have been found to affect one or more of the wound healing phases (Demirci *et al.*, 2014). Basically in the first phase —inflammation phase — pro-inflammatory cytokines and growth factors are released by cells which include fibroblasts, macrophages, neutrophils, keratinocytes and endothelial cells. Macrophages are attracted to the wounded area to get rid of dead tissue and prevent infection by bacteria. Migration and proliferation of keratinocytes (re-epithelization) and fibroblasts (granulation) form the second stage of wound healing. The third stage involves the production of collagen and scar formation (Ebeling *et al.*, 2014). Antimicrobial characteristics and antioxidant properties are other factors that contribute to wound healing (Houghton *et al.*, 2005).

Wounds are caused by a number of factors, including cuts, abrasions, gunshots, crush injury and so on. Also, fire, heat, radiation, chemicals and sunlight are the causes of burn wounds. When wounded an opening or a break of the skin results. Wounds differ according to the underlying causes, they can be open or closed wounds and depend on how the wound heals they are categorized into acute and chronic wounds. In open wounds bleeding is visible and blood escapes the body, these include incised wounds, laceration wounds, abrasions, puncture wounds, penetration wounds, gunshot wounds, avulsions, cuts and fish hook wounds. Blood remains in the body in closed wounds, it only escapes the circulatory system, closed wounds include bruises, blood tumor, crush wounds etc (Mittal *et al.*, 2013). Acute wounds results from cuts or surgical incisions, they heal within expected time frame (Alam *et al.*, 2011). However if healing does not occur within the normal time frame, pain and swelling will be produced at the wound site by inflammatory mediators (Mittal *et al.*, 2013). Chronic wounds are wounds that have failed to heal within a normal time frame and they, therefore, start to cause complications and their healing process becomes delayed. Sometimes even after healing they re-occur regularly (Alam *et al.*, 2011). They are a result of degradation of growth factors essential for wound repair. Proteases inactivate the growth factors by proteolytic cleavage. Chronic wounds have high levels of reactive oxygen species released by neutrophils within the wound environment (Eming *et al.*, 2007). Infections and conditions like diabetes mellitus are some of the causes of these chronic wounds (Alam *et al.*, 2011) and they may lead to multiple organ failure or death. Antibiotics, antimicrobial ointments, and pain medications are used for wound care and maintenance (Reddy *et al.*, 2012).

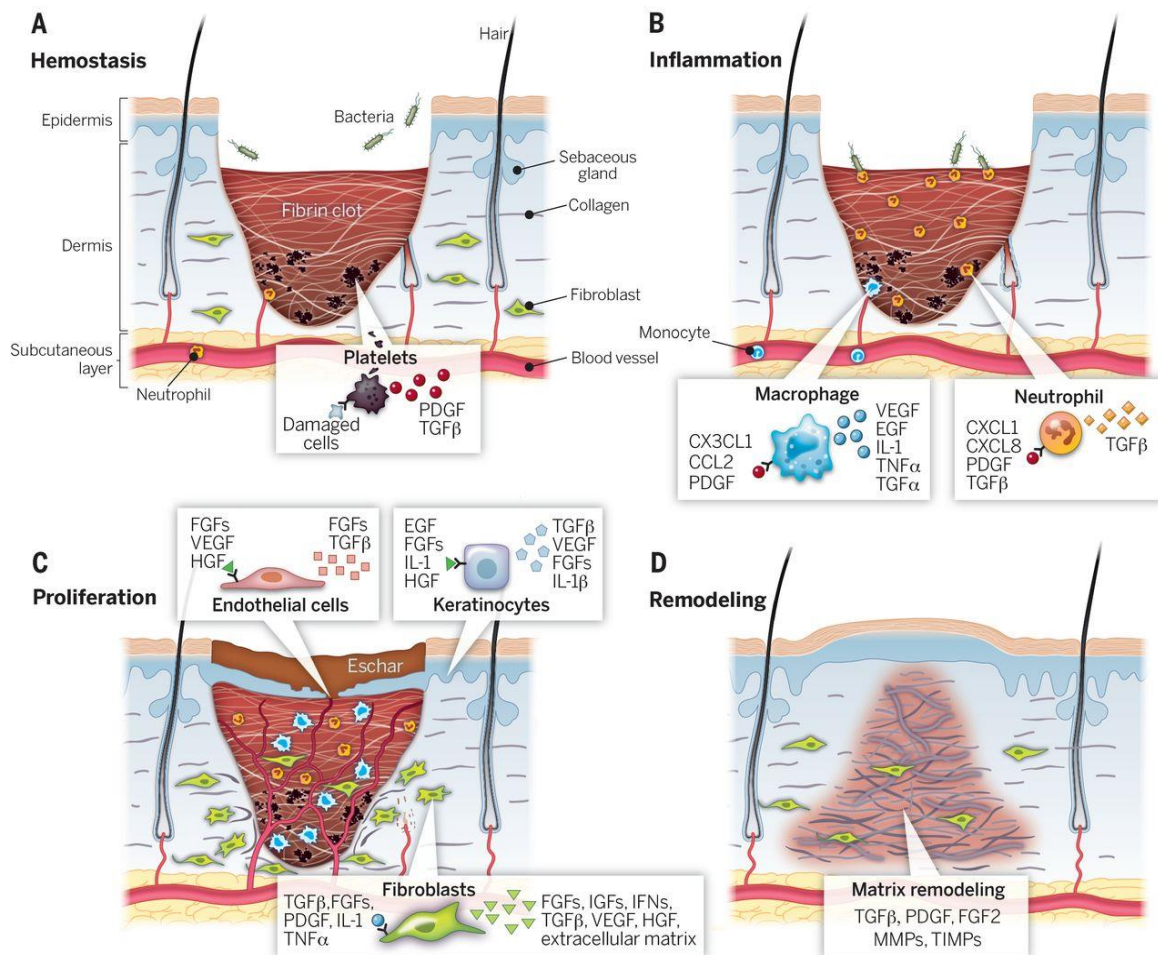
There are many factors that can cause a delay in the process of wound healing resulting in impairment or improper healing. These include improper diet (figure 4 A), having an infection at the wound site, drugs, elderly age, lack of oxygen supply and tissue perfusion at the wound site and conditions such as diabetes mellitus. There are known nutrients that assist in the wound healing process, these include Vitamins (A, C, E), bromelain, zinc, protein, glucosamine, arginine and glutamine, and they play different roles in the wound healing process. Also, the bioactive compounds isolated from plants have been shown to have an effect on the wound healing process (Mittal *et al.*, 2013). They promote and enhance wound repair in a natural way by using a number of mechanisms to induce healing and regenerate lost tissue (Figure 4 B) (Thakur *et al.*, 2011).



**Figure 4: Improper diet with insufficient nutrients can lead to a chronic wound where there is no formation of collagen in the wound site and wound contraction does not occur (A), with proper diet and sufficient nutrients (B).....(Demling, 2009)**

### 2.3.1 Phases of wound healing

There are three phases that occur during the process of wound healing, these are the inflammatory phase, proliferation phase, and remodeling phase (as shown in figure 5). These phases work in conjunction with each other to make sure that wound repair occurs accordingly. They are able to perform this work through the presence and actions of activated platelets, neutrophils, and macrophages. Clotting of blood and platelet aggregation, inflammatory response, collagen formation, re-epithelialization, and remodeling are all the steps involved in wound healing (Govindarajan *et al.*, 2007).



**Figure 5: Phases of wound healing include platelet aggregation and clotting of blood (A), then inflammatory phase (B), collagen formation and re-epithelialization through the proliferation phase (C) and finally remodeling phase (D)**

<http://www.sciencemag.org/content/346/6212/941/F1.expansion.html>

### 2.3.1.1 Inflammatory phase

This is the first phase that occurs immediately after the injury, it also includes haemostatic phase (Figure 5A). It lasts between 24 – 48 hours but at times, it may last upto two weeks. Through this phase, the wound swells and becomes painful. These are the signs of inflammation. Neutrophils and monocytes migrate to the surrounding tissue due to an increase in vascular permeability. During this phase blood loss is stopped at the wound site, this loss is due to the clotting of blood induced by vasoconstriction and platelet aggregation (Alam *et al.*, 2011). Phagocytosis of bacteria by macrophages (Figure 5B) and removal of dead tissue from the wound area occurs (Ebeling *et al.*, 2014). Migration and division of cells —fibroblasts, epithelial and endothelial— involved in the proliferative phase take place following the release of growth factors and prostaglandins by macrophages. Prostaglandins serve as the final mediators of acute inflammation. Reactive

oxygen species are also released into the wound environment. The manner in which the wound heals depends on the amount of infiltrating leukocytes into the wound area (Eming *et al.*, 2007).

#### **2.3.1.2 Proliferation phase**

This is the second phase of wound healing. It occurs subsequent to the inflammatory phase and it lasts 2 days to 3 weeks. Stimulation and migration of wound fibroblasts occur during this phase hence proliferation also occurs. Granulation, contraction, and epithelialization are the three steps involved in this phase (Figure 5C). A layer of collagen (commonly type III) is produced by the fibroblast cells in the first step of this phase. Collagen form cross-links with each other to provide strength and stability. Fibroblasts have been reported to produce a number of substances crucial for wound repair with collagen being one of the substances hence migration of these cells within this phase across the wound site. Within the contraction step, the wound area is reduced by the wound edges pulling together towards the wound. Epithelialization is the replacement of dead tissue with the new healthy cells. In this last step of this phase epithelial cells that are not damaged de-attach from the edges of the wound, starts reproducing and then they migrate down and across the wound forming a monolayer over the wounded site (Alam *et al.*, 2011), they also secrete cytokines responsible for cellular migration and proliferation. Keratinocytes form the protective outer layer. Later on, in this phase type III collagen is replaced by the production of type I collagen which is stronger than type III (Ghosh and Gaba, 2013). Enzymes from fibroblasts, neutrophils and macrophages break down this collagen and it gets digested by proteases released by these cells (Tsala *et al.*, 2013).

#### **2.3.1.3 Remodelling Phase**

This is the third phase of wound healing; it lasts up to 2 years (Figure 5D). Collagen type I is produced. Intermolecular linking of collagen by specific enzymes occurs and this thus increases the tissue's tensile strength, therefore, the scar tissues become as strong as the original tissue (Alam *et al.*, 2011; Mittal *et al.*, 2013). Fibroblasts and macrophages are eliminated and the supply of blood to the wound area is decreased (Tsala *et al.*, 2013).

### **2.3.2 Parameters used to assess wound healing activity**

There are *in vivo* and *in-vitro* methods that are used for determination of phases of the wound healing process. *In vivo* methods include animal models —incision and excision—, and also dead space and burn wound models can be used from time to time (Sharma *et al.*, 2013). With the incision model, wound tensile strength is measured; it involves creating abdominal cuts of equal sizes on the paravertebral area of the animal. This method is usually used for histopathological examination. Excision model involves frequently capturing images of the wound size in order to calculate wound closure. A cut on the skin is created within the dorsum region of an animal; this model is used for histological assessment. Dead space model entails creating a cut in the skin of the animal in a form of a pouch where the desired material such as polypropylene is inserted under the skin inside the pouch. Dry weights of granulation tissue and/or hydroxyproline content are some of the parameters studied for this model.

Burn wound model is where the hot molten wax at 80<sup>0</sup>C is poured over the back of an animal that is under anesthesia and was starved overnight. Parameters considered for this model are wound contraction and epithelialization stage (Tsala *et al.*, 2013).

*In vitro* models are used to investigate the behavior of cells—fibroblasts and keratinocytes—important in wound healing process and tissue repair. Boyden chamber base transmembrane and scratch wound assay are models used to study migration and invasion of cells. Microfluidic chambers and exclusion zone assays are the new methods that have emerged as other alternative screening assays (Tsala *et al.*, 2013).

### **2.3.3 Inflammation and wound healing**

Growth factors that are essential for the tissue growth or repair after an injury are generated through the acute inflammatory response, hence, if the inflammatory response is delayed, chronic inflammation may be the end result thereby preventing wound repair. Through research, it has been indicated that plant extracts with anti-inflammatory activities also have potential as wound healing agents (Mittal *et al.*, 2013).

### 2.3.4 Wound healing and antioxidants

Wound healing may be affected or delayed by the production of free radicals at or around the wound site. The free radicals are produced by activated platelets, neutrophils, and macrophages during the inflammatory phase. The body's immune system produces these free radicals to destroy any invading microorganisms at the wound site (figure 6) however overproduction these free radicals may cause damage to the tissues (Ghosh and Gaba, 2013) by lipid peroxidation, breakage of DNA and enzyme inactivation. Thus, agents that display significant antioxidant activities may be used to protect the cells from being damaged by the excess of free radicals thereby stimulating wound healing process (Barku *et al.*, 2013; Mittal *et al.*, 2013). Synthetic compounds have been suspected to be degraded by high concentrations of ROS; therefore, there is a great need to find alternatives that will be less affected by the ROS levels (Reddy *et al.*, 2012). Phenolic compounds have been reported to possess antioxidant properties (Tsala *et al.*, 2013) and antioxidant property is believed to be one of the important components of wound healing.

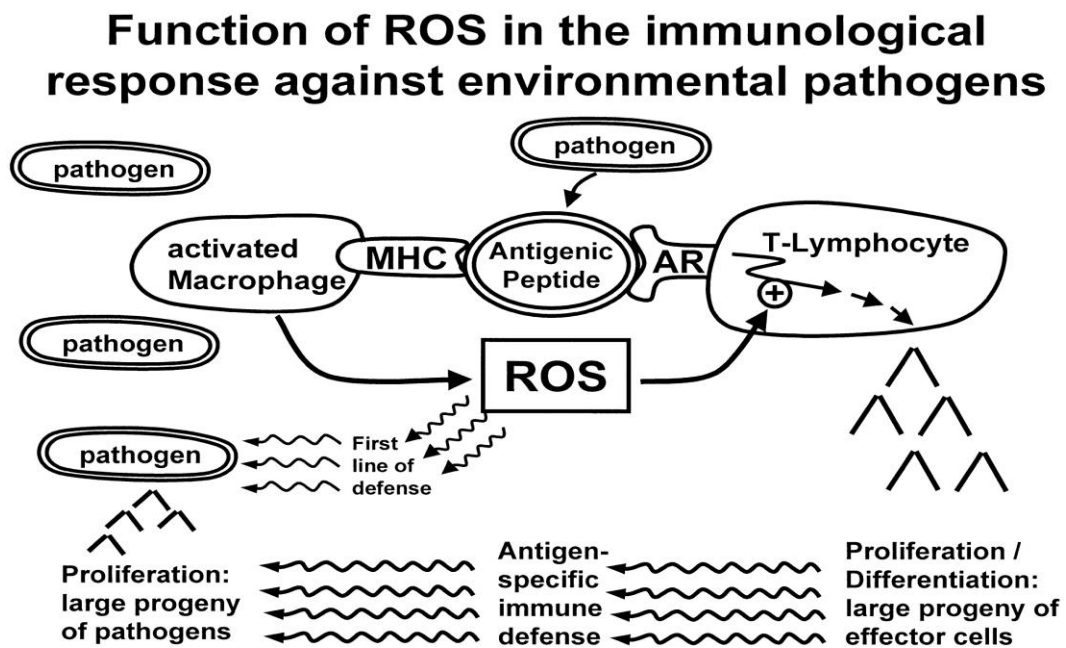


Figure 6: The body's immune system produces reactive oxygen species during an injury to destroy pathogenic organisms (Droge, 2001)

## 2.4 Plant species

Use of plants for medicinal purposes is not an uncommon phenomenon for humankind across races, culture and generations. Plants were directly used by almost 80% of world's population as a source of medicine before synthetic drugs were developed but after research, they were found to contain substances that can be used for the preparation of drugs hence discovery of medicinal drugs. Currently, more concentrated extracts from plants are used for scientific testing to find chemicals or nutrients present in them. It has been shown that crude plant extracts are less toxic than the synthetic products; therefore, there is an increased interest for the population to use plants for medicinal purposes. However, some of the plants can have adverse effects on human population hence it is important to conduct experiments that determine the safety of the plants on animal or human population to avoid the use of these plants for any medicinal purposes. Below are some of the plants used traditionally for medicine in KwaZulu-Natal, South Africa. It is important to look at their nature, where they are found, how they grow, compounds isolated from them and ailments they are known to treat. Plants species used in this study include:

### 2.4.1 *Achyranthes aspera*

This perennial/annual plant is from a family of *Amaranthaceae*, and is 0.4-2m in height. It is commonly known as devil's horsewhip. The leaves are elliptic or broadly ovate (Figure 7). The plant grows in moist soil therefore; it prefers to grow in light sandy, medium loamy and heavy clay soils (Singh, 2009). It is widely distributed along roadsides and open fields in tropical and subtropical regions as a weed (Amrutia *et al.*, 2011). Saponins, betaine, achyranthine, amino acids,  $\beta$ -ecdsonone, lauric acid, myristic acid, steroids, triterpenoids, flavonoids are the chemical constituents that have been isolated from this plant (Bhosale *et al.*, 2012). Tannins were isolated from this plant and they were found to contribute to the plants wound healing effect (Edwin *et al.*, 2008). It is known to be used in the treatment of piles, itching, blood diseases, vomiting, abdominal pains, dysentery (Singh, 2009), snake bites (Raji, 2013). Achyranthine is an alkaloid that has been found to possess anti-inflammatory and antioxidant activity, and the roots are used as astringent to wounds (Ahmed and Nahor, 2012).



**Figure 7: *Achyrathes aspera* (Srivastava, 2014)**

#### **2.4.2 *Alternanthera sessilis***

It belongs to a family *Amaranthaceae*; it is a perennial herb that grows upto 1m height throughout the tropical and subtropical regions in the world (Figure 8). It is found in wetlands and uplands but it prefers wet conditions. It is usually found growing in damp shady areas along roadsides on a variety of soil types (Singh, 2009; Subhashini *et al.*, 2010). It bears short-petioled simple leaves and small white flowers (Subhashini *et al.*, 2010). Flavonols, triterpenoids, steroids, and tannins are some of the important constituents isolated from *A.sessilis* (Rajani *et al.*, 2011). In medicine, it is used for treating diarrhea, dysentery, intestinal cramps, fever, wounds, congestion, asthma and snake bites. But the conditions that the plant treats are different amongst different areas (Singh, 2009). The plant is also known to have anti-inflammatory, antioxidant and wound healing activities (Mrinmay and Ashok, 2014).



**Figure 8: *Alternanthera sessilis* (Mrinmay and Ashok, 2014)**

### 2.4.3 *Amaranthus dubius*

This plant species belongs to a family *Amaranthaceae*, it is considered to be a morphologically unusual allopolyploid (Figure 9) but it is very close to other species of *Amaranthus*. It is considered as a weed in rice crops, its leaves are eaten as vegetables in Venezuela (Rodriguez *et al.*, 2011). The species of *amaranthus* are known to contain bioactive compounds such as saponins, phytosterols, squalene, and polyphenols. Being an important source of minerals, *A.dubius* consumption might prevent and improve diseases such as osteoporosis, anemia (Quintero *et al.*, 2011). Also, it has been reported in treating fever, haemorrhage and stomach ache (Alegbejo, 2013).



Figure 9: *Amaranthus dubius* (Grubben, 2004)

### 2.4.4 *Amaranthus hybridus*

*A. hybridus* belongs to a family *Amaranthaceae*, it is commonly known as pigweed. It is an erect perennial herb that grows up to 6 feet high (Figure 10). It grows annually. It is mostly found in waste places, cultivated fields and barnyards. The leaves have wavy margins; they are alternate petioled, rough, hairy and dull green. The flowers are small with greenish or red terminal panicles (Akubuywo *et al.*, 2007). Alkaloids, flavonoids, saponin, tannins, phenols, hydrocyanic acid and ptycic acid are the constituents isolated from this plant species according to Akubuywo *et al.*,(2007). In medicine, the plant is used to treat ulcers, dysentery, haemorrhage of the bowel and the leaves are used to reduce tissue swelling (Singh and Sheoran, 2011). It is also used as an antidote for snake and scorpion bite (Shah *et al.*, 2006), for intestinal bleeding and excessive menstruation (Omodamiro and Jimoh, 2015).



**Figure 10: *Amaranthus hybridus* (Shahet *al*, 2006)**

#### **2.4.5 *Amaranthus spinosus***

Commonly known as spiny amaranth, belongs to *Amaranthaceae* family. It is an annual or perennial herb that is found growing in wastelands and roadsides on sandy, loamy, heavy, basic, acidic and neutral soil (Figure 11). However the plant often requires moist soil to grow and it cannot grow in the shade. Alkaloids, glycosides, saponins, phytosterols, phenolics, flavonoids and tannins are some of the constituents isolated from *A. spinosus* (Antara, 2012). In medicine it is used as diuretic, antsnake venom, antileprotic, anti-gonorrheal, anti-inflammatory, antioxidant, anthelmintic, antidiabetic and antipyretic. Externally the plant is used to treat ulcerated mouth, vaginal discharge, nose bleeds, wounds, eczema, burns and boils (Alegbejo, 2013; Gul *et al.*, 2011). According to Barku *et al.*,(2014) the plant possess antioxidant, anti-inflammatory, antimicrobial and wound healing activities.



**Figure 11: *Amaranthus spinosus* (Kumar *et al*, 2014)**

#### 2.4.6 *Asystasia gangetica*

This is from *Acanthaceae* family, it is commonly known as creeping foxglove. It is known to spread over ground growing from 300mm to 600mm in height. It grows fast and it is widely distributed from tropical Asia to Africa (Ezike *et al.*, 2008) and the leaves are oval-shaped with a rounded base with a dark green colour. The flower is cream-white with purple markings (Figure 12) and the fruit is a club-shaped capsule. Alkaloids, tannins, steroidal aglycones, saponins, flavonoids and terpenoids are some of the chemical constituents of *A. gangetica* (Mohan *et al.*, 2010). In KwaZulu-Natal this plant is used as a vegetable and possess antioxidant properties (Tillo *et al.*, 2012) and in medicine it is used for swellings, fever, epilepsy, stomach aches, heart disease, snakebite and also applied on women abdomen to help during birth (Hamid *et al.*, 2011; Kumar *et al.*, 2011). It has also been reported to be used for intestinal worms and asthma and possess anti-inflammatory activities (Akah *et al.*, 2003).



Figure 12: *Asystasia gangetica* (Tillooet *al.*, 2012)

#### 2.4.7 *Bidens pilosa*

Also known as blackjack is from a family of *Asteraceae*, it is a herbaceous plant widely distributed in Africa in tropical and subtropical regions and it is widely used (Frida *et al.*, 2008). It grows to a height of upto 1.5m with yellow flowers (Figure 13). A number of studies have reported the isolation of flavonoids, sterols, terpenoids, phenylpropanoids, acetylenes and hydrocarbons in the extracts of *B. pilosa* (Bartolome *et al.*, 2013; Silva *et al.*, 2011). In medicine, it is used to treat various diseases such as diabetes, hepatitis and inflammation (Horiuchi and Seyama, 2006). In Uganda, the sap from crushed leaves is used to speed up clotting of blood in fresh wounds. It is also used to treat a headache, ear

infections, kidney problems (Ezeonwumelu *et al.*, 2011). The Zulu people use the aqueous preparations of the leaves to treat diarrhea, colic and dysentery (Silva *et al.*, 2011). Bartolome *et al.*, (2013) has reported its use for digestive disorders, infectious diseases, wounds and as an anti-inflammatory and antioxidant agent. Hassan *et al.*, (2011) reported on wound healing potential and inhibition of prostaglandin synthesis.



**Figure 13: *Bidens pilosa* (Yang, 2014)**

#### **2.4.8 *Buddleja saligna***

Also known as false olives, *B. saligna* comes from a family of *Buddlejaceae*. It is found usually growing on rocky hillsides or along watercourses. This is a small to a medium evergreen tree with grayish green foliage; it grows upto a height of 10m (Adedapo *et al.*, 2009). The bark is grey-brown in colour and the leaves are opposite and oblong in shape with an olive green top and whitish underneath (Figure 14). It prefers to grow in sunny conditions but in moist soil. Alkaloids, saponins, tannins, reducing sugars are some of the major constituents found in the extracts of *B. saligna* (Verdoorn, 1963). The fruits are not tasty but they are safe to eat. In many parts of the world these wild olives are used to lower blood pressures but in South Africa they are used to treat colic, coughs, sore eyes, urinary problems (Adedapo *et al.*, 2009).



**Figure 14: *Buddleja saligna* (Solomon, 2009-2014)**

#### **2.4.9 *Bulbine natalensis***

Belongs to a family of *Asphodelaceae*, it is commonly known as rooiwortel. This is a tender evergreen perennial plant that is widely distributed in the northern and eastern parts of South Africa (Yakubu and Afolayan, 2009). The flowers are yellow and star-shaped while the leaves are broad, sharp-pointed and yellow-green (Figure 15). The leaves are filled with clear gel similar in appearance and consistency to the *Aloe Vera* gel (Pather *et al.*, 2011). The stem of the plant has been shown to contain tannins, anthraquinones, saponins, glycosides and alkaloids (Yakubu and Afolayan, 2009). In medicine, it is used to treat wounds, burns, rashes, itches, cracked lips, ringworm and herpes. Vomiting and diarrhea are relieved by the extracts from the roots (Mosa *et al.*, 2011; Coopoosamy, 2011). A study conducted by Pather *et al.*, (2011) demonstrated wound healing potential of *Bulbine natalensis*.



**Figure 15: *Bulbine natalensis* (Yakubu *et al.*, 2008)**

#### 2.4.10 *Carpobrotus dimidiatus*

*Carpobrotus dimidiatus* belongs to a family *Mesembryanthemaceae*; it is a robust trailing plant that is also known as natal sour figs. It is found along the coast on sandy dunes from Eastern Cape to KwaZulu-Natal. The plant has green, fleshy, three-sided leaves (Figure 16). It may spread over the ground to form a cover that is resistant to drought and salt spray. Numerous small, brown, shiny seeds are embedded in a slimy sourish pulp. In medicine; the juice from the plant is used for a sore throat, thrush, digestive troubles, diarrhea and dysentery. It has also been shown to help with skin problems such as sores and rashes. There is also a report on the presence of tannins (Nonjinge, 2007).



Figure 16: *Carpobrotus dimidiatus* (Nonjinge, 2007)

#### 2.4.11 *Centella asiatica*

This is a perennial creeper that is widely distributed throughout the world. It belongs to a family of *Apiaceae* commonly known as marsh pepperwort. It is the most valuable medicinal herb that has been used for centuries in ayurvedic medicine for the treatment of many skin conditions (Chippada *et al.*, 2011). It grows upto 30cm in height and in swampy areas (Gohil *et al.*, 2010). The leaves are fan-shaped, yellowish green, thin, alternate with long petioles (Figure 17). The plant grows horizontally through its green to red stolones which combine with each other and roots underground (Orhan, 2012). Triterpene saponins such as asiaticoside, sapogenin, Asiatic acid, madecassoside and madecassic acid are the major bioactive constituents isolated in *C. asiatica* (Anilkumar, 2010) with asiaticoside being the main active constituent responsible for wound healing( Ruszymah *et al.*, 2012). Alkaloids, flavonoids, glycosides, saponins, tannins are other chemical constituents isolated but triterpenoids and flavonoids are known to have remarkable anti-inflammatory activity

(Vangalapati and Chippada, 2011). It is used in medicine for treating skin conditions such as eczema, for treating anemia, asthma, blood disorders, bronchitis, fever, urinary discharge and mental fatigue (Anilkumar, 2010). It has been demonstrated to accelerate wound healing (Brinkhaus *et al.*, 2000) and has anti-inflammatory and antioxidant activities (George *et al.*, 2009).



**Figure 17: *Centella asiatica* (Orhan, 2012)**

#### **2.4.12 *Ceratotheca triloba***

This plant belongs to the family *Pedaliaceae* and is commonly known as wild foxglove. It is found in summer in areas like roadsides. The leaves are soft and green (Figure 18) and they are divided into 3 lobes, the flowers are pink with red stems or sometimes the flowers are white with yellowish-green stems (Mohanlall, 2010). According to Mohanlall *et al.*, (2011) phlobatannins, saponins, steroids and terpenoids were the major constituents isolated from extracts of *C. triloba* and it was noted that there was no detection of flavonoids, tannins or cardiac glycosides. In medicine, it is used in the treatment of stomach cramps, nausea, fever, diarrhea, painful menstruation (Lewu and Afolayan, 2009) and insect infestations of the skin (Naidoo *et al.*, 2012).



**Figure 18: *Ceratotheca triloba* (taken at Amanzimtoti, 2014)**

#### **2.4.13 *Chenopodium album***

Also known as the fat hen, this wild plant belongs to a family *Chenopodiaceae*. It grows upto 250cm in height. The stems are angular, branched and brownish yellow (Figure 19). The leaves are alternate, petioled, deep green to light green (Bassett and Crompton, 1978). Flavonoid, alkaloids, isoflavonoid, polyphenol, saponins are some of the constituents found in *C. album* (Agrawal *et al.*, 2014; Khoobchandani *et al.*, 2009). The plant is used in medicine as a laxative, anthelmintic against round and hook worms, and it is used as a purifier. It is also used to treat intestinal ulcers and burns (Usman *et al.*, 2010). It also enhances memory, improves appetite and used for anorexia, cough, diarrhea, oedema, eye diseases (Agrawal *et al.*, 2014).



**Figure 19: *Chenopodium album* (Agrawal *et al.*, 2014)**

#### 2.4.14 *Cleome monophylla*

*Cleome monophylla* commonly known as spindle-pod comes from the family *Capparaceae*. It is an annual herb that is widely spread in tropical and subtropical regions. It grows upto 1m tall and the stem is covered with glandular hairs. It's got simple entire leaves (Figure 20) and it is found in dry savanna grassland but it tolerates a wide range of soil types. The plant is used to treat swellings, headache, sores, roots, cough, ulcers, boils and wounds (Jansen, 2004a).



Figure 20: *Cleome monophylla* (Hyde *et al*, 2016)

#### 2.4.15 *Dichrostachys cinerea*

This plant species belongs to a family *Fabaceae*, it is commonly known as sickle bush. It is a deciduous thorny shrub or small rounded tree found in tropical and subtropical regions that grow upto 12m high. The leaves are bipinnate, each pinna bearing a gland. The flowers are 2.5cm long and are composed of an upper and a yellow bottom (Figure 21). It grows on heavy soils and occurs in central, southern and tropical Africa. Phytochemical studies performed have revealed the presence of tannins, sterols, triterpenes, polyphenols and flavonoids (Aworet-Samseny *et al.*, 2011). *D. cinerea* is used to treat leprosy, dysentery, headache, syphilis, toothache (Mishra *et al.*, 2009), jaundice, inflammations, fever, asthma, body ache, chest problems, ulcers, wounds and eye diseases (Babu *et al.*, 2011). It has been demonstrated to have anti-inflammatory activities (Aworet-Samseny *et al.*, 2014; Hassan *et al.*, 2012).



Figure 21: *Dichrostachys cinerea* (Hurinanthan, 2009)

#### 2.4.16 *Ekerbegia capensis*

Also known as Cape ash, it is from *Meliaceae* family. It is an evergreen tree that grows upto 20m tall (Figure 22). It is widely distributed in Africa. According to Spatulaet *al*, (2010) the phytochemical screening of *E. capensis* showed a strong presence of saponins and steroid glycosides. Alkaloids, tannins, carotenoids, flavonoids, anthraquinones and anthrocyanosides were weakly present in the extract. The plant is used for a headache, fever, cough, skin diseases, diarrhea(Irungu *et al.*, 2014) and heartburn, respiratory complaints, abscesses, boils and pimples(Murata, 2008).



Figure 22: *Ekerbegia capensis* (Dlamini, 2004)

#### 2.4.17 *Elytropappus rhinocerotis*

Commonly known as rhinoceros bush, this shrub belongs to *Asteraceae* family. It is a single-stemmed plant (Figure 23) that grows up to 2m in height. The bark is smooth and grayish and it is widely spread in the Cape floristic region (Maiko, 2010). It is commonly found on dry clay flats and slopes. Major constituents isolated include cardiac glycosides, saponins, tannins and reducing sugars but not alkaloids (Dekker *et al.*, 1988). In medicine, this plant species is used to treat acidity in young children and digestive disorders in the adult. It is also used for the treatment of colic, wind and diarrhea (Levyns, 1935). It is also used for stomach cancer, fever and stimulates sweating (Gakuba, 2009).



Figure 23: *Elytropappus rhinocerotis* (Bergh, 2006)

#### 2.4.18 *Emex australis*

Belongs to a family *Polygonaceae*, it is commonly known as devils thorn. It is an annual broadleaf plant. It develops as a rosette and the leaves are alternate and oval with wavy edges (Figure 24). In South Africa, it is a weed of cereal crops and roadways.



Figure 24: *Emex australis* (Parsons and Cuthbertson, 1992)

#### **2.4.19 *Ficus sur***

This is a monoecious fig tree that belongs to *Moraceae* family; it is widely distributed throughout South Africa. It is a freestanding or occasionally epiphytic tree that grows up to a height of 25m along river banks, streams and forest margins (Figure 25). It produces green to dark red figs containing up to 3000 flowers. Figs are mainly from the leafless branches on the larger branches but some can be from smaller branches (Zachariades, 1994). In traditional medicine, it is used to treat wounds, toothache, eye problems, general body pain, lung and throat problems, cough, sore throat, diarrhea, stomach and chest pain (Eldeen *et al.*, 2005; Lumbile and Mogotsi, 2008). Flavonoid glycosides, alkaloids, phenolic acids, steroids, saponins, tannins, triterpenoids have been isolated from this plant (Sirisha *et al.*, 2010).



**Figure 25: *Ficus sur* (Hankey, 2003)**

#### **2.4.20 *Galinsoga parviflora***

*Galinsoga parviflora* is an annual herb that belongs to one of the largest families of the plant kingdom which is *Asteraceae*. This herb grows up to 80cm high with strong opposite branches. It grows on sandy fields, waste places and cultivated fields (Meric and Dane, 2005). It is considered to be a common weed in several crops that are important (Figure 26). It grows well in moist soil and it also prefers high-intensity light (Damalas, 2008). It is used for diarrheal disorders (Yadav and Tangpu, 2008) but previous studies have also shown its use as an anti-inflammatory agent. It has also been shown to heal wounds and skin problems, prevents scurvy bleeding and treats colds and flu (Bazylko *et al.*, 2012).

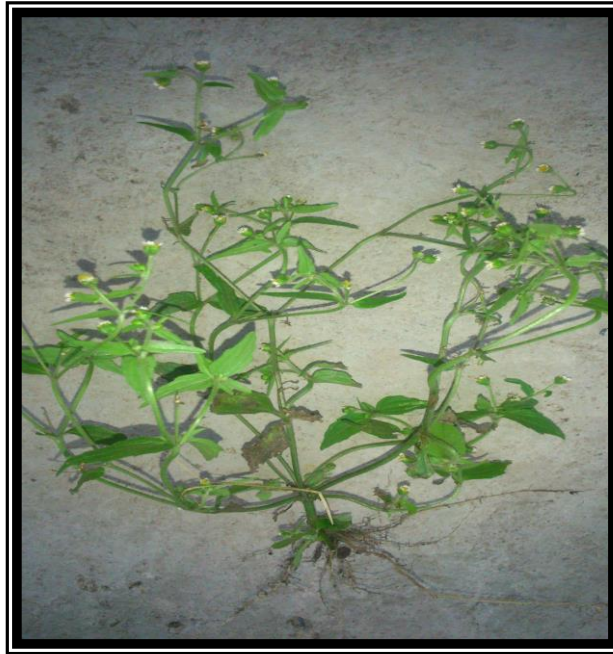


Figure 26: *Galinsoga parviflora* (taken by N Mzindle at Amanzimtoti, 2014)

#### 2.4.21 *Guilleminea densa*

It is from a family *Amaranthaceae*; it is found growing on roadsides. It is commonly known as small matweed. This perennial herb grows up to 5cm high in sunny, open, disturbed areas. The flowers are yellowish cream to off-white or translucent, they are mixed with leaves (Hyde and Wurstein, 2002). The leaves are narrowly elliptic with long matted white hairs on the lower surface (Singh, 2009). It forms a dense spreading mat on the ground (Figure 27). No information available on biological properties or chemical constituents of *G. densa*, however, it has been reported to be used traditionally for nausea and diarrhea (Sibandze, 2009).



Figure 27: *Guilleminea densa* (Hyde & Wursten, 2002)

#### **2.4.22 *Gunnerea perpensa***

Also known as river pumpkin, it belongs to *Gunneraceae* family. This is a robust wetland herb (Figure 28) that lives in marshy areas extending from eastern and northern parts of South Africa and tropical Africa (Xuma and Naidoo, 2007). It grows upto 1m in shallow water. A phenylpropanoid glycoside (Z-venusol) has been identified as the major constituent isolated from *G. perpensa* but also Celastrin has been reported as the constituent that might be present in the extracts of this plant (Simelane *et al.*, 2010). It has been used mostly by the Zulu people traditionally as a medicinal plant for a long time (Khan *et al.*, 2004). It is used to induce labor, assist in the expulsion of the uterus, to treat stomach troubles, rheumatic fever, swellings, menstrual pain, stomach bleeding and dressings of wounds (Watt and Breyer-Brandwyk, 1962; Nkomo *et al.*, 2010; Simelane *et al.*, 2010). It has been shown to stimulate the growth of fibroblast during wound healing and possess antioxidant activity (Steenkamp *et al.*, 2004).



**Figure 28: *Gunnerea perpensa* (Glen, 2005)**

#### **2.4.23 *Heteropyxis natalensis***

*Heteropyxis natalensis* is also known as the lavender tree, it comes from the family *Heteropyxidaceae*. It grows approximately 10 metres in height (Shode *et al.*, 2005). It is known to have a very strong aromatic odour that is emitted from the leaves during summer. The leaves are small, simple, narrow, oblong, and elliptic and are arranged spirally (Figure 29). It grows on the north-eastern part of South Africa on the coastal and inland regions extending from the tree's place of origin which is KwaZulu-Natal (Van Vuuren and Viljoen, 2008). It occurs in bushveld, forest margins and also on rocky hillsides.

It has leafy branches and due to regular flaking, the bark has a distinctive mottled appearance. Yellow flowers followed by small dry capsules are some of the characteristics of this plant (Frum, 2006).  $\beta$ -ocimene, 1.8-cineole, limonene and linalool are some of the major constituents of the plant (Van Vuuren, 2007). Limonene from this plant has been shown to display 5-lipoxygenase inhibitory activity (Baylec and Racine, 2003). It is used in medicine to treat respiratory and bleeding disorders. The leaves are used in herbal tea and they also used with roots to treat worms in stock, also, a bleeding nose can be healed by inhaling the steam from a decoction of the roots (Dlamini and Hankey, 2002). Frum and Viljoen, 2006 reported on the anti-inflammatory activity of this plant.



**Figure 29: *Heteropyxis natalensis* (Van Vuuren and Viljoen, 2008)**

#### **2.4.24 *Hibiscus sabdariffa***

*Hibiscus sabdariffa* belongs to *Malvaceae* family; it is commonly known as a rosette. It is an annual shrub that has a deep penetrating taproot and it grows upto 3.5m in height. The stems are smooth, cylindrical and they are dark green to red. The leaves are green with reddish veins. Flowers are yellow or buff with a rose or maroon eye (Mahadevan *et al.*, 2009). The stem leaves and branches can also be purple and the calyx is reddish purple (Figure 30). Active compounds that have been isolated from this plant include protocatechuic acid, anthocyanin (Reanmongkol and Itharat, 2007) but also alkaloids, tannins, saponins; flavonoids are all present in the extracts of *H. sabdariffa*. It is used in medicine for the treatment of abscesses, bilious conditions, cancer, cough, fever, heart ailments, necrosis, dyspepsia, 'hangover' and hypertension (Mungole and Chaturvedi, 2011) and also prevention of cardiovascular disease (Christian *et al.*, 2006). Antioxidant

and anti-inflammatory properties of this plant have been reported (Christian *et al.*, 2006; Lin *et al.*, 2007).



**Figure 30: *Hibiscus sabdariffa* (Simpson, 2006)**

#### **2.4.25 *Justicia flava***

Also known as yellow justicia, the plant belongs to *Acanthaceae* family. This is an erect perennial herb that is about 450mm high; it is widely spread in tropical and Southern Africa (Burkill, 1985). It is found growing in disturbed areas in full sun or semi-shady areas. It can grow in a wide range of soils but can also withstand dry conditions. It produces yellow flowers (Figure 31) and it covers the ground (Froneman, 2008). Tannins, steroids, flavonoids, alkaloids are the chemical constituents isolated from this plant. In medicine, it is used in the treatment of swellings, oedema, gout, menstrual pains, pulmonary troubles, diarrhea, dysentery, nasopharyngeal infections, coughs, paralysis, fever, epilepsy, skin infections and disorders (Burkill, 1985; Agyare *et al.*, 2013). Increased rate of wound closure and the antioxidant property has been demonstrated by Agyare *et al.*,(2013).



**Figure 31: *Justicia flava* (Froneman, 2008)**

#### **2.4.26 *Leonotis leonurus***

*Leonotis leonurus* is a shrub that is found mostly in South Africa. It belongs to Lamiaceae family. It is commonly known as wild dagga. The plant is commonly found at forest margins, on rocky hillsides and riverbanks. It grows to about 5m high. The base is thick and the branches appear to be pale brown in colour. The leaves are hairy with flowers that are bright orange (Figure 32) and tubular in shape. Leonurine, diterpenoids, flavonoid aglycones, sterols, tannins, alkaloids, saponins are some of the major constituents that have been isolated from *L. leonurus*. *L. leonurus* has been documented to treat many conditions including influenza, tuberculosis, coughs, jaundice, muscular cramps, skin diseases, sores, bee and scorpion stings, menstrual disorders, headache, parasites in animals, wounds, asthma, diabetes, obesity, cancer, piles, viral hepatitis, arthritis, bladder and kidney disorder and hypertension (Kenechukwu, 2004; Maphosa *et al.*, 2012; Mazimba, 2015). Anti-inflammatory and antioxidant properties have been demonstrated (Maphosa *et al.*, 2012; Mazimba, 2015).



**Figure 32: *Leonotis leonurus* (Hurinanthan, 2009)**

#### **2.4.27 *Momordica balsamina***

*Momordica balsamina* is commonly known as African cucumber, it belongs to *Cucurbitaceae* family. This is a perennial herb with soft stems and tendrils that climb up forests, boundary fields or shrubs. The leaves are green and the fruits produced are dark green when unripe and bright to deep orange (Figure 33) when ripe (Hassan and Umar, 2006). Tannins, saponins, diterpenes, lectins have been isolated from *M. balsamina*. It is used in the treatment of skin disease, and also as a tranquillizer in mental illness (Geidam *et al.*, 2004). In some other countries the plant is used for the reproduction of blood that

was lost during labour as well as milk purification (Hassan and Umar, 2006). In addition Ilangoet *al*,(2010) reported on the traditional use of this plant including wound healing, leprosy, piles, jaundice, gout and rheumatism. Furthermore, the plant demonstrated wound healing property *in vivo* and Bot *et al*,(2007) reported on the anti-inflammatory and antioxidant activities.



**Figure 33: *Momordica balsamina* (Hurinanthan, 2009)**

#### **2.4.28 *Oxygonum sinuatum***

It belongs to a family *Polygonaceae* and it is commonly known as ‘Double thorn’. It is widely distributed in South Africa growing in fields and waste grounds. This is an erect annual herb that spreads over the ground. It grows upto 1m tall. The stems are green to red-brown and the leaves are reddish, fringed with long hairs at the apex and they have an acidic taste. Flowers are either white or pink (Figure 34). Medicinally the plant is used to treat boils, tonsillitis, fungal infections and eye infections (Jansen, 2004b).



**Figure 34: *Oxygonum sinuatum* (Wursten, 2009)**

#### 2.4.29 *Pelargonium* sp

*Pelargonium* species also commonly known as geranium belongs to a family *Geraniaceae*. There are 250 *Pelargonium* species and approximately 80% occur in South Africa, mainly in winter. These small perennial or annual shrubs have a wide variety of growth habits. Leaves may be covered with fine hairs and may be rough, sticky or have a velvety texture, they may be in different shapes and sizes. In some species, the leaves edges may appear 'curly' or 'crisped'. Flowers may be white, mauve, pink, pale yellow, lavender or burgundy (Figure 35). They grow in low humidity areas within short grassland or with shrubs or trees. Chemical analyses of *Pelargonium* sp. show that about 65 secondary metabolites are isolated including phenolic acids, cinnamic acids, tannins, flavonoids and coumarins. In South Africa, *Pelargonium* species are used in medicine to treat diarrhea, dysentery, fever, tonsillitis, gastroenteritis, haemorrhage, kidney and bladder disorders, skin disorders, tuberculosis and coughs (Saraswathi, 2011; Avila *et al.*, 2013). The plant is also used for wound healing, reports on anti-inflammatory and antioxidant properties have been obtained (Avila *et al.*, 2013; Boukhatem *et al.*, 2013).



Figure 35: *Pelargonium* sp. (Avila *et al.*, 2013)

#### 2.4.30 *Physalis viscosa*

Also known as stairhair ground cherry, it belongs to *Solanaceae* family. It is a rhizomatous perennial herb with a hairy stem that grows upto 40cm tall. The leaves are oval, smooth with toothed edges. The flowers are bell-shaped and they are yellow with dark centres (Figure 36). It can grow in semi or no shade area. In medicine, it is used as a diuretic and

also traditionally used to treat fever, inflammations of the bladder, sore throat, earache, diabetes, asthma, hepatitis, abdominal pain, boils, sores, constipation, malaise and wounds. Withanolides are the major chemical constituents that have been isolated from the plant however phytosterols and flavonol glycoside have also been reported (Mahalakshmi and Nidavani, 2014; Silver *et al.*, 1993).



**Figure 36: *Physalis viscosa***

<http://www.pfaf.org/user/Plant.aspx?LatinName=Physalis+viscosa>

#### **2.4.31 *Portulaca oleracea***

*Portulaca oleracea* is a herbaceous plant that belongs to a family *Portulacaceae*. It is commonly known as purslane. It is widely distributed all over the world (Jagan *et al.*, 2002). It can grow upto 40cm in height and it is an annual herb with reddish stems that form mats with leaves and yellow flowers (Figure 37). The major bioactive constituents isolated from *P. oleracea* have been shown to be flavonoids, coumarins, monoterpene glycoside, anthraquinones, saponins, tannins, triterpenoids and alkaloids (Jagan *et al.*, 2002; Lee *et al.*, 2012). For medicinal purposes the main use of the herb is as a sedative, it is also used to treat conditions related to the eye as well as conditions such as bacillary dysentery, dysuria, mastitis, boils, impetigo, sore nipples, ulcers of the mouth, fevers, abscesses, swellings and urinary disorders (Chan *et al.*, 2000; Sonja *et al.*, 2009; Masoodi *et al.*, 2011). Anti-inflammatory, antioxidant and wound healing activities of this plant have been shown (Chan *et al.*, 2000; Jagan *et al.*, 2002; Masoodi *et al.*, 2011).



**Figure 37: *Portulaca oleracea* (Dixon, 2012)**

#### **2.4.32 *Senna occidentalis***

Commonly known as cassia senna, it belongs to *Leguminosae* family. This weed is distributed throughout tropical and subtropical regions of the world with yellow flowers (Figure 38). It is found mixed with cultivated crops such as soybean, corn etc (Barbosa-Ferreira *et al.*, 2005). Sennoside, anthraquinone glycoside, flavonoid glycosides, sterols, saponins and tannins are some of the chemical compounds that have been isolated from *S. occidentalis*. Fever, menstrual problems, tuberculosis, diuretic anemic, sore eyes, rheumatism, hematuria, asthma, leprosy, skin diseases, blood purifier are some of the conditions that the plant is known to be used for. In addition it is used as an antidote for poisons (Sheeba *et al.*, 2009; Sini *et al.*, 2010; Vijayalakshmi *et al.*, 2013). Anti-inflammatory activities of this plant have also been reported by Basha *et al.*, (2011).



**Figure 38: *Senna occidentalis* (Vijayalakshmi *et al.*, 2013)**

#### **2.4.33 *Solanum nodiflorum***

This is a common weed that belongs to *Solanaceae* family; it is commonly known as white nightshade. It has star-shaped flowers that grow in small clusters at the forks of the upper leaves that ripen to form green to black round berries (Figure 39). Medicinal uses of this plant include treatment of skin diseases, ringworms, diarrhea, heart disease and leprosy. Steroidal alkaloids and anthocyanins are chemical constituents that have been reported so far to be present in this plant (Babalola *et al.*, 2012).



**Figure 39: *Solanum nodiflorum* (Lange, 2006)**

#### **2.4.34 *Sonchus oleraceus***

The plant belongs to the family *Asteraceae*, it is commonly known as sow thistle. It is found in disturbed areas such as oil sites and along roadsides with heavy traffic (Xiong, 1997). Leaves are bluish green in colour (Figure 40) and the stems are hollow and produce a milky sap that can be observed when broken. Sesquiterpene lactones, sesquiterpene glucosides, flavonoid glucosides, luteolin, cynaroside, quercetin are some of the phytochemicals that have been reported to be found in *S. oleraceus*. Traditionally, it is used to treat fever, toothache, stomach disorders, liver disease, inflammation, wounds, sores, rashes, stops bleeding and prevents infection (Singh, 2010; Jimoh *et al.*, 2011; Prichoa *et al.*, 2011). The plant has been shown to possess anti-inflammatory and wound healing properties (Jimoh *et al.*, 2011).



Figure 40: *Sonchus oleraceus* (Reaume, 2010)

#### 2.4.35 *Syzygium cordatum*

Belongs to *Myrtaceae* family, it is commonly known as water berry. It is a medium sized tree that grows upto 15m tall. It has a rough brown bark and the leaves are bluish green, broad and circular (Figure 41). Streams, forest margins, or swampy spots are the places where *S. cordatum* is found. It is found occurring along the stream banks from KwaZulu-Natal (Van Wyk *et al.*, 1997). It is used to treat respiratory ailments, tuberculosis, stomach ache, wounds and diarrhea (Van Wyk *et al.*, 1997; Chalannavar *et al.*, 2011). Musabayane *et al.*,(2005) demonstrated that it can also be used for the treatment of mild diabetes. Triterpenoids, tannins, phenolics, flavonols are some of the constituents isolated from *Syzygium cordatum* (Ndhlala *et al.*, 2008).



Figure 41: *Syzygium cordatum* (Orwa *et al*, 2009)

#### 2.4.36 *Taraxacum officinale*

*Taraxacum officinale* belongs to *Asteraceae* family; it is commonly known as dandelion. This is a perennial herb that is deeply rooted without a stem (Figure 42), the root is thick and the leaves form basal rosettes at the soil level (Cavieres *et al.*, 2008). Among the most important compounds in dandelion are sesquiterpene lactones, phenylpropanoids, triterpenoid saponins and polysaccharides. Sterols, tannins, phenolics and flavonoids have also been reported (Chaitanya *et al.*, 2013). It is used to treat abscesses, reduces eye inflammation, treats liver, gall bladder, kidney disorders, skin disorders and cancer (Yarnell and Abascal, 2009). It has also been reported in the treatment of abdominal complaints, cardiac complaints, migraine, jaundice, breast and uterus cancer (Jeon *et al.*, 2008; Chaitanya *et al.*, 2013) as well as controlling burning sensation of urine, treatment of menstrual pain, respiratory infections, toothaches, diabetes, constipation and swelling (Martinez *et al.*, 2015). Anti-inflammatory and antioxidant properties have been demonstrated (Jeon *et al.*, 2008).



Figure 42: *Taraxacum officinale* (Hurinanthan, 2009)

#### 2.4.37 *Tetradenia riparia*

*Tetradenia riparia* is a dioecious shrub that belongs to *Lamiaceae* family; it is also known as ginger bush. It grows upto 3m high in wooded hillsides and stream banks. The plant occurs from KwaZulu-Natal south coast through to Swaziland, Mozambique, Zimbabwe and Malawi (Phillipson and Steyn, 2008). The leaves are fuzzy green and pungent (Figure 43). Diterpenes, phytosterols,  $\alpha$ -pyrones are the major chemical constituents isolated from *T. riparia*. Traditionally it is used for the treatment of colds and flu, bronchitis, stomach

upsets, mouth ulcers, diarrhea, fevers and headaches. Wound healing potential of *Tetradenia riparia* has been investigated (Coopoosamy and Naidoo, 2011).



**Figure 43: *Tetradenia riparia* (Aubrey, 2001)**

#### **2.4.38 *Tulbaghia violacea***

Commonly known as wild garlic, it belongs to *Alliaceae* family. It is originally found in KwaZulu-Natal and Eastern Cape in South Africa. It is a small bulbous herb that grows in rocky grasslands (Kubec *et al.*, 2002). It grows very fast upto a height of 0.5m in dry soil (Olorunnisola *et al.*, 2012). The leaves are bluish grey with shoots of a lavender purple flower (Figure 44). It is used to treat fever, colds, asthma, stomach problems, oesophageal cancer, tuberculosis, gastroenteritis, abdominal pain, acute inflammation and also as a snake repellent (Bungu *et al.*, 2008). According to Hutchings *et al.*, (1996); steroidal saponins have been isolated from this plant. Biological activities of this plant including anti-inflammatory and antioxidant properties have been demonstrated (Zheng and Wang, 2001; Gaidamashvili and Van Staden, 2002).



**Figure 44: *Tulbaghia violacea***  
(<http://stores.ebay.com.au/exotictropicalplants>)

The following table (Table 1) is a summary of all the plants that were investigated, their chemical constituents, that gives them the properties they have to alienate diseases or inhibit some pathways or processes leading to specific conditions and also the documented medicinal uses for these plants in rural areas.

**Table 1: List of plant species used in this study and their medicinal uses**

<b>Plant Species</b>	<b>Bioactive compounds</b>	<b>Traditional medicinal use</b>	<b>References</b>
<i>Achyranthes aspera</i>	Achyranthine, glucosides, saponins, flavonoids, alkaloids, steroids, tannins	Stomach complaints, boils, menstrual bleeding, coughs, abscesses, chest pain, wounds piles, itching, blood diseases, vomiting, abdominal pains and snake bites	Iwalewa <i>et al.</i> , 2007 Edwin <i>et al.</i> , 2008 Ahmed and Nahor, 2012 Bhosale <i>et al.</i> , 2012
<i>Alternanthera sessilis</i>	Carotenoids, steroids, alkaloids, flavonoids, tannins saponins, terpenoids	Diarrhea, dysentery, intestinal cramps, fever, wounds, congestion asthma and snake bites	Iwalewa <i>et al.</i> , 2007 Singh, 2009 Kumarasamyraja <i>et al.</i> , 2012 Mrinmay and Ashok, 2014
<i>Amaranthus dubius</i>	Saponins, phytosterols, squalene polyphenols	Anaemia, osteoporosis, fever, stomach ache, haemorrhage	Quintero <i>et al.</i> , 2011 Alegbejo, 2013
<i>Amaranthus hybridus</i>	Alkaloids, flavonoids, saponin, tannins, phenols, hydrocyanic acid, ptytic acid	Ulcers, Dysentery, hemorrhage, Swelling, Antidote for snake and scorpion bites, intestinal bleeding and excessive menstruation	Shah <i>et al.</i> , 2006 Akubuywo <i>et al.</i> , 2007 Singh and Sheoran, 2011 Omodamiro and Jimoh, 2015
<i>Amaranthus spinosus</i>	Alkaloids, glycosides, saponins, phytosterols, phenolics, flavonoids, tannins	Ulcerated mouth, vaginal discharge, nose bleeds, wounds, fever, urinary troubles, sores, boils, eczema, burns	Gul <i>et al.</i> , 2011 Antara, 2012 Alegbejo, 2013 Sudha and Mathangi, 2013
<i>Asystasia gangetica</i>	Alkaloids, tannins, steroidal aglycones, saponins,	Swellings, fever, epilepsy, stomach aches, heart	Akah <i>et al.</i> , 2003 Mohan <i>et al.</i> , 2010

	flavonoids, triterpenoids	disease, snake-bite, intestinal worms, asthma	Hamid <i>et al.</i> , 2011 Kumar <i>et al.</i> , 2011
<i>Bidens pilosa</i>	Chakones, polyacetylenes, flavonoids, terpenoids, tannins, phenylpropanoids	Jaundice, eye, colic, bronchial problems, rheumatism, inflammation, digestive disorders, wounds diabetes, hepatitis, headache, kidney problems, diarrhea, dysentery, infectious diseases	Jager and Van Staden, 2005 Horiuchi and Seyama, 2006 Iwalewa <i>et al.</i> , 2007 Ezeonwumelu <i>et al.</i> , 2011 Silva <i>et al.</i> , 2011 Bartolome <i>et al.</i> , 2013
<i>Buddleja saligna</i>	Tannins, saponins, alkaloids, reducing sugars, flavonoids	Colic, coughs, sore eyes, urinary problems, colds, sores, thrush, diabetes, tuberculosis	Verdoon, 1963 Adedapo et al, 2009
<i>Bulbine natalensis</i>	Tannins, anthraquinones, saponins glycosides, flavonoids, alkaloids	Wounds, burns, rashes, itches, cracked lips, ringworm, herpes, vomiting, diarrhea	Yakubu and Afolayan, 2009 Coopoosamy, 2011 Mosa <i>et al.</i> , 2011
<i>Carpobrotus dimidiatus</i>	Tannins, malic acid, hyperoside, citric acid	Wounds, burns, toothache, sore throat, thrush, diarrhea, dysentery, digestive troubles, It has skin problems such as sores and rashes	Nonjinge, 2007 Hurinanthan, 2009 Fawole <i>et al.</i> , 2010
<i>Centella asiatica</i>	Saponins, Alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids	Skin conditions, asthma, blood disorders, bronchitis, fever, urinary discharge, improves memory, anemia	Brinkhaus <i>et al.</i> , 2000 Anilkumar, 2010 Chippada <i>et al.</i> , 2011 Vangalapati and Chippada, 2011 Ruszymah <i>et al.</i> , 2012
<i>Ceratotheca triloba</i>	Phlobatamins, saponins,	Stomach cramps, nausea,	Mohanlall, 2010

	steroids, terpenoids	fever, diarrhea, painful menstruation, insect infestations of the skin	Mohanlall <i>et al.</i> , 2011 Naidoo <i>et al.</i> , 2012
<i>Chenopodium album</i>	Flavonoid, alkaloids, isoflavonoid, polyphenol, saponins	Laxative, antihelmintic, blood purifier, intestinal ulcers, burns, enhances memory, improves appetite, cough, diarrhea, oedema, eye diseases	Khoobahandani <i>et al.</i> , 2009 Usman <i>et al.</i> , 2010 Agrawal <i>et al.</i> , 2014
<i>Cleome monophylla</i>		Swellings, headache, sores, cough, ulcers, wounds	Jansen, 2004a
<i>Dichrostachys cinerea</i>	Alkaloids, saponins, triterpenoids, $\beta$ -amyrim, $\beta$ -sitosterol, flavonoids, tannins	Abdominal pains, diarrhea, coughs, bacterial infections, headache, toothache, leprosy, dysentery, inflammations, fever, asthma, bodyaches, chest problems, ulcers, wounds and eye diseases	Jager and Van Staden, 2005 Iwalewa <i>et al.</i> , 2007 Mishra <i>et al.</i> , 2009 Babu <i>et al.</i> , 2011
<i>Erkebegia capensis</i>	Saponins, steroid, glycosides, flavonoids, triterpenoids	Coughs, chest pains, headache, dysentery fever, cough, diarrhea, heartburn, abscesses, boils and pimples	Spatula, 2012 Irungu <i>et al.</i> , 2014 Iwalewa <i>et al.</i> , 2007 Murata, 2008
<i>Elytropappus rhinocerotis</i>	Cardiac glycosides, saponins, tannins, reducing sugars saponins, tannins	Indigestion, dyspepsia, ulcers, stomach cancer, appetite stimulant, bitter tonic, fever, stimulate sweating, diarrhoea	Levyns, 1935 Van Wyk, 2008 Gakuba, 2009
<i>Emex australis</i>	No information available related to its chemical constituents or biological activity		
<i>Ficus sur</i>	flavonoid glycosides, alkaloids, phenolic acids, steroids, saponins, coumarins, Tannins, triterpenoids	Skin diseases, wounds, cough, influenza, toothache, sore throat, stomach and chest pains	Eldeen <i>et al.</i> , 2005 Lumbile and Mogotsi, 2008 Sirisha <i>et al.</i> , 2010
<i>Galinsoga parviflora</i>	No information available related to its chemical constituents	Wounds, scurvy bleeding, colds and flue, skin problems, diarrhoea	Yadav and Tangpu, 2008 Bazylo <i>et al.</i> , 2012
<i>Guilleminea densa</i>	No information available	Treatment of diarrhea,	Mathabe <i>et al.</i> , 2006

	with regards to its chemical constituents	nausea	Sibandze, 2009
<i>Gunnerea perpensa</i>	Phenylpropanoid glucoside (Z-venusol), celastrin, succinic acid, lactic acid, benzoquinones	Cancerous sores, facilitates child-birth, painful joints, rheumatism, swellings, stomach ailments, menstrual pain, wounds, and psoriasis	Watt and Breyer-Brandwyk, 1962 Van Wyk, 1997 Iwalewa <i>et al.</i> , 2007 Simelane <i>et al.</i> , 2010
<i>Heteropyxis natalensis</i>	$\beta$ -ocimene, 1.8-cineole, limonene, linalool	Colds, weaning, roots for nose bleeds, bleeding gums, menorrhagia, respiratory disorders	Frum, 2006 Iwalewa <i>et al.</i> , 2007 Van Vuuren and Viljoen, 2008 Van Wyk, 2008
<i>Hibiscus sabdariffa</i>	Photocatechuic acid, anthocyanin, alkaloids, tannins, saponins, flavonoids	Abscesses, bilious conditions, cancer, cough, fever, neurosis, dyspepsia, hypertension, hangover, prevention of cardiovascular disease	Christian <i>et al.</i> , 2006 Reanmangkol and Itharat, 2007 Mungole and Chaturvedi, 2011
<i>Justicia flava</i>	Tannins,steroids, flavonoids, alkaloids	Swellings, oedema, gout, menstrual pains, pulmonary troubles, diarrhea, dysentery, nasopharyngeal infections  coughs, paralysis, fever, epilepsy, skin infections, and disorders	Burkill, 1985 Agyare <i>et al.</i> , 2013
<i>Leonotis leonurus</i>	Diterpenoids, leonurine flavonoid aglycones, phenolic compounds, sterols, tannins, alkaloids, saponins	Coughs, colds, influenza, asthma, bronchitis, high blood pressure, headache, viral hepatitis, skin disorders and cramps, dysentery tuberculosis, jaundice, sores, bee and scorpion stings, menstrual disorders, internal	Kenechukwu, 2004 Iwalewa <i>et al.</i> , 2007 Van Wyk, 2008 Maphosa <i>et al.</i> , 2012 Mazimba, 2015

		parasites in animals, wounds, diabetes, obesity, cancer, piles, arthritis, bladder and kidney disorder and hypertension	
<i>Mormodica balsamina</i>	Tannins, saponins, diterpenes, lectins, glycosides, steroids	Regenerate blood lost during labour, purify milk, skin disease, tranquillizer in mental illness, healing wounds, leprosy, piles, jaundice, gout, rheumatism	Geidam <i>et al.</i> , 2004 Hassan and Umar, 2006 Ilango <i>et al.</i> , 2010
<i>Oxygonum sinuatum</i>	No information available related to its chemical constituents	Boils, tonsillitis, fungal infections, eye infections	Jansen, 2004b
<i>Perlagonium</i>	Phenolic, compounds, cinnamic acids, flavonoids, coumarins, tannins, alkaloids, terpenoids	Skin disorders, coughs, gastroenteritis, fever tuberculosis, Diarrhea, dysentery, hemorrhage, tonsillitis, gastroenteritis, kidney and bladder disorders, coughs	Saraswathi, 2011 Avila <i>et al.</i> , 2013 Boukhatem <i>et al.</i> , 2013
<i>Physalis viscosa</i>	Withanolides, phytosterols, flavonol glycoside	fever, gout, malaise, wounds inflammations of the bladder, sore throat, earache, diabetes, asthma, hepatitis, abdominal pain, boils, sores, constipation	Silva <i>et al.</i> , 1993 Mahalakshmi and Nidavani, 2014
<i>Portulaca oleracea</i>	Flavonoids, coumarins, monoterpene, alkaloids, saponins, tannins, anthraquinones, cardiac glycosides	Gastric sedative, bacillary dysentery, dysuria, mastitis boils, impetigo sore nipples, ulcers of the mouth, fevers, abscesses, swellings and urinary disorders, inflammation of the eyes	Chan <i>et al.</i> , 2000 Sonja <i>et al.</i> , 2009 Masoodi <i>et al.</i> , 2011 Lee <i>et al.</i> , 2012
<i>Senna occidentalis</i>	Sannoside, Anthraquinone, Glycoside, Flavonoid glycosides, Tannins sterols, saponins, alkaloids,	Fever, menstrual problems, tuberculosis, Diuretic anemic, Sore eyes, Rheumatism, Asthma,	Sheeba <i>et al.</i> , 2009 Sini <i>et al.</i> , 2010 Basha <i>et al.</i> , 2011 Vijayalakshmi

	terpenes	Leprosy, haematuria skin diseases, blood purifier, an antidote for poisons	<i>etal.</i> ,2013
<i>Solanum nodiflorum</i>	Steroidal alkaloids, anthocyanins	Painful abdominal upset, Toothache, Ulcers, Coughs Asthma, Rheumatism Swellings, ringworms, diarrhea, heart disease and leprosy	Iwalewa <i>et al.</i> , 2007 Babalola <i>et al.</i> , 2012
<i>Sonchus oleraceus</i>	Sesquiterpene lactones, sesquiterpene, flavonoid glycosides, luteolin, cynaroside, saponins, steroids, alkaloids, tannins, flavonoids, coumarins	Fever, Inflammation, Detoxifies blood, toothache, stomach disorders, wounds, sores, rashes, stops bleeding and prevents infection	Singh, 2010 Jimoh <i>et al.</i> , 2011 Prichoa <i>et al.</i> , 2011
<i>Syzygium cordatum</i>	Phenylpropanoids, flavonoids, sesquiterpenes oleanolic acid, tannins, phenolics	Coughs, diarrhea, tuberculosis, stomach ache respiratory ailments, wounds, diabetes	Van Wyk <i>et al.</i> , 1997 Ndhlala <i>et al.</i> , 2008 Chalannavar <i>et al.</i> , 2011 Musabayane, 2012
<i>Taraxacum officinale</i>	Sesquiterpene lactones, phenylpropanoids, triterpenoid, saponins, sterols, tannins, phenolics, flavonoids	Abscesses, reduces eye inflammation, treats liver, gall bladder, kidney disorders, skin disorders and cancer , abdominal complaints, cardiac complaints, migraine, jaundice, breast and uterus cancer, controls burning sensation of urine, menstrual pain, respiratory infections, toothaches, diabetes, constipation, swelling	Jeon <i>et al.</i> , 2008 Yarnell and Abascal, 2009 Chaitanya <i>et al.</i> , 2013 Martinez <i>et al.</i> , 2015
<i>Tetradenia riparia</i>	Diterpenoids, phytosterols, $\alpha$ -pyrores	Coughs, Sore throat, Malaria, fever, Stomach	Iwalewa <i>et al.</i> , 2007 Cooposamy and

		upsets, colds and flu, bronchitis, mouth ulcers, diarrhea, fevers, headaches	Naidoo, 2011
<i>Tulbaghia violacea</i>	Alkyl cysteine sulfoxides, flavones, flavonols, steroidal saponins	Fever, Headache, Painful joints, Asthma, tuberculosis colds, stomach problems, oesophageal cancer, gastroenteritis, abdominal pain, acute inflammation, snake repellent	Hutchings <i>et al.</i> , 1996 Iwalewa <i>et al.</i> , 2007 Bungu <i>et al.</i> , 2008 Van Wyk, 2008

## 2.5 Bioactive compounds with anti-inflammatory and wound healing properties

As shown in Table 1, there are some common secondary metabolites that are found in the investigated plant species, which may be responsible for several biological activities mentioned in the table. These plant compounds have been found to have an ability to act as anti-inflammatory and wound healing agents. Flavonoids, triterpenoids, sterols, tannins, alkaloids, chalcone, anthraquinone, sesquiterpenoids, curcumin, coumarins, polyphenolic compounds, carotenes, limonoids, are some of the secondary metabolites isolated from plants capable of modifying the activities of inflammatory cells (Iwalewa *et al.*, 2007). This is the major group of phenolic compounds (Okem, 2011). Below are some of these secondary metabolites discussed in detail.

### 2.5.1 Phenolic compounds

Phenolics are compounds that have an aromatic ring with a hydroxyl group/substitute. They include those metabolites derived from the condensation of acetate units and those produced by conversion of aromatic amino acids. Phenolic compounds from plants are produced by the phenylpropanoid pathway and they represent a large group of defensive compounds that have a phenol moiety. They are the most widely occurring amongst all phytochemicals. They range from phenolics, quinones, chalcones, stilbenes, anthocyanins, anthochlors, benzofurans, chromones, coumarins, flavonoids, isoflavonoids, neoflavonoids, stilbenoids and tannins (Okem, 2011). They exhibit a wide range of physiological properties such as anti-allergenic, anti-atherogenic, anti-inflammatory,

antimicrobial, antioxidant, antithrombotic, cardioprotective and vasodilatory effects (Aberoumand and Deokule, 2008).

Sterols and polyphenols have been reported to exhibit antioxidant activities therefore, they scavenge free radicals thereby reducing lipid peroxidation which in turn reduces cell necrosis and improves vascularity (Mittal *et al.*, 2013).

#### **2.5.1.1 Flavonoids**

These are the major group of phenolic compounds that are widely distributed in the plant; they are found in different parts of the plant such as in fruits, seeds, flowers, leaves etc. Except for forming a part of the normal diet of humans they are also known to play a major role in treating medical conditions. They have biological activities including anti-inflammatory, antioxidant activities and wound healing properties. When an injury occurs an increase in the production of reactive oxygen (ROS) species is observed and this overproduction of ROS affects the wound healing process. Microbial infection also adds to the problem. Flavonoids have been reported to improve vascularity by reducing lipid peroxidation in that way increasing the strength of collagen fibres. They also prevent cell damage and act as antimicrobials; therefore, promoting wound healing process by increasing wound contraction and rate of epithelialisation (Ghosh and Gaba, 2013; Mittal *et al.*, 2013). Two phenolic rings are observed on the structure of flavonoid, these rings are linked through a heterocycle ring. The structure is made up of 15 carbons. Flavones, flavonols, flavanones, chalcones, flavonols, anthocyanidins, isoflavones and dihydroflavonols are the major classes of flavonoids (Bodenstein and Du Toit, 2012). They have been shown to have similar activities to synthetic non-steroidal anti-inflammatory agents; therefore, certain members of flavonoids can be used to treat inflammatory pain disorders (Iwalewa *et al.*, 2007). They have also been suspected to decrease the release of histamine from the inflammatory cells— basophils and mast cells.

#### **2.5.1.2 Tannins**

Tannins are also part of phenolic compounds; they act as astringents and are found in herbs used for healing purposes. Their astringent property is mainly for wound contraction and increasing rate of epithelialisation (Tsala *et al.*, 2013). They have also been found to

act as free radical scavengers and have antimicrobial properties thereby promoting wound healing process (Ghosh and Gaba, 2013). Tannins also cause a local anti-inflammatory effect by promoting capillary vasoconstriction which in turn decreases vascular permeability (Edwin *et al.*, 2008). They play a major role in wound healing.

### **2.5.2 Terpenes and terpenoids**

Terpenes contain one or more double bonds. These hydrocarbons are a result of condensation of isoprene units. Monoterpenes and sesquiterpene are the two categories of terpenes. Besides being linear, terpenes can be monocyclic or bicyclic molecules. In medicinal plants, the monoterpenes and sesquiterpenes are widely distributed and the sesquiterpene constitutes secondary metabolites that may exhibit stress-induced compounds. Terpenoids are terpenes that consist of oxygen analogues. A methyl group is replaced or shifted by an oxygen molecule in various functional groups. These oxygenated compounds belong to a number of different chemical groups including alcohol, aldehydes, esters, ketones, oxides and phenols (Seatholo, 2007). These compounds have been reported to be responsible for wound contraction and a high rate of epithelialization; this is due to their astringent and antimicrobial activity (Mittal *et al.*, 2013).

#### **2.5.2.1 Saponins**

These are glucosides of triterpenes or steroids, which include the group of cardiac glycosides and steroidal alkaloids. They have water-soluble sugar residues linked therefore they can either be hydrophilic or hydrophobic. Saponins are non-volatile compounds that are widely spread in all plant species. *Liliaceae* family is where steroidal saponins are commonly found. Upon wounding  $\beta$ -glucosidase enzyme breaks down the saponins stored in the plant vacuole to monodesmosidic compounds (Okem, 2011). They are referred to as steroidal glycosides and triterpenes consisting non-polar aglycones coupled with one or more monosaccharide moieties. They have soap like behaviour in aqueous solutions. The steroidal saponins have 3 methyl groups removed while triterpenoid saponins have all 30 carbon atoms retained. They are used in medicine as a health tonic, aphrodisiac and galactagogue (Rohit *et al.*, 2012). These compounds have also been reported to be responsible for wound contraction and a high rate of epithelialization; this is due to their antioxidant and antimicrobial activity (Mittal *et al.*, 2013).

### 2.5.3 Alkaloids

These chemical compounds contain nitrogen and they are of basic nature (Ameyaw and Duker-Eshun, 2009). Classes of alkaloids include pyridine, pyrrolidine, tropane, indolizidine, quinoline, isoquinoline, phenanthrene, phenethylamine, indole, purine and terpenoid groups. They differ in a way they exist in plant cells, some exist in free form, as organic salts form and a few exist in inorganic salt form (Yubin *et al.*, 2014). Organic solvents are usually used for their extraction especially the free and salt alkaloids (Woolley, 2001) however organic salts are usually more water soluble. The complex cyclic structure that they possess provides them with the significant biological activities including antimalarial, antimicrobial, antihyperglycemic, anti-inflammatory and pharmacological effects (Ameyaw and Duker-Eshun, 2009). They are suspected to promote early phases of wound healing. They increase migration of fibroblasts to the wounded site thereby accelerating healing process (Tsala *et al.*, 2013).

### 3. METHODOLOGY

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#### 3.1 Collection and preparation of plant material

Plant species were collected from Durban, KwaZulu-Natal province of South Africa. Once collected they were identified and verified using taxonomic keys by Professor H. Baijnath and deposited at the Ward Herbarium University of KwaZulu-Natal as voucher specimens. Leaves from the plant specimens were removed and washed with distilled water to get rid of any foreign material that might be present and they were dried in an oven (Memmert B. Owen Jones limited, South Africa) at 25°C for 24-48 hours (Jeremy and Whiteman., 2003). Once samples were dry they were milled to a fine powder with a grinder (Retsch GmbH, West Germany) and stored in labelled Schott bottles in a cool dark place until further use.

#### 3.2 Preparation of crude plant extracts

The solvent used during the extraction procedure is important to obtain successful isolation of compounds from plant materials. Water is a solvent mainly used by traditional healers and apart from water, methanol is a solvent chosen for the extraction of many medicinal plants because it has shown to provide more consistent anti-inflammatory activities when compared to other known solvents (Lin *et al.*, 1999). Therefore, aqueous and methanolic extracts of the dried plant material were prepared according to the procedure outlined by Jeremy and Whiteman, (2003) with minor modifications.

##### 3.2.1 Aqueous extracts

Approximately 50 g of dried plant material was added to 200 ml distilled water, this mixture was stirred for 24-48 hours in a shaker (Infors HT, Germany). The resulting mixture was then centrifuged at 8000 rpm for 10 minutes (Eppendorf centrifuge 5810R, Germany), it was then filtered through Whatman No. 1 filter paper and the supernatant was collected. The supernatant was freeze-dried (Virtis Benchtop Freeze Dryer) after being frozen in a biofreezer (Snijders Scientific, Holland) at -80°C. This was done to concentrate the sample. The material that resulted after freeze drying was used as a stock and working solutions were prepared from it for testing.

### **3.2.2. Methanolic extracts**

Approximately 50 g of dried plant material was added to 80% methanol, this mixture was stirred for 24-48 hours in a shaker (Infors HT, Germany). The resulting mixture was filtered through Whatman No.1 filter paper and the supernatant was collected. Buchi RE Rotoevaporator was used to concentrate the sample by removing the solvents through evaporation. The rotor evaporator was connected to a water bath (Buchi 461) set at a temperature of 50°C to facilitate the evaporation process. The supernatant was freeze-dried (Virtis Benchtop Freeze Dryer) after being frozen in a biofreezer (Snijders Scientific, Holland) at -80°C. The resulting sample after freeze drying was used as a stock and working solutions were prepared from it for testing.

The methanolic plant extracts were diluted in either methanol and the aqueous extracts were diluted in distilled deionized water to give concentrations of 1000 µg/ml, 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml and 10 µg/ml.

### **3.3 Anti-inflammatory activity using lipoxygenase assay**

Lipoxygenase inhibitor screening assay kit (Cayman Chemical, USA) was used to assess the anti-inflammatory activity of plant extracts as outlined by Sircar *et al.*, (1983) and Evans (1987).

#### Principle of the method

The enzyme 5-lipoxygenase catalyses the oxidation of unsaturated fatty acids containing 1-4pentadiene structures. The enzyme has arachidonic acid as its substrate but it can also accept linoleic acid. Linoleic acid is converted to hydroperoxy linoleic acid, which is then evaluated by the appearance of a conjugate diene at 234 nm using a spectrophotometer. Therefore, 5-lipoxygenase activity is evaluated by the by-product at 234 nm. A decrease in the initial reaction rate is an indication of the inhibitory activity of the sample (Baylac and Racine, 2003).

## Protocol

Nordihydroguaiaretic acid (NDGA) known inhibitor of soybean lipoxygenase, was used as a control. An assay buffer was used throughout the assay and Arachidonic acid was used as a substrate. Assay buffer was used to dissolve the samples to produce a starting concentration of 1000 µg/ml. The standard assay mixture was prepared by adding 10 µl of plant extract solution, to 90 µl of lipoxygenase enzyme mixed in diluted Assay Buffer (pH 7.4). The negative controls were prepared by adding 90 µl of lipoxygenase enzyme with a solvent without the addition of the plant extracts. This was done in a 96-well plate (Greiner, Germany).

The addition of 10 µl of Arachidonic Acid (substrate) diluted with 25 µl of potassium hydroxide and 950 µl of HPLC-grade water initiated the reaction. After the addition of a substrate the 96-well plate was placed in a shaker for five minutes. 100 µl of Chromogen was prepared by mixing equal volumes of developing reagent 1 and developing reagent 2 was added to each well to stop enzyme catalysis and develop a reaction. The 96-well plate was then covered with a plate cover and placed on a shaker (IKA MTS digital, Germany) for five minutes. The plate cover was then removed and the absorbance was read at 490-500 nm using an ELISA plate reader (Digital Analogues Systems, Italy). The percentage inhibition of the enzyme activity was calculated by comparing with the control using the following equations:

$$\% \text{ Inhibition} = \frac{[\text{IA} - \text{Inhibitor}]}{\text{IA}} \times 100$$

Where IA represents 100% Initial activity

### **3.4 Antioxidant activity using DPPH radical scavenging assay**

The ability of the plant extracts to have free radical scavenging activity was determined using 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH) assay described by Blois, (1958) with minor modifications.

A sample stock solution (1 mg/ml in methanol) was prepared. Different concentrations (500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml, and 10 µg/ml) were made by diluting the stock solution with methanol. Rutin found in the buckwheat plant *Fagopyrum esculentum* was used as a positive control.

Four hundred microliters of 0.1 mM DPPH in methanol was added to 1 ml of various concentrations of the test solution, methanol and Rutin in test tubes. Methanol with DPPH served as a negative control and Rutin with DPPH served as a positive control. 1 ml of methanol plus plant extract was used as a blank. The reaction mixtures were allowed to react at room temperature in the dark for 30 minutes. Varian Cary 1E UV-Visible spectrophotometer (Biochrom Libra S21, Cambridge) was used to measure the absorbance values at 518 nm. Each sample was evaluated in triplicate. The absorbance decreases with increasing free radical scavenging activity. The percentage inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

### 3.5 Safety evaluation of plant extracts

#### 3.5.1 Brine shrimp lethality assay

The safety of the extracts was evaluated using brine shrimp larvae test described by Meyer *et al*, (1982) with few modifications.

Artificial seawater was prepared by mixing (23g NaCl, 11g MgCl<sub>2</sub>.6H<sub>2</sub>O, 4g Na<sub>2</sub>SO<sub>4</sub>, 1.3g CaCl<sub>2</sub>.2H<sub>2</sub>O, 0.7gKCL) with 1 L of distilled water. The mixture was stirred using a magnetic stirrer (Stuart heat-stir, USA) and a stir bar to dissolve the contents. The pH of the seawater was adjusted to 9.0 using Na<sub>2</sub>CO<sub>3</sub> to avoid the risk of death of *Artemia* larvae. Twenty-five milligrams of class C *Artemia salina* eggs (Natures Petland, Durban, South Africa) was added to about 250 ml of prepared artificial seawater in a hatching chamber. This was incubated at room temperature for 24 hours. After 24 hours, 1.5 ml of yeast solution was added to the hatching chamber to feed the larvae. After 48 hours of incubation, the moving larvae were visibly counted and 10 larvae of brine shrimp were placed in each well of a 24-well plate and filled up with artificial seawater to about 5 ml. A drop of yeast suspension (3 mg in 5 ml seawater) was added each well. One hundred microlitres of plant extracts at various concentrations (1000 µg/ml, 500 µg/ml, and 100 µg/ml) was assayed in triplicate. Artificial seawater with methanol was used as a control.

The plates were then incubated at room temperature for 24 hours after which dead larvae were counted and percentage death determined by the following equation:

$$Mm_{ct} = \frac{N_{Mm}}{N_o} \times 100$$

Where  $Mm_{ct}$  is the mortality of individuals in time  $t$  [%],  $N_{Mm}$  is average number of died individuals,  $N_o$  is initial number of living individuals put into every concentration at the test start.

### **3.5.2 Cytotoxicity**

#### *1. Culturing of cells*

The mouse fibroblast cell line (3T3 NIH) was used in this study. The cells were kindly supplied by Dr C. Snyman (the University of KwaZulu-Natal, School of Botany/Biochemistry, South Africa) maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal bovine serum and antibiotics (Pen/Strep) kept in T-flasks (Greiner, Germany). Upon arrival cells were viewed under the inverted microscope (Nikon TMS, Japan) to check for viability. The cells were then incubated at 37°C in a humidified incubator (Snijders Hepa, United Scientific group, Cape Town South Africa) with 5% CO<sub>2</sub>. Once they were 80% confluent they were subcultured and the stock cultures were stored in a bio-freezer at -80°C until required for use. Cell culturing procedures were performed under laminar flow cabinet containing UV light (Scientific Engineering INC) and the cabinet was sterilized with 70% ethanol (Merck, South Africa) before being used.

#### *2. Maintenance of cells*

Maintenance of cells was performed according to the protocol of Freshney, (1987). Filter sterilized (0.22 µm) Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum supplemented with antibiotics (penicillin, 100 U/ml and streptomycin 100 U/ml) was used to aseptically culture the cells into 75 cm<sup>2</sup> tissue culture flasks (T75) (Greiner, Germany). Cells were then incubated in a humidified incubator (Snijders Hepa, United Scientific group, Cape Town South Africa) under 5% CO<sub>2</sub> atmosphere at 37°C. The culture flasks were examined for colour changes and turbidity in the media on a daily basis and for cell growth. The media were changed every second day. When the cells were 80% confluent, flasks were rinsed with phosphate buffered saline (PBS) and cells were

enzymatically detached with Trypsin-EDTA solution for five minutes. Trypsin is inactivated using serum-containing medium; therefore, the medium was added. And cell suspension was concentrated by centrifugation at 1.2 rpm for 3 minutes (Eppendorf centrifuge 5702, Germany). The cell pellet was resuspended in a small volume of fresh media (DMEM containing antibiotics and 10% FBS). The resuspended cells were then divided into separate flasks containing fresh culture media to produce a single cell suspension. These flasks were then incubated in a humidified incubator (Snijders Hepa, United Scientific group, Cape Town South Africa) at 37°C with 5% CO<sub>2</sub>.

### *3. Storage of the cells*

Cells were pelleted and washed twice with pre-warmed phosphate buffered saline, pH 7.2 and cooled on ice. 1 ml DMSO, 7 ml DMEM and 2 ml FBS solution was prepared as a cryoprotecting agent; this was also placed on ice. About 3 ml of the cryoprotecting agent were added to the pellet. Equal volumes of cell suspension were added to cryotubes (Corning, South Africa). The tubes were transferred to thermos flask and kept overnight at -20°C. The cells were then transferred to a -80°C bio-freezer and stored.

### *4. Regeneration of cells*

As described by Freshney, 1987. Cells were removed from the -80°C, biofreezer; they were rapidly thawed in a 37°C waterbath (Julabo TW2, Germany). The vials were sprayed with 70% ethanol and placed under the laminar. Cells were transferred to T75 culture flasks containing complete media. The cells were viewed under the inverted microscope (Nikon TMS, Japan) and incubated at 37°C in a humidified incubator (Snijders Hepa, United Scientific group, Cape Town South Africa) with 5% CO<sub>2</sub>.

### *5. Effect of plant extracts on 3T3 NIH fibroblast cells*

Effect of both aqueous and methanolic plant extracts on proliferation and viability of 3T3 NIH cells was assessed by using 3-(4, 5-dimethylthiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric according to protocols described by Mossmann, 1983. The test is based on the ability of cells to reduce the yellow dye MTT to a dark purple formazan product. Basically, the MTT reagent enters the cells and passes into the mitochondrion where it is reduced to an insoluble, dark purple formazan product. After the cells are

solubilized in an organic solvent, the solubilized formazan product can then be measured spectrophotometrically.

To seed the cells they were first trypsinized (by adding 4 ml of trypsin to the flasks and incubated for 2 minutes) and then transferred to centrifuge tubes to concentrate the cells by centrifuging at 1.2 rpm for 3 minutes to obtain a pellet. The acquired pellet was then resuspended in the small volume (2 ml) of fresh DMEM (with 1% antibiotics and 10% FBS). Equal volumes of cell suspension and trypan blue [Biowhittaker, Wakersville (USA)] (v/v 1:1) were mixed and the cell density to be seeded was counted using a haemocytometer. Only the viable (translucent) cells that lay within or that touched, the left or top boundary was counted. Cells were seeded at a density of  $1 \times 10^4$  cells/well in triplicate by diluting the cell concentration using DMEM with 10% FBS, after which 100  $\mu$ l of the cell suspension was dispensed into 60 wells of a 96 well plate (Cellstar, Greiner, Germany). The cells were incubated for 24 hours at 37°C and 5% CO<sub>2</sub> to allow them to adhere to the bottom of the wells before treatment begins. A stock solution of 1mg/ml plant extracts diluted in phosphate buffered saline, pH 7.2 was prepared and further 10 fold-dilutions were prepared from it to obtain desired concentrations (100  $\mu$ g/ml and 50  $\mu$ g/ml) for each well. The sample extracts were filtered through a 0.22  $\mu$ m membrane filter and 20  $\mu$ l of the extracts at different concentrations were added to each well. Cells with untreated cells were used as negative controls and Camptothecin was used as a positive control. Sterile distilled water was added to the outer row of wells. The 96 well plate was then incubated at 37°C and 5% CO<sub>2</sub> for further 48 hours. After 48 hours of incubation, the supernatant was replaced with 20  $\mu$ l of MTT solution (5 mg/ml in phosphate buffered saline, pH 7.2) (Sigma, St Louis, USA) and incubated for 4 hours at 37°C and 5% CO<sub>2</sub>. After 4 hours of incubation, 100  $\mu$ l of Dimethyl sulfoxide (DMSO) was added to each well to solubilize the deposited formazan formed at the bottom of each well and further incubated for 1 hour at 37°C and 5% CO<sub>2</sub>. The absorbance values were read at 570 nm using a microplate reader, the percentage of viable cells at particular concentrations of extract was calculated by using the following formula:

$$\% \text{ Viability} = \frac{A_T}{A_C} \times 100$$

Where  $A_T$  is the absorbance of treated cells and  $A_C$  is the absorbance of untreated cells at 570nm.

### 3.6 Scratch wound assay

Since the proliferation of fibroblast cells is important in tissue repair, a useful method to test for wound healing activity *in vitro* is essential. Scratch wound assay is a test characterized by a scratch “wound” created on the cell monolayer. The test is based on the migration of cells to cover a wounded area. The cell monolayer increases the concentration of growth factors and cytokines at the wound edge (Balekar *et al.*, 2012) therefore enabling cells to move toward the opening to close the gap until new cell-cell contacts are created again. This assay is said to mimic to a certain level migration of cells *in vivo*. The assay was performed according to the previously reported and standardized protocol by Liang *et al.* (2007). 3T3 NIH fibroblast cells were used for this assay.

The assay was carried out in 24 well, flat-bottomed microtitre plates (Costar, Corning, USA). The plates were marked with horizontal lines using a sharp object to trace the point of observation. The cells were trypsinized and a concentration of  $2 \times 10^5$  cells/ml (in DMEM containing 10% FBS) were seeded in each well. The cells were incubated in a humidified incubator (Snijders Hepa, United Scientific group, Cape Town South Africa) under 5% CO<sub>2</sub> at 37°C overnight. The cells were then examined under an inverted microscope (Nikon TMS, Japan) for the formation of a confluent monolayer. Once the confluent monolayer was formed, media was discarded and a linear scratch was created in a monolayer using a sterile 100 µl pipette tip from top to bottom of culture plates (tangentially to the horizontal lines marked on the plate). Culture plates were then washed with phosphate-buffered saline (PBS) to remove any cellular debris. Fresh media (250 µl) and 50 µl of plant extracts were added to the wells except the wells that served as controls where cells were untreated. Fetal Bovine Serum (10% FBS) was used as a positive control (Kashyap *et al.*, 2013). Scratches were viewed under the inverted microscope and images were captured at 10 X magnification using a digital camera (Moticam 2500) attached to the microscope (Nikon TMS, Japan) at 0 hours. The plates were then incubated in a humidified incubator (Snijders Hepa, United Scientific group, Cape Town South Africa) under 5% CO<sub>2</sub> at 37°C. After 18 hours of treatment, images were taken and computer software was used to measure the width of the scratch area both at 0 hour and 18 hours and these were recorded. The experiment was done in triplicate. The percentage migration rate using the

changes in the wounded area over time after the application of the plant extracts was calculated using the following equation:

$$\% \text{ Migration} = \frac{\text{Area at 0hr} - \text{Area at 18hr}}{\text{Area at 0hr}} \times 100$$

### **3.7 Statistical Analysis**

Data was entered and captured using Microsoft Excel (2007). Anova was used to evaluate statistical analysis. Results were expressed as mean $\pm$ SD and all experiments were carried out in triplicates.

## 4. RESULTS

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### 4.1 Anti-inflammatory activity

Lipoxygenase inhibitor screening assay kit was used to screen for any lipoxygenase inhibition, an enzyme responsible for prostaglandin biosynthesis, which leads to inflammation. It is noteworthy that almost all aqueous extracts showed good anti-inflammatory activity (Table 2). Aqueous extracts exhibited high percentage inhibition of lipoxygenase with most percentages above hundred percent when compared to methanolic extracts. Nordihydroguaiaretic acid (NDGA) which was used as a positive control, inhibited lipoxygenase enzyme by 129%. *Amaranthus dubius* and *Portulaca oleracea* were found to have the best activities with the highest percentage inhibition of 139% and 154% compared to the positive control. However, *Galinsoga parviflora* and *Syzygium cordatum* had the least effect on the enzyme inhibition with percentages of -2% and 34%. *Gunnera perpensa* was found to have a moderate anti-lipoxygenase activity of 71%. *Bidens pilosa*, *Buddleja saligna* and *Taraxacum officinale* were found to have significant percentage inhibition ranging from 90%-94%. Percentage inhibitions for methanolic extracts were slightly lower than those of aqueous extracts ranging from 17%-93%. Fourteen of the methanolic extracts had significant percentage inhibition ranging from 70%-100%. Seven of the extracts had moderate inhibition between 50% and 69%. Thirteen of the methanolic extracts exhibited extremely lower percentage inhibition of lipoxygenase ranged from 17%-45%. Compared to NDGA, which was shown to have a percentage of 122%, the methanolic extracts results were lower. *Amaranthus spinosus* had the highest percentage inhibition among all the methanolic extracts and *Galinsoga parviflora* had the lowest. It was noted that in both the aqueous and methanolic extracts *Galinsoga parviflora* exhibited the least inhibition of the enzyme. The results showed that aqueous extracts contributed much more to the 5-lipoxygenase activity than the methanolic extract.

**Table 2: Effects of aqueous and methanolic extracts on the inhibition of lipoxygenase (5-LOX) enzyme tested at a concentration of 1mg/ml using a lipoxygenase inhibitor screening assay kit**

Plant species	% Inhibition	
	Aqueous extracts	Methanolic extracts
<i>Achyranthes aspera</i>	95.2 ± 11.38	27.6 ± 13.71
<i>Alternanthera sessilis</i>	99.0 ± 1.48	87.2 ± 2.79
<i>Amaranthus dubius</i>	139.8 ± 15.20	70.4 ± 5.05
<i>Amaranthus hybridus</i>	100.6 ± 0.78	44.4 ± 5.77
<i>Amaranthus spinosus</i>	112.9 ± 6.08	93.4 ± 11.54
<i>Asystasia gangetica</i>	116.7 ± 8.34	66.3 ± 0.72
<i>Bidens pilosa</i>	102.2 ± 18.31	17.9 ± 14.43
<i>Buddleja saligna</i>	91.4 ± 16.69	ENA
<i>Bulbine natalensis</i>	100.6 ± 11.38	88.8 ± 6.49
<i>Carpabrotus dimidiatus</i>	122.6 ± 10.61	77.0 ± 11.54
<i>Centella asiatica</i>	118.3 ± 15.20	41.3 ± 1.44
<i>Ceratotheca triloba</i>	104.9 ± 15.91	ENA
<i>Chenopodium album</i>	115.1 ± 3.04	76.5 ± 22.37
<i>Cleome monophylla</i>	103.3 ± 9.12	76.0 ± 5.77
<i>Dichrostachys cinerea</i>	110.8 ± 12.16	36.2 ± 5.77
<i>Ekerbegia capensis</i>	103.8 ± 19.02	45.4 ± 11.54
<i>Elytropappus rhinocerotis</i>	103.8 ± 8.34	22.4 ± 12.27
<i>Emex australis</i>	129.0 ± 6.08	75.5 ± 10.82
<i>Ficus sur</i>	103.8 ± 11.38	55.1 ± 9.38
<i>Galinsoga parviflora</i>	-2.2 ± 48.72	17.3 ± 5.05
<i>Guilleminea densa</i>	116.1 ± 12.16	90.8 ± 2.16
<i>Gunnera perperna</i>	71.5 ± 8.34	66.8 ± 1.44
<i>Heteropyxis natalensis</i>	101.6 ± 3.82	58.2 ± 12.27
<i>Hibiscus sabdariffa</i>	124.2 ± 6.79	90.3 ± 2.89
<i>Justicia flava</i>	106.0 ± 6.86	57.7 ± 0.00
<i>Leonotis leonurus</i>	113.5 ± 6.86	89.8 ± 10.82
<i>Mormodica balsamina</i>	121.0 ± 12.94	79.1 ± 1.44
<i>Oxygonum sinuatum</i>	108.6 ± 6.08	63.3 ± 3.61
<i>Perlagonium</i>	109.2 ± 8.41	41.3 ± 5.77
<i>Physalis viscosa</i>	105.9 ± 9.90	62.8 ± 10.10
<i>Portulaca oleracea</i>	154.3 ± 9.90	ENA
<i>Senna occidentalis</i>	114.0 ± 19.80	86.2 ± 5.77
<i>Solanum nodiflorum</i>	111.8 ± 12.16	37.2 ± 2.89
<i>Sonchus oleraceus</i>	111.8 ± 12.16	ENA
<i>Syzygium cordatum</i>	40.5 ± 6.65	78.6 ± 3.62
<i>Taraxacum officinale</i>	94.7 ± 13.65	27.0 ± 8.66
<i>Tetradenia riparia</i>	101.6 ± 2.26	37.8 ± 6.49
<i>Tulbaghia violacea</i>	100.6 ± 8.41	88.8 ± 9.38
<b>NDGA (Positive control)</b>	129.1 ± 7.57	121.9 ± 8.66

Values are mean±SD of two experiments ENA – Extract Not Available for testing

## 4.2 Antioxidant activity

The antioxidant activities of thirty-eight selected medicinal plants were evaluated by the DPPH free radical scavenging assay. The methanolic plant extracts were found to be more effective in scavenging DPPH free radicals than the water extracts (Table 3). All the methanolic extracts exhibited scavenging ability ranging from 60%–104%. *Asystasia gangetica*, *Ficus sur*, *Heteropyxis natalensis*, *Hibiscus sabdariffa*, *Pelargonium* sp. showed significantly high free radical scavenging abilities ranging from 101%–104%. Twenty-four extracts also demonstrated significant activities and their scavenging activity was in the range of 90%-99%. *Amaranthus spinosus*, *Bulbine natalensis*, *Leonotis leonurus*, *Solanum nodiflorum*, *Tulbaghia violacea* showed moderate activities ranging from 68%-90% respectively. It was observed that for most extracts both water and methanolic, as the concentration decreased the percentage scavenging activity also decreased. *Heteropyxis natalensis* and *Hibiscus sabdariffa* exhibited scavenging ability even at the lowest concentration of 10µg/ml. The higher values of methanolic extracts suggest that the extracts contain a higher concentration of active compounds. Some of the methanolic extracts proved to be more effective compared to a positive control (Rutin). Aqueous extracts displayed remarkably lower activities than methanolic extracts. Thirty-one aqueous extracts had a scavenging capacity ranging from 22%—59%, and seven extracts had moderate activity ranged from 60%–70%. Aqueous extract of *Bidens pilosa* was shown to have the highest antioxidant activity amongst all the other water extracts. Compared to the positive control (Rutin), the water extracts had very low scavenging capability.

**Table 3: DPPH free radical scavenging activity of aqueous and methanolic extracts of 38 South African medicinal plants at different concentrations**

Plant Species	% Scavenging activity					
	1000µg/ml	500µg/ml	250µg/ml	100µg/ml	50µg/ml	10µg/ml
<i>Achyranthes aspera</i>	42.5±1.01	35.6±1.10	31.2±1.79	26.7±2.21	10.3±1.26	-2.5±2.49
	94.2±0.29	93.7±0.19	94.3±0.58	92.6±0.50	71.8±3.04	13.8±2.37
<i>Alternanthera sessilis</i>	47.8±2.50	38.4±1.07	28.2±0.36	11.9±2.10	6.5±1.34	-3.2±0.88
	95.0±0.41	91.0±0.41	94.5±0.38	70.5±0.46	37.0±1.30	4.4±1.25
<i>Amaranthus dubius</i>	51.0±0.34	38.3±3.26	26.4±0.90	6.3±1.05	-3.8±1.60	-5.5±2.52
	96.7±0.59	96.2±0.42	95.0±0.68	74.7±4.95	39.8±5.10	4.0±0.26
<i>Amaranthus hybridus</i>	31.7±5.28	14.2±1.31	-10.8±1.05	-29.2±10.25	-45.9±1.60	-47.4±0.50
	95.9±0.31	95.0±0.71	95.4±0.21	93.9±0.84	70.5±0.91	10.3±1.17
<i>Amaranthus spinosus</i>	59.3±0.90	47.5±0.31	45.3±4.90	35.9±0.50	19.4±1.51	1.5±4.08
	68.3±2.92	34.1±1.41	19.4±1.81	9.8±2.84	3.9±1.25	-0.8±0.96
<i>Asystasia gangetica</i>	40.8±1.44	38.4±2.70	34.8±2.11	29.4±17.25	11.7±1.12	2.9±2.09
	101.4±0.59	98.9±0.07	97.4±0.23	94.8±1.34	62.9±0.78	9.2±1.70
<i>Bidens pilosa</i>	70.4±2.09	59.0±2.54	57.4±0.95	43.2±0.44	34.3±0.08	9.3±1.54
	95.4±0.23	95.7±0.51	94.8±0.28	93.2±0.51	81.3±0.08	13.0±1.35
<i>Buddleja saligna</i>	59.2±3.09	45.0±0.47	35.6±1.31	21.9±0.83	11.3±1.94	-2.5±1.41
	ENA	ENA	ENA	ENA	ENA	ENA
<i>Bulbine natalensis</i>	42.7±3.61	30.7±0.81	12.2±1.89	1.3±0.42	-2.4±1.58	-2.7±0.98
	86.8±0.54	53.3±0.41	32.2±1.24	12.9±1.40	8.6±0.62	0.5±1.45
<i>Carpobrotus dimidiatus</i>	45.5±2.12	40.3±1.15	32.7±0.78	19.8±1.91	10.8±1.29	1.5±2.09
	97.1±0.47	95.8±0.49	95.3±0.72	85.5±3.01	46.1±1.55	5.5±1.33
<i>Centella asiatica</i>	55.9±3.91	40.6±1.48	32.7±0.20	11.3±1.86	1.1±1.95	-7.3±1.77
	96.1±1.24	94.1±1.75	94.5±0.53	88.1±0.49	47.4±2.29	12.0±2.04
<i>Ceratotheca triloba</i>	50.8±1.05	45.2±0.88	39.2±0.65	31.3±0.64	17.8±1.18	4.1±0.76
	ENA	ENA	ENA	ENA	ENA	ENA
<i>Chenopodium album</i>	27.2±6.60	13.7±2.05	3.8±1.10	1.2±1.81	0.0±2.68	-4.4±0.48
	94.9±1.07	70.3±0.77	40.9±1.98	17.7±2.00	8.4±0.69	4.2±0.65
<i>Cleome monophylla</i>	38.0±1.54	23.0±0.93	9.9±0.58	2.4±1.60	-0.1±3.05	-1.6±1.91
	94.3±0.53	74.5±0.69	45.6±1.48	26.1±1.06	13.9±3.89	5.7±3.73
<i>Dichrostachys cinerea</i>	45.0±2.50	42.8±1.19	30.5±2.66	19.3±1.68	11.5±1.34	-0.5±2.33
	96.0±0.75	86.7±1.47	55.1±0.93	29.7±1.10	17.3±0.80	1.1±1.39
<i>Ekerbegia capensis</i>	37.4±2.82	30.3±0.81	27.6±0.09	17.8±1.25	7.2±1.12	-6.1±0.42
	94.3±0.71	94.5±0.88	94.1±1.05	93.6±0.54	68.2±2.46	12.1±1.59
<i>Elytropappus rhinocerotis</i>	55.1±2.66	40.8±2.41	30.8±1.49	31.2±0.97	23.4±1.13	1.2±2.31
	95.4±0.54	94.4±0.25	95.4±0.85	96.2±0.32	93.9±1.10	24.9±0.90
<i>Emex australis</i>	47.0±1.47	39.0±0.84	27.0±0.15	16.0±0.70	4.3±1.85	-2.4±1.39
	98.3±0.41	95.3±0.06	68.0±0.79	32.0±3.38	15.6±1.47	1.9±0.64
<i>Ficus sur</i>	30.5±1.36	27.6±1.59	26.2±0.63	8.8±0.44	0.2±0.35	-6.5±1.05
	102.8±0.70	97.1±0.61	94.8±1.33	93.7±0.53	93.1±1.41	38.1±7.95

<i>Galinsoga parviflora</i>	22.7±0.73	26.6±2.88	5.4±1.61	-5.9±2.21	-15.5±3.95	-17.3±3.65
	95.9±0.59	94.1±0.29	82.6±2.21	35.4±2.82	16.6±7.31	4.2±0.23
<i>Guilleminea densa</i>	46.1±2.25	35.2±0.29	21.4±0.49	8.1±6.11	0.4±1.13	-4.9±2.01
	94.9±0.29	70.2±1.46	40.8±1.75	23.2±3.07	11.1±0.59	0.3±0.50
<i>Gunnera perpensa</i>	68.5±1.56	62.2±1.02	50.8±2.23	41.5±2.38	39.0±1.86	37.9±2.22
	95.7±0.60	94.7±1.77	95.5±0.74	95.3±0.24	95.3±0.39	65.9±0.91
<i>Heteropyxis natalensis</i>	69.7±1.17	69.4±0.83	58.2±0.71	54.6±0.58	56.6±3.72	42.9±2.83
	101.7±5.04	101.1±2.09	98.4±0.32	95.8±0.55	94.7±0.46	64.8±1.42
<i>Hibiscus sabdariffa</i>	45.5±2.30	37.9±1.59	26.5±1.73	11.7±1.63	5.3±1.98	-2.9±1.13
	104.4±1.17	93.2±0.40	64.0±0.91	28.9±4.27	18.6±4.13	2.8±0.83
<i>Justicia flava</i>	40.6±1.25	31.4±1.56	25.9±1.18	5.1±2.95	-6.2±2.26	-30.2±2.64
	95.2±1.16	94.2±1.13	91.5±0.00	67.5±24.90	29.5±1.28	0.1±2.98
<i>Leonotis leonurus</i>	38.7±3.01	26.6±1.31	13.3±1.17	7.9±0.94	6.2±0.17	3.2±2.79
	68.1±1.38	35.9±0.46	20.8±0.91	9.7±0.90	7.0±3.25	0.6±0.81
<i>Mormodica balsamina</i>	67.1±0.91	57.6±1.49	46.5±2.51	27.2±1.57	11.3±4.23	2.9±2.41
	98.2±1.89	94.8±0.85	84.3±4.16	38.6±4.33	21.8±1.59	4.6±2.52
<i>Oxygonum sinuatum</i>	56.2±0.92	43.7±2.37	36.9±3.22	30.9±1.22	16.5±3.20	0.4±1.79
	97.4±0.36	95.3±0.33	84.3±2.03	37.7±2.90	19.4±1.67	2.9±1.59
<i>Perlagonium sp.</i>	46.2±1.04	42.2±3.89	43.2±0.80	43.3±0.48	37.4±1.71	4.7±2.01
	100.6±0.50	98.1±0.39	97.0±0.49	96.9±0.77	96.9±0.54	58.3±5.08
<i>Physalis viscosa</i>	46.3±0.93	37.0±1.30	31.6±1.44	18.8±0.96	5.7±1.35	-8.4±3.18
	95.1±0.17	94.9±0.38	94.0±0.85	55.3±3.50	32.0±1.08	6.1±0.21
<i>Portulaca oleracea</i>	31.7±0.36	17.7±2.11	7.3±1.59	6.3±1.92	2.0±1.55	1.1±1.87
	ENA	ENA	ENA	ENA	ENA	ENA
<i>Senna occidentalis</i>	46.8±3.03	34.9±1.54	14.7±3.06	-8.7±2.39	-16.58±0.58	-23.0±3.49
	96.3±0.86	73.7±1.62	45.1±1.52	23.4±3.47	16.2±3.09	4.5±4.21
<i>Solanum nodiflorum</i>	57.5±0.61	47.5±0.31	45.3±4.90	35.9±0.50	19.1±1.01	3.8±0.00
	89.8±1.46	86.9±3.51	78.3±1.96	26.1±1.55	13.9±0.10	-0.8±1.56
<i>Sonchus oleraceus</i>	24.5±1.38	19.4±5.02	2.9±5.93	-19.1±1.56	-26.4±1.75	-36.4±1.17
	ENA	ENA	ENA	ENA	ENA	ENA
<i>Syzgium cordatum</i>	65.9±4.09	50.6±2.32	48.9±2.84	34.1±3.52	30.9±2.66	7.2±3.27
	99.0±0.20	97.2±0.63	95.0±0.44	95.3±0.63	95.5±0.61	48.1±1.46
<i>Taxacum officinale</i>	65.6±1.62	54.5±2.51	50.2±4.26	44.8±1.14	31.1±0.99	7.3±1.22
	96.4±0.13	95.9±0.81	93.3±0.35	92.8±0.62	81.7±8.74	13.3±0.56
<i>Tetradena riparia</i>	55.9±0.67	44.9±1.04	39.5±1.51	33.2±1.55	20.2±1.24	4.6±3.90
	98.0±0.31	97.0±0.58	94.6±0.31	94.1±0.76	91.9±0.76	25.8±0.59
<i>Tulbaghia violacea</i>	32.1±2.02	17.1±8.42	-5.4±1.05	-23.7±4.70	-34.6±1.63	-38.6±0.71
	76.4±2.34	43.7±1.18	27.9±1.79	16.6±2.64	8.5±1.30	1.7±2.31
Rutin	96.0±0.78	93.2±0.78	91.0±0.77	88.1±0.47	83.4±2.63	13.4±1.87
	95.3±0.48	93.8±0.66	94.1±0.41	92.8±0.74	92.8±0.08	27.4±1.42

Values in blue = aqueous extracts; values in red = methanolic extracts; Values are mean±SD of three experiments; ENA = Extract Not Available

## 4.3 Safety evaluation

### 4.3.1 Brine shrimp lethality test

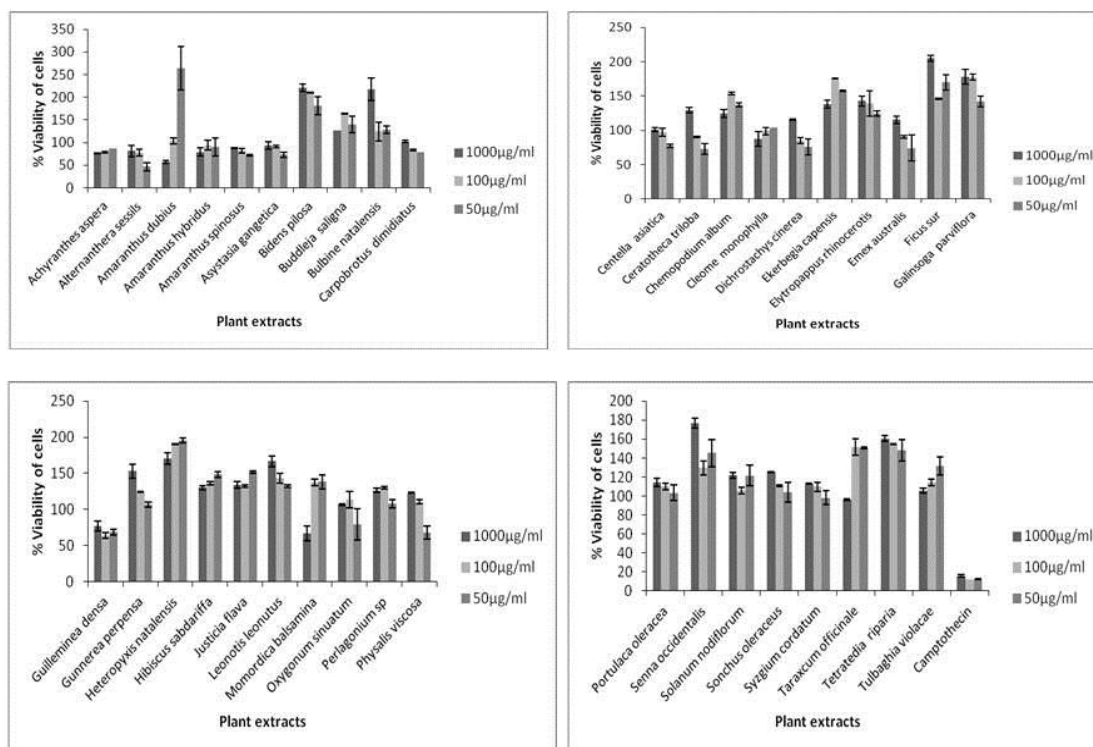
The cytotoxic activity of the plant extracts was determined using brine shrimp lethality test. Aqueous and methanolic extracts of the thirty-eight studied plants were evaluated for their activity against *Artemia salina nauplii* at different concentrations of 1000 µg/ml, 500 µg/ml and 200 µg/ml. Only one methanolic plant extract— *Hibiscus sabdariffa*—was considered to be toxic to *Artemia salina* at all the concentrations after 24 hours of incubation with the plant extracts. It displayed 80% mortality. Among all other extracts, no concentration of extracts was found to be detrimental to brine shrimp eggs. Also, in the negative control group, no mortality was observed.

### 4.3.2 Cytotoxicity

MTT assay was performed using 3T3 NIH fibroblast cells by treating them with various concentrations of aqueous and methanolic extracts. The assay is based on the idea that toxic chemicals affect basic functions of cells and that damage to the cells is a measure of toxicity. Investigating toxicity of the plant is important in evaluating if the plant can still be used for further biological screening. The assay was performed as a percentage of relative cell viability against various concentrations of the extracts. Percentages below 50% are regarded as cytotoxicity; results indicated that most of the extracts were not toxic to the cells. Most had percentage viability of more than 76% for aqueous extracts and 90% for methanolic extracts at the highest concentration of 1 mg/ml. Some extracts were shown to decrease percentage viability at high concentrations; this implies that caution must be taken when using infusions of these plant species for treating wounds. Other extracts were shown to stimulate the growth of fibroblast cells at high concentrations but also improved viability was observed at lower concentrations. Also, a variation of results was observed at 100 µg/ml.

Twenty-seven aqueous extracts were found to stimulate (>100% viability) the growth of cells at the highest concentration of 1 mg/ml and percentage viability was observed to be decreasing with decreasing concentration (Figure 45). The percentage viabilities ranged

from 76%-221%. Highest stimulation of cell growth was observed with *Bidens pilosa* (221%). Ten extracts were shown to be toxic at the highest concentration with *Amaranthus dubius* and *Mormodica balsamina* having the lowest percentage viability of 53% and 67% respectively, and as the concentration of the extracts decreased improved viability of cells was observed.

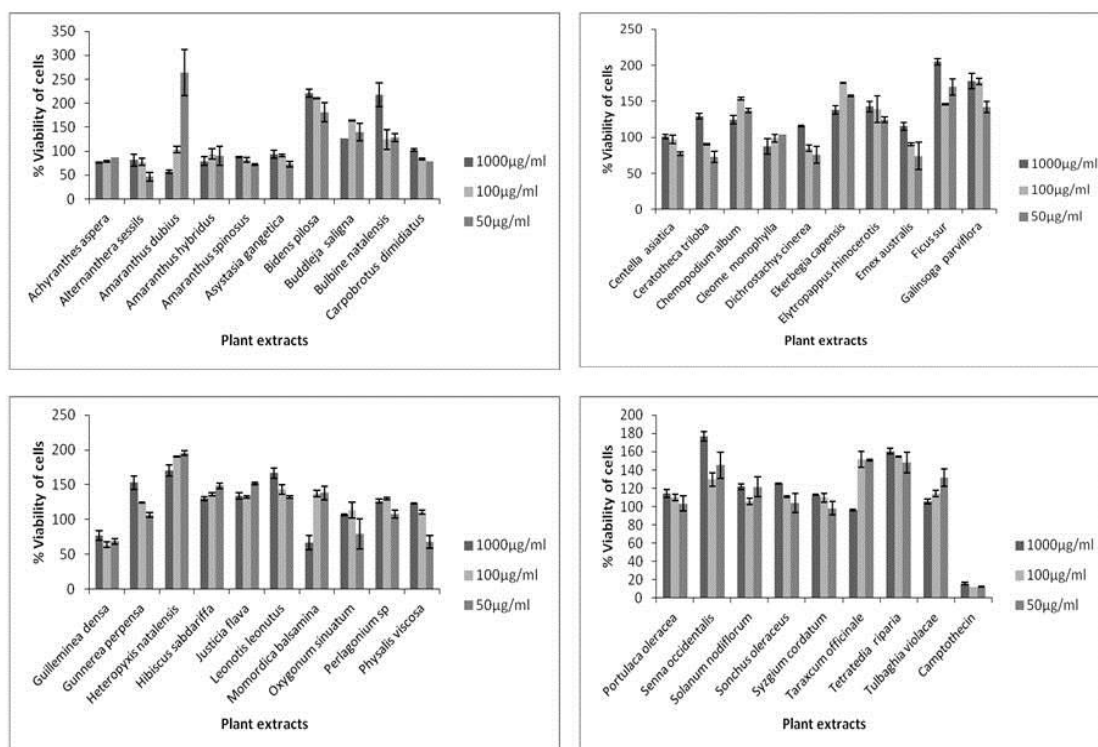


**Figure 45: Graphs showing the effect of aqueous extracts at three different concentrations on 3T3 NIH fibroblast cells after 24 hours with Camptothecin as the positive control**

Values are mean±SD of three experiments

As demonstrated in Figure 46, twenty-two methanolic extracts were shown to stimulate the growth of fibroblast cells significantly at the highest concentration of 1 mg/ml with percentages ranging from 103%-204%; however toxicity of *Solanum nodiflorum* was observed with a percentage viability of 46% at the highest concentration but as the concentration decreased the viability of cells improved upto 143%. Highest stimulation of cell growth was observed with *Dichrostachys cinerea* with a percentage viability of 205%. Ten extracts were shown to be lowering viability of cells at high concentration and as

concentration decreased improved viability was observed. *Alternanthera sessilis*, *Amaranthus spinosus*, *Carpobrotus dimidiatus*, *Centella asiatica*, *Dichrostachys cinerea*, *Elytropappus rhinocerotis*, *Emex australis*, *Galinsoga parviflora*, *Justicia flava*, *Leonotis leonurus*, *Oxygonum sinuatum*, *Physalis viscosa* stimulate the growth of fibroblast cells in both the aqueous and methanolic extracts.



**Figure 46: Graphs showing the effect of methanolic extracts at three different concentrations on 3T3 NIH fibroblast cells after 24 hours with Camptothecin as the positive control**

Values are mean±SD of three experiments

Methanolic extracts of *Buddleja saligna*, *Ceratotheca triloba*, *Portulaca oleracea*, *Sonchus oleraceus* were not available for testing.

#### 4.4 Scratch wound assay

Scratch wound healing assay is a method that shows the role of plant extracts in healing wounds. Methanolic and aqueous extracts were evaluated for changing the speed of migration of the 3T3 NIH fibroblast cells. The migration of cells was studied at 0 and 18

hour intervals and the closing of the scratch was monitored manually by imaging at these different time intervals. Compared to untreated cells, an increase in the number of fibroblast cells in the exposed area of the treated cells was observed and a significant migration rate was also noted. FBS was used as a positive control and cells exposed to FBS showed complete closure after 18 hours of treatment.

The results showed that the migration rate of cells supplemented with extracts was faster than the control culture (Table 4). The methanolic extracts were found to be more effective in the migration of fibroblasts than the corresponding aqueous extracts. Both aqueous and methanolic extracts of *Amaranthus hybridus*, *Justicia flava*, *Leonotis leonurus*, and *Senna occidentalis* demonstrated highest percentage wound closure. Nine aqueous extracts were shown to have the highest migration rate ranging from 77%-86% when compared to the untreated cells with a percentage migration rate of 24%. *Sonchus oleraceus* (86%), *Justicia flava* (85%) and *Dichrostachys cinerea* (85%) had the highest percentage of migration rate when compared to the positive control (10% FBS) of 84%. Seven extracts had moderate wound closure ranging from 58%-69% and the rest of the aqueous extracts had the lowest migration rate ranging from 12%-56% with *Oxygonum sinuatum* (12%) and *Syzygium cordatum* (23%) being the lowest amongst all the aqueous extracts.

Eleven methanolic extracts had the highest migration rate ranging from 75%-100% respectively. Methanolic extracts of *Senna occidentalis* and *Hibiscus sabdariffa* significantly induced migration rate of fibroblast cells when compared to the positive control (10% FBS) that had migration rate of 96% respectively, complete wound closure was observed with these extracts after 18 hours of treatment. Nine extracts had moderate percentage wound closure ranging from 60%-69%. The lower migration rate of the rest of the extracts was observed with percentages ranging from 11%-56% with *Dichrostachys cinerea* having the lowest migration rate of 12%. There was no migration of cells with *Solanum nodiflorum*; instead, the extract was toxic to the cells, therefore, ended up killing them but the aqueous extract demonstrated a significant migration rate of the cells. Figure 47a, b and 48a, b illustrates the migration rate of fibroblast cells treated with both aqueous and

methanolic extracts and figure 49 demonstrates the comparison between migration rate of cells treated with aqueous extracts and those treated with methanolic extracts.

**Table 4: Percentage wound closure of 3T3 NIH mouse fibroblast cells over 18 hour period of treatment with aqueous and methanolic plant extracts at a concentration of 1 mg/ml; 10% FBS was used as a positive control.**

Plant Species	Area between the scratch ( $\mu\text{m}^2$ )				% Migration rate after 18 hours	
	Aqueous		Methanolic		Aqueous	Methanolic
	0 h	18 h	0h	18 h		
<i>Achyranthes aspera</i>	212 159.8	113 800.1	141 536.0	72 620.2	46.4±0.42	48.6 ± 6.01
<i>Alternanthera sessilis</i>	95 751.2	64 516.9	101 274.9	13 590.2	32.8±4.74	86.8 ± 3.11
<i>Amaranthus dubius</i>	127 977.2	60 762.0	127 481.9	46 901.7	53.0 ± 2.62	63.5 ± 3.54
<i>Amaranthus hybridus</i>	103 099.5	23 430.1	125 535.2	8262.5	77.3 ± 0.49	94.8 ± 5.44
<i>Amaranthus spinosus</i>	107 490.9	46 292.9	175 646.6	101 240.4	54.3 ± 4.95	42.9 ± 3.89
<i>Asystasia gangetica</i>	104 581.2	18 969.9	118 647.3	36 618.8	81.9 ± 1.27	69.3 ± 0.71
<i>Bidens pilosa</i>	142 001.2	99 644.0	147 061.8	29 959.7	30.5 ± 7.21	79.3 ± 11.46
<i>Buddleja saligna</i>	130 752.9	52 429.1	ENA	ENA	59.9 ± 2.55	ENA
<i>Bulbine natalensis</i>	89 083.1	27 711.3	157 985.0	49 237.0	69.3 ± 3.75	69.1 ± 6.15
<i>Carpobrotus dimidiatus</i>	93 353.1	57 763.6	140 216.3	50 140.9	39.7 ± 7.28	64.0 ± 2.12
<i>Centella asiatica</i>	135 429.9	60 778.0	225 642.2	131 870.7	55.1 ± 0.28	41.7 ± 1.47
<i>Ceratotheca triloba</i>	199 027.0	109 032.5	ENA	ENA	42.3 ± 14.50	ENA
<i>Chenopodium album</i>	169 753.8	76 699.3	117 818.9	37 649.5	54.6 ± 2.80	68.4 ± 1.90
<i>Cleome monophylla</i>	221 957.7	91 712.9	174 418.1	74 654.7	58.8 ± 0.85	57.3 ± 1.48
<i>Dichrostachys cinerea</i>	122 001.6	19 064.1	164 693.1	138 026.1	84.5 ± 1.56	11.5 ± 7.14
<i>Ekerbegia capensis</i>	184 793.6	120 888.9	176 655.9	118 800.7	34.8 ± 3.20	32.9 ± 0.72
<i>Elytropappus rhinocerotis</i>	85 498.0	55 742.1	196 148.1	101 970.0	34.6 ± 4.88	48.1 ± 0.21
<i>Emex australis</i>	88 095.2	16 793.6	128 279.5	46 334.5	77.6 ± 2.69	64.1 ± 2.80
<i>Ficus sur</i>	96 586.7	35 315.9	140 396.7	48 384.3	63.4 ± 1.56	65.5 ± 2.19
<i>Galinsoga parviflora</i>	177 045.8	81 026.8	98 894.2	42 141.1	54.1 ± 1.63	56.9 ± 5.52
<i>Guilleminea densa</i>	155 735.4	81 537.2	104 524.9	26 668.2	48.2 ± 3.82	75.1 ± 8.91
<i>Gunnera perpensa</i>	87 887.8	61 709.3	124 187.9	66 809.7	30.0 ± 5.59	45.5 ± 4.81
<i>Heteropyxis natalensis</i>	134 965.7	83 393.5	143 608.1	107 795.3	38.7 ± 2.69	24.9 ± 3.46
<i>Hibiscus sabdariffa</i>	133 164.4	47 108.1	74 585.6	0	64.7 ± 0.42	100 ± 0.00
<i>Justicia flava</i>	100 158.3	16 175.7	107 891.6	8 106.4	84.9 ± 6.43	93.2 ± 4.88

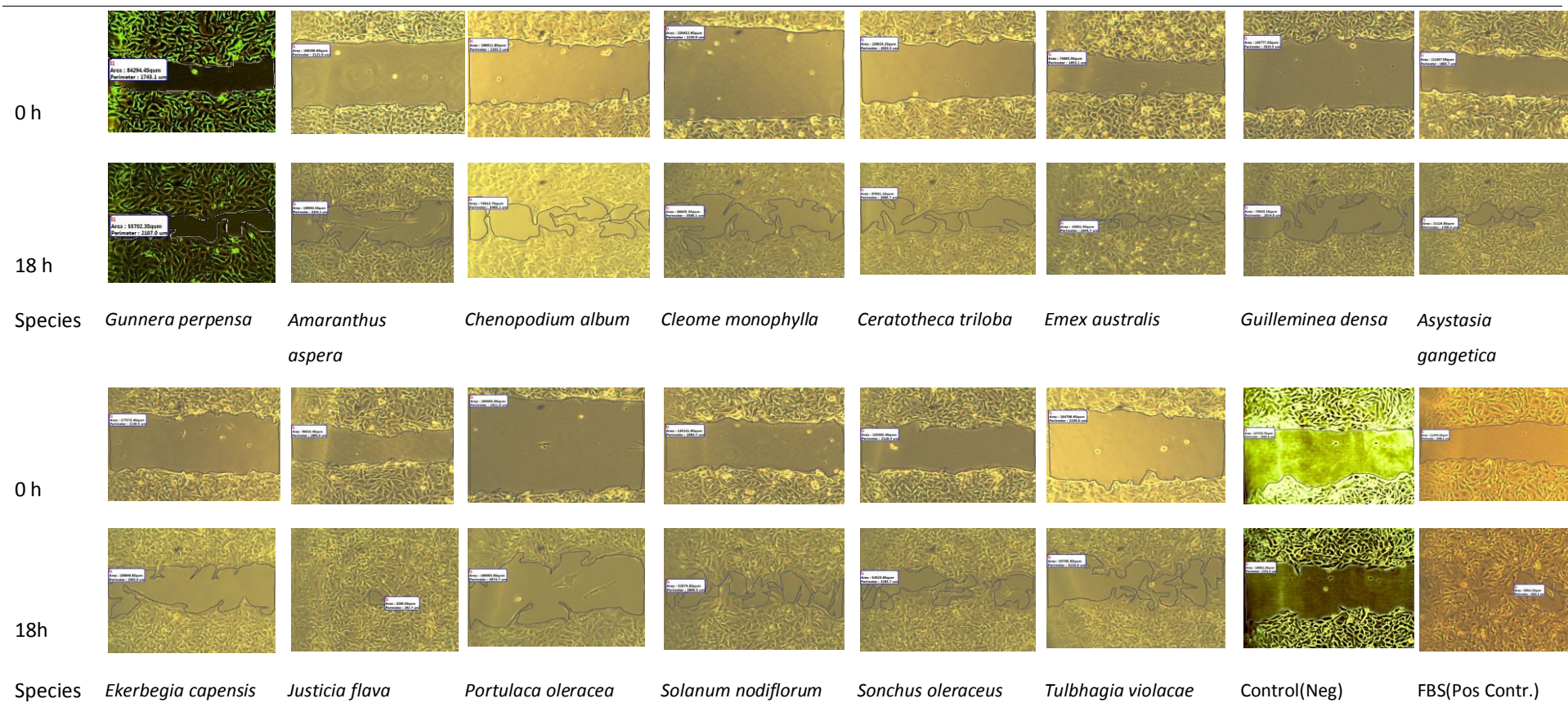
<i>Leonotis leonurus</i>	176 361.7	32 258.9	138 307.8	32 217.7	82.5 ± 8.13	76.8 ± 9.90
<i>Mormodica balsamina</i>	204 046.9	85 151.3	73 729.1	17 322.7	58.4 ± 3.75	76.6 ± 3.04
<i>Oxygonum sinuatum</i>	137 572.7	112 679.6	218 624.6	98 631.0	12.2 ± 4.24	54.6 ± 3.28
<i>Perlagonium sp.</i>	140 454.2	62 248.1	198 950.7	171 032.3	55.6 ± 3.11	14.1 ± 4.88
<i>Physalis viscosa</i>	60 611.5	14 175.2	137 489.3	83 027.5	76.7 ± 0.92	33.3 ± 0.14
<i>Portulaca oleracea</i>	284 432.2	181 890.6	ENA	ENA	36.1 ± 2.66	ENA
<i>Senna occidentalis</i>	179 831.8	42 285.5	150 872.1	69 003.8	76.5 ± 0.64	100 ± 1.13
<i>Solanum nodiflorum</i>	135 333.1	43 813.3	121 968.3	126 494.6	67.8 ± 3.14	-3.9 ± 5.37
<i>Sonchus oleraceus</i>	140 883.4	20 113.2	ENA	ENA	86.1 ± 5.87	ENA
<i>Syzgium cordatum</i>	85 865.5	65 363.6	138 336.0	55 068.3	23.3 ± 18.10	60.2 ± 0.00
<i>Taxacum officinale</i>	147 866.0	105 077.7	148 514.5	37 421.4	28.9 ± 0.14	75.3 ± 5.66
<i>Tetradena riparia</i>	124 723.4	83 853.0	129 205.4	39 615.0	33.9 ± 4.74	69.4 ± 0.46
<i>Tulbaghia violacea</i>	229 959.8	99 683.3	154 912.6	38 802.4	56.4 ± 3.11	74.9 ± 1.27
<b>Control (Untreated)</b>	171 568.2	131 337.5	124 234.7	61 059.4	23.5 ± 0.07	50.9 ± 0.49
<b>10% FBS</b>	99 442.6	16 613.1	119 977.6	5 190.2	83.7 ± 4.9	95.7 ± 6.08

Values are mean±SD of three experiments

Area between scratch represent average area

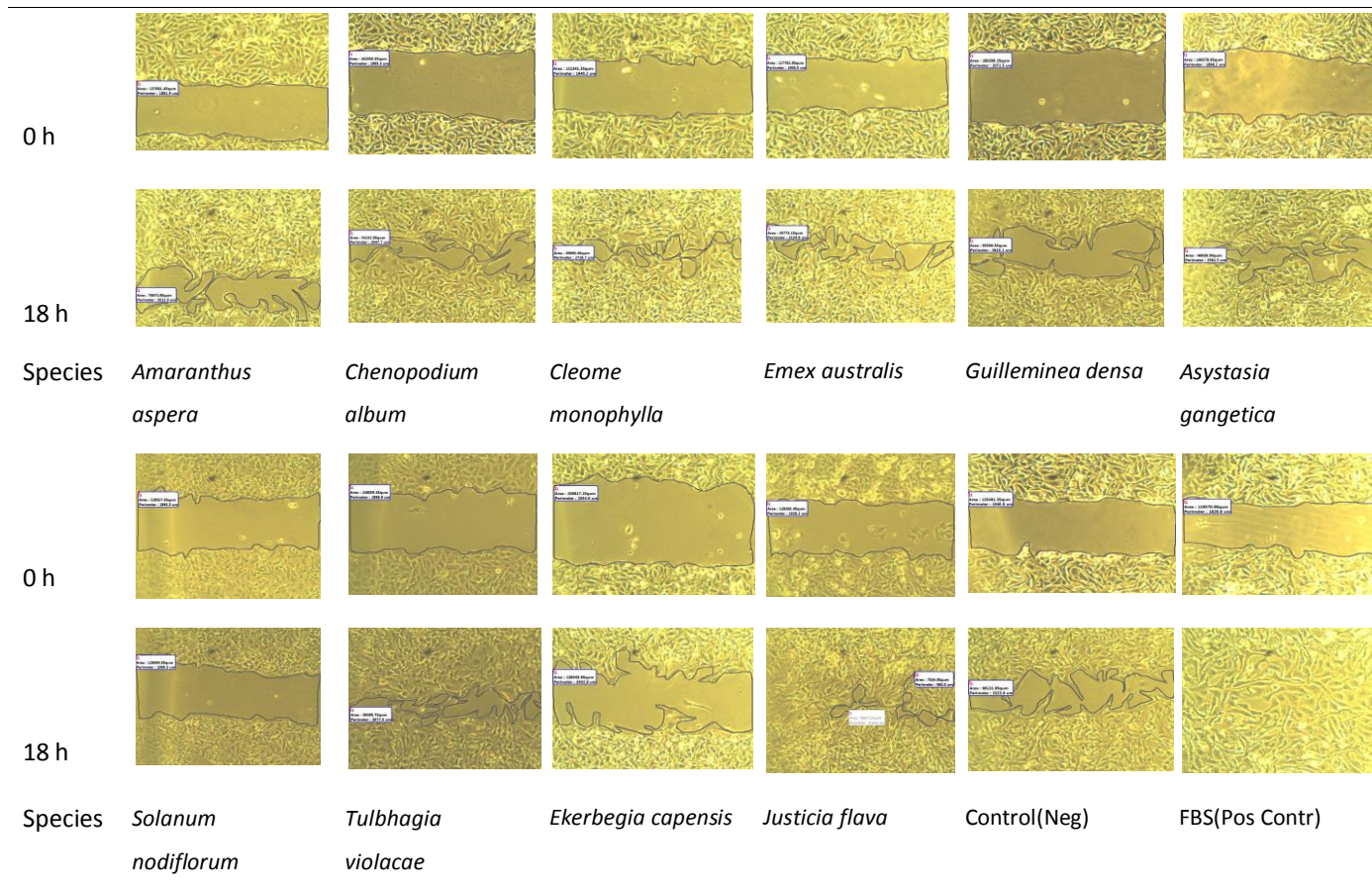
Methanolic extracts of *Buddleja saligna*, *Ceratotheca triloba*, *Portulaca oleracea*, *Sonchus oleraceus* were not available for testing.

0hr								
18hrs								
Species	<i>Alternanthera sessilis</i>	<i>Oxygonum sinuatum</i>	<i>Physalis viscosa</i>	<i>Syzgium cordatum</i>	<i>Bulbine natalensis</i>	<i>Bidens pilosa</i>	<i>Galinsoga parviflora</i>	<i>Leonotis leonurus</i>
0hr								
18hrs								
Species	<i>Ficus sur</i>	<i>Heteropyxis natalensis</i>	<i>Hibiscus sabdariffa</i>	<i>Senna occidentalis</i>	<i>Taraxcum officinale</i>	<i>Tetradenia riparia</i>	<i>Mormodica balsamina</i>	<i>Perlagonium</i>
0hr								
18hrs								
Species	<i>Amaranthus hybridus</i>	<i>Amaranthus spinosus</i>	<i>Buddleja saligna</i>	<i>Centella asiatica</i>	<i>Carpobrotus dimidiatus</i>	<i>Dichrostactach cinerea</i>	<i>Amaranthus dubius</i>	<i>Elytropappus rhicocerotis</i>

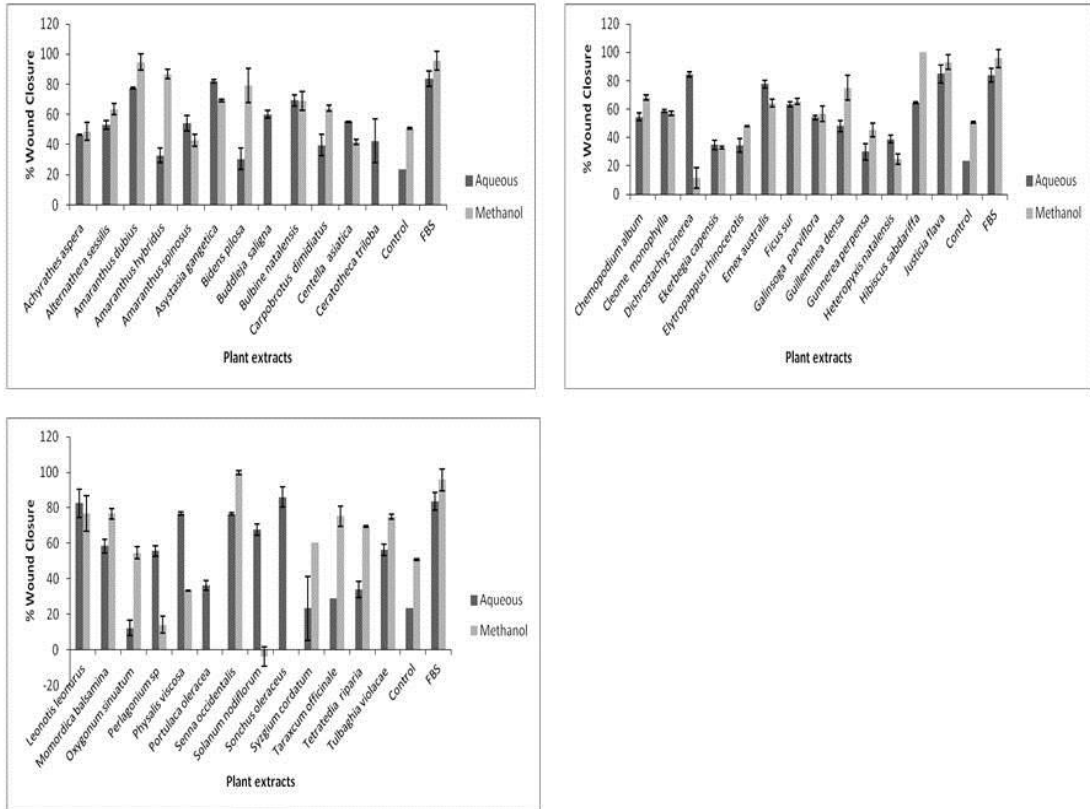


**Figure 47: Migration of 3T3 NIH mouse fibroblast cells at 0 and 18 hours after incubation with the aqueous extracts at a concentration of 1mg/ml, which displayed the highest migration rate measured by quantifying the total distance that the cells moved from the edges of the scratch towards the centre; 10% FBS was used as a positive control. Images were taken at 10x magnification using an inverted microscope (Nikon TMS, Japan). Data expressed as mean  $\pm$  SD (n = 3).**

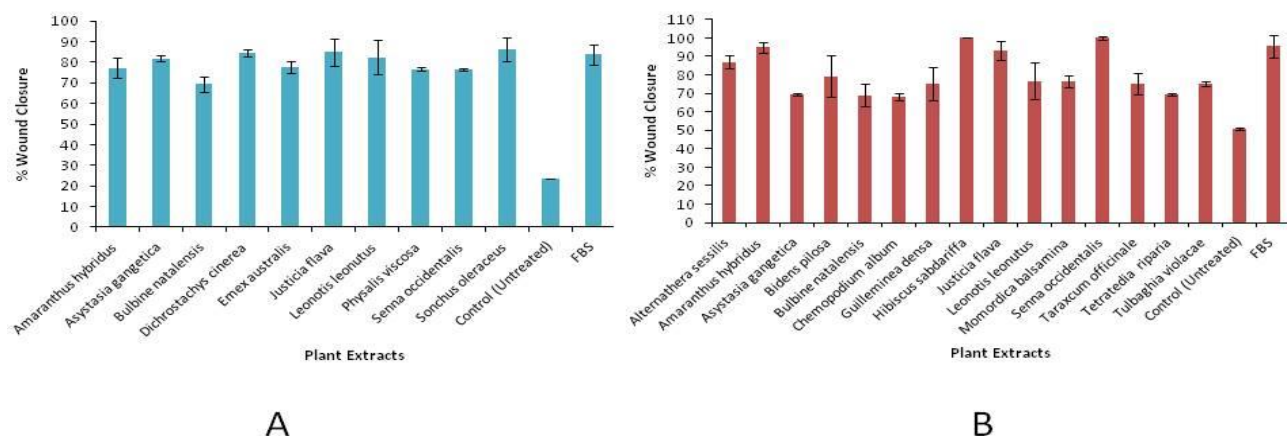
0 h								
18 h								
Species	<i>Alternanthera sessilis</i>	<i>Oxygenum sinuatum</i>	<i>Physalis viscosa</i>	<i>Syzgium cordatum</i>	<i>Bulbine natalensis</i>	<i>Bidens pilosa</i>	<i>Galinsoga parviflora</i>	<i>Leonotis leonurus</i>
0 h								
18 h								
Species	<i>Ficus sur</i>	<i>Heteropyxis natalensis</i>	<i>Hibiscus sabdariffa</i>	<i>Senna occidentalis</i>	<i>Taraxcum officinale</i>	<i>Tetradenia riparia</i>	<i>Mormodica balsamina</i>	<i>Perlagonium</i>
0 h								
18 h								
Species	<i>Amaranthus hybridus</i>	<i>Amaranthus spinosus</i>	<i>Centella asiatica</i>	<i>Carpobrotus dimidiatus</i>	<i>Dichrostactachys cinerea</i>	<i>Amaranthus dubius</i>	<i>Elytropappus rhiccerotis</i>	<i>Gunnera perpensa</i>



**Figure 48:** Migration of 3T3 NIH mouse fibroblast cells at 0 and 18 hours after incubation with the methanolic extracts at a concentration of 1mg/ml, which displayed the highest migration rate measured by quantifying the total distance that the cells moved from the edges of the scratch towards the centre; 10% FBS was used as a positive control. Images were taken at 10x magnification using an inverted microscope (Nikon TMS, Japan). Data expressed as mean  $\pm$  SD (n = 3).



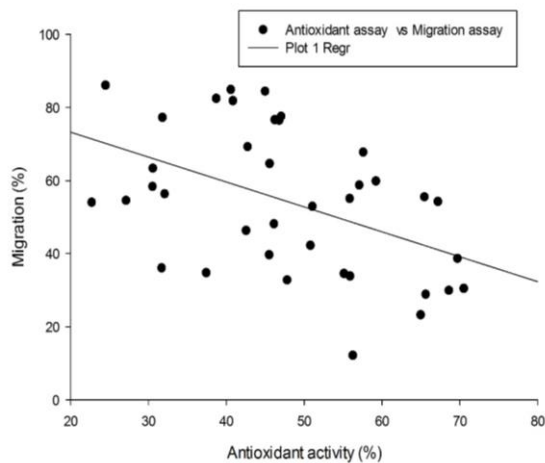
**Figure 49: Graphs displaying the percentage wound closure of 3T3 NIH Mouse Fibroblast cells over 18 hour period of treatment with aqueous and methanolic plant extracts**



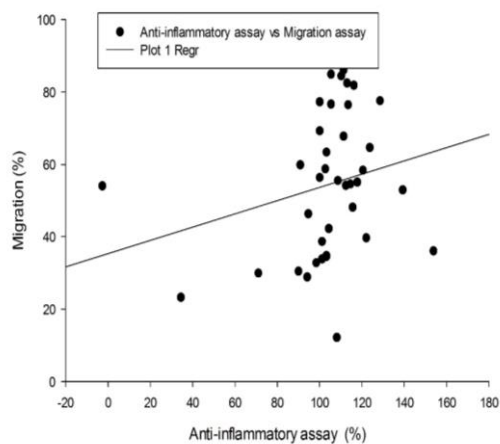
**Figure 50: Graphs showing aqueous (A) and methanolic extracts (B) that exhibited high percentage wound closure over 18 hours of treatment using 3T3 NIH mouse fibroblast cells**

#### 4.5 Statistical analysis

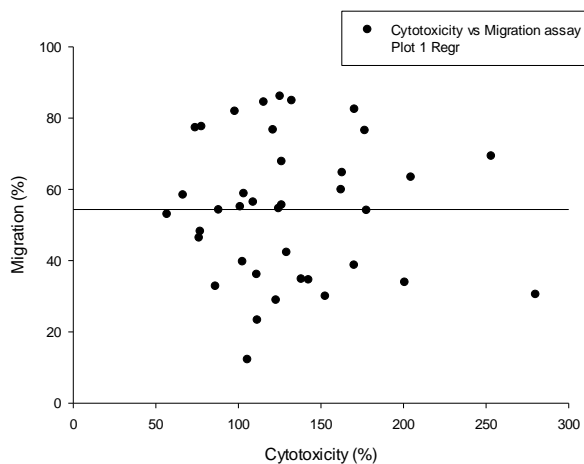
Relationships between antioxidant, anti-inflammatory and cytotoxicity activities of the tested medicinal plants were analysed using ANOVA. The results showed that for aqueous extracts there was moderately negative correlation between antioxidant activity and cell migration, with an r-value of -0.46. A poor positive relationship between anti-inflammatory activity and cell migration was also noted, with an r value of 0.23. And between cytotoxicity and cell migration, a poorly positive correlation was observed with r=0.14 (**Fig. 52 A, B, C**)



(A)



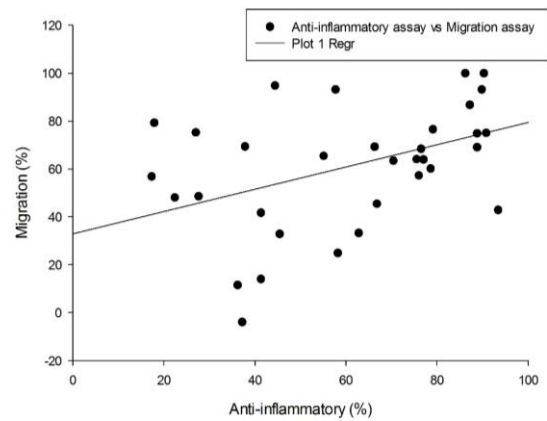
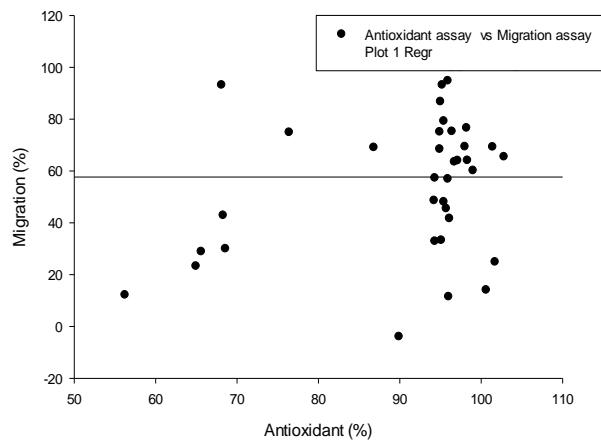
(B)



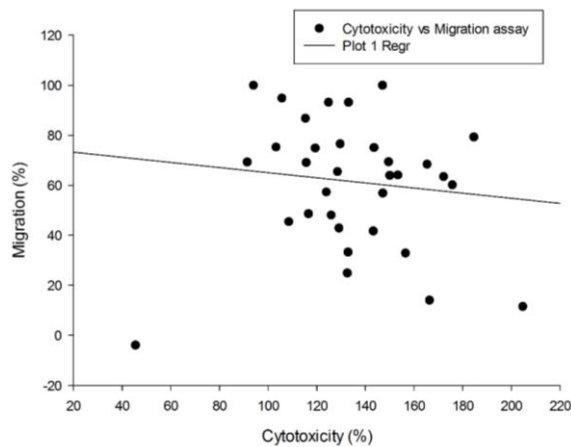
(C)

**Figure 51: Graphs showing the relationship between antioxidant activity vs cell migration (A), anti-inflammatory activity vs cell migration (B) and cytotoxicity vs cell migration(C) in aqueous extracts using ANOVA**

In methanolic extracts, there was no relationship between antioxidant activity and cell migration,  $r$  value = -0.02. However there was moderately positive correlation between anti-inflammatory activity and cell migration ( $r= 0.46$ ). A poor negative relationship was also observed between cytotoxicity and cell migration ( $r=-0.12$ ). **Fig. 53 (D, E, F)**



(D)(E)



(F)

**Figure 52: Graphs showing the relationship between antioxidant activity vs cell migration (D), anti-inflammatory activity vs cell migration (E) and cytotoxicity vs cell migration(F) in methanolic extracts using ANOVA**

#### 4.5.1 Summary tables

Summary tables with activities of all the tests carried out (Table 5 and 6) and a table showing the relationship between anti-inflammatory activity and wound closure (Table 7) are necessary to identify plants with most beneficial effects and plants with useful properties across the tests and to further illustrate if there is any correlation between inflammation and wound healing. Ranks were used to identify the best plants within each test conducted and thus compare their activities or properties. From the summary tables, it is observed that

some plants are able to inhibit enzyme responsible for inflammation as well as scavenge free radicals that may be due to an injury and hence enhance wound healing. However, there are some plants shown which are unable to scavenge free radicals but are still able to block lipoxygenase and allow wound healing to occur. *Asystasia gangetica*, *Bulbine natalensis*, *Emex australis*, *Hibiscus sabdariffa*, and *Leonotis leonurus* were found to be the best extracts to inhibit lipoxygenase as well as close wounds the fastest in both extracts.

**Table 5: Review of aqueous extracts activities for all tests investigated as well as their ranks to identify relationship between tests**

Plant extracts	% Inhibition	Rank	% Scavenging Activity	Rank	% Viability	Rank	% Wound closure	Rank
<i>Sonchus oleraceus</i>	118.8	7	24.5	37	122	21	86.1	1
<i>Justicia flava</i>	106.0	18	40.6	28	130.1	13	84.9	2
<i>Dichrostachys cinerea</i>	110.8	16	45.0	24	87.7	30	84.5	3
<i>Leonotis leonutus</i>	113.5	13	38.7	29	134.2	12	82.5	4
<i>Asystasia gangetica</i>	116.7	9	40.8	27	93.5	28	81.9	5
<i>Emex australis</i>	129.0	3	47.0	18	142.6	10	77.6	6
<i>Amaranthus hybridus</i>	100.6	29	31.8	32	79.3	32	77.3	7
<i>Physalis viscosa</i>	105.9	19	46.2	20	126.4	16	76.7	8
<i>Senna occidentalis</i>	114.0	12	46.8	19	114.1	23	76.5	9
<i>Bulbine natalensis</i>	100.6	29	42.7	25	217.2	2	69.3	10
<i>Solanum nodiflorum</i>	111.8	15	57.6	9	177	5	67.8	11
<i>Hibiscus sabdariffa</i>	124.2	4	45.5	22	170.4	6	64.7	12
<i>Ficus sur</i>	103.8	22	30.5	34	77.7	33	63.4	13
<i>Buddleja saligna</i>	91.4	35	59.2	8	126.5	15	59.9	14
<i>Cleome monophylla</i>	103.3	25	57.1	10	124.5	19	58.8	15
<i>Momordica balsamina</i>	121.0	6	30.5	35	166.6	7	58.4	16
<i>Tulbaghia violacae</i>	100.6	29	32.1	31	160.6	8	56.4	17
<i>Perlagonium sp</i>	105.9	19	65.4	6	126.4	16	55.6	18
<i>Centella asiatica</i>	118.3	8	55.8	13	102.5	25	55.1	19
<i>Chemopodium album</i>	115.1	11	27.1	36	129.1	14	54.6	20
<i>Amaranthus spinosus</i>	112.9	14	67.2	4	88	29	54.3	21
<i>Galinsoga parviflora</i>	-2.2	38	22.7	38	204.9	3	54.1	22
<i>Amaranthus dubius</i>	139.8	2	51.1	15	56.7	37	53	23
<i>Guilleminea densa</i>	116.1	10	46.1	21	177.9	4	48.2	24
<i>Achyranthes aspera</i>	95.2	33	42.5	26	76.1	35	46.4	25
<i>Ceratotheca triloba</i>	104.9	21	50.8	16	101.1	26	42.3	26

<i>Carpobrotus dimidiatus</i>	122.6	5	45.5	23	15.9	38	39.7	27
<i>Heteropyxis natalensis</i>	101.6	27	69.7	2	152.8	9	38.7	28
<i>Portulaca oleracea</i>	154.3	1	31.7	33	122.8	20	36.1	29
<i>Ekerbegia capensis</i>	103.8	22	37.4	30	115.4	22	34.8	30
<i>Elytropappus rhinocerotis</i>	103.8	22	55.1	14	138.4	11	34.6	31
<i>Tetratedia riparia</i>	101.6	27	55.9	12	96.4	27	33.9	32
<i>Alternanthera sessilis</i>	99.0	32	47.8	17	81.7	31	32.8	33
<i>Bidens pilosa</i>	102.2	26	70.5	1	221.1	1	30.5	34
<i>Gunnerea perpensa</i>	71.5	36	68.6	3	76.9	34	30	35
<i>Taraxcum officinale</i>	94.7	34	65.6	5	113	24	28.9	36
<i>Syzgium cordatum</i>	40.5	37	65.0	7	125.4	18	23.3	37
<i>Oxygonum sinuatum</i>	108.6	17	56.2	11	66.7	36	12.2	38

**Table 6: Review of methanolic extracts activities for all tests investigated as well as their ranks to identify relationship between tests**

Plant extracts	% Inhibition	Rank	%Scavenging Activity	Rank	% Viability	Rank	% Wound closure	Rank
<i>Justicia flava</i>	57.7	21	95.2	22	133	15	93.2	5
<i>Dichrostachys cinerea</i>	36.2	29	96	16	204.6	1	11.5	33
<i>Leonotis leonutus</i>	89.8	4	68.1	34	124.8	22	93.2	5
<i>Asystasia gangetica</i>	66.3	18	101.4	4	91.4	33	69.3	14
<i>Emex australis</i>	75.5	15	98.3	7	153.3	8	64.1	18
<i>Amaranthus hybridus</i>	44.4	24	95.9	17	105.6	29	94.8	4
<i>Physalis viscosa</i>	62.8	19	95.1	23	132.8	16	33.3	29
<i>Senna occidentalis</i>	86.2	8	96.3	14	93.9	31	100	1
<i>Bulbine natalensis</i>	88.8	5	86.8	31	115.6	26	69.1	15
<i>Solanum nodiflorum</i>	37.2	28	89.9	30	45.5	34	-3.9	34
<i>Hibiscus sabdariffa</i>	90.3	3	104.4	1	147	12	100	1
<i>Ficus sur</i>	55.1	22	102.8	2	128.5	20	65.5	17
<i>Cleome monophylla</i>	76.0	14	94.3	27	123.9	23	57.3	22
<i>Momordica balsamina</i>	79.1	10	98.2	8	129.6	18	76.6	9
<i>Tulbaghia violacae</i>	88.8	5	76.4	32	119.3	24	74.9	12
<i>Perlagonium sp</i>	41.3	25	100.6	5	166.3	5	14.1	32
<i>Centella asiatica</i>	41.3	25	96.1	15	143.2	14	41.7	28
<i>Chemopodium album</i>	76.5	13	94.9	25	165.3	6	68.4	16
<i>Amaranthus spinosus</i>	93.4	1	68.3	33	129.1	19	42.9	27
<i>Galinsoga parviflora</i>	17.3	34	95.9	17	147.1	11	56.9	23
<i>Amaranthus dubius</i>	70.4	16	96.7	12	172.1	4	63.5	20
<i>Guilleminea densa</i>	90.8	2	94.9	25	143.5	13	75.1	11
<i>Achyranthes aspera</i>	27.6	30	94.2	29	116.5	25	48.6	24

<i>Carpobrotus dimidiatus</i>	77.0	12	97.1	11	150	9	64	19
<i>Heteropyxis natalensis</i>	58.2	20	101.7	3	132.5	17	24.9	31
<i>Ekerbegia capensis</i>	45.4	23	94.3	27	156.4	7	32.9	30
<i>Elytropappus rhinocerotis</i>	22.4	32	95.4	20	125.9	21	48.1	25
<i>Tetradedia riparia</i>	37.8	27	98	9	149.5	10	69.4	13
<i>Alternanthera sessilis</i>	87.2	7	95	24	115.3	27	86.8	7
<i>Bidens pilosa</i>	17.9	33	95.4	20	184.5	2	79.3	8
<i>Gunnerea perpensa</i>	66.8	17	95.7	19	108.4	28	45.5	26
<i>Taraxacum officinale</i>	27.0	31	96.4	13	103.2	30	75.3	10
<i>Syzigium cordatum</i>	78.6	11	99	6	175.7	3	60.2	21
<i>Oxygonum sinuatum</i>	86.2	8	97.4	10	93.9	31	100	1

**Table 7: Relationship between wound closure and inhibition of lipoygenase for both aqueous and methanolic extracts**

Plant extracts	Aqueous extracts		Methanolic extracts	
	% Inhibition	% Wound closure	% Inhibition	% Wound closure
<i>Achyranthes aspera</i>	95.2	46.4	27.6	48.6
<i>Alternanthera sessilis</i>	99.0	32.8	87.2	86.8
<i>Amaranthus dubius</i>	139.8	53.0	70.4	63.5
<i>Amaranthus hybridus</i>	100.6	77.3	44.4	94.8
<i>Amaranthus spinosus</i>	112.9	54.2	93.4	42.9
<i>Asystasia gangetica</i>	116.7	81.9	66.3	69.3
<i>Bidens pilosa</i>	102.2	30.5	17.9	79.3
<i>Buddleja saligna</i>	91.4	59.9	ENA	ENA
<i>Bulbine natalensis</i>	100.6	69.3	88.8	69.1
<i>Carpobrotus dimidutus</i>	122.6	39.7	77.0	64.0
<i>Centella asiatica</i>	118.3	55.1	41.3	41.7
<i>Ceratotheca triloba</i>	104.9	42.3	ENA	ENA
<i>Chemopodium album</i>	115.1	54.6	76.5	68.4
<i>Cleome monophylla</i>	103.3	58.8	76.0	57.3
<i>Dichrostachys cinerea</i>	110.8	84.5	36.2	11.5
<i>Ekerbegia capensis</i>	103.8	34.8	45.4	32.9
<i>Elytropappus rhinocerotis</i>	103.8	34.6		
<i>Emex australis</i>	129.0	77.6	75.5	64.1
<i>Ficus sur</i>	103.8	63.4	55.1	65.5
<i>Galinsoga parviflora</i>	-2.2	54.1	17.3	56.9
<i>Guilleminea densa</i>	116.1	48.2	90.8	75.1
<i>Gunnerea perpensa</i>	71.5	30.0	66.8	45.5
<i>Heteropyxis natalensis</i>	101.6	38.7	58.2	24.9
<i>Hibiscus sabdariffa</i>	124.2	64.7	90.3	100.0
<i>Justicia flava</i>	106.0	84.9	57.7	93.2
<i>Leonotis leonutus</i>	113.5	82.5	89.8	76.8
<i>Momordica balsamina</i>	121.0	58.4	79.1	76.6
<i>Oxygonum sinuatum</i>	108.6	12.2	63.3	54.6
<i>Perlagonium sp</i>	109.2	55.6	41.3	14.1
<i>Physalis viscosa</i>	105.9	76.7	62.8	33.3
<i>Portulaca oleracea</i>	154.3	36.1	ENA	ENA
<i>Senna occidentalis</i>	114.0	76.5	86.2	54.1
<i>Solanum nodiflorum</i>	111.8	67.8	37.2	-3.9
<i>Sonchus oleraceus</i>	111.8	86.1	ENA	ENA
<i>Syzgium cordatum</i>	40.5	23.3	78.6	60.2
<i>Taraxcum officinale</i>	94.7	28.9	27.0	75.3
<i>Tetratedia riparia</i>	101.6	33.9	37.8	69.4
<i>Tulbaghia violacae</i>	100.6	56.4	88.8	74.9
<b>Positive control</b>	<b>129.1</b>	<b>83.7</b>	<b>121.9</b>	<b>95.7</b>

## 5. DISCUSSION

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The results of this whole investigation suggest that the plant extracts studied contain bioactive compounds with biological properties. Plants have been tested and found to have a wide spectrum of compounds (secondary metabolites) that are able to manage or treat various diseases. Flavonoids, alkaloids, phenols, tannins, terpenoids, saponins and steroids are some of the compounds that are usually isolated from the plant species. The presence of these phytochemicals in the plant species could be a solution to the use of toxic drugs for the alleviation of inflammatory diseases and wound healing. Two solvents were used for extraction, water and methanol. Antioxidant, cytotoxicity and scratch assay results revealed that methanol is more effective for extracting the compounds responsible for activity within these assays. Isolation of the bioactive compounds is dependent on the solvent used during extraction (Lin *et al.*, 1999). Higher amounts of polyphenols in methanolic extracts are suspected to be the cause of high activity when compared to the aqueous extracts. It has also been demonstrated that aqueous extracts contain an enzyme called polyphenol oxidase which degrades polyphenols thereby decreasing their activity whereas this enzyme is inactive in the methanolic extracts. Organic solvents have also been found to infiltrate the plant tissue easier than the inorganic solvents (e.g. water) for extraction purposes (Balekar *et al.*, 2012) because they possess polar properties, hence, water soluble components in the plant material will be dissolved in the solvent.

### ***Anti-inflammatory activity***

The results showed that the aqueous extracts had a huge effect in inhibiting the activity of lipoxygenase enzyme which is a key enzyme required in the arachidonic pathway for the production of prostaglandins and most methanolic extracts had low effect when compared to the control (NDGA). Aqueous extracts exhibited higher inhibition of the enzyme than the methanolic extracts (Table 2). The possibility of enhanced activities of 5-Lipoxygenase was observed in some of the aqueous extracts. Phenols and terpenoids have been reported as being active in inhibiting 5-lipoxygenase activity (Albano and Miguel, 2010). It has been noted

that amongst all the bio-active compounds isolated from plants, flavonoids are the major ones that serve as anti-inflammatory agents by inhibiting the lipoxygenase and cyclooxygenase pathways (Agnihotri *et al.*, 2010). Flavonoids from plants exhibit almost similar activities to the NSAIDS that are used for inflammatory diseases (Iwalewa *et al.*, 2007). Plants that possess antioxidant properties can also be used as anti-inflammatory agents since it has been reported that lipoxygenases are sensitive to antioxidants due to the formation of a substrate (lipid hydroperoxide) that is required for the lipoxygenase catalysis being inhibited especially in the methanolic extracts (Shailasree *et al.*, 2013). It was shown that aqueous extracts of *P. viscosa*, *T. violacea*, *G. perpensa* as well as methanolic extract of *S. nodiflorum*, *H. sabdariffa*, *M. balsamina*, *A. aspera*, *C. dimidiatus*, *G. perpensa* and *O. sinuatum* were able to act as both anti-inflammatory and antioxidant agents, Table 5 and 6, ranks were used to categorize extracts according to their activity (i.e. high, moderate and low). These results suggested that the plant extracts were capable of inhibiting lipoxygenase preventing the process of Arachidonic acid pathway to take place and scavenging any free radicals produced in excess by activated platelets, neutrophils and macrophages which may be due to an injury. All these plant species consist of common secondary metabolites which have been reported to play a major role in inhibition of lipoxygenase enzymes as well as scavenging of free radicals thus reducing inflammation and promoting wound healing as they are mostly used traditionally for the treatment of wounds. Poor activity of the methanolic extracts could be because only one pathway of inflammation was explored therefore if other pathways of inflammation are investigated improved activity could be observed. Furthermore mostly bioactive compounds of aqueous extracts are required for inhibition of lipoxygenase enzyme than methanolic extracts.

### ***Antioxidant activity***

DPPH was the method used for evaluation of antioxidant activities of the plants. In methanol solution, the DPPH free radical produces a violet color and in the presence of an antioxidant, it gets reduced and disappears then turns yellow (Choi *et al.*, 2002). Results suggested that methanol was the best solvent for extracting compounds responsible for scavenging free radicals compared to water although plants are different; a completely different solvent

might be suitable for each plant since different plants possess different compounds with different polarities. This ability of the methanolic extracts to possess high antioxidant activities compared to the aqueous extracts is that methanol is a strong hydrogen bond accepting solvent (Miguel, 2010) and methanol has both polar and nonpolar properties hence it is able to extract all compounds in solution. Significant free radical scavenging activities in methanolic extracts were observed. Results also showed that even at lowest concentrations methanolic extracts were still able to scavenge free radicals but the activity was dose dependent as the concentration of the extracts decreased also the antioxidant activity decreased (Table 3). It has been reported that the antioxidant activities of plant extracts are due to the presence of phenolic compounds (Albano and Miguel, 2010). Oxidative stress caused by overproduction of free radicals results in tissue damage thereby contributing to the development of a number of disorders including inflammation. Plants have been shown to possess antioxidant properties since some of the constituents are able to scavenge free radicals thereby preventing or reducing oxidative stress (Iwalewa *et al.*, 2007). Compounds from the plant extracts that have antioxidant properties donate electrons or supply protons to the DPPH free radicals, therefore, DPPH acts as both the probe and oxidant (Amoo *et al.*, 2012), they act either as reducing agents, hydrogen donors and singlet oxygen quenchers (Kahkonen *et al.*, 1999). Based on activities with ranks (Table 5 and 6), it was demonstrated that there was a close relationship between scavenging activity and wound closure time in aqueous extracts of *S. nodiflorum*, *G. densa*, *E. capensis* as well as methanolic extracts of *H. sabdariffa*, *M. balsamina*, *T. officinale*, *C. dimidiatus*, *O. sinuatum*. Hence results indicated that the plant species with antioxidant properties possessed wound healing properties as well. During an injury, free radicals are produced to kill any microorganisms that may be present to the wounding site, however these may be produced in excess causing tissue damage therefore it is necessary to have a compound to act as an antioxidant thereby preventing tissue damage that might be caused to the wound. Results suggest that these plants can be used as both antioxidants and wound healing agents. Alkaloids, tannins, saponins, flavonoids and phenolic compounds have been isolated from all these plant species and these chemical compounds are understood to be playing a major role in scavenging free radicals therefore acting as antioxidants. Apart from scavenging free radicals, they have also been found to be associated with anti-inflammatory processes, tissue repair and wound

healing (Amponsah *et al.*, 2013) which further confirm the findings from this study. Furthermore there are reports on traditional use of these plant species for wound treatment, swellings, sores as well as skin disorders thus demonstrating common medicinal properties between them. Essentially antioxidants speed up the healing process by destroying the reactive oxygen species released by the neutrophils during an inflammatory response hence plants with high levels of antioxidants are used for wound treatment.

### ***Safety and toxicity***

In this assay nauplii of *Artemia salina* are used for testing. They are treated with plant extracts at different concentrations. After 24 hours of treatment percentage death of brine shrimps is measured. This assay is very convenient in monitoring biological activities of natural products (Baravalia *et al.*, 2012). The results demonstrated that the plant extracts tested were not toxic to the brine shrimps at all concentrations. No mortality rate was observed. The results imply that no compounds extracted possessed ovicidal or larvicidal properties. Therefore, these results encourage the use of these plant extracts for the management of inflammation and wound healing.

The effect of the plant extracts on the viability of 3T3 NIH mouse fibroblast cells was assessed by the MTT assay. After exposing the cells to the plant extracts at different concentrations the results showed that the extracts stimulated the growth of fibroblast cells at the highest concentration of 1000 µg/ml except aqueous extract of *Guilleminea densa*, *Taraxacum officinale*, *Balsamina mormodica*, *Amaranthus dubius*, *Amaranthus hybridus*, *Cleome monophylla* and methanolic extract of *Solanum nodiflorum* that showed cytotoxic effects at high concentrations (Figure 45 and 46). Thus, this implies that care should be taken when using these plant extracts for biological purposes such as wound healing (Balekar *et al.*, 2012). High levels of free radicals will damage tissues consequently cells leading to reduced wound healing, therefore it is important during healing to have compounds that can act as antioxidants as well as anti-inflammatory agents to prevent or minimize cell damage. Increased viability of cells was observed with plant extracts possessing antioxidant as well as anti-inflammatory activities, in addition extracts which stimulated fibroblast cells were shown

to enhance wound healing. According to Table 5 and 6, it was observed that aqueous extracts of *A. gangetica*, *A. hybridus*, *S. nodiflorum*, *F. sur*, *E. rhinocerotis*, *B. pilosa* as well as methanolic extracts of *E. australis*, *Perlagonium*, *Centella asiatica*, *C. dimidiatus*, *E. rhinocerotis*, *T. riparia* and *S. cordatum* were able to stimulate the growth of fibroblast cells by destroying free radical species that might have been present thereby allowing cells to grow and reproduce. A number of extracts also showed a relationship between anti-inflammatory activity and viability of cells, these include *L. leonurus*, *H. sabdariffa*, *M. balsamina*, *Perlagonium*, *C. album*, *A. aspera*, *C. triloba*, *E. capensis*, *T. riparia*, *A. sessilis*, *B. pilosa*, *G. perpensa*, *P. viscosa*, *F. sur* and *T. officinale*. If an extract inhibits lipooxygenase to perform its function, inflammation will not occur therefore immune response cells will not secrete as much of free radicals thereby damage to cells will not happen hence allowing cells to proliferate. There was also correlation observed between viability of cells and wound closure. Plants that stimulated growth of fibroblast cells promoted wound closure; it was also observed that there was also a decreased percentage wound closure where plant extracts were found to be toxic to the cells. If extracts are toxic to the cells they will die and as a result wound healing will be impaired leading to a chronic wound.

### ***Wound healing properties***

When studying cell migration in vitro, a scratch assay is generally used. The assay involves the creation of a scratch or artificial gap with a pipette tip on a confluent monolayer of cells. The gap or scratch created will close by allowing the cells on the edges of the gap to move towards the scratch until they form contacts with each other. During an injury cells heal in the same manner as in the assay, they move towards the wound protrude, migrate towards each other and close the wound. The second phase of wound healing which is proliferation and migration of either keratinocytes or fibroblasts is covered in this assay (Balekar *et al.*, 2012). Fibroblast cells have an ability to migrate and close wounds; therefore, they are very important in wound repair hence they were chosen for this study (Hostanska *et al.*, 2012).

Aqueous and methanolic extracts were evaluated for changing the rate of migration of 3T3 NIH mouse fibroblast cells. The migration of cells was studied between 0 to 18 hours with

images taken at these different time intervals. Some of the extracts demonstrated a significant increase in a number of fibroblast cells in the exposed area when compared to the control (untreated cells); therefore, the plant extracts stimulated proliferation and migration of fibroblast cells. The results also suggested that there was higher migration rate of fibroblast cells compared to the untreated control. Figure 47 a, b and Figure 48 a, b represent the images of plant extracts that restored the cells to a confluent or near confluent state within 18 hours of treatment. To exclude the possibilities that wound closure could be due to proliferation FBS was used. In previous studies it has been shown that wound healing is associated with anti-inflammatory activities due to the presence of flavonoids; however the exact compounds responsible for wound healing are still unknown. Migration of cells or proliferation of migrated cells could be the reason of increased population of cells in the scratched area (Fronza *et al.*, 2009). Amongst the plants listed by Alam *et al.*, (2011); Reddy *et al.*, (2012); Pawar and Toppo, (2012) which are known to be used for management and treatment of wounds none of the plants in this study were evaluated. It is known that using certain plants that possess anti-inflammatory and antioxidant properties can enhance the rate of wound healing (Jaiswal *et al.*, 2004). Based on ranks in Table 5 and 6, a correlation was observed between anti-inflammatory and wound healing activities in some of the plant extracts, these included the aqueous extracts of *E. australis*, *S. occidentalis*, *Perlagonium*, *C. triloba*, *H. natalensis*, *A. sessilis*, *G. perperna*, *T. officinale* as well as *S. cordatum* and the methanolic extracts of *L. leonorus*, *M. balsamina*, *C. asiatica* as well as *C. album*. During wound healing the bio-active compounds isolated from plant extracts play three major roles, they act as antimicrobials at the wounded site, furthermore they suppress the increase of free radicals produced by the innate immune system to destroy the invading microorganisms at the wound site during the inflammation phase thereafter an increase in cell proliferation and enhanced collagen production occurs (Ghosh and Gaba, 2013). These plant species contain chemical compounds that affect one or more phases of wound healing thereby allowing healing to occur efficiently. From table 7 it was observed that both extracts of *Asystasia gangetica*, *Bulbine natalensis*, *Emex australis*, *Hibiscus sabdariffa*, and *Leonotis leonurus* had good anti-inflammatory activities as well as good wound healing activities, suggesting that these plants can be used to formulate anti-inflammatory or wound healing

agents. They also appeared to stimulate the growth of fibroblast cells causing proliferation to occur thus faster migration rate.

### ***Literature supporting findings of this study***

Literature reported that most of the plants investigated are used traditionally for inflammation and wound healing purposes, these include *Achyranthes aspera*, *Alternanthera sessilis*, *Amaranthus spinosus*, *Bidens pilosa*, *Bulbine natalensis*, *Centella asiatica*, *Galinsoga parviflora*, *Gunnera perpensa*, *Cleome monophylla*, *Dichrostachys cinerea*, *Ekerbegia capensis*, *Ficus sur*, *Justicia flava*, *Leonotis leonurus*, *Mormodica balsamina*, *Pelargonium sp*, *Physalis viscosa*, *Portulaca oleracea*, *Senna occidentalis*, *Solanum nodiflorum*, *Sonchus oleraceus*, *Syzygium cordatum* and *Taraxacum officinale*. Results from this study confirmed and supported the use of some of the plants for wound healing purposes and the work conducted correlates with research that has been done for some of the plants as potential anti-inflammatory and wound healing agents however most tests done so far have been conducted *in vivo* furthermore treatment periods used for previous studies were not the same with the current study ( i.e. in previous studies incubations were for days and in this study incubation period was hours) in addition solvents used in former studies are not the same as solvents used in this study, therefore results may vary substantially due to these factors and when comparing results these factors should be considered, nevertheless there are some similarities observed from the findings. Amrutia *et al*, (2011) reported that the extracts of *Achyranthes aspera* were shown to control the release of various mediators in both the early and late phase of inflammation; results from this study confirmed the potential of *A. aspera* as an anti-inflammatory agent since the aqueous extract was shown to inhibit the lipoxygenase enzyme by 95.2%. Furthermore research conducted by Edwin *et al*, (2008) and Firku *et al*,(2012) demonstrated the wound healing potential of this plant using rats, a moderate wound closure ( 47%) in fibroblast cells was observed in this study , differences in results could be because of different solvents used or different stages that compounds affect during wound healing *in vivo* and *in vitro*.

Good anti-inflammatory, antioxidant and wound healing effects of *Alternanthera sessilis* were observed in this study, these results correlate with work conducted by Mrinmay and Ashok, (2014); Phusrisom *et al*,(2013); Rajani and Gupta, (2011)who demonstrated anti-inflammatory, antioxidant and wound healing abilities of *A. sessilis* . Anti-inflammatory activities of *Amaranthus hybridus* were demonstrated by Omodamirot and Jimoh, (2015)in rats, their work was in agreement with work from this study where the aqueous extract of this plant showed good anti-inflammatory activity *in vitro*. Wound healing, antioxidant and anti-inflammatory activities of *Amaranthus spinosus* were demonstrated by Barku *et al*,(2014) by showing that tannins, flavonoids and terpenoids were the major constituents responsible for these properties, similar results were observed in this study, the plant possessed good anti-inflammatory, antioxidant and moderate wound closure. *Asystasia gangetica* was found to have good anti-inflammatory activities; results were in agreement with the study conducted by Akah *et al*, (2003). Wound healing, antioxidant and anti-inflammatory activities of *Bidens pilosa* were reported by Ezeonwumelu *et al*, (2011) and Hassan *et al*,(2011), high antioxidant, high anti-inflammatory as well as high wound healing activities of aqueous extract of this plant were observed in this study, large difference observed between anti-inflammatory activities of aqueous and methanol extracts could be because bio-active compounds required for lipooxygenase inhibition are extracted more in water than methanol. Pather *et al*, (2011) demonstrated wound healing potential of *Bulbine natalensis*, results from this study also showed a good wound healing property of *B. natalensis*. Many studies have been conducted to show the wound healing potential of *Centella asiatica* including Ruszymah *et al*, (2012) however wound healing ability of this plant was not properly demonstrated in this study. Hassan *et al*, (2012) demonstrated anti-inflammatory activity of *Dichrostachys cinerea* and no research has been conducted on wound healing potential of this plant although it has been reported to be used traditionally for wound healing, aqueous extract of this plant in this study was shown to have good wound healing ability and anti-inflammatory activity which then confirms the traditional use of the plant. Aqueous extract of *Galinsoga parviflora* was shown to stimulate the growth of fibroblast cells in this study, these results agreed with those reported by Bazylko *et al*, (2012) where good anti-inflammatory activities was shown to accelerate healing of wounds and was found to have an active constituent that inhibits cyclooxygenase enzyme. Steenkamp *et al*, (2004) found that *Gunnera perpensa*

stimulates the growth of fibroblast cells in wound healing. A study conducted by Nkomo *et al*, (2010) displayed some anti-inflammatory activities of the plant; former studies are in agreement with this study where good anti-inflammatory activities are also observed.

Aqueous extract of *Heteropyxis natalensis* was found to have good anti-inflammatory activity; this was in agreement with a study carried out by Frum and Viljoen (2006) who also demonstrated the anti-inflammatory activities of *H. natalensis*. Although studies have reported the use of *Hibiscus sabdariffa* against inflammatory diseases, Lin *et al*, (2007) revealed that there was no anti-inflammatory effect observed for this plant species, however results from this study showed that *H. sabdariffa* has a good potential as an antioxidant, an anti-inflammatory and wound healing agent, variation in results mean that more investigations need to be conducted for verification purposes. Agyare *et al*, (2013) showed that *Justicia flava* increased the rate of wound closure; similarly results from this study demonstrated the wound healing ability of this plant. Good antioxidant, anti-inflammatory as well as wound healing activities were observed with *Leonotis leonurus*, which has been shown to have medicinal properties against many skin conditions, results supported a study done by Mazimba, (2015), the plant has also been reported to be traditionally used for skin ailments, these results hence support these beliefs. *Mormodica balsamina* was shown to have good wound healing property; this work supports research conducted by Ilango *et al*, (2010) which demonstrated the potential of this plant as a wound healing agent. Anti-inflammatory activities of *Pelargonium* species were investigated by Boukhatem *et al*, (2013), results from this study showed that aqueous extract of this plant has potential as an antioxidant, anti-inflammatory as well as wound healing agent and no toxicity has been related to the plant. Although *Physalis viscosa* has been documented to have anti-inflammatory properties no work had been conducted to show its inflammatory and wound healing potentials, nevertheless results from this study have shown that aqueous extract of this plant could be a potential as an anti-inflammatory and wound healing agent. Chan *et al*, (2000); Jagan *et al*, (2002) demonstrated the anti-inflammatory activities of *Portulaca oleracea* which was also supported by the results from this study that demonstrated good anti-inflammatory activities and Masoodi *et al*, (2011) showed that the plant accelerated wound healing due to its antioxidant property which was also investigated however low wound healing activity was

observed, could be because of the solvent used or stage of wound healing that's affected. Good anti-inflammatory activities of *Senna occidentalis* were observed in this study, these results supported research conducted by Basha *et al*, (2011), Sheeba *et al*, (2009) illustrated that the plant had potential as a wound healing agent, these findings were supported by the results from this study which showed good wound healing activity.

Jimoh *et al*, (2011) demonstrated the wound healing property of *Sonchus oleraceus*, similar results were observed in this study, plant also possessed some wound healing properties and the antioxidant capability of the plant was investigated by Prichoa *et al*, (2011) which was however found to be very low in this study, this could be because of the solvent used. Jeon *et al*, (2008) demonstrated antioxidant and anti-inflammatory activities of *Taraxacum officinale*; however, wound healing property has not been investigated nevertheless results from this study demonstrated the methanolic extract of the plant to be a good potential as a wound healing agent. Coopoosamy and Naidoo, (2011) demonstrated the potential of *Tetradenia riparia* as a wound healing agent, their findings were in agreement with the results from this study which demonstrated healing potential of the methanolic extract of the plant. *Tulbaghia violacea* was reported by Olorunnisola *et al*, 2012 to possess cytotoxicity and antioxidant activities, therefore, might be a potential as a wound healing agent through killing free radicals and preventing cell death. Results from this study showed *T. violaceae* as a potential anti-inflammatory, antioxidant and wound healing agent, it was also shown to be stimulating the growth of fibroblast cells which is necessary during wound healing process.

### ***Correlation between wound closure and anti-inflammatory activity***

From the results it was observed that *Amaranthus dubius*, *Asystasia gangetica*, *Bidens pilosa*, *Buddleja saligna*, *Carpobrotus dimidiatus*, *Chenopodium album*, *Dichrostachys cinerea*, *Emex australis*, *Ficus sur*, *Guilleminea densa*, *Hibiscus sabdariffa*, *Physalis viscosa*, *Syzygium cordatum*, *Taraxacum officinale* and *Tulbaghia violacea* demonstrated good anti-inflammatory and wound healing activities, positive correlations were shown between anti-inflammatory activity and wound closure, indicating a relationship between inflammation and wound healing, meaning that one factor affects another or relies on the other, this thus

confirms role of inflammation as it is the first step during wound healing process. Therefore, plants that possess anti-inflammatory activities can also serve as wound healing agents. It was also noted that for all the assays *Syzygium cordatum* exhibited low activities for aqueous extracts than the methanolic extracts nevertheless the plant still possessed good antioxidant, anti-inflammatory and wound healing activities.

With all the plants tested it has been reported that they possess chemical constituents that contribute to their properties, these include flavonoids which are known to be mostly involved in the antioxidant and anti-inflammatory properties of the plants. Also, tannins have been documented to contribute to the wound healing potential of the plants.

#### *Limitations of the study*

Not all the plants in this study have been investigated for anti-inflammatory and wound healing properties although they have been reported for these purposes due to limitation of availability of certain plants in the season. Phytochemical tests will need to be conducted in order to verify that a certain biological property is due to the secondary metabolites isolated. Since wound healing was done *in vitro*, animal studies have to be done to confirm the effects of these plant extracts *in vivo*. Use of more than one cell line could be recommended since it is known that cells are different therefore their sensitivity to plant extracts is also different

## 6. CONCLUSION

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This study was aimed at justifying the traditional use of aqueous plant extracts in the treatment of inflammation and wound healing as plants have played a great role in health care. The traditional plants used for medicinal purposes still exert a great deal of importance to people who still cannot afford modern treatments and also in development or discovery of new drugs, therefore, it is extremely important that these plants are studied, their chemical constituents are screened and tested for anti-inflammatory activities and wound healing properties. Protective strategies for the healing of wounds based on resolving the pro- and anti-inflammatory pathways involved in tissue repair be developed to protect regenerative tissue from being damaged by the ROS filled environment of the chronic wound.

There was a positive correlation observed between anti-inflammatory activities and wound healing properties in some of the methanolic extracts tested, this was however expected given that inflammation is the first phase of wound healing therefore, agents that are able to alleviate inflammation must also be able to induce wound healing. Among plants that were investigated, extracts of *Alternanthera sessilis*, *Bidens pilosa*, *Bulbine natalensis*, *Carpobrotus dimidiatus*, *Dichrostachys cinerea*, *Ficus sur*, *Justicia flava*, *Leonotis leonurus*, *Mormodica balsamina*, *Physalis viscosa*, *Senna occidentalis*, *Sonchus oleraceus*, *Syzygium cordatum* and *Taraxacum officinale* confirmed their traditional use as indicated in **Table 2**, this was made evident by their ability to stimulate growth of fibroblasts leading to rapid and effective wound closure.

Therefore compounds of the plant extracts that were found to have anti-inflammatory activities and wound healing properties have to be identified and characterized in order to develop cheaper and less toxic drugs. Methanolic extracts of *Alternanthera sessilis*, *Guilleminea densa*, *Hibiscus sabdariffa*, *Oxygonum sinuatum*, *Syzygium cordatum* and *Tulbaghia violacea* demonstrated the best antioxidant, anti-inflammatory activities as well as wound healing properties, they also proved to be non-toxic to the cells instead they stimulated their growth hence combination of these plants may be prepared to develop formulations or substitutes to the already available wound healing agents, furthermore in future studies the mechanism in

which the compounds affect the activation and release of pro-inflammatory mediators and the stages of wound healing have to be reviewed in order to identify the exact stage of the healing process that a particular plant or part of influences.

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## APPENDIX A

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### PREPARATION OF REAGENTS

#### Antioxidant Activity

##### Preparation of Flavonoid

The flavonoid chosen was Rutin which is a potent antioxidant, is found in the buckwheat plant *Fagopyrum esculentum*. Rutin hydrate (HPLC grade) was purchased from Sigma. A stock solution of 1000 g/ml was prepared which was diluted to 500 g/ml, 250 g/ml, 100 g/ml, 50 g/ml, and 10 g/ml with ethanol.

#### Media and reagents for continuous cell line culture

##### 1. Dulbecco's Modified Eagle's Medium

With 4.5g/l glucose, 0.110/L Sodium pyruvate with L-glutamine (Highveld Biological, South Africa).

##### 2. Foetal Bovine Serum (FBS)

Was filter sterilized which preserve the biological integrity of serum. The serum was aliquoted into 10ml sterile flasks and kept frozen (-20°) until use. (Highveld Biological, South Africa).

##### 3. Penicillin/ Streptomycin (Pen/Strep)

Mixture contained 100mg/ml penicillin G sodium and 100mg/ml streptomycin sulphate in double reverse osmosis water. One ml aliquots were frozen (-20°C) until use (Highveld Biological, South Africa).

##### 4. Phosphate Buffered Saline (PBS)

#### Ingredients per 1 000 ml

Sodium Chloride 8g

Potassium Chloride 0.2g

Potassium dihydrogen phosphate 0.12g

Sodium hydrogen phosphate 0.91g

Double distilled water (ddH<sub>2</sub>O) 1000 ml

Mix Sodium Chloride, Potassium Chloride, Potassium dihydrogen phosphate, Sodium hydrogen phosphate in one litre of double distilled water. And was equilibrated to pH 7.2 filter sterilized and autoclaved aliquoted into 100ml sterile McCartney's Bottles and kept (4°C) until use. (Merck NT, South Africa)

### **5. Dimethyl sulphoxide (DMSO)**

Was pharmaceutical grade (tested in tissue culture) was filter sterilized and aliquoted into 100ml sterile Dark Bottles and kept away from direct sunlight and excessive temperature until use. (Highveld Biological, South Africa).

### **6.70% ethanol**

Contained 70ml Analar grade ethanol (100%) and 30ml distilled water was combined to provide general tissue culture disinfectant (Merck NT, South Africa).

### **7. Complete Culture Media (CCM)**

Contained 20 ml FCS, 10ml NEAA, 2ml Pen/Step mixture in 68ml DMEM and stored in sterile bottle at 4°C.

### **8. Reagents for Microscopy**

0.2 % Trypan Blue Solution (w/v) was made in PBS pH 7.4. Biowhittaker, Walkersville, USA.

### **Reagents for Cytotoxicity assays**

1. 3-{4, 5-dimethylthylthiazol-2-yl}-2, 5 diphenyl tetrazolium bromide (MTT) reagent was prepared by dissolving 5 mg MTT in 1 ml PBS (pH 7.5). The solution was filter sterilized and stored in the fridge. (Sigma –Aldrich, South Africa)

### **Reagents for Lipoxygenase screening assay**

#### 1. Lipoxygenase inhibitor screening assay buffer

3ml of Assay Buffer concentrate was diluted with 27ml of HPLC-grade water. The final buffer at pH7.4 was used for dilution of samples and 15-LO standard.

#### 2. Chromogen

Equal volumes of developing reagent 1 and 2 supplied with the kit were mixed in a test tube.

#### 3. 15-Lipoxygenase standard

10  $\mu$ l of the supplied enzyme was transferred to another vial and diluted with 990 $\mu$ l of Assay buffer

#### 4. Arachidonic Acid

25  $\mu$ l of linoleic acid was transferred to another vial and 25  $\mu$ l of potassium hydroxide was added. The solution was mixed and further diluted with 950 $\mu$ l of HPLC-grade water to achieve a final concentration of 1mM.