PHARMACOLOGICAL SCREENING OF TRADITIONAL MEDICINAL PLANTS USED AGAINST SKIN AILMENTS IN THE FREE STATE, SOUTH AFRICA

By

Valeria Makhosazana Xaba

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Supervisor: Dr L.V. Komoreng

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DECLARATION

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Valeria Makhosazana Xaba (Miss)

DEDICATION

This work is dedicated to all the members of my family, the pillars of my strength, and a special dedication goes to my father, Linda Xaba, the wind beneath my wing, and to my mother, Letticia Xaba, a positive motivating force in my life. I am proud to be your daughter and I hope I have made you proud.

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Abstract

The skin is the largest organ of the body and it is protected by a composition of layers. It consists of three main protective layers namely epidermis, dermis and subcutaneous layer, also known as a fat layer. Its most crucial function is playing a key immunity role in protecting the body by forming a part of a defence and mechanical barrier to the surrounding environment, and thereby preventing invasion by pathogens. The skin is colonized by indigenous microbial flora which comprises of a broad variety of species, among them are *Staphylococccus* sp., *Propionibacteria, Diptheroids*, *Micrococcus*, *Bacillus* species and some fungal species which sustain the health of the skin. The skin can still be susceptible to injuries that allow opportunistic microbial agents to enter the skin. Skin diseases vary from mild conditions which are likely to have an effect on the skin's appearance and can lead to severe conditions which cause disfigurement, disability, and distress or even lead to death. An ethnobotanical survey was conducted and plants used against skin infections were collected and documented. Bark, stem, roots, rhizomes, corms and bulbs were reported to be the most commonly used plant parts. The survey indicated an oral intake of the decoction or concoction preparation. The study documented 22 plant species used by the traditional healers and herbalists of the Free State Province of South Africa for the treatment of wounds and skin infections. Eight frequently used plants, namely *Pentanisia prunelloides, Cotyledon orbiculata, Hermannia depressa, Dioscorea sylvatica, Lycopodium clavatum, Merwilla plumbea, Eucomis bicolar, Eucomis autumnalis* and *Xysmalobium undulatum* were investigated for the presence of secondary metabolites, antimicrobial, antioxidant and anti-inflammatory properties.

Most plant species tested positive for the presence of saponins, flavonoids, tannins and terpenoids. Saponins were detected in *Pentanisia prunelloides* extracts, *Hermannia depressa* and *Cotyledon orbiculata* aqueous stem extracts, and *Xysmalobium undulatum* aqueous and ethanol extracts. Flavonoids were present in almost all plants, particularly *P. prunelloides* aqueous and ethanol extracts, and aqueous extracts prepared from *H. depressa, X. undulatum*, and *C. orbiculata* stem. Tannins were detected in aqueous, ethanol, methanol and acetone extracts prepared from *C. orbiculata* stem, *P. prunelloides* and *H. depressa*. *P*. *prunelloides* showed a high content of total flavonoids (40.43%), total alkaloids (84.8%), and

saponins (19%). Tannins had an absorbance value of 16.54 mg. Total contents found in *H. depressa* were flavonoids (9.70%), alkaloids (7.0%) and saponins (5%). *X. undulatum* showed small and limited amounts of total content values, flavonoids (4.50%), alkaloids (6.7%), and saponins (9%). The presence of most general phytochemicals might be responsible for the plants' therapeutic and pharmacological effects.

P. prunelloides ethanolic extract showed the best activity against *Bacillus pumilus* and *Staphyloccocus aureus* (0.098-0.52 mg/ml). Methanol extracts showed the least activity, but a progressive inhibition of 0.325 mg/ml against *P. aeruginosa* was observed. *H. depressa* acetone extract showed the best activity against *B. pumilus* at 0.098 mg/ml. Aqueous extract displayed good activity against *E. coli*, *S. aureus* and *P. aeruginosa* with the MIC values of 0.098, 0.36 and 0.195 mg/ml, respectively. *Lycopodium clavatum* acetone extract displayed good minimum inhibition against *S. aureus* (0.39 mg/ml), and against *P. aeruginosa* (0.098 mg/ml).

Concerning antifungal activity, the best inhibition was observed with *P. prunelloides* organic solvents (0.049 mg/ml). *H. depressa* extracts also showed low MIC values (0.049 mg/ml-0.33 mg/ml).

The total phenolic content was determined and recorded as gallic acid equivalents. Extracts that showed the highest phenolic content were *H. depressa*, *C. orbiculata*, *Dioscorea slyvatica*, *Eutumnalis bicolar* and *L. clavatum*. *H. depressa* methanolic extract had the highest phenolic content at 2.09±0.07 mg GAE/g, followed by *C. orbiculata* acetone extract at 1.48±0.64 mg GAE/g. Acetone and ethanol extracts of *E. bicolar* and *L. clavatum* displayed good total phenolic content ranging from 0.92±0.13 to 1.50±0.13 mg GAE/g. For DPPH scavenging activity, *C. orbiculata* methanol extract with an IC_{50} value of 0.10±0.03 µg/ml, followed by *D. slyvatica* aqueous extract (0.12±0.03 µg/ml). The total capacity of antioxidant using Phosphomolybdenum assay was also investigated with gallic acid as a frame of reference. The best activity was found in *D. sylvatica* ethanol extracts with an IC_{50} value of 0.04±0.03 µg/ml. Concerning anti-inflammatory activity using 5-Lipoxygenase assay, *L. clavatum* and *C. orbiculata* exhibited a higher anti-inflammatory activity than that of NDGA and inhibited 5-LOX. *L. clavatum* ethanol

extract displayed the best activity (0.02±0.08 µg/ml). *C. orbiculata* ethanol extract also exhibited great activity at 0.09±0.02 µg/ml.

Keywords: Skin diseases, antibiotic resistance, medicinal plant use, antimicrobial, antioxidant and anti-inflammatory.

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5-LOX-5- Lipoxygenase

AA- Arachidonic acid

AIDS- Acquired immunodeficiency syndrome

BHA- Butylated hydroxyanisol

BHT- Butylated hdroxytolune

CA MRSA- community- associated methicillin-resistant *Staphylococcus aureus*

CDC- Centres for disease control and prevention

cSTTIs- complicated skin and soft tissue infection

DMSO- Dimethylsulfoxide

DPPH- 1-1-diphenyl-2-picryl hydrazyl

GAE mg/g- Gallic acid equivalents

HCUP- Healthcare cost and utilization project

HIV- Human immunodeficiency virus

$^{\cdot}$HO- hydroxyl radical

INT- *p*-iodonitrotetrazolium violet

MIC- Minimum inhibitory concentrations

MRSA- Methicillin resistant *Staphylococcus aureus*

NDGA- Nordihydroguaiaretic acid

NIS- Nationwide inpatient sample

NO- Nitric oxide

NSAIDs- Non-steroidal anti-inflammatory drugs

($O_2^{\cdot-}$) - Superoxide anion

OTC- Over-the-counter

PVP-1- Polyvinyl pyrrolidone iodine

ROO- Peroxyl radical

ROS- Reactive oxygen species

SAID- steroidal anti-inflammatory drugs

SSTIs- Skin and soft tissue infection

uSSTIs- uncomplicated skin and soft tissue infection

WHO- World Health Organization

Chapter 1

Skin and Soft Tissue Infections

1. Introduction

1.1. Skin as the protective barrier for the host

The skin is the most important and largest organ of the body and it is protected by a composition of layers. Hence it is defined as a complex organ that is able to resist infections based on its properties (Nester et al., 2004). According to Fonacier et al. (2010) and Dryden (2010), the skin is one of the largest immunologic organs.

The skin consists of three main protective layers namely epidermis, dermis and subcutaneous layer, also known as a fat layer (Weideman, 2007; Torok & Conlon, 2005; Davis et al., 2005). The epidermis, which is the outermost layer, is directly contiguous with the environment **(Figure 1.1)**. It is a superficial thin layer composed of epithelial cells and embedded keratin. The skin reproduces the protective epidermis every 30 days. It functions without blood vessels and is said to regenerate in the absence of scar tissue (Tortora & Grabowski, 1996; Weideman, 2007). The epidermis, being first in the line, is supported by the stratum corneum which is a desiccated layer that protects from injuries and microbial invasion (Ong et al., 2002, McNeil et al., 2014). Infection of the skin occurs by invasion, damage and infection. This may have an effect on the anatomical layer. The dermis is the layer of skin beneath epidermis, with a deeper thicker portion composed of tight woven connective tissues. It functions in the presence of blood vessels and is responsible for the skin's elasticity, strength and regenerate with scar formation (McNeil et al., 2014). As an overlying portion, the subcutaneous layer is composed of fat layers, fascia and muscle which helps insulate the body from heat and cold and serves as an energy storage area (Torok & Conlon, 2005; Weideman, 2007; Dryden, 2010).

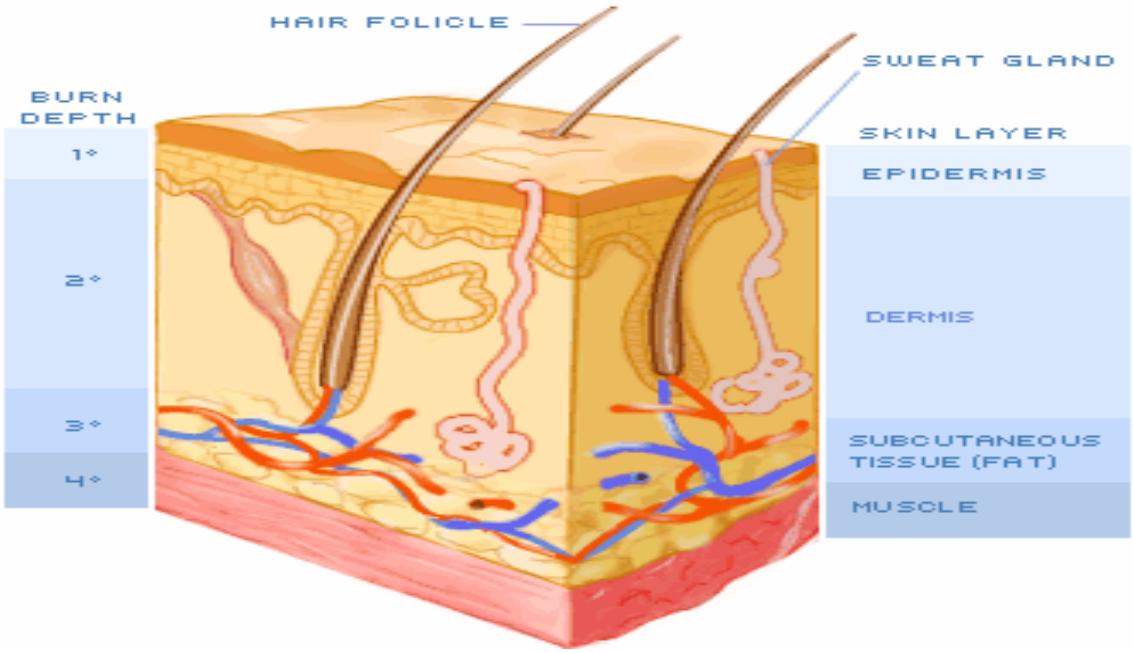

Figure 1.1: Shows the main protective skin layers (http://www.skinhealing.com/2/2 skinburnscars.shtml)

The skin covers the whole surface of the body, both internally and externally (Cutting, 2001, Weideman, 2007). The skin's responses to the environment is that it is a large contributor to external and internal factors. Its most crucial function is playing a key immunity role in protecting the body by forming a part of a defence and mechanical barrier to the surrounding environment, and thereby preventing invasion by pathogens. It also aids in sustaining microorganisms that influence human health and disease (Ong et al., 2002; Nester et al., 2004; Torok & Conlon, 2005; Grice et al., 2009). Functioning as a protectant organ of the inner tissues and layers, the skin is colonized by indigenous microbial flora which comprises of a broad variety of species, among them are *Staphylococcus* sp., *Propionibacteria, Diptheroids*, *Micrococcus*, *Bacilli* sp. and some fungal species (Torok & Conlon, 2005; Dryden, 2010; Davis et al., 2005). These microorganisms, referred to as normal flora, sustain the health of the skin and act as competitive inhibitors of pathogenic microbes (van Hemmen, 2000; Bowler et al., 2001; Nester et al., 2004; Kowsalya, 2012).

Although skin serves as a protective barrier, it can still be susceptible to injuries that allow opportunistic microbial agents to enter the skin (Ong et al., 2002; Torok &

Conlin, 2005; Kowsalya, 2012). For example, *Staphylococcus epidermidis*, a good example of a beneficial commensal bacteria of the skin, may have fatal consequences if it breaches the skins' integrity and enters the blood circulation (Cutting, 2001; Kowsalya, 2012). This type of breaching can make an entry route for the microbial flora to generate an infection which can invade deep underlying tissues (Cooper & Lawrence, 1996; O'Dell, 1998; Kingsley, 2001; Weideman, 2007). Examples of such infections include leg ulcers, burns and surgical or traumatic wounds which mostly allow entry and colonization of a wide range of bacteria (Dryden, 2010). The most common early colonizers of burn wounds include *S. aureus, Escherichia coli* and *Streptococci* sp. (Pandit & Gore, 1997; von Eiff et al., 2002; Bagdonas et al., 2003). Their pathology is provoked by the fact that they penetrate and invade unburned underlying subcutaneous tissues to form a myriad of abscesses in variable sizes (Pruitt et al., 1998; DiNubile & Lipsky, 2004; Weideman, 2007). Investigations have been conducted and reports show that *S. aureus* is a single causative bacterium in approximately 25 to 30% of cutaneous abscesses. It has also been frequently isolated in superficial infections. According to Armstrong et al. (1995) and Hansis (1996), it is impossible to differentiate between causative pathogenic and non-pathogenic species in polymicrobially infected wounds, since the responsible microorganisms or etiologic agents cannot be simplified because of the presence of the mixed pathogens; commensal or facultative and potentially virulent agents.

Another group of colonizers such as *Pseudomonas aeruginosa*, along with *Enterococcus* sp. and *Bacilli* sp. cause invasive burn infections in compromised hosts and injection drug users (Pruitt et al., 1998; DiNubile & Lipsky, 2004; Dryden, 2010; Kowsalya, 2012). Other pathogens including those among the β-haemolytic *Streptoccoci* group A, *Kliebsiella pneumoniae*, *Acinetobacter buamanni* and *Candida albicans* cause a wide variety of diseases (Bisno & Stevens, 1996; Stevens et al., 2005; Torok & Conlin, 2005, Dryden, 2010; Wang et al., 2014).

1.2. Diseases of the skin

Skin diseases vary from mild conditions which are likely to have an effect on the skin's appearance and can lead to severe conditions which cause disfigurement, disability, and distress or even lead to death (Maseleno & Hasan, 2012). Skin diseases such as wounds and burns are recently regarded as one of the most dangerous diseases and infections leading to a high mortality rate. A number of common ailments are accountable for the vast majority of the skin disease burden (Hay et al., 2006; 2014), and these ailments cannot be easily diagnosed due to their broad variety (Hu et al., 2011).

Wound and skin infections represent the invasion of tissues by bacteria, fungi or viruses. Skin infections may be grouped according to causative organisms, soft tissues involved or clinical syndrome (DiNubile & Lipsky, 2004). Issues such as epidemiology, pathogenesis and prognosis of the infection are also considered. The microorganisms that typically infect wounds and skin depend on what is present in the environment, the state of person's immune system and the depth of the wound. According to Grice et al. (2009), microbes are reported to have an impact in the pathophysiology of many skin disorders. *Staphylococcus* sp. is considered to be the most problematic bacteria that can cause a variety of skin infections, some of which are severe and even life threatening (Klimlek, 1985; Page & Beatti, 1992; Mayhall, 1993; Nichols & Smith, 1994; Haneke, 1997; Giacometti et al., 2000; Davis et al., 2005). According to a study done by Boehncke et al. (2005), *S. aureus* is carried by a measurable percentage of the world's population, and this constitutes a risk factor for the progression of localized skin, soft tissue and systemic infections.

According to a number of studies undertaken by Davis et al. (2005), Bickers et al. (2006), Brooks & Jefferson (2012) and Davis (2014), skin conditions pose a very significant financial burden in the form of physician visits, hospital care, prescription drugs and over-the-counter (OTC) products for treating or managing skin conditions, and indirect costs caused by productivity losses. Davis et al. (2005) argues that OTC bandage products are efficient and that they contain antimicrobials. They have been used for decades for quicker healing processes, such as covering and protecting wounds, but they may not be effective in managing and reducing the

invasion of bacteria in wounds. Moreover, these products pose a disadvantage to the underprivileged communities in developing countries since they are inaccessible and expensive, and there is a shortage of appropriate products used in treating wounds and skincare (Brooks & Jefferson, 2012). Furthermore, Bickers et al. (2006) mentions that their inaccessibility has led to a significant burden on health status and the quality of life.

According to Mckoy (2011), the underdeveloped or developing countries have limited or no skincare specialists and patients often travel to developed countries for consultation and treatment. The lack of affordability for skin treatment products has led to more than 3000 identified varieties of skin diseases, where symptoms range from simple burning to severe itchiness and deep wounds (Robson, 1997; McGcuckin et al., 2003; Bickers et al., 2006; Hay et al., 2006). These conditions, subsequently, lead to physical disfigurement and associated emotional and social affection. Such disfigurement, disability can cause morbidity (Hay et al., 2006; 2014). Although mortality rates for skin diseases are relatively low, they cause an enormous burden and pose a negative impact on the quality of life, as they are persistent and are difficult to treat (De Wet et al., 2013).

1.3. Different types of skin infections

Skin infection occurs as a result of exposure to pathogenic microbes that invade the skin and cause an infection (Pattanayak & Sunita, 2008). According to Bowler et al. (2001), infection is generated when virulence factors of a single or number of microorganisms in a wound outcompete the natural host habitats which are commensal to the immune system. Some skin infections are generally easy to diagnose, involving a number of limited predictable pathogens and respond well to antibiotics (Robson; 1997; DiNubile & Lipsky, 2004). However, some skin infections can be complicated and difficult to treat.

There are several types of skin infections. The type of infection often depends on the cause. Wound infections can be classified into, skin and soft tissue infection (SSTIs). SSTIs are defined by Wang et al. (2014) as common infections or infections occurring beneath the skin or the tissue beneath the skin and affect all age

groups (Torok & Conlin, 2005). SSTIs represent an inflammatory response of microbial invasion on the epidermis, dermis and subcutaneous tissues (Dryden 2010). According to Dryden (2010), SSTIs can be classified according to the anatomical site of infection or according to their microbial aetiology or by severity.

Most people will experience only one episode of SSTIs during their lifetime, recurrence can occur in certain individuals (Torok & Conlin, 2005). SSTIs range from mild infections, such as pyoderma, to serious life threatening infections, such as necrotizing fasciitis. Most SSTIs can heal on their own but some are serious and deadly and require medical treatment (Wang et al., 2014). Moreover, some of them cause persistent swelling, bacterial resistance, recurrent events, and side effects due to the long-term use of medical treatment. SSTIs can be 'complicated STTIs' (cSTTIs) or 'uncomplicated SSTIs' (uSSTIs); uSSTIs are very common and can be extreme (Dryden, 2010; Wang et al., 2014). According to Eron et al. (2003), Dryden (2010), Ghafur & Shareek (2012), Ong et al. (2012) and Wang et al. (2014), SSTIs are classified into 5 categories, comprising superficial uncomplicated infection (includes impetigo, erysipelas and cellulitis), complicated necrotizing infection, infections associated with bites and animal contact, surgical site infections and infections in the immunocompromised host.

1.3.1. Uncomplicated SSTIs

The uSSTIs include abscesses, impetiginous lesions, and cellulitis. Many traumatic wound infections can also be considered uncomplicated when they are as the result of common skin colonizers (Giordano et al., 2007). These infections are caused by Gram-positive microorganisms (DiNubile & Lipsky, 2004). Lymphangitis, cellulitis and erysipelas infections have been found in streptococci isolates. β-heamolytic streptococci is able to produce a variety of toxins that may affect the soft tissues due to its virulence potential (Dryden, 2010).

1.3.1.1. Impetigo

Impetigo is an infection occurring on the epidermis, which is caused by *S. aureus* or group A streptococci (Torok & Conlin, 2005). Group A streptococcus was formerly the cause of impetigo, however, recent studies also revealed the presence *Staphylococcus* isolates (Torok & Conlin, 2005). Sites of infection are observed on

the face and hands, with accompanied symptoms. Impetigo starts on exposed surfaces appearing as small vesicles that postulate rapidly and ruptures readily; the purulent discharge dries and forms crusts which form a golden-yellow scab. The purulent discharge is characterized by dry, yellow, stuck on crusts, pruritus, and scratching of lesions, which results in further spreading of the infection (Ghafur & Shareek, 2012).

1.3.1.2. Erysepelas

Erysepelas is a painful lesion with bright red, edematous, indurated appearance with raised and sharply demarcated borders. This kind of infection is superficial and involves prominent lymphatic involvement (Ghafur & Shareek, 2012). It is caused by group A streptococcus. Symptoms are accompanied by fever. Other predisposing factors include lymphoedema, venous stasis, obesity, diabetes mellitus, alcohol abuse and nephrotic syndrome. Leucocytosis is also common. Infants, young children and older adults are the most commonly affected (Ghafur & Shareek, 2012).

1.3.1.3. Cellulitis

Cellulitis Is an acute spreading infection that involves subcutaneous tissue. The condition is manifested by local sign of inflammation and, in most case, by fever and leucocytosis. It is most commonly caused by *S. aureus* and streptococci species (Torok & Conlin, 2005; Ghafur & Shareek, 2012). Group A β-haemolytic streptococci can cause aggressive cellulitis (DiNubile & Lipsky, 2004). Trauma and underlying skin lesion often lead to the development of cellulitis (Ghafur & Shareek, 2012). The *H. influenza* and *Pneumococcus* have also been reported to cause the condition.

1.3.2. Complicated necrotizing infection

Complicated SSTIs characteristically involve deeper skin structures or coexist in patients with comorbidities or immune suppression (Nichols, 1999; Giordano et al., 2007). Included in this category are necrotizing fasciitis, psoriasis, eczema, infected ulcers, burns and major abscesses.

1.3.2.1. Necrotizing fasciitis

Necrotizing fasciitis is described by Ghafur & Shareek (2012) as a disease condition which spreads rapidly in the fascial planes of connective tissue, resulting in tissue necrosis. It is a deep-seated infection of the subcutaneous tissue that results in a progressive destruction of fascia and fat (Bisno & Stevens, 1996). Any part of the body may be infected, but it is most common on legs (Ghafur & Shareek, 2012). Other sites of infection include groin areas, abdominal wall, and postoperative wounds.

Necrotizing infections cause cell death (Wang et al., 2014). *Streptococcus pyogenes* has been found as the leading bacteria, but any other, either alone or together (polymicrobial) can cause the disease. According to Stevens et al. (2005), necrotizing fasciitis can be divided into Type 1 (which is polymicrobial) and Type 2 which is caused by Group A streptococcus or in combination with other organisms, particularly *Staphylococcus*. Fungi are also isolated in necrotizing infections. It is said that the organism mostly get the entry route via trauma, surgery and ulcers (Casali et al., 1980).

1.3.2.2. Psoriasis

Psoriasis is known to affect more than 2% of the world's population. It is characterized by symptoms of scaly, red cutaneous plaques which lead to inflammatory infiltrates and epidermal hyperproliferation (Robert & Kupper, 1999). Psoriasis occurs in stereotypical sites such as umblici, gluteal creases, occiputs, elbows and knees. Microbes are predicted to play a role in the pathophysiology of many common dermatoses with predilection for specific skin sites eg, atopic dermatitis, psoriasis, acne, seborrheic dermatitis (Grice et al., 2009). It has been linked with bacterial and fungal infections of the skin (Robert & Kupper, 1999; Grice et al., 2009). The sites of infection include the outer elbows, knees and lower back, where repetitive trauma is consistent (Grice et al., 2009). Other sites include umbilici, occiputs and gluteal creases (Boehncke et al., 2005).

1.3.2.3. Eczema/ Atopic dermatitis

Eczema, also referred to as atopic dermatitis, is a chronic inflammatory skin disease. It is classified under secondary infections, which are responsible for further infections in chronic skin conditions (DiNubile & Lipsky, 2004). The recurrent infections such as skin lesions which are as a result of bacterial, viral and fungal pathogens trigger this type of disease (Ong et al., 2002). Approximately 30% individuals infected with atopic dermatitis are found to have bacterial or viral infections of the skin (Christophers & Henseler, 1987; Ong et al., 2002). House dust mite, *Dermatophagoides pteronyssinus*, is reported to be responsible for atopic dermatitis (Dryden, 2010). According to Robert & Kupper (1999) and Leung (2000), atopic dermatitis is often found frequently in families suffering from asthma and allergic rhinitis. Sites of infection are on the inner bend of the elbow. It is accompanied by scaly dry skin and itchiness (Grice et al., 2009). The prevalence of atopic dermatitis is associated with *Staphylococcus aureus* infections which have led to an increased colonisation on atopic skin lesions (Bunikowski et al., 2000).

1.3.3. Infections associated with bites and animal contact

Bite wounds are ubiquitous throughout the world. Animal bites are a public health issue, with up to 2% of the population being bitten each year (Thomas & Brook, 2011). Several studies have evaluated a broad range of pathogens isolated from various human and animal bite infections. Human bites are reported by Dryden (2010) as life threatening and can result in serious soft tissue infection with *Streptococcus anginosus* (52%), *S. aureus* (30%), *Eikenella corrodens* (30%) and *Candida* sp. (8%) found in the SSTIs. Human saliva can contain up to 109 organisms per ml, and there may be 190 different bacterial species present (Thomas & Brook, 2011). The most common pathogens in cats and dogs are *Pasteurella* sp., *Staphylococcus* and *Streptococcus* sp. (Thomas & Brook, 2011).

1.3.4. Surgical site infection

Surgical site infections (SSIs) are serious operative complications that occur in approximately 2% of surgical procedures and account for 20% of healthcare-associated infections (de Lissovoy et al., 2009). SSIs are devastating and common complications of hospitalization, occurring in 2% to 5% of patients undergoing

surgery in the United States (Anderson, 2011). Over the past 50 years, the frequency of surgical procedures has increased, procedures have become more invasive, with a greater proportion of operative procedures including insertion of foreign objects, and procedures are performed on an increasingly morbid patient population. Clinical findings from Nationwide Inpatient Sample (NIS) from the Healthcare Cost and Utilization Project (HCUP, 2005) conducted findings of 7 categories of surgical procedures ranging from neurological, cardiovascular, colorectal, skin, subcutaneous tissue, breast, gastrointestinal, orthopaedic, and obstetric and gynaecologic surgery.

According to Torok and Conlin (2005), surgical wounds are divided into superficial (skin or soft tissues) and deep (involving fascia or muscle). They may be susceptible to microbial invasion and contamination (Raahave et al., 1986; Bowler et al., 2001). *Staphylococcus aureus* is the most common cause of SSI, with 20% to 37% of SSI cases reported in the community hospitals that report to the Centers for Disease Control and Prevention (CDC) (Anderson, 2011).

Trauma is among the most common underlying event for all wounds and it may be considered to be accidental or intentionally induced (Robson, 1997; Giacometti et al 2000; Bowler et al., 2001; Sen et al., 2009). It is hospital-acquired and is grouped according to how it is acquired, i.e. surgically, use of intravenous medical devices or the result of a disease process such as chronic ulcer on the foot of a diabetic patient (as host), and perioperative antibiotics (Robson, 1997; Torok & Conlin, 2005).

1.3.5. Infections in the immunocompromised hosts

Diseases of the skin and mucous membrane were among the first recognized clinical manifestations of Acquired Immunodeficiency sydnrome (AIDS) (Tschachler et al., 1996; Mckoy, 2013). Tschachler et al. (1996) argues that over the past decade it has become increasingly clear that cutaneous disorders, are not only associated with terminal immunodeficiency but also occur throughout the course of Human Immunodeficiency Virus (HIV) infection. Reports by Tschachler et al. (1999) and (2013) also show that more than 90% patients develop skin or mucous membrane conditions at some time in the course of HIV/AIDs infection and, in many instances,

the skin is the first organ to be affected. Such infections and diseases of the skin have led to high mortality rates among patients who are hospitalized (Weideman, 2007). Moreover, difficulties which involve using treatment, especially antibiotics, are experienced in the case of absence of an intact immune system (Dryden, 2010).

1.4. Causes of skin infections

The causal effects of wounds and skin infections are reported to be both genetic and environmental factors (Davis, 2014). High humidity, heat and poor sanitation and hygiene levels have also been associated as causal factors, with an increased risk of fungal and bacterial skin infections (Tschachler, 1996; De Wet et al., 2013). Studies undertaken by Mckoy (2011) and Davis (2014) reported that the effects of climate change may have a negative impact due to the rising temperatures which have shifted the behaviour and distribution of disease vectors. Hot and humid climatic conditions are problematic especially in the developing countries, namely in Sub-Saharan Africa where it was found that over 78 million people were infected with *Tinea capitis*, a superficial skin infection affecting the scalp (De Wet et al., 2013). Such vectors are facilitated by insects, hurricanes, storms, and flooding. Overpopulation leading to overcrowding and hygiene practices have been investigated and reported to influence the transmission of infection (Mckoy, 2011; De Wet et al., 2013). Other causes include allergens, weakened immune system, the contents of the skin lipids, pH, sweat and sebum secretion, vascular insufficiency, disrupted venous or lymphatic drainage, diabetes mellitus, cellulitis, presence of foreign body, accidental or surgical trauma, obesity, poor hygiene and certain immunodeficiencies (DiNubile & Lipsky, 2004; Grice et al., 2009; Davis, 2014).

According to Bowler et al. (2001) and Davis, (2014), opportunistic pathogens and nosocomial infections are important causes of infection in burn wounds due to a compromised skin barrier. DiNubile & Lipsky (2004) strongly emphasises that wound infections are highly diverse in their aetiology, clinical manifestations and severity. Infections caused by fungal pathogens, pose a threat especially in patients with HIV/AIDS, diabetes, and open wounds (Hurinanthan, 2009).

1.5. Prevalence

Skin infections and diseases are on an alarming rate and have become a burden, especially chronic wounds which are the most common in many countries (McGuckin et al., 2003). Skin infections are all over the world, and they amount to an approximation of 34% of all known and occupational diseases affecting people of all ages, from children to elderly people (Abbasi et al., 2010; De Wet et al., 2013). Studies by Mckoy (2011) reported that, in developing countries, the prevalence of skin diseases has the same prevalence as in the developed countries. A ratio of 3 to 4 million people per dermatologist has been estimated in urban areas of South Africa (Mckoy, 2011). Skin diseases in developing countries are most dominant with Buruli ulcer (*Mycobacterium ulcerans* infection), Chagas disease (American trypanosomiasis), leishamaniasis, leprosy (Hansen's diseases), lymphatic filariasis, onchocerciasis, trachoma and yaws. WHO (2005 & 2011) stresses the need to address skin diseases in rural and developing countries especially those with serious economic, social and health burdens.

People with HIV/AIDS are mostly susceptible to various skin disorders such as bacterial infections or tropical ulcers. According to Mckoy (2013), the burden and epidemic of HIV/AIDS in Africa and all over the world has brought critical issues on the skin conditions. In Africa, India and the western Pacific, tropical ulcers are observed and are associated with a combination of bacterial, unidentified spirochetes, and along with exposure of stagnant water and mud. A study by Hay and Fuller (2011) indicated that 50 to 80% of skin diseases depend on whether or not there is a local endemic disease such as scabies and *Tinea capitis*. *Tinea capitis* is a very common and contagious fungal disease and has been observed to spread extensively especially in homes and schools (De Wet et al., 2013). Skin ailments present a major health burden in both developed and undeveloped countries. For example, in the US, skin infections caused by methicillin-resistant Staphylococcus aureus (MRSA) result in approximately 12600 hospitalizations while the invasion MRSA results in approximately 94 360 infections and 18 650 deaths each year, a rate which exceeds that of AIDS (Sen et al., 2009; De Wet et al., 2013; Davis, 2014). A negative impact on the public health and economy of United States has been seen due to the threat of chronic wounds, which represent a large fraction. One out of

three people are affected (Davis, 2014). Each year about 18 650 deaths are reported, along with 126 000 hospitalizations have been observed due to the causal agent, methicillin-resistant *S. aureus* (MRSA).

In South Africa, over 19, 500 fire related deaths are reported annually and they rank among the 15 leading causes of death among youngsters aged between 5 to 29 years. Burns are reported to cause serious public health problems due to global increase in burn mortality rates (Weideman, 2007; De Wet et al., 2013). De Wet et al. (2013) suggested that burn victims were not only susceptible to serious conditions but were actually exposed to fatal *Pseudomonas aeruginosa* infections. Burn injuries are said to cause mechanical disruptions which allow environmental microbes to invade the deeper tissues (Weideman, 2007, Grice et al., 2009). Kowsalya (2000) and Bowler et al. (2001) estimated that approximately 50-75% of deaths due to infections are related to burn injuries. Eczema is one of the leading and the largest allergen which is said to be related to contact dermatitis with a prevalence of 0.7 to 18.6% (De Wet et al., 2013; Davis, 2014. Exposure to Herpes simplex and zoster (26%), from sunlight (17%), acne (7%), skin cancer (1%), benign skin growths (4%), fungal nail infections (8%), skin ulcers (< 1%) and autoimmune conditions such as psoriasis, lupus, and vitiligo (< 1%), and melanoma (<1) were found to be the most prevalent (Davis, 2014). Leg and pressure ulcer infections are reported to constitute approximately 30% of total number of isolates in non-infected individuals (Brook & Frazier, 1990; Bowler & Davies, 1999). *S. aureus* was the prevalent isolate in diabetic foot ulcer, together with other aerobes such as *Staphylococcus epidermidis*, *P. aureus*, and *Enterococcus* (Haneke, 1997). A study conducted in 2004 cited skin diseases among the top 15 groups of medical conditions (Bickers et al., 2006).

1.6. Management of skin infections

According to Cutting (2001), before a wound care decision can be taken, there are certain considerations that one should take into account, including how safe is the treatment and its effectiveness. The use of antibiotics is one of the effective methods, especially in treating wounds and controlling bacteria (Robson, 1997),

however, the development of resistance against certain antibiotics has been observed. According to Holzheimer (2001), antibiotics should show low toxicity, a low incidence of allergy, and should form part in the selection of virulent organisms. Antibiotics and surgical drainage are the basis of treatment for *Staphylococcus* infections, but the resistance evoked by the strains to multiple agents has caused complications on the choice of treatment or therapy (Cutting, 2001; Dryden, 2010). Iodine, such as polyvinyl pyrrolidone iodine (PVP-1), has been proven to be one of the effective antiseptics and it plays a role in managing infections with bactericidal agents, also against MRSA (Goldenheim, 1993; Drosou et al., 2003; Khan, 2005). Antibiotic prophylaxis plays an important role in prevention of wound infections and is often used when the infection is severe, for example in the case of cellulitis (Bowler et al., 2001). Bowler et al. (2001) warns that this kind of systemic antibiotic therapy should not be further used unless the infected site shows apparent features of the current infection. Further emphasis is that, the over-use of such topical creams may have cytotoxic effects on the fibroblasts, and may cause burning sensations and staining of the skin. Among others is silver sulfadiazine, which is a well known antibiotic. Silver sulfadiazine has excellent antimicrobial spectrum of activity, low toxicity, ease of application and is thought to minimize pain. Reports show that it has inhibiting potentials by targeting DNA replication and modification of the cell membrane in *S. aureus, E. coli, Kliebsella* sp., *Pseudomonas* sp.*, Proteus, Enterobacteriaceae* and *Candida albicans* (McGuckin et al., 2003). Consequently, it may cause a transient leukopenia, 5 to 15% incidence on large burn wounds. Not only does it contribute to side effects but it is also expensive and inaccessible.

Topical antibiotics in wound management are said to generate the rise of resistant microorganisms, and apart from the resistant strains, there has been issues of hypersensitivity reactions being delayed with the use of topical antibiotics (Huovinen et al., 1994; Bowler et al., 2001). Therefore, it is of paramount significance that a clear understanding on antibiotic guidelines work and how one can opt for the empirical treatment (Cutting, 2001). Wounds can be cleansed with the use of hydrogen peroxide, acetic acid, hexidine gluconate and some concentrations of povidine-iodine (McGuckin et al., 2003; Brooks & Jefferson, 2012). There are other topical creams and antiseptics which could heal wounds and facilitate the treatment

faster. According to Davis et al. (2005), occlusive bandages help facilitate healing processes, since they have the ability to absorb a few drops of blood and decrease protruding wound fluid. There are numerous antibiotics that have been incorporated into bandage pads to prevent infection from *S. aureus* in minor cuts, scrapes, and burns (Davis et al., 2005). Among them are mupirocin and fusidic acid, hydrocolloid, polyurethane or hydrogel which are specifically used in the treatment of Staphylococcal infections and nose and ear infections, including MRSA (Cutting, 2001). The above mentioned topicals are safe and less toxic to humans, however, evidence shows an increasing bacterial resistance to mupirocin (Pappa, 1990). Moreover, according to a study done by Mulaudzi et al. (2013), on the investigation of the effects of anti-inflammatory drugs such as non-steriodal anti-inflammatory drugs (NSAIDs) are associated with renal and gastrointestinal toxicity. Hydrocolloid dressing was reported to be the only material that could control the spread of *S. aureus* and *P. aeruginosa,* and it has the ability to adhere to the skin surrounding the wound, thereby acting as a physical barrier (Davis et al., 2005). Hydrocolloid is one of the most important compounds used also as a botanical product with its bioactive molecules in the treatment of wounds (Lall & Kishore, 2014). Polyhexamethylene biguanide is also recommended in reducing burn wound pathogen *P. aeruginosa* from entering the wounds, in the case of partial thickness wounds.

Other antibiotics used in the treatment of SSTIs include cotrimoxazole, daptomycin, clindamycin, etc. All these antibiotics have re-emerged as a good choice for mild to moderate SSTIs due to the community-associated methicillin-resistant *S. aureus* (CA MRSA). Clindamycin has been reported to be a good anti-staphylococcal activity aiding in soft tissue penetration and toxin suppressing activity (Ghafur & Shareek, 2012). Cotrimoxazole has been reported to have side effects such hyperkalaemia in elderly people. The use of broad-spectrum antibiotics raises a problem of emergence of resistant microorganisms, the expensive costs and effectiveness, and the fact that these treatments require hospital care (Cutting, 2001).

Aside from the use of antibiotic therapy, studies undertaken by Brooks & Jefferson (2012) report that normal saline, procaine spirit, distilled water and boiled water have been evidently used to clean vulnerable or compromised skin. However, Brooks &

Jefferson (2012) warns that caution should be taken, particularly, in developing countries where sources of water are obtained from bore holes, wells, rivers or lakes, and ponds since water can be likely polluted by animal and human waste, and by chemicals and heavy metals. This sort of problem can cause a rise in skin and wound infections. It is, therefore, crucial to implement effective treatment strategies that will result in significant personal and public health gains (Hay et al., 2006; 2014).

1.7. Physiology of wound healing

Wound healing is a complicated and complex process that involves at least four different cell types. The process is commonly referred to as occurring in "phases". The main phases include homeostasis, inflammation or re-epithelization, proliferation or granular tissue formation and maturation or remodelling (Diegelmann & Evans, 2004; Steenkamp et al., 2004; Shenoy et al., 2009). Homeostasis is when bleeding occurs right after tissue injuries, resulting in disruption of blood vessels and clotting (Chen & Rogers, 2007). Inflammation phase, also known as the re-epithelization, involves the body's natural response to trauma (Advanced tissue, 2015). It plays a role in repairing of tissues and restoration of function, hence being the most crucial result of injury (Chen et al., 2007). In the proliferation phase, the wound heals and begins to build new granulation tissue, the indication of reddish or pink wound which shows healing as there is enough supply of nutrients and oxygen (Advanced tissue, 2015). During the proliferative phase, there is formation of the epithelium to cover the wound surface with concomitant growth of granulation tissue to fill the wound space. Granulation tissue formation involves proliferation of fibroblasts, deposition of collagens and other extracellular matrices, and development of new blood vessels (Chen et al., 2007). The new tissue is formed in the maturation or remodelling phase. Once the new tissue within the wound is formed, the remodeling phase begins, it maintains tissue restoration, structural integrity and functional competence (Chen et al., 2007). According to studies undertaken by McGcuckin et al. (2003), Nestor et al. (2004) and Marwah et al. (2007), when the process of homeostasis or inflammation is impaired, sites of the infection increases, although microorganisms have mixed or beneficial effect on the granulation and epithelialization stages of wound healing.

1.8. The use of traditional medicine for wound care

The World Health Organization (WHO) defines traditional medicine as the sum total of the knowledge, skills and practices that are based on theories, beliefs and experiences used in the maintainance of health (WHO, 2000). Traditional medicine plays a vital role in the lives of thousands of South Africans everyday. Approximately 80% of black South Africans make use of traditional medicine (Holdstock, 1978; Mander, 1998; Dold & Cocks, 2002; Street & Prinsloo, 2012), accounting for some 26.6 million consumers (Mander, 1998). Traditional medicine is necessary for treating a wide range of diseases, some of which may not be effectively treated by Western medicine. The main source of traditional medicine is indigenous plants (Kong et al., 2003; Rao & Arora, 2004; Shai et al., 2008).

Approximately 3000 indigenous plant species are used as medicines by South African traditional healers (De Wet et al., 2013). Medicinal plants play an important role in the treatment of skin ailments (Weideman, 2007; De Wet et al., 2013). Several plants have been investigated for treatment and management of skin ailments (Hutchings et al., 1996; Grierson & Afolayan, 1999; Mabona & van Vuuren, 2013; De Wet et al., 2013). In the Eastern Cape 8 plant species were documented for treating skin skin problems by the Xhosa people whilst in Western Cape Province, 5 plant species were documented (De Wet et al., 2013). Studies conducted by Hutchings et al. (1996) reported on on 190 Zulu medicinal plants that are used to treat various skin disorders.

1.9. Aim of this study

The aim of this study was to conduct an ethnobotanical survey of traditional medicinal plants used against skin ailments in the Free State Province of South Africa and to screen the collected plants for antimicrobial, antioxidant and anti-inflammatory activities.

1.10. Objectives

The objectives of this study were:

- To conduct qualitative and quantitative phytochemical analysis on plants used by traditional healers against skin ailments.
- To screen medicinal plants for antibacterial and antifungal activities.
- To screen traditional medicinal plants used against skin ailments for their anti-inflammatory properties using 5-lipoxygenase assay.
- To screen plants used against skin ailments for the presence of antioxidants (Free radical scavenging activity).

Chapter 2

Ethnobotanical survey of medicinal plants used in the treatment of skin infections

2.1. Introduction

Since the human civilization era, plant products, animals and minerals have been the centre and main source of all drugs (Wabe et al., 2011). Hence, the term "ethnobotany" as the study of botany of primitive human race (Mahmood et al., 2011). The term 'ethnobotany' entails modern and indigenous use of medicinal plants in which there is a relationship between people and plants (Balik & Cox, 1996; Wabe et al., 2011). Medicinal plants have been used since immemorial times, as natural products. They have been used for their healing properties. WHO (2003) defines traditional medicine as the knowledge and belief systems which are practiced in the use of mineral, plant and animal based remedies, and spiritual therapies which can prevent, heal and maintain well being. The use of traditional herbal healing has been a valuable aspect of human culture (Weideman, 2007).

The use of herbs as complementary and alternative medicines has increased in the last 20 to 25 years (Kaur & Arora, 2009). According to WHO (2005), Kaur & Arora (2009), Wabe et al. (2011) and Yadav & Agarwala (2011), the use of traditional medicine is relied upon by 65- 80% of the World's population for their primary healthcare needs. Herbal medicines have been a crucial aspect in the culture and tradition of the African people (Fennell et al., 2004). Today, most people still prefer the use of traditional medicines simply because of the healing properties of traditional medicinal plants provided by herbalists (Weideman, 2007). The African continent has a long history of medicinal plants use, serving societies with traditional medicine with therapeutic and primary health care needs. In African countries, a population of 90% has been reported to be solely reliant on medicinal plants as a source of drugs (Hostettman et al., 2000). It is estimated that about 25 000 species of medicinal plants, ranging from 4 to 20%, are being traded globally (Schippman et al., 2002).

Approximately 3000 plant species in South Africa are reported by various cultural groups as part of their *materia medica* (Louw et al., 2002; van Wyk et al., 2009; van Wyk & Viljoen, 2011). Traditional herbal healing is widely practiced throughout South Africa and, in estimation, about 70% of the South Africans regularly use medicinal plant based medicine (Taylor & van Staden, 2001; Weideman, 2007). Research also shows that over 3 million people in South Africa consult with traditional healers for primary healthcare purposes (Bhat & Jacobs, 1996; Coopoosamy et al., 2012; Mabona & van Vuuren, 2013). In South Africa, especially in the rural areas, traditional medicine is very crucial and serves as the only reliant source of primary healthcare (Fennell et al., 2004). The eastern part of the Free State is rural with poor and low socio-economic status and lack of employment. The majority of people are still dependent on farming systems and cultural beliefs, including use of traditional medicinal plants. Many traditional healers in this Province are known to heal many ailments including blood pressure, ulcers, skin infections, diarrhoea, diabetes, tuberculosis and others using herbal remedies. Apart from the cultural significance, herbal medicines are generally more accessible and affordable (Fennell et al., 2004). Traditional medicinal plants are presumed to be safe, cheaper and with less side effects. This has urged researchers to look for local products, in the form of medicinal plants, that have proved to be effective, safe, inexpensive and culturally acceptable (Wabe et al., 2011).

Although the knowledge and experience of these herbalists have not been scientifically documented, according to Koduru et al. (2007), the need for accurate scientific documentation of the knowledge system must be considered since there is an increase in factors such as the rapid rate of deforestation and loss of biodiversity. Approximately 20% of plants which are found in the world have been screened for the presence of antimicrobial properties and have thus far received a large recognition and validation (Coopoosamy et al., 2010; Coopoosamy & Naidoo, 2012). With the promising new drugs medicinal plants seem to attain, more plants are being investigated for other potential activities such as anthelminthic, antioxidants, anti-inflammatory, and other crucial bioactive compounds. Hence, the study of ethnobotany is crucial for the documentation of knowledge obtained from the

herbalists and to acquire a clear scientific validation of the plants used medicinally (Igoli et al., 2005).

2.2. Aim of this study

The aim of this chapter was to document and reveal the ethnobotanical information of traditional medicinal plants used in the treatment of skin infections and related diseases.

2.3. Materials and methods

2.3.1. Study Area

This study was conducted in the Free State Province (**Figure 2.1**). The Free State is an inland bean-shaped Province of South Africa. It is the third biggest Province; covering about 10.6% of the country's total area and practices most of the commercial farming of South Africa (South African Government, 2014). The farming in the eastern part of the Province includes both stock and crop farming. The eastern Free State is covered by multitude of biomes; eastern Free State Sandy Grassland, Basotho Montane Shrubland, Northern Drakensberg Highland Basalt Grass (Mucina & Rutherford, 2006). The area of study includes mainly 2 vegetation types which cover the whole interior, the Eastern Free State Sandy Grassland and Basotho Montane Shrubland. These biomes cover the Free State Province, Lesotho and marginally into KwaZulu-Natal, with foothills facing the west side along the vicinities of Drakensberg, Golden Gate and Klerkspruit catchment. The area reaches an altitude of 1520 – 1800 m, even altitudes of 2020 m in some places of the region. Phuthaditjhaba is part of the eastern Free State, located on the foot of Maluti Mountains, naturally grassland (Taylor, 2005). The area consists of cool to very cold winters and warm to hot summer days. This area experiences snow falls almost every year. Rainfall is common after afternoon thunderstorms which happen frequently on hot summer days (Lonely Planet Publications, 2004). The Drakensberg Mountains receive the greatest amount of rainfall and have the steepest slopes of the Upper Orange River catchment (Compton & Maake, 2007). The area is a Summer-rainfall region with an annual rainfall of more than 720 mm, along the Maloti Mountains range and can receive rainfall of more than 1400 mm

particularly in wet years (Mucina & Rutherford, 2006). The area is characterized by high rate of unemployed rural dwellers.

Figure 2.1: Map showing the Free State Province, Thabo Mofutsayana District. Circle indicates the study area. Map copied from (http//:www.freestatetourism.org).

2.3.2. Ethnobotanical survey

Ethnobotanical survey was conducted from March 2014 to August 2014. The interviews were conducted using a structured questionnaire and discussed on an individual basis and explained by an interpreter, conveying messages in Sesotho or IsiZulu. This was due to a misinterpretation of the English language. A total of 10 informants included 2 traditional healers, 4 herbalists and 4 vendors, comprising 8

females and 2 males were interviewed. Interviews were conducted at the herbal markets in the Free State Province and other commercial specimens were purchased at Durban *Muthi* market.

2.3.3. Plant collection and identification

Plants were collected around different areas (Mangaung, Tseseng, Tseki, Bluegumbosch), with the assistance of the herbalists and traditional healers (**Figure 2.2**). Traditional healers/herbalists provided the local names of the plants used, identification of scientific names was done by Dr E.J.J. Sieben. Voucher specimens were prepared and deposited at the Herbarium of the University of the Free State, QwaQwa Campus.

Figure 2.2: Plant collection with the herbalist.

2.4. Intellectual property agreement statement

All the traditional healers and herbalists who contributed information to this project during the ethnobotanical survey were adequately financially rewarded with further informed consent and agreements should be documented not for commercial purposes, but to serve as enlightenment to the community and the entire Free State Province on the plants used for the treatment of skin infections in the area.

2.5. Results and discussions

Eight females with ages ranging between 50 to 65 years, and 2 males aged 35 to 55 years were interviewed. A total of 22 plant species belonging to 18 families were reported to be currently used in the treatment of wounds and skin infections (**Table 2.1**). Plants belong to the families Acoraceae, Aizoaceae, Apiaceae, Apocynaceae, Asparagaceae, Amaryllidaceae, Aloaceae, Asphodelaceae, Crassulaceae, Dioscoreaceae, Fabaceae, Hyacinthaceae, Hypericaceae, Hypoxidaceae, Lycopodiaceae, Poaceae, Rubiaceae and Sterculiaceae. From the 22 plant species, eight frequently used medicinal plants, namely *Pentanisia prunelloides* (**Figure 2.3**)*, Cotyledon orbiculata* (**Figure 2.4**)*, Hermannia depressa* (**Figure 2.5**)*, Dioscorea sylvatica* (**Figure 2.6**)*, Lycopodium clavatum* (**Figure 2.7**)*, Merwilla plumbea* (**Figure 2.8**)*, Eucomis bicolar* (**Figure 2.9**)*, Xysmalobium undulatum* (**Figure 2.10**) and *Eucomis autumnalis* were selected for phytochemical analysis and pharmacological screening. The information that was obtained included the local names of the plants used, their local uses, and parts of plant used, mode of preparation, composition and administration. It was observed that some plants have more than one common names. This is due to the fact that the same plant is called in different ways by different communities. The traditional healers/ herbalists also provided information stating the harvesting methods, and the cultivation of plants. Traditional healers cultivate these medicinal plants by digging out the roots and bulbs or corms, this is cultivated in the back of their gardens. Bulbs are the major plant part used, followed by root, leaves and flowers in the treatment of skin disorders in most ethnobotanical studies obtained. According to the traditional healers, bulbs or tubers are regarded as the most valuable material as they claim that they contain the highest concentration of potent healing agents (Louw et al., 2002). The bark, leaves or roots are generally harvested, whereas leaves of herbs are normally used to prepare medicine (Louw et al., 2002).

Bark, stem, roots, rhizomes, corms and bulbs were reported to be the most commonly used plant parts. It is stated that the underground parts of the plant have higher amount of bioactive compounds than other parts noted (Li et al., 2015). Leaves and fruits were the least used. The survey indicated an oral intake of the decoction or concoction preparation. Decoctions and infusions are the most

frequently used methods of preparation (Koduru et al., 2007). Infusion was reported to be the best effective method of preparation because it only requires less ingredients, which is just an addition of boiled water to the plant material (Wabe et al., 2011). Application methods varied from cleaning wounds with aqueous extracts prepared from the plants and dressing the injured skin/wound with poultice.

According to a study undertaken by Marwah et al. (2007), the traditional ways of using herbs to heal wounds are remarkably similar, either as a poultice or expressed juice from fresh plants. Moreover, according to Samy & Gopalakrishnakone (2010), traditional healers prepare a wide range of healing juices, crude extracts, paste and tinctures from various herbs by using water extracts. Nethathe and Ndip (2011) reported that the use of water extract is very effective and should be noted because traditional medicine is mainly prepared as decoctions or infusions in water which are taken orally.

According to Mander (1998), non-sustainable harvesting threatens the survival of valuable medicinal plant species. If the whole plant is heavily exploited, the plant species could be threatened and that would lead to extinction. The traditional healers and herbalists mentioned that they conserve their valuable medicinal plants by cultivating them in their gardens and yards so that they be readily available for harvesting. This supports sustainable utilization of medicinal plants.

Table 2.1: Table shows the list of medicinal plants used for the treatment of wounds and skin infections in the Free State Province, South Africa.

Family	Scientific names	Common names	Plant part used	Medicinal uses	Applications and Preparations
Acoraceae	*Acorus calamus* L.	Sweet flag (E), uKhalumuzi (Z).	Rhizome	Stomach ulcers, chest complaints, asthma, headache, appetite stimulant, antibacterial, mucous congestion, diarrhoea	Alcoholic extracts are generally used. The dried or candied rhizomes may be directly chewed or taken as an infusion in boiling water.
Aizoaceae	*Carpobrotus edulis* (L.) L.B. Bolus	Sour fig/Cape fig (E), Umgongozi/ Ikhambi-lamabulano (Z), Hottentosvyg (A).	Leaves/roots	Oral and mouth ulcers, burns, bruises, scrape, cuts, eczema, dermatitis and other skin conditions.	The fresh juice is taken orally or gargled. The leaf pulp may be applied to treat skin wounds and infections. The leaf juice is astringent and mildly antiseptic. It is used internally, to treat diarrhoea, dysentery and stomach cramps, laryngitis, sore throat and mouth infections. A sore throat can be relieved by chewing a leaf tip and swallowing the juice. The leaf juice is used as a lotion for burns, sunburn and other skin ailments.
Apiaceae	*Alepidea amatymbica Eckl. & Zeyh. Var. amatymbica*	Larger tinsel (E), iKhathazo (Z), Iqwili (X).	Roots/ Rhizomes	Inflammation, bleeding wounds, burns, etc.	Roots and rhizome are smoked for mild sedation and vivid dreams or in powdered form and sniffed. The fresh roots and rhizomes may be chewed, while roots are boiled and decoction is taken orally. Fresh rhizome is also applied externally as a

					styptic.
Apocynaceae	*Xysmalobium undulatum* (L.) W.T. Aiton	White Bush (E), Ishongwe (Z), Poho-tsehla (S).	Entire plant/roots and leaves.	Topically to treat sores and wounds (anti-diarrhoeal, spasmolytic, wound-healing.	The powdered root is used to treat wounds and abscesses, as an anti-diarrhoeal and snuff. Dry powdered root and extracts of the root are apparently an excellent remedy for painful menstrual cramps and have an antispasmodic action. Used as a vermifuge in children, also as a decongestant and for headaches. Both roots and leaves are boiled and decoction is taken orally,
Asparagaceae	*Asparagus africanus* (Lam.) Oberm.	uMathunga (X)).	Roots	Sores and wounds, flatulence and colic. Cleanses the blood assist in HIV/AIDs patients.	Decoctions of warmed bulbs in water or milk are usually administered orally for several weeks.
Amaryllidaceae	*Haemanthus albiflos* Jacq.	Paintbrush (E), uZaneke (Z), uMathunga (X), Poeierkwas (A).	Roots	Sores and wounds.	Sun-dried roots are powdered and infused in water and then taken orally until the patient is cured
Aloaceae	*Aloe ferox* Mill.	Bitter aloe (E) Umhlaba (Z), Ikhala (X).	Leaves and roots	Dermatis, cutaneous, disorders of skin, burns, jaundice. Also effective against toothache, earache and oral and vaginal thrush, skin and wound healing.	The fresh juice from root infusion is taken orally or gargled. Leaves are also boiled in hot water, cooled and filtered and half-cup is taken orally. The leaf pulp may be applied to treat skin wounds and infections such as burns, bruises, scrape, cuts, sunburn, eczema, dermatitis and other skin conditions. The leaf is used as a laxative, emetic. Leaves

					approximately three.
Asphodelaceae	*Aloe aristata* Haw.	Umathithibala (Z)	Leaf	Treatment of infections, internal parasites, digestive ailments, dressing to wounds.	Aloe is mixed with water to ash wounds and sores for a refreshing effect. Also used as a laxative. The inner pulp is used externally to relieve skin discomforts.
Crassulaceae	*Cotyledon orbiculata* L.	Pig's Ear (E), Morianna wa di-tsebe/ sereledi (S)	Leaves/ Stem	Inflammation, removes warts, treats epilepsy, internal parasites, skin ailments and various diseases.	Fleshy leaves have been used to treat corn and warts. The juice of the leaves is used as drops for earache and tootache and as hot poultice for boils and inflammation. Fresh leaf juice by half cup doses are taken 2 or 3 times a day. Leaves are eaten as vermifuge and applied as a hot poultice to treat boils, earache and inflammation. Roots and rhizomes are boiled in water for external use to treat acne and other skin diseases. Also used in combination with *Pentanisia prunelloides* to treat eczema. For warts, leaf is heated until very hot and placed on the swollen part of the body to remove warts.
Dioscoreaceae	*Dioscorea elephantipes* (L'Her.) Engl.	Elephant's foot (E), Ingweva (Z).	Whole plant	Sores, wounds, syphilis, peptic ulcers.	The whole plant is placed in water for 3 days before boiling. Peeled or grated root is rubbed on the skin. Can be used as a contraceptive
Dioscoreaceae	*Dioscorea sylvatica* (kunth) Eckl.	Wild Yam (E) Ingefu/	Whole plant	Skin problems.	Water heated in the scooped out

		Uskolpati (Z), Usikolipati (X), Skilpadknol (A).			tuber is used to treat sores and wounds in humans and animals. The fresh peeled rhizome is rubbed on the skin. Infusions are taken. During pregnancy to ensure, as oral contraceptives, nervous spasms.
Fabaceae	*Elephantorrhiza elephantina* (Burch.) Skeels	Elephant's foot/Mosquito plant/ (E), Mositsane (S), Intolwane (Z).	Tuber/corm	Sunburn, acne, burns and rash.	The grated root is steeped in water for 24 hours or more then strained and ready for external use. For internal use it must be boiled for 10 minutes and small quantities must be taken for 3 times a day. If treating acne, the face is held in the vapour arising from a warm infusion. The underground parts are used to treat sunburn, and root infusions for acne.
Fabaceae	*Afzelia quanzensis* Welw.	Lucky bean (E), umDlavusa (Z), umHlavusi (X), Peulmahonie (A).	Bark/root	Burns and warts	Cold water infusions of the powdered bark are taken orally.
Hyacinthaceae	*Eucomis bicolar* Baker.	uMbola (Z).	Bulbs	Skin ailments, burns, and wounds.	Decoctions and infusions are prepared.
Hyacinthaceae	*Eucomis autumnalis* (Mill.) Speta. Chitt	Pineapple flower/Pineapple lily (E), uMakhandakantsele (Z), uMathunga (X), wildepynappel/ krulkoppie (A).	Bulbs/ Roots	Sores and wounds.	Decoctions of warmed bulbs in water or milk are usually administered orally for several weeks. Sun-dried roots are powdered and infused in water and then taken orally until the patient is cured.

Hyacinthaceae (formerly part of the Liliaceae –lily family of perrenia bulbous herbs)	*Merwilla plumbea* (Lindl.) Speta (Blue squil) formerly known as *Scilla natelsensis* (Wild squill).	Wild squill/ Blue hyacinth (E), Inguduza (Z)	Bulbs	Treatment of skin conditions. Bulb decoctions used as enemas and purgatives, for boils, sprains and fractures.	Ointments from fresh bulbs are used externally to treat various skin ailments like boils and sores. Ash from burnt plants and bulbs are used in powdered form to rub on cuts and scratches, over sprains and fractures. Decoctions/Infusions are made from bulbs, warmed gently and taken orally till the patient is cured.
Hypericaceae	*Hypericum aethiopicum*	John's Wort/Two days (E), uNsukumbili (Z), Bohoho (S),	Leaves/Barks	Stomach ulcers and complaints, fever, backache, anti-depressant, diuretic.	The grated plant is boiled for 20 minutes and then strained and must be taken for 3 times a day. Alternatively half a cup of boiling water must be poured over two spoons of the powdered plant and then strained after 10 minutes.
Hypoxidaceae	*Hypoxis hemerocallidea* Fisch.Mey.&Ave & Ave-Lall.	African potato (E), Inkomfe (Z), Ilabatheka (X).	Corm	Immune stimulant, mouth and oral ulcers, colds and flu, cancer and tumours.	The corm is diced, boiled and taken internally. The dosage depends on the discretion of the person with sores.
Lycopodiaceae	*Lycopodium clavatum* L.	Clubmoss/ Belly powder (E), uMnwele (Z).	Whole plant	Wounds, sores, warts and other skin ailments.	Whole part grounded into powder, boiled and taken internally or roots applied externally on burns.
Poaceae	*Themeda triandra forssk.* var. burchellii (Hack.) Stapf	Seboku (S).	Leaves	Eczema, skin allergies.	Infusion of ground leaves and water is drunk for stomach pains.
Rubiaceae	*Pentanisia prunelloides* (Klotzsch ex Eckl. & Zeyh.) Walp. Var *prunelloides*	Wild verbena/broad leaved Pentanisia (E). Icimamlilo (Z), Setimamollo (S), Sooibrandbossie (A).	Root	Root decoctions are taken orally or as enemas and also applied externally for burns, swellings..	The heated leaf is used as a poultice for boils and other accessible inflammations. i.e., earache. The fleshy part of the leaf is applied to soften and

					remove hard corns and warts. The Basotho use a dried leaf as a protective charm for an orphan child and as a plaything. Also used in combination with *Dicoma anomala* to treat insect stings and bites.
Sterculiaceae	*Hermannia depressa* N.E.Br.	Doll's Rose (E), seletjhane (S).	Root	Headache, headaches and wound healing.	Roots applied externally for burns and swellings and inflamed wounds.

Figure 2.3*: Pentanisia prunelliodes* (Klotzsch ex Eckl. & Zeyh.) Var *prunelloides*

Figure 2.4*: Cotyledon orbiculata* L.

Figure 2.5*: Hermannia depressa* N.E.Br

Figure 2.6*: Dioscorea sylvatica* (Kunth) Eckl.

Figure 2.7*: Lycopodium clavatum* L.
(http//:///hiveminer.com/Tags/pteridofita/Lycopdium sp.)
(https://farm2.static.flickr.com/1682/23895521919_7daa7d88cc_b.jpg)

Figure 2.8*: Merwilla plumbea (Scilla natalensis)* (Lindl.) Speta

Figure 2.9*: Eucomis bicolar* Baker*.*

Figure 2.10*: Xysmalobium undulatum* (L.)

2.6. Conclusions

Ethnobotanical information revealed that inflamed wounds, eczema, bites, ringworm, boils, septic sores and burns were common among the South Africans. This study documented 22 plant species used by the traditional healers and herbalists of the Free State Province for the treatment of wounds and skin infections. The ethnobotanical survey revealed infusions, decoctions, poultice or juice from fresh plants as traditional ways of using herbs to treat skin infections. The aqueous extracts of collected medicinal plants are used to clean and disinfect wounds. The ethnobotanical survey conducted in this study provided us with information and knowledge on use of traditional medicinal plants. It also indicated that a single medicinal plant can cure or heal more than one ailments. This could lead to a high demand of medicinal plants which can lead to plants becoming endangered, rare or threatened due to unsustainable harvesting methods. This also shows that, in spite of modern civilization and access to modern medicine, the people of Free State are still dependent on the practices of traditional treatment.

All the interviewed traditional healers mentioned that there is no incidence of poisoning or worsening since patients administer already prepared decoctions, where they buy prepared medicines rather than preparing their own. This is to avoid misinterpretation of instructions and preparations. They also emphasized the importance of regulating the dosages to be administered to a patient in order to prevent lethal side effects. There is, however, still a lack of knowledge amongst the traditional healers, street vendors and the public about the safety and efficacy of medicinal plants. If such matters are not considered seriously, there could be an increase in the rate of death due to toxicity of these medicinal plants.

Chapter 3

Phytochemical Analysis

3.1. Introduction

In the early 19th century, the first isolation of active compounds such as morphine, strychnine, quinine, etc., has marked a tremendous effect on the importance of medicinal plants' use and modern medicinal plants research. In 1945, more emphasis was on plant derived drugs with a huge development of chemistry and microbial fermentation and, in this period, plant metabolites were investigated from a phytochemical and chemotaxanomic perspective (Saxena et al., 2013). Trease and Evans (1989) differentiates plant metabolites into primary and secondary metabolites. Primary metabolites are defined by their importance to plants, e.g. nucleic acids and carbohydrates (Ndlovu, 2009), while the secondary metabolites include those compounds that are of medicinal value to man.

Phytochemicals are defined as biologically active, naturally occurring chemical compounds found in plants, which are beneficial to human health and are attributed to macronutrients and micronutrients (Saxena et al., 2013; Gnanaraja et al., 2014). Plants have evolved secondary biochemical pathways that allow them to synthesize a component of chemical compounds from primary metabolic functions of the plant, hence the term 'secondary metabolites' (Yadav & Agarwala, 2011; Omotayo & Borokini, 2012; Sathya et al., 2013). According to Cowan (1999), the so-called 'secondary metabolites' encompass a major contribution to the specific odours, tastes and colours of plants. The response is interrelated to specific environmental stimuli, such as herbivore-induced damage, pathogen attacks against bacteria, fungi and viruses, or nutrient depravation (Kennedy & Wightman, 2011; Saxena et al., 2013). They are produced and used by the plants for protection because they are synthesized as part of the defence system of plants, for example, phytoalexins are produced when the plants are attacked by bacteria or fungi, hence leading to their anti-fungal and anti-bacterial properties (Ndlovu, 2009; Samy & Gopalakrishnakone, 2010; Kennedy & Wightman, 2011). Such antimicrobial properties are synthesised during the secondary metabolism of the plant (Sathya et al., 2013).

More than 4,000 phytochemicals are known and recorded, they are classified by protective function, physical characteristics and chemical characteristics (Saxena et al., 2013). Secondary metabolites include saponins, tannins, essential oils, peptides, flavonoids, alkaloids, coumarines, cardiac glycosides and other chemical compounds, which most of them possess curative properties and are said to produce a physiological action in the human body (Omotayo & Borokini, 2012; Saxena et al., 2013; Gnanaraja et al., 2014).

3.2. Types of secondary metabolites

Based on their chemical structure, secondary metabolites can be divided into the following groups:

3.2.1. Alkaloids

Alkaloids are regarded as the second largest group which is found in all organisms, and is said to contain a large number of medicines. They are characterized by water-soluble compounds under acid conditions and have lipophilic properties under neutral and basic conditions (Verpoorte, 1998). According to Gnanaraja et al. (2014), alkaloids function in the defence systems and elements which protect against predation by herbivores. Alkaloids are deterrent to herbivores because of their toxicity, analgesic, anti-inflammatory, anaesthetic and adaptogenic properties (Kennedy & Wightman, 2011; Yadav & Agarwala, 2011; Gnaranaja et al., 2014). They are the most pharmacologically active and therapeutically significant plant substances (O'Doherty et al., 2010; Omotayo & Borokini, 2012). These activities assist in the alleviation of pains and inflammation, which are a form of a developed resistant mechanism against many diseases.

3.2.2. Tannins

Tannins are said to encompass a very diverse group of oligomers and polymers, making them to be complex as they are heterogeneous with polyphenolic compounds (Saxena et al., 2013). With such a capacity, they are reversible and irreversible, forming complexity in proteins, polysaccharides (cellulose, hemillulose, and pectin), alkaloids, nucleic acids and minerals. This results in reduction in digestibility of such macromolecules and thus inhibition of microbial growth

(Omotayo & Borokini, 2012). The role of inhibiting oxidation properties has been reported and is said to be due to the presence of gallic and diagallic acids (Omotayo & Borokini, 2012). On a structural basis, they are characterized into gallotannins, ellagitannins, complex tannins and condensed tannins (Cowan, 1999; Omotayo Borokini, 2012; Saxena et al., 2013). Generally, tannins are attributed to their ability to bind proteins with anti-tumor promoting effects and are known universal antitumor agents (Mazid et al., 2011). Tannins are found in a number of plants and they possess antimicrobial, anti-inflammatory, antiseptic, anti-phagocytic, anti-tumor, anti-neoplastic, astringent and antioxidant properties (Cowan, 1999; O'Doherty et al., 2010; Omotayo & Borokini, 2012; Lekganyane et al., 2012; Saxena et al., 2013).

3.2.3. Cardiac glycosides

Cardiac glycosides are cardioactive compounds belonging to triterpenoids class of compounds (Brian et al., 1985). Their inherent activity resides in the aglycone portions of their sugar attachment. Cardiac glycosides have been used for over two centuries as stimulants in cases of cardiac failure, and are said to increase the force of myocardiac contraction (Awonyika et al., 2007; Omotayo & Borokini, 2012). According to Brian et al. (1985), cardiac glycosides exert their hypotensive effect by inhibiting $Na^{+}K^{+}$ ATPase, act on the heart muscles and increase renal flow (diuresis), smooth muscle of the vascular system and neural tissue. Hence, these effects are reported to have an indirect influence on the mechanical and electrical activities of the heart, vascular resistance and capacitance modification (Omotayo & Borokini, 2012). Glycosides are also known to lower blood pressure (Yadav & Agarwala, 2011). Plants that contain such compounds are regarded as the most important causes of livestock poisoning (Botha & Penrith, 2008). However, it is reported that some plants contain non-toxic glycosides which are able to get hydrolized to release phenolics which are in turn toxic to microbial pathogens (Aboaba et al., 2006).

3.2.4. Flavonoids

Flavonoids are defined as polyphenolic compounds which are found to be the most important components in a human diet (Gnanaraja et al., 2014). Universally, they are present in small constituents in flowering plants, particularly in plants used as foods (Miean & Mohamed, 2001; Saxena et al., 2013). Examples of foods rich in

flavonoids are spices, green tea, wine, and fruits and vegetables. There is a wide array of flavonoids which are produced by plants, these include sesquiterpenoid alcohols, triterpernoids, quinic acid and caffeates (Samy & Gopalakrishnakone, 2010). Flavonoids are reported to have therapeutic and preventative properties against diseases (Saxena et al., 2013). In addition, flavonoids have been reported to exert several biological activities such as antimicrobial, cytotoxicity, anti-inflammatory, and antitumor (Cowan, 1999; Gowri & Vasantha, 2010; O'Doherty et al., 2010; Saxena et al., 2013). Flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and viruses (Gowri & Vasantha, 2010).

3.2.5. Saponins

This class is said to be widely distributed in the botanical kingdom, with pharmacological and biological activities (Wengfeng & Kun, 2011; Gnanaraja et al., 2014). Compounds found in saponins are a group of glycosylated steroids, triterpenoids and steroid alkaloids. Generally, they are known for their stable foam in aqueous solutions such as soap, hence the name "saponin", hemolytic activity, cholesterol binding properties and bitterness (Gowri & Vasantha, 2010; O'Doherty et al., 2010; Yadav & Agarwala, 2011; Saxena et al., 2013). Due to their high antimicrobial properties, they protect plants from insect attack. Saponins have pharmacological effects and are responsible for anti-inflammatory, molluscicidal, antimicrobial, anti-spasmodic, antidiabetic, anticancer, hypocholesterolemic, antioxidant, analgesic, anthelminthic, antitussive and cytotoxic activities (Wengfeng & Kun, 2011; Saxena et al., 2013; Gnanaraja et al., 2014). Although they are toxic, their consumption by humans may be beneficial in heart disease reduction by binding with plasma membrane and cholesterol (Price et al., 1987).

3.2.6. Terpenoids

Terpenoids can be classified according to the number of isoprene units, e.g. hemiterpene, monoterpenes, sesquiterpenes, diterpernes, sesterpenes, triterpenes, tetraterpenes and sesquiterpernes (Kennedy & Wightman, 2011). Terpenoids are known to range from toxic to being entirely edible, making them repellent or deterrent to insects and mammals. They also play an ecological role in the ecosystem, and

they possess mammalian cholinesterase inhibitory and antimicrobial properties (Kennedy & Wightman, 2011). Terpenoids are known to be present in a host of spices, flavors, and foods that make up essential components in our diets, for taste and healthy eating (Samy & Gopalakrishnakone, 2010; Kennedy & Wightman, 2011). The bitterness in Chilli peppers is due to the presence of terpenoid capsaicin.

3.3. Aim of this study

The aim of this chapter was to perform the phytochemical analysis in order to identify the major phytoconstiuents present in plants used against skin ailments in the Free State Province.

3.4. Phytochemical analysis

The presence of alkaloids, flavonoids, terpenoids, saponins, anthraquinones, cardiac glycosides and tannins was determined individually (**Figure 3.1**) by following standard procedures to identify the constituents as described by Harborne (1973), Trease and Evans (1989), Sofowara (1993), and Edeoga et al. (2005).

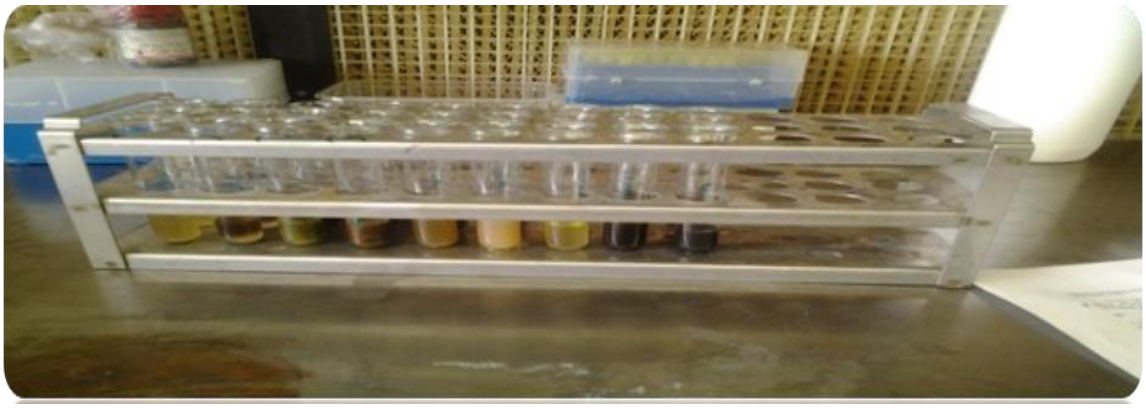

Figure 3.1: Phytochemical analysis, showing test tubes with different plant extracts.

3.4.1. Test for alkaloids

An amount 0.5 g of the powdered root material was stirred in 5 ml of 1% aqueous hydrochloric acid, heated on a water bath and filtered. One millimetre of the filtrate was then treated with few drops of Mayer's reagent and a second portion was treated with Dragendroff's reagent. Turbidity of precipitation with either of those

reagents was taken as preliminary evidence for the presence of alkaloids in the extract (Harborne, 1973).

3.4.2. Test for tannins

In the test for tannins, 0.5 g of dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue black colouration as indication of tannins (Sofowara, 1993).

3.4.3. Test for saponins

An amount of 2 g of powdered material was boiled in 20 ml of distilled water in a water bath and then filtered. Ten millilitres of the filtrate was then mixed with 5 ml of distilled water and then shaken vigorously and observed for a stable persistent froth. The frothing was then mixed with 3 drops of olive oil, shaken and observed for the formation of emulsion as indication of saponins (Harborne, 1973).

3.4.4. Test for flavonoids

One grams (1 g) of the powdered material was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was then filtered and 4 ml of the filtrate was shaken vigorously with 1 ml of dilute ammonia solution. A development of yellow colouration was an indication of the presence of flavonoids (Sofowara, 1993).

3.4.5. Test for steroids

In this test, 2 ml of acetic anhydride was added to 0.5 g of ethanolic extract with a 2 ml concentrated H_2SO_4. The colour change from violet to blue indicated the presence of steroids (Harborne, 1973).

3.4.6. Test for terpenoids

Five millilitres of plant extract was dissolved in 2 ml chloroform and a 3 ml H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface was an indication of terpenoids (Harborne, 1973).

3.4.7. Test for cardiac glycosides

In this test, 5 ml of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1ml of concentrated H_2SO_4. A brown ring of the interface showed an indication of a deoxysugar characteristic of cardenolides. A violet ring appeared below the brown ring, while in the acetic acid layer, a greenish ring may form throughout the thin layer (Trease & Evans, 1989).

3.4.8. Test for anthroquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid (H2SO4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes (Harborne, 1973).

3.5. Quantitative analysis of secondary metabolites

The total contents of phytochemicals were determined in order to evaluate the quantity of the presence of phytochemicals found in different plants. Results were obtained using spectrophotometric methods and some were obtained by the process of drying and weighing crude extracts until a constant weight of dry mass was obtained.

3.5.1. Determination of total flavonoids

The determination of flavonoids was done as described by Boham and Kocipaiabyazan (1974). About 5 g of the aqueous plant extract was weighed and emptied into a flask. Subsequently, 100 ml of 80% methanol was poured into the extract at room temperature. The upper layer of the content was decanted, then another 100 ml 80 % aqueous methanol was poured again. Both collections were filtered and transferred into crucibles. The crucibles were weighed as empty vials before the solutions were transferred. The solution were then evaporated to dryness on a water bath at 45ºC until a dry and constant weight was obtained for each.

3.5.2. Determination of total alkaloids

In determining the quantification of alkaloids, the method by Harborne (1973) was performed. Five grams of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 h. This was filtered and the extract concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop by drop to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue, which is the alkaloid, was dried and weighed. The filtrate (alkaloids) was calculated as percentage of the dried fraction.

3.5.3. Determination of total tannins

About 500 mg of the sample was weighed into a 50 ml plastic bottle. Fifty millilitres of distilled water was added into the sample and shaken for 1 h in a mechanical shaker. This was then filtered into a 50 ml volumetric flask and made up to the mark. About 5 ml of the filtrate was then pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was read at 120 nm within 10 min (van Burden and Robinson, 1981).

3.5.4. Determination of total saponins

The samples were ground and 20 g of each powdered materials were put into a conical flask and 100 ml of 20% aqueous ethanol was added. The samples were then heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was then transferred into a 250 ml separate funnel and 20 ml of diethyl ether added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty millilitres of n-butanol was then added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a

water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated (Obadoni and Ochuko, 2001).

3.6. Results and discussions

3.6.1. Phytochemical screening

Results for qualitative analysis of secondary metabolites present in the studied plants are presented in **Table 3.1**. Most plant species tested positive for the presence of saponins, flavonoids, tannins and terpenoids. Alkaloids, cardiac glycosides, anthroquinones and steroids were the least present phytochemicals. Saponins were detected in *P. prunelloides* extracts, *H. depressa* and *C. orbiculata* aqueous stem extracts, and *X. undulatum* aqueous and ethanol extracts. The leaves of *C. orbiculata* were reported to contain tannins, saponins, triterpene steroid, reducing sugar and cardiac glycosides in a study undertaken by Amebeoku et al. (2007).

Table 3.1: Qualitative analysis of phytochemical constituents found in plants used against skin ailments in Free State.

Plant name	Extract	Tannins	Terpenoids	Saponins	Flavonoids	Cardiac glycosides	Alkaloids	Anthroquinones	Steroids
P. prunelloides	MeOH	+	-	+	-	+	+	-	-
	EtOH	-	-	+	+	-	-	-	-
	Acetone	+	-	+	-	+	-	-	-
	H_2O	+	-	+	+	+	-	-	+
H. depressa	MeOH	+	-	-	-	-	+	-	-
	EtOH	+	+	-	-	+	+	+	+
	Acetone	+	-	-	-	-	+	-	-
	H_2O	-	-	+	+	-	-	-	-
X. undulatum	MeOH	-	-	-	-	-	-	-	-
	EtOH	-	-	-	+	-	+	-	-
	Acetone	-	-	-	-	-	-	-	-
	H2O	+	-	-	-	-	+	-	-
C orbiculata (Stem)	MeOH	+	-	-	-	+	-	-	-
	EtOH	+	-	-	-	+	-	-	-
	Acetone	+	+	-	-	-	+	-	-
	H_2O	+	-	+	+	-	-	-	-
C. orbiculata (Leaves)	MeOH	+	-	-	-	-	+	+	-

	EtOH	-	-	-	-	-	+	+	-
	Acetone	-	+	-	+	-	+	+	-
	H_2O	-	+	-	--	-	-	-	-
E. autumnalis	**MeOH**	-	-	-	-	-	-	-	-
	EtOH	-	-	-	+	-	-	-	-
	Acetone	-	-	-	-	-	-	-	+
	H_2O	+	+	-	-	+	-	-	+
E. bicolar	**MeOH**	+	+	-	+	-	-	-	-
	EtOH	+	+	-	-	-	-	-	-
	Acetone	+	-	-	+	+	-	-	-
	H_2O	-	+	+	+	+	-	-	-
D. sylvatica	**MeOH**	-	+	+	-	-	+	-	-
	EtOH	-	+	-	-	-	+	+	-
	Acetone	-	+	-	+	-	-	+	-
	H_2O	-	-	-	-	-	-	+	+
L. clavatum	**MeOH**	+	-	-	+	+	-	-	-
	EtOH	+	-	-	+	-	+	-	+
	Acetone	+	+	-	+	-	-	-	-
	H_2O	+	+	+	+	-	-	-	-
M. plumbea	**MeOH**	+	+	-	+	-	-	+	-
	EtOH	+	+	+	+	-	-	-	-

	Acetone	-	-	+	-	+	-	+	-
	H_2O	+	-	-	-	+	+	-	+

+: Present; - : Absent.

M. plumbea methanolic extract displayed the presence of tannins, terpenoids, flavonoids and anthroquinones, whereas saponins were detected in acetone and ethanol extracts. Phytochemical screening of *M. plumbea* bulbs revealed the presence of of saponins and bufadienolides in a study by Sparg et al. (2002). Lower (1985) and Bruneton (1995) reported that saponins have both analgesic and anti-inflammatory properties. In our study, flavonoids were present in almost all plants, particularly *P. prunelloides* aqueous and ethanol extracts, and aqueous extracts prepared from *H. depressa, X. undulatum*, and *C. orbiculata* stem. Amusan et al. (2007) performed phytochemical analysis on *X. undulatum* and observed the presence of flavonoids, glycosides, polyphenols, saponins, and sterols in the plant.

Alkaloids were present in *P. prunelloides* methanol extract, *D. sylvatica* alcoholic extracts, *H. depressa* ethanol extract, *C. orbiculata* methanol and acetone stem extracts and *L. clavatum* ethanol extracts. The genus of *Lycopodium* is known to be rich in alkaloids with high toxicity, this may also contribute to the antimicrobial activity of the *L. clavatum* extracts (Orhan et al., 2007). Alkaloid fractionation of *L. clavatum* was performed, and the content of alkaloid was analysed by capillary gas chromatography-mass spectrometry (GS-MS) and identified lycopodine as the major alkaloid (Orhan et al., 2007). It is reported that the *Lycopdium* genus also contains various phenolic acids such as dihydrocaffeic, vanillic syringic, p-hydroxy- benzoic, p-coumaric, ferulic acid and phenolic acids which are known to possess antimicrobial activity against a variety of microorganisms.

Anthroquinones were only detected in *C. orbiculata* stem and *E. autumnalis*. Methanolic and ethanol extracts of *E. bicolar* displayed the presence of tannins, terpenoids, and flavonoids. The presence of terpenoids shows that plants may be active against bacteria; this shows that it could be effective against any bacterial infections (Sheikh et al., 2013).

Cardiac glycosides were only detected in *P. prunelloides* aqueous, methanol and ethanol extracts. Moreover, the methanol extract prepared from *L. clavatum* displayed the presence of tannins, flavonoids and cardiac glycosides. Cardiac glycosides are known to lower blood pressure (Yadav & Agarwala, 2011). Harborne

& Baxter (1993) reported that, in small doses, cardiac glycosides are used medicinally for controlling congestive heart failure. In general, it is reported that concentrations of cardiac glycosides in plants are very low, lower than 1% (Bruneton, 1995). Hence, it may be possible to use plant material without any fatality. It is important, however, that the concentration of the cardiac glycoside is known, for proper treatment (Reid et al., 2005).

Tannins were detected in aqueous, ethanol, methanol and acetone extracts prepared from *C. orbiculata* stem, *P. prunelloides* and *H. depressa*. The presence of tannins suggests the ability of the plant to function as anti-diarrhoec and anti-haemorrhagic agents (Awoyinka et al., 2007). Steroids were only detected in *P. prunelloides* aqueous and *E. autumnalis* acetone extracts.

3.6.2. Quantitative analysis

Quantitative analysis of secondary metabolites present in plants is presented in Table 3.2. *P. prunelloides* showed a high content of total flavonoids (40.43%), total alkaloids (84.8%), and saponins (19%). Tannins had an absorbance value of 16.54%. *C. orbiculata* (stems) also showed the quantity of total content of flavonoids (3.0%), alkaloids (6.6%), and saponins (2%). Total phytochemical contents found in *H. depressa* were flavonoids (9.70%), alkaloids (7.0%) and saponins (5%). *X. undulatum* showed small and limited amounts of total phytochemical content values; flavonoids (4.50%), alkaloids (6.7%), and saponins (9%). *E. autumnalis* showed a low content of total flavonoids (15.25%), total alkaloids (10.5%), total tannins 0.70% and saponins (4%). *E. bicolar* showed a low content for flavonoids at 0.618 mg, 5.3% for alkaloids, tannins (absorbance at 0.441 nm), and saponins at 8%. *D. sylvatica* revealed a low content for flavonoids (1.0%), alkaloids (9.9%), tannins (1.11%) and saponins (9%). *L. clavatum* also detected a low concentration for flavonoids at 1.0%, 2.9% for alkaloid content, saponins 5% and tannins with an absorbance at 1.0%. Concerning *M. plumbea*, flavonoids content was 2.4%, alkaloids had 1.5%, tannins with an absorbance at 0.16%and saponins had 2%. The high contents found in the presented phytochemicals indicate that the metabolites may be used for wound healing or skin theraphy especially flavonoids, alakoloids

and saponins. The presence of most general phytochemicals might be responsible for the plants' therapeutic and pharmacological effects. The given or presented values indicate that there are secondary metabolites found in the mentioned plants.

Table 3.2: Quantification estimation of phytochemical present in plants used against skin infections in Free State.

Plant name	Total flavonoids Dry matter calculated in %	Alkaloids Dry matter calculated in %	Total tannins absorbance measured in %	Saponins Dry matter calculated in %
P. prunelloides	40.43	84,8	16.54	19
C. orbiculata **(Stems)**	3.0	6,6	1.93	2
C. orbiculata **(Leaves)**	2.42	8,1	3.75	1
H. depressa	9.70	7,0	1.73	5
X. undulatum	4.52	6,7	1.13	9,8
E. autumnalis	1.52	10.5	0.70	4
E. bicolar	6.18	5.3	1.71	8
D. sylvatica	1.0	9.9	1.11	9
L. clavatum	1.0	2.9	1.10	5
M. plumbea	2.40	1.5	0.16	2

Steroids and cardiac glycosides not determined.

3.7. Conclusions

The screening of the plants for the presence of phytochemicals was very crucial, as it gives us an indication of the presence of bioactivity a plant could possess. The qualitative and quantitative analysis of the phytochemicals showed that such plants can be of medicinal value to humans.

Chapter 4

Pharmacological screening

4.1. Introduction

In the 1890's, an introduction of a very successful synthetic drug, aspirin, was born (Rainsford, 2004; Mahdi, 2010). During that era, it was one of the synthetic drugs derived from natural compounds and was particularly the first example of using nature as a lead for new synthetic drugs (Verpoorte, 1998). It was not until the late 1800's when other scientists discovered that the willow bark contains salicylic acid, which is an active ingredient used to make aspirin (James, 2004; Rainsford, 2004). About 12 000 secondary metabolites which are plant-derived agents are known and have been isolated (Samy & Gopalakrishnakone, 2010). At least 30 plant based drugs are reported to be used worldwide, they are compounds isolated from higher plants and are synthetically modified (Weideman, 2007), drugs such as quinine, aspirin, picrotoxin, and reserpine (Borokini & Omotayo, 2012). Plants have, thus, become sources of bioactive agents, which can be continued to be utilized in the production and preparation of synthetic drugs.

Natural products found in medicinal plants include physiologically active chemicals that over the years have been used in traditional medicine for the treatment of various illnesses. These physiologically active ingredients are reported to be active against plant and human pathogenic microorganisms (Rojas et al., 2003). Such biological activity has made these plants to be used in traditional medicinal practices (Rojas et al., 2003; Shai et al., 2008; Sathya et al., 2013). The active ingredients present in these plants act directly or indirectly in the prevention or treatment of diseases, hence, the plants are used to maintain health by stimulating immune responses or by preventing diseases (Ndlovu, 2009). The use of conventional antimicrobial agents has led to an increase in resistance of pathogens, hence the use of medicinal plants is on an increase (Omotayo & Borokin, 2012). Subsequently, Kim et al. (1995) and Alagesaboopathi (2011) suggested methods that can be used to reduce resistance to antibiotics.

4.1.1. Antibacterial screening

The wide transfer of microbial pathogens is much higher in the majority of people in Africa, than those of First World countries, due to improper monitoring of sanitation and hygiene levels (Fennell et al., 2004; Shai et al, 2008; Sathya et al., 2013). This has, therefore, led to an exposure to many infections and diseases, where a high susceptibility to bacterial infections is established (Sathya et al., 2013). Local and indigenous plants are often the only available means of treating such infections, hence, the pharmacological screening is crucial as the screening and the knowledge help scientists and researchers to target plants which may be medicinally useful (Fennell et al., 2004). Microorganisms resistant to most antibiotics are rapidly spreading, hence there is an urgent need for novel antibiotics (Sumthong, 2007). The rates of some communicable diseases have started to increase again as a result of the rise in antibiotic resistance (Kowsalya, 2012). The search for new and effective antimicrobial agents may alleviate the difficulties associated with patient outcome and treatment of antibiotic resistant infections (Kowsalya, 2012). It has been documented that the rate of infection with strains such as *E. coli, K. pneumoniae, S. aureus* and *C. albicans* has increased in immuno-compromised patients worldwide (Aremu et al., 2010). The *S. aureus* is an opportunistic organism which is said to become pathogenic when the immune system of the patient is compromised. The *S. aureus* has been reported by Lekganyane et al. (2012) in diabetic wounds and foot ulcers. According to Bannister et al. (2000), these organisms continue to invade at the initiation sites of the infection.

4.1.2. Antifungal screening

Pathogenic fungi are reported to be the most resistant to majority of therapies used against other infections (Aremu et al., 2010). A significance of the fungal cell wall is that it is a unique structure containing vital and complex mannoproteins, chitins and glucans that are essential for the survival of fungi (Sumthong and Verpoote, 2007). Fungal infections are resistant to antifungal medications that are designed to cure them, and this phenomenon is a concern for invasive infections with the fungas *Candida*. The *C. albicans* is one of the earliest and opportunistic pathogens that commonly affects immunologically compromised patients with infections associated with HIV/AIDS (Fennell et al., 2004; Duarte et al., 2005; Essop, 2005; Hurinanthan,

2013). South Africa, has a high population of immunocompromised patients due to HIV/AIDS pandemic, opportunistic infections such as candidosis caused mainly by *C. albicans* are common. It has been recorded that 36 – 85% of HIV infected patients suffer from candidosis, in which *C. albicans* is the major etiological agent (Larhisini, 2006). Up to two thirds of HIV infected individuals display signs of oral candidosis (Shai et al., 2008). Consequently, antifungal therapy is playing a vital role in healthcare and the screening of traditional medicinal plants in search of novel antifungal drugs is now frequently performed (Motsei et al., 2003; Buwa & van Staden, 2006; Webster et al., 2008; Ncube et al., 2011; Ghuman & Coopoosamy, 2011; Amoo et al., 2013; Orhan et al., 2013).

4.1.3. Aim of this study

The aim of this chapter was to carry out dilution bioassays which are standard methods used to compare the inhibition efficiency of antimicrobial agents with the minimum inhibitory concentration (MIC) values of plant extracts used against skin ailments in the Free State.

4.2. Materials and methods

4.2.1. Preparation of extracts

Plants were collected through surveys with the traditional healers and herbalists of the Free State Province. The collected plant material was cut into smaller pieces and dried in oven at 40°C, (**Figure 4.1 and 4.2**). The dried plant material was ground into fine powders using a blender (**Figure 4.3**). An amount of 40 g each of the powdered plant material was extracted with 400 ml acetone, ethanol, methanol and distilled water, respectively (**Figure 4.4**) and left in the shaker for 24 hours (**Figure 4.5**). The extracts were then filtered through a Whatman No. 1 filter paper and filtrates were taken to dryness using a rotary-evaporator (**Figure 4.6**), until a constant dry weight was obtained.

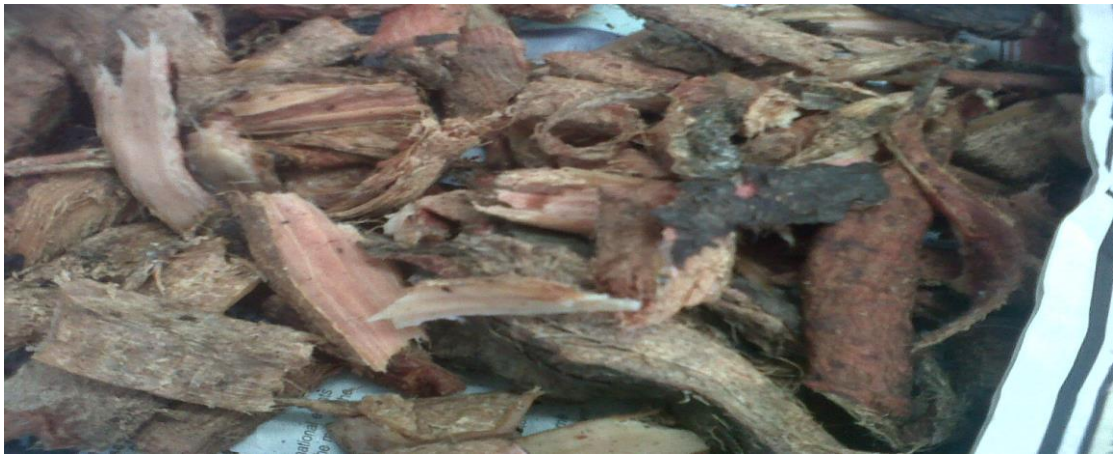

Figure 4.1: Fresh plants were collected and air dried.

Figure 4.2: Air-dried plants were cut into smaller pieces.

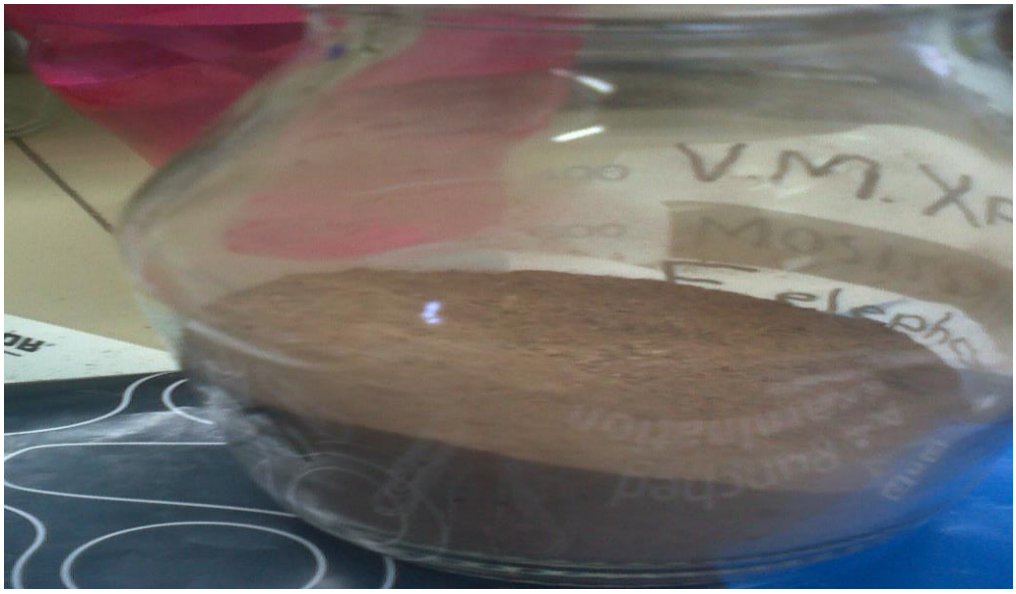

Figure 4.3: Fine powder kept and stored in jars to preserve freshness.

Figure 4.4: The powdered plant material was extracted with the respective solvents.

Figure 4.5: Plant material with solvents were left in the shaker for 24 hours.

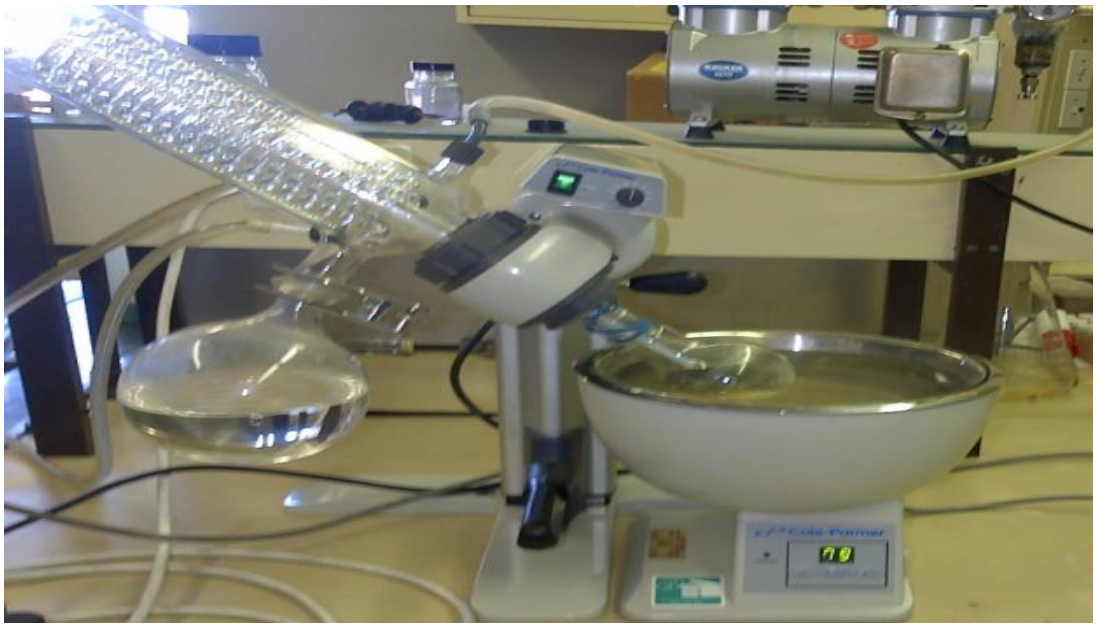

Figure 4.6: The solvents were then dried using a rotary-evaporator.

4.2.2. Antibacterial screening

Five pathogenic bacterial strains: *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 1958), *S. aureus* (ATTC 6538), *B. pumilus* (ATCC 14884) and *K. pneumoniae* (ATTC 13047) were selected and used in the study.

The microplate method of Eloff (1998) was used to determine the MIC values for plant extracts with antibacterial activity (**Figure 4.1**). Residues of plant extracts were dissolved at 50 mg/ml with the extracting solvents. All extracts were initially tested at

12.5 mg/ml in 96 well microplates and serially diluted two fold to 0.098 mg/ml, after which 100 µl bacterial cultures were added to each well. The suspension cultures were prepared by inoculating 20 ml Mueller-Hinton (MH) broth with 20 µl of bacterial stock cultures and incubated at 37ºC in a water bath and left on an orbital shaker for 24 hours. The antibiotic neomycin was included as a standard in each assay. Neomycin (Sigma-Aldrich, Germany) (100µg/ml) (100µl: 0.4 mg/ml) was used as a positive control, to dissolve plant extracts and bacteria-free wells. The microplates were incubated at 37ºC for 24 hours. As an indicator of bacterial growth, 40 µl *p*-iodonitrotetrazolium violet (INT) (Sigma) dissolved in water at 0.2 mg/ml was added to the wells and incubated 37ºC for 30 minutes. The MIC values were recorded as the lowest concentrations of the extract that completely inhibited bacterial growth, i.e. a clear well.

Figure 4.7: Image showing the preparation for the antimicrobial bioassay.

4.2.3. Antifungal screening

Two fungal strains, such as *Trichophyton mucoides* and *Candida albicans* were used to test for antifungal activity. The broth microdilution test with modifications was performed (Espinell-Ingroff and Pfaller 1995; Motsei et al., 2003). Four millilitres of sterile saline were added to approximately 400 µl of 24-hour-old fungal cultures. The absorbance was read at 530 nm and adjusted with sterile saline to match that of a 0.5 McFarland standard solution. From the prepared stock cultures, a 1:1000 dilution with broth (e.g. 10 µl stock culture: 10 ml broth) was prepared. One hundred microlitres of broth were added to each well of a 96-well microplate. All water extracts were dissolved at 100 mg/ml. Water extracts were initially tested at 25 mg/ml whereas organic solvent extracts were tested at 6.25 mg/ml in 96 well microplates. One hundred microlitres of the water extract were added to well (A) and serially diluted from (A) by taking 100 µl into (B). This two-fold dilution was continued down the plate and 100 µl from the last well (H) were discarded. In the case of organic solvent extracts 25 µl of the extracts were added to 175 µl broth and serially diluted. Three replicates were prepared for each extract. All the wells were then filled with 100 µl of stock cultures. Amphotericin B was used as a positive control for this experiment and the following controls were prepared: wells containing broth only, fungal strain with no extract, and serial dilutions of Amphotericin B with the fungi at the recommended inhibitory concentrations. The microplates were incubated overnight at 37°C. As an indicator of bacterial growth, 40 µl *p*-iodonitrotetrazolium violet (INT) (Sigma) dissolved in water at 0.2 mg/ml was added to the wells and incubated 37ºC for 30 minutes.

4.3. Results and discussions

4.3.1. Antibacterial screening

Results for antibacterial activity are presented in Table 4.1. The antibacterial activity for *P. prunelloides* ethanolic extract showed the best activity against *B. pumilus* and *S. aureus* with MIC values ranging between 0.098-0.52 mg/ml. The best inhibition by the ethanolic extracts could be due to a bioactive compound- palmatic acid in the plant (Madikizela et al., 2013). Minimal inhibition of 1.82 mg/ml was observed against *P. aeruginosa* (**Figure 4.8**). *P. prunelloides* methanol extracts showed the

least activity, but a progressive inhibition of 0.325 mg/ml against *P. aeruginosa* was observed (**Figure 4.9 and Figure 4.10**). Acetone extracts exhibited good MIC value at 0.42 mg/ml against *S. aureus* (**Figure 4.11**). In a study undertaken by Madikizela et al. (2013), *Pentanisia* ethanolic leaves and root extracts showed good activity (0.195-0.78 mg/ml) against *K. pneumoniaea and S. aureus*. Low activity was detected with aqueous and methanol extracts. The low activity values in some of the extracts tested in this study could be due to the impure form and or low concentration of the active compounds (Rabe & van Staden, 1997). Water extracts generally exhibit poor pharmacological activities in many cases (Ellof, 1998; Ncube et al., 2011). This is explained by the fact that most of the active constituents found in plants are non-polar and water extracts mainly constitute of polar components (Ncube et al., 2011). Although traditional healers often use water or alcohol for extracting medicinal plants, studies have shown that the use of water for plant material extraction naturally limits the type and amount of compounds extracted (Eloff, 1998b). This probably indicates that dosage is important with regard to which solvent is being used. The relatively higher inhibitory activities were attributed to the presence of terpenoids which have been detected in crude extracts during a qualitative test study, although the compounds have not been isolated and tested individually (van Wyk, 2008).

Table 4.1: Antibacterial activity of traditional medicinal plant extracts used against skin ailments in the Free State (MIC values in mg/ml).

Plant name	Plant part used	Extract yield (g)	Extract	Bacterial strains				
				K.p	***B.p***	***E.c***	***S.a***	***P.a***
P. prunelloides	Roots	98.65	Ace	0.78	0.65	0.78	0.42	3.38
		105.18	EtOH	0.812	0.098	0.52	0.52	1.56
		112.30	MeOH	2.08	1.822	2.148	1.822	0.325
		185.34	d H_2O	3.125	3.125	3.125	3.125	6.25
X. undulatum	Roots	198.68	Ace	2.34	2.08	3.125	3.125	1.07
		124.54	EtOH	2.08	1.56	1.56	3.125	0.42
		181.39	MeOH	3.125	3.125	5.21	6.25	1.56
		179.23	d H_2O	12.5	6.32	10.42	4.30	12.5
H. depressa	Roots	177.32	Ace	3.125	0.098	6.25	2.21	0.36
		106.27	EtOH	2.60	5.21	4.68	1.3	0.26
		174.55	MeOH	2.60	1.56	1.56	1.07	1.3
		131.03	d H_2O	3.125	3.125	0.098	0.36	0.195
C. orbiculata	Stem	143.96	Ace	6.25	6.25	6.25	6.25	1.56
		139.65	EtOH	5.21	6.25	6.25	2.15	6.25
		135.42	MeoH	6.25	6.25	6.25	6.25	3.125

		148.95	d H_2O	6.25	6.25	6.25	6.25	6.25
C. orbiculata	Leaf	176.62	Ace	2.08	1.56	1.56	3.125	0.78
		174.34	EtOH	6.25	3.125	1.56	6.25	6.25
		163.24	MeOH	2.60	3.125	3.125	3.125	0.78
		181.32	H_2O	4.70	4.70	1.56	1.56	1.56
E. autumnalis	Root	134.34	EtOH	3.125	3.125	3.125	3.125	3.125
		143.21	MeOH	12.5	12.5	12.5	12.5	12.5
		146.94	d H_2O	12.5	12.5	12.5	12.5	12.5
		123.65	Ace	12.5	12.5	12.5	12.5	12.5
D. sylvatica	Whole plant	173.24	Ace	3.125	6.25	12.5	6.25	3.125
		184.36	EtOH	6.25	6.25	6.25	6.25	3.125
		163.24	MeOH	3.125	3.125	3.125	3.125	3.125
		167.64	d H_2O	12.5	12.5	12.5	12.5	12.5
E. bicolar	Root	123.65	Ace	6.25	6.25	6.25	6.25	3.125
		143.24	EtOH	3.125	3.125	3.125	3.125	12.5
		135.32	MeOH	12.5	12.5	12.5	6.25	12.5
		143.21	D H_2O	6.25	6.25	12.5	12.5	6.25
L. clavatum	Whole plant	153.32	Ace	6.25	3.125	12.5	0.39	0.098

		143.62	EtOH	3.125	1.56	1.56	1.56	6.25
		148.32	MeOH	12.5	12.5	1.56	1.56	6.25
		155.24	d H_2O	12.5	6.25	0.78	3.125	12.5
M. plumbea	Roots	143.65	Ace	6.23	8.333	2.08	6.25	3.125
		137.36	EtOH	3.125	4.167	6.25	0.78	3.125
		143.65	MeOH	2.088	3.125	6.25	12.5	12.5
		153.64	d H_2O	12.5	6.25	6.25	4.167	8.33

K.p., Kliebsella pneumoniae, B.p., Bacillus pumullis, E.c., Escherichia coli, S.a., Staphyloccocus aureus, P.a., Pseudomonas aeruginosa. Ace., acetone, EtOH., ethanol, MeOH., methanol., d H_20., distilled water.

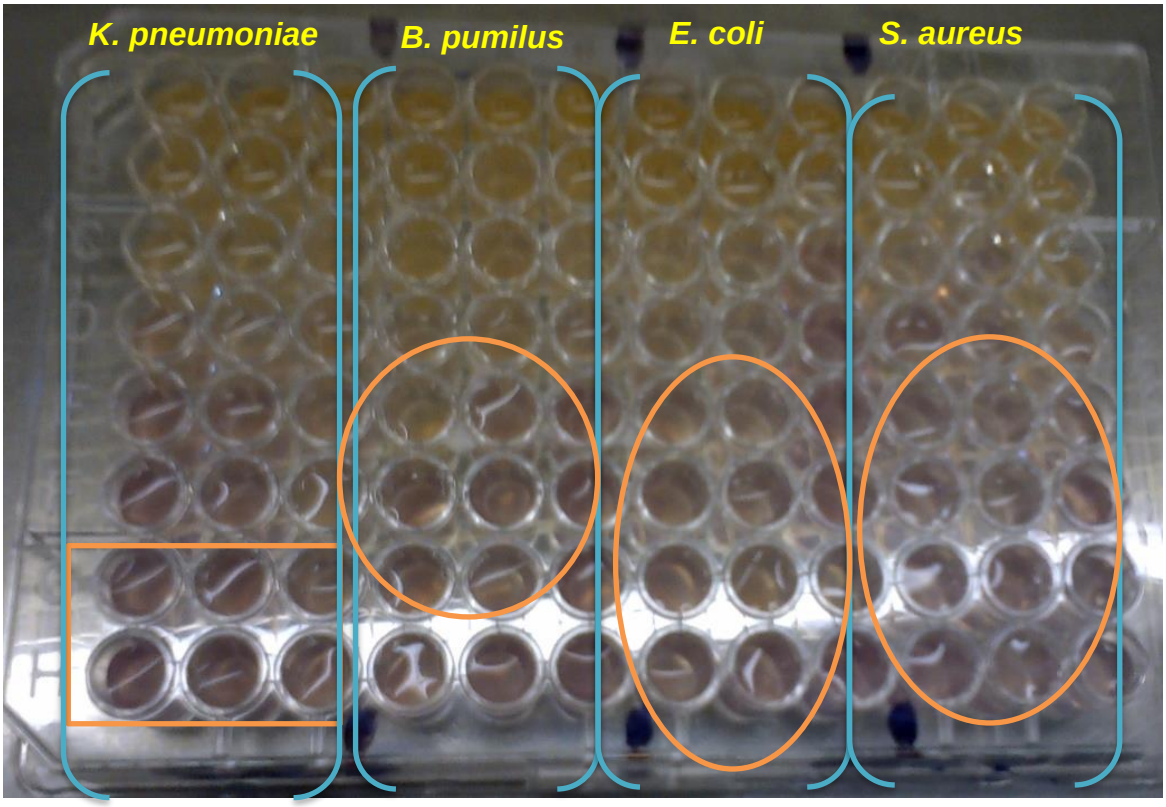

Figure 4.8: Ethanolic extract of *P. prunelloides* showing MIC values of 0.098 and 0.52 mg/ml against *B. pumilus* and *S. aureus.* Orange circle indicates clear wells. Orange square indicates pink wells, showing bacterial growth.

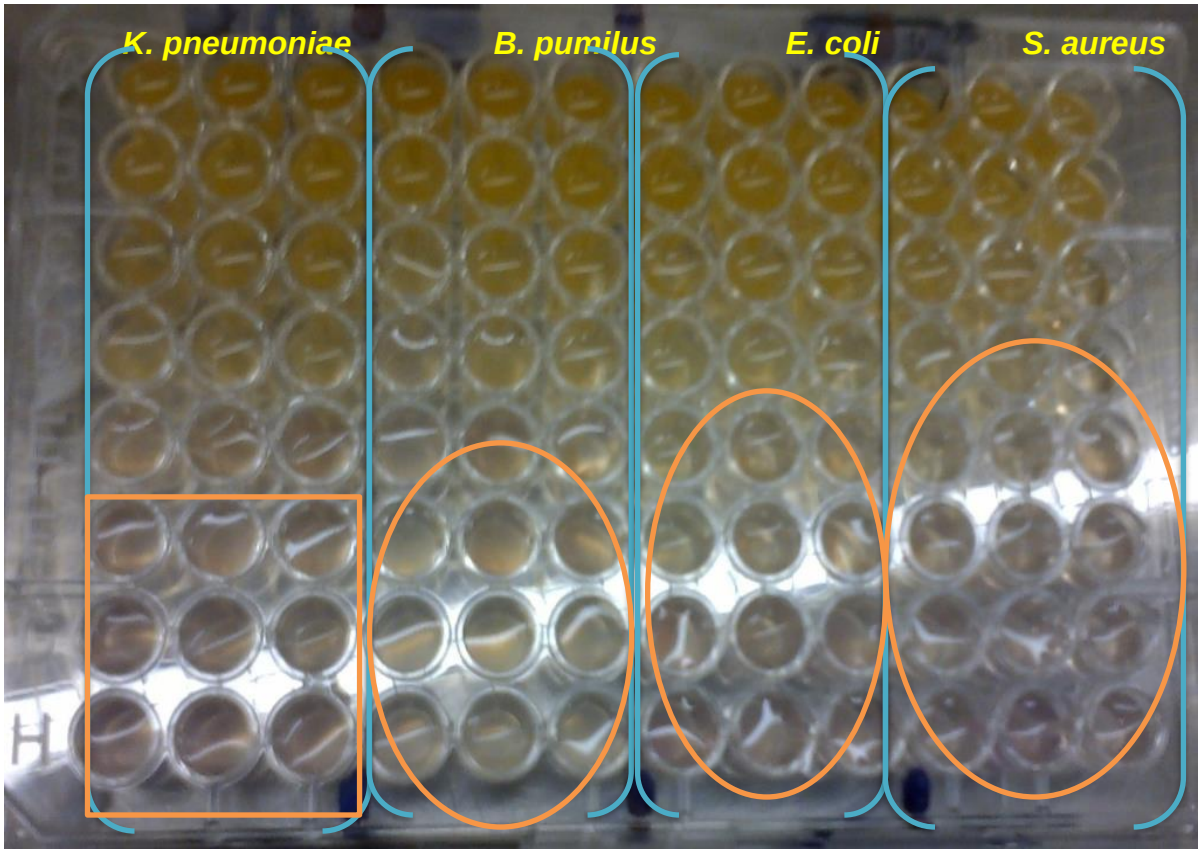

Figure 4.9: Methanolic extracts of *P. prunelloides* with MIC values ranging between 0.098 – 0.78 mg/ml against *B. pumillis* and *S. aureus.* Orange circle indicates clear wells. Orange square indicates pink wells, showing bacterial growth.

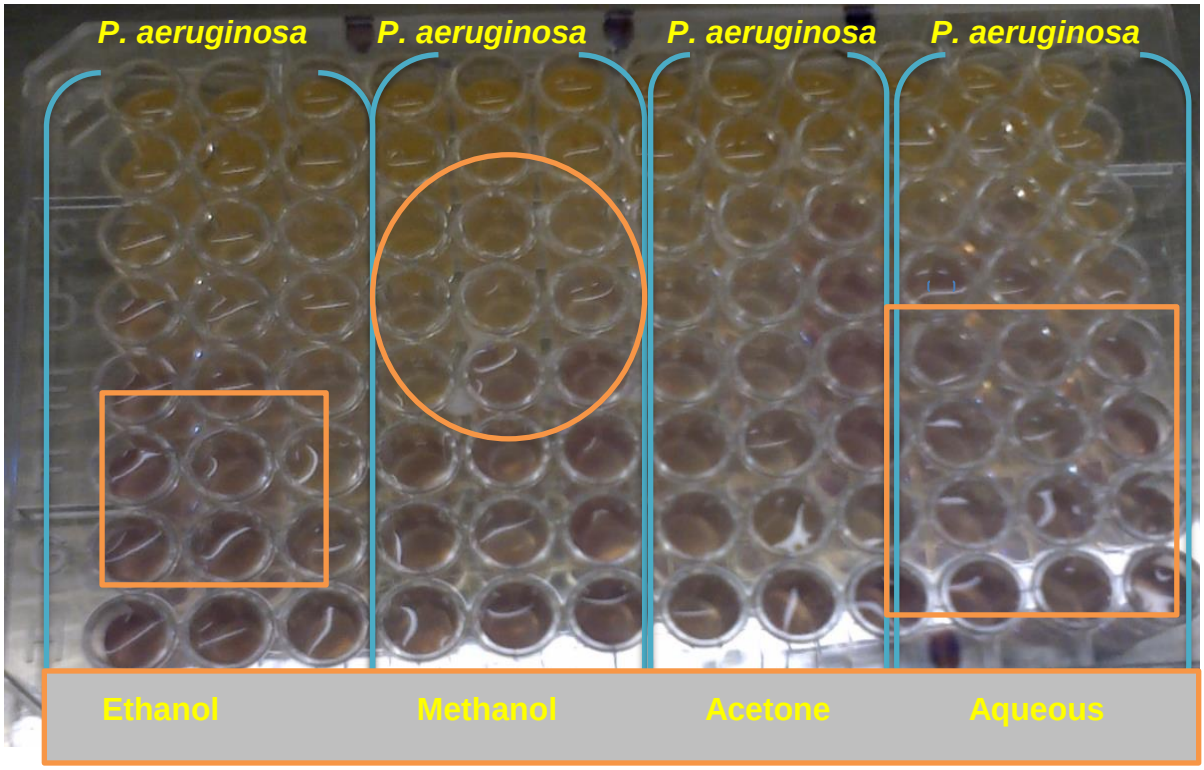

Figure 4.10: Methanolic extracts of *P. prunelloides* with an MIC value 0.325 mg/ml against *P. aeruginosa*. Orange circle indicates clear wells. Orange square indicates pink wells with bacterial growth.

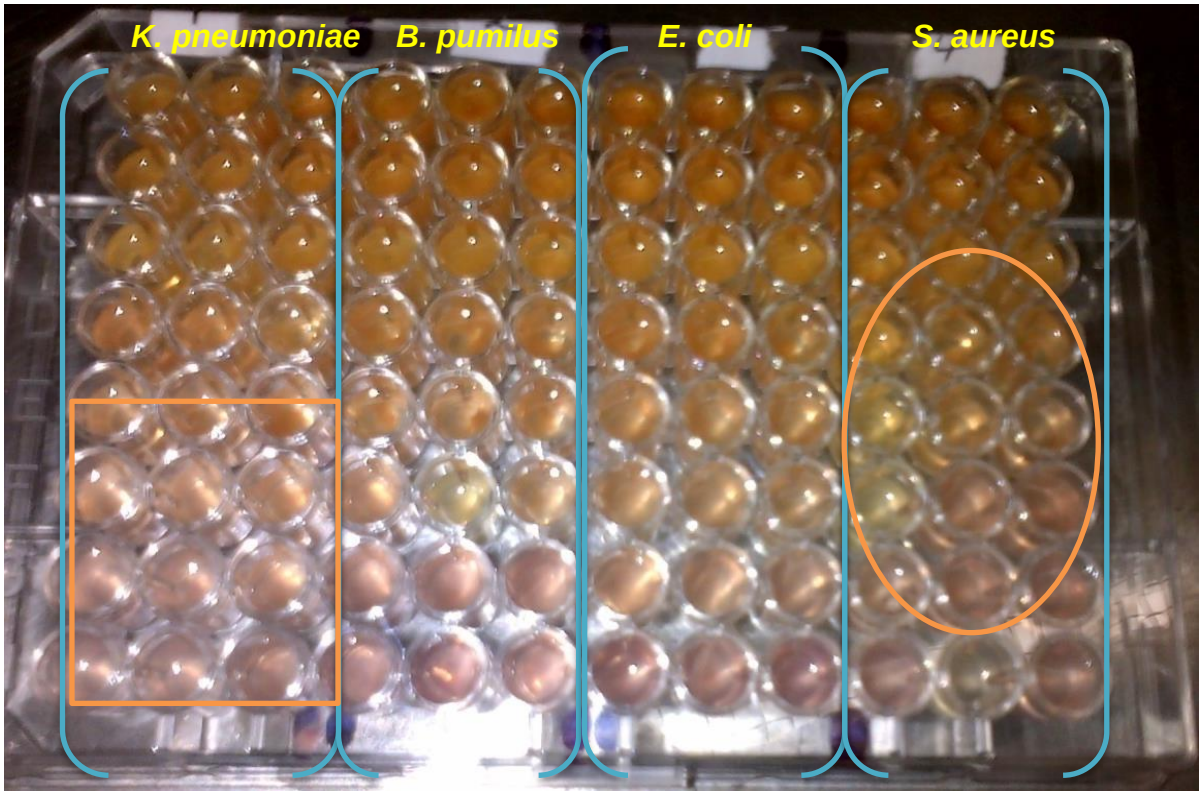

Figure 4.11: Acetone extracts of *P. prunelloides*, lowest inhibition was observed against *S. aureus* at 0.42 mg/ ml. Orange circle indicates clear wells. Orange square indicates pink wells with bacterial growth.

H. depressa acetone extract showed the best activity against *B. pumilus* at 0.098 mg/ml. The acetone and ethanol extracts displayed a good activity against *P. aeruginosa* with MIC values ranging between 0.26 – 0.36 mg/ml. The good inhibitory concentrations for *H. depressa* were due to the presence of tannins, alkaloids (7.0%), and cardiac glycosides in ethanol, methanol and acetone extracts. The aqueous extract displayed good activity against *E. coli*, *S. aureus* and *P. aeruginosa* with the MIC values of 0.098, 0.36 and 0.195 mg/ml, respectively. This could be due to the saponin (5%) and flavonoid (9.70%) contents found in the aqueous extracts. Essop et al. (2008) obtained moderate inhibition with ethyl acetate and ethanol extracts displaying MIC values ranging between 0.78 to 3.90 mg/ml against *S. aureus* and *B. subtillis*. The results confirm that the selected *Hermannia* species were effective against *S. aureus* and therefore they can be utilized in the treatment of bacterial infections (Essop, et al., 2008).

Concerning *C. orbiculata,* all stem extracts (acetone, ethanol, methanol and aqueous) displayed low or poor activity against all the test microorganisms, whereas acetone and methanol leaf extracts exhibited good activity against *P. aeruginosa* (0.78 mg/ml). A previous study by Aremu et al. (2010) also showed poor inhibition by *C. orbiculata var. obiculata* and C*. orbiculata var. dactylopsis* against *B. subtilis*, *S. aureus, E. coli*, *K. pneumoniae*, and fungal strain *C. albicans*.

The *L. clavatum* acetone extract displayed good minimum inhibition against *S. aureus* with an MIC value of 0.39 mg/ml, and best inhibition at 0.098 mg/ml against *P. aeruginosa*. Good activity was also observed against *E. coli* with an MIC value of 0.78 mg/ml. The good activity could be attributed to the phytochemicals that were detected in the extracts.

M. plumbea (syn*. Scilla natalensis*) ethanolic extract exhibited a good activity with an MIC value of 0.78 mg/ml against *S. aureus*. According to Fennell et al. (2004), the fact that extracts are more active against Gram-positive bacteria can be attributed to the fact that the cell wall of Gram-positive bacteria is easier to penetrate than that of Gram-negative bacteria. Gram- positive are more susceptible, having only an outer peptidoglycan layer which is not an effective permeability barrier. The cell walls of Gram-negative organisms, which are more complex than the Gram-positive act as a diffusional barrier and that makes them less susceptible to the anti-microbial agents than are Gram- positive bacteria (Nostro et al., 2000; Hodges, 2002; Tadeg et al., 2005).

4.3.2. Antifungal screening

The results for antifungal screening are presented in Table 4.2. The best inhibition was observed with *P. prunelloides* organic solvents displaying MIC values of 0.049 mg/ml for all extracts (**Figure 4.12**). *H. depressa* alcoholic extracts also showed great MIC values (0.049 -0.33 mg/ml) against both fungal strains (**Figure 4.13**).

X. undulatum acetone extract showed good inhibition against both test microorganisms with an MIC value of 0.78 mg/ml. Poor activity was detected with

water extract. These results supported the investigations that *X. undulatum* aqueous extract does not exhibit antimicrobial activity (Vermaak et al., 2014).

C. orbiculata acetone, methanol and ethanolic stem extracts showed a very good inhibition against both fungal strains at 0.78 mg/ml (**Figure 4.14**). Concerning the leaf extracts, good antifungal activity was observed (0.13- 0.39 mg/ml). Different results were observed by Aremu et al. (2010) where *Cotyledon* species exhibited low inhibition (MIC values at 6.25 -12.5 mg/ml).

E. autumnalis acetone extracts revealed good inhibition against C. *albicans* and *T.* mucoides (MIC values at 0.39 mg/ml). In a study by Motsei et al. (2003), *E. autumnalis* extracts (ethanol, ethyl acetate and hexane) exhibited poor MIC values (>8.35 mg/ml) against *C. albicans* (ATCC 10231). Good activity was detected with *D. sylvatica* acetone and methanolic extracts with MIC values 0.39 mg/ml. The *M. plumbea* ethanolic extract displayed a significant MIC value against *C. albicans* at 0.78 mg/ml.

Table 4.2: Antifungal activity of traditional medicinal plants used against skin ailments in the Free State (MIC values in mg/ml).

Plant name	Part used	Extract Yield (g)	Extract	Fungal strain	
				C.a	*T.m*
P. prunelloides	Root	98.56	Ace	0.049	0.049
		105.20	EtOH	0.049	0.049
		110.24	MeOH	0.049	0.049
X. undulatam	Root	185.34	d H_2O	6.25	4.16
		200.73	Ace	0.78	0.78
		124.54	EtOH	3.125	5.21
		180.62	MeOH	6.25	6.25
H. depressa	Root	179.12	d H_2O	6.25	4.166
		177.32	Ace	0.30	0.33
		106.30	EtOH	0.39	0.195
		173.73	MeOH	0.049	0.049
		131.07	d H_2O	1.56	3.125
C. orbiculata	Stem	146.98	Ace	0.78	1.56
		174.56	EtOH	0.78	1.56
		170.09	MeOH	0.78	1.56
		186.98	d H_2O	6.25	1.56

E. autumnalis	Root	200.98	Ace	0.39	0.39
		189.65	EtOH	1.56	1.56
		175.34	MeOH	2.08	2.08
		171.58	d H_2O	3.125	3.125
D. sylvatica	Whole plant	168.57	Ace	0.39	0.39
		134.45	EtOH	1.56	2.08
		109.87	MeOH	0.39	0.39
		197.65	d H_2O	1.56	3.125
L. clavatum	Whole plant	175.36	Ace	3.125	3.125
		127.95	EtOH	1.56	2.60
		143.89	MeOH	3.125	3.125
		145.36	d H_2O	3.125	3.125
M. plumbea	Root	168.32	Ace	5.20	2.08
		154.62	EtOH	0.78	1.56
		187.65	MeOH	3.125	3.125
		189.32	d H_2O	6.25	6.25

C.a.,Candida albicans., T.m.,Trichophyton mucoides. Ace., acetone, EtOH., ethanol, MeOH., methanol., d H_20., distilled water.

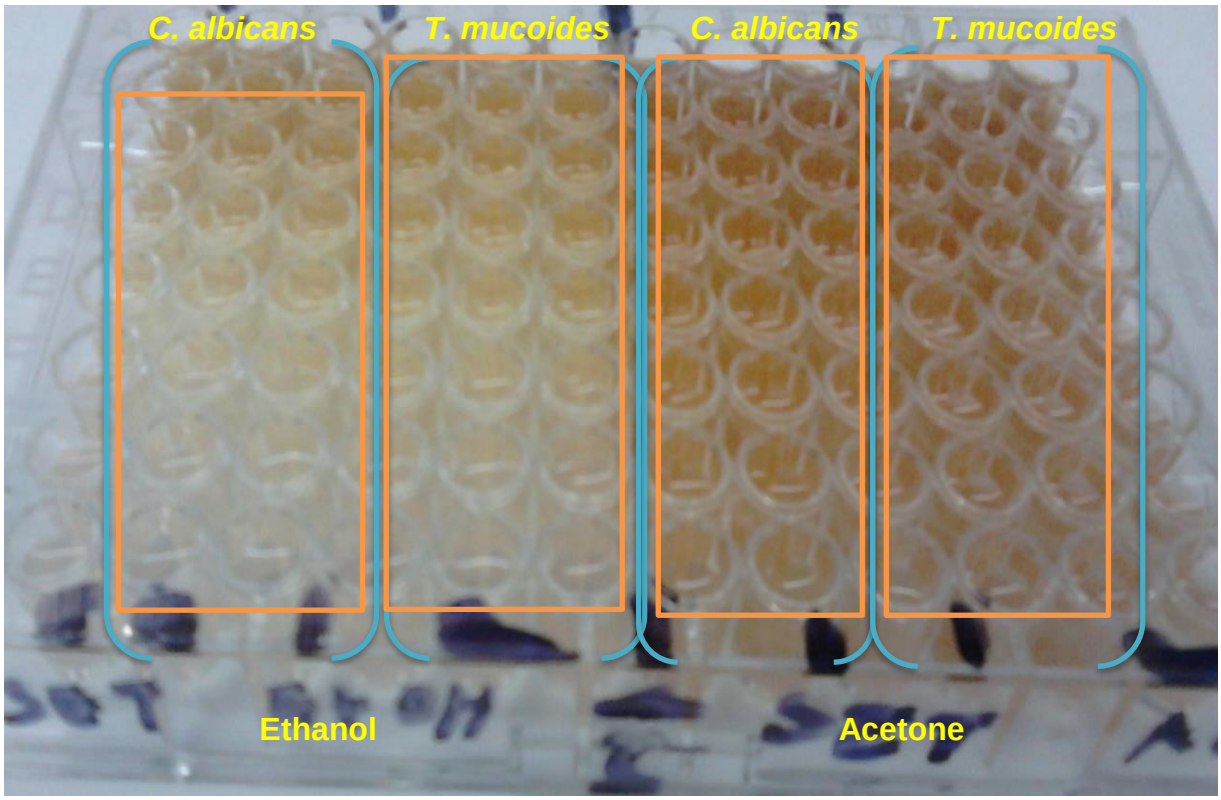

Figure 4.12: Ethanol and acetone extracts. Lowest minimum inhibition was observed at 0.049 mg/ml for *P. prunelloides* against two fungi, *C. albicans* and *T. mucoides*. Orange squares indicating clear wells and inhibition at the lowest concentration

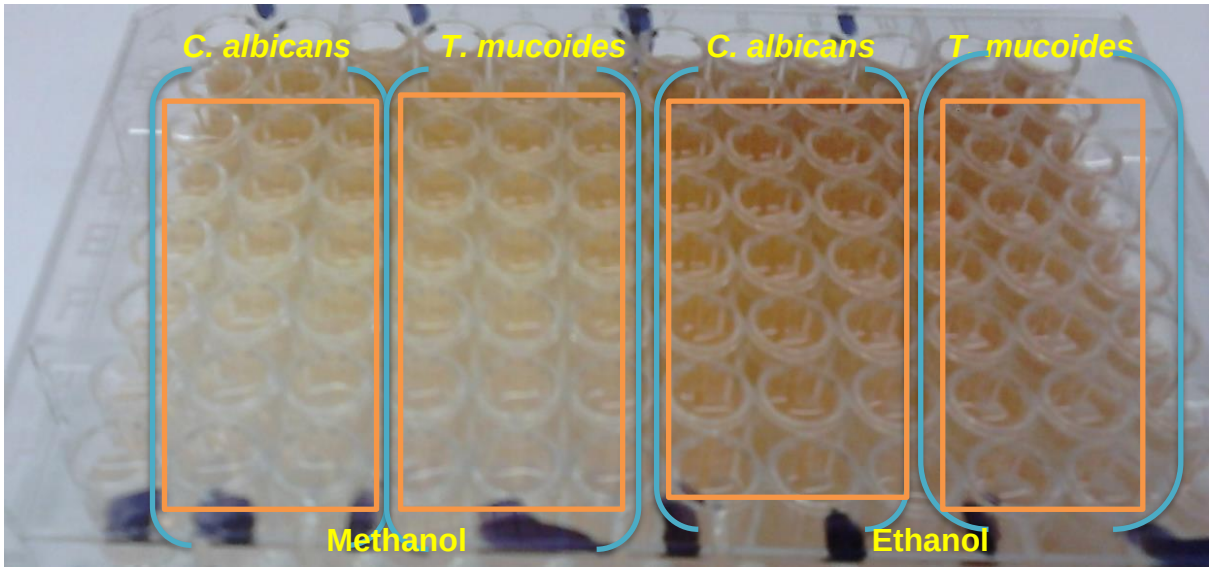

Figure 4.13: Alcoholic extracts of *H. depressa* showing MIC at 0.049 mg/ml. Orange squares indicate clear wells and inhibition at the lowest concentration.

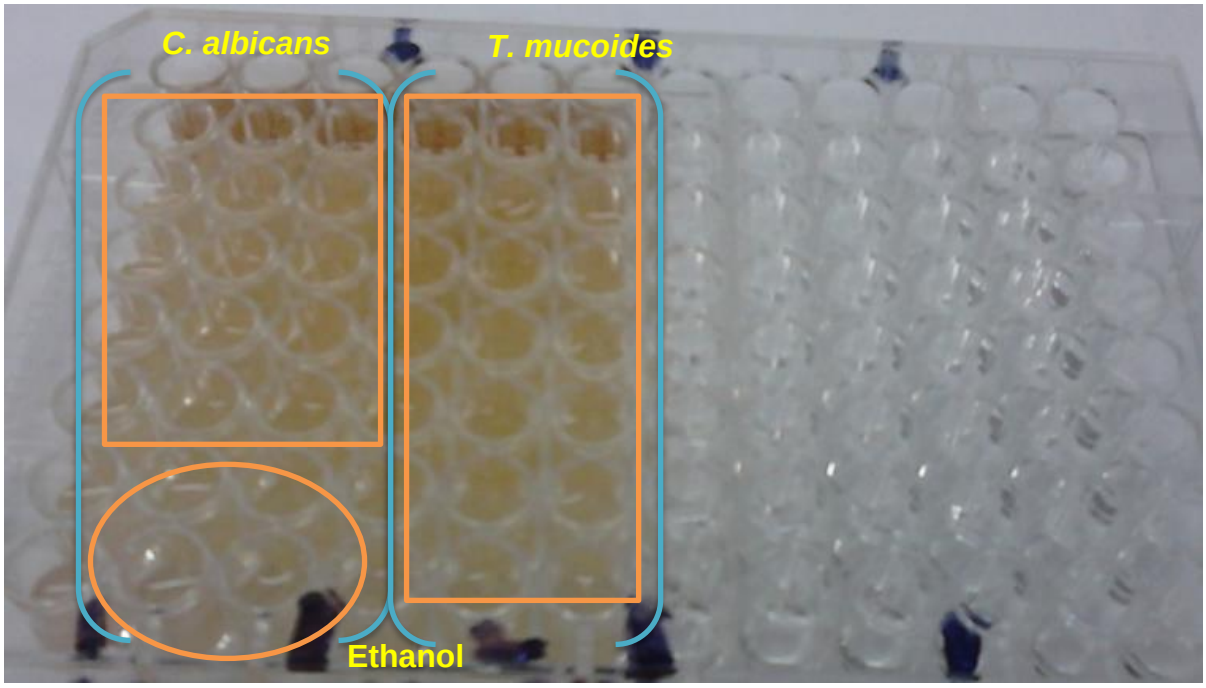

Figure 4.14: Ethanol and methanol extracts of *C. orbiculata* showing lowest inhibition concentration at 0.78 mg/ml. Orange squares indicating clear wells and inhibition at the lowest concentrations. Orange circle indicating pink wells, with bacterial growth.

4.4. Conclusions

The results indicated that the extracts of plant species tested have inhibitory effects against both Gram-negative and Gram-positive bacteria, as well as fungal strains, hence, they have displayed some activity. The results have also revealed the presence of medicinally important constituents in the plants studied. It further shows that the plants do contain bioactive compounds due to the presence of the phytochemicals found to be present and detected. In addition, several studies also confirmed that the presence of phytochemicals contribute to medicinal properties and can therefore cure or eliminate the human pathogens. Ethanol and acetone were the most active extracts for both antibacterial and antifungal screening, while aqueous extracts exhibited either low or poor activity against the test microorganisms. Minimal inhibition was noteworthy among *H. depressa* and *L. clavatum.* Plant extracts tested in this study could, therefore, be used as a good source of useful remedies. These results validate the claims made by the traditional healers that these medicinal plants are useful as treatment for various skin ailments.

Chapter 5

IN VITRO ANTIOXIDANT OF DPPH RADICAL SCAVENGING ACTIVITY AND TOTAL CAPACITY

5.1. Introduction

5.1.1. Reactive Oxygen Species

There has been a counteraction between living organisms which have developed a complex antioxidant network of reactive species which may be detrimental to human life (Prior et al., 1998; Ndlovu, 2009). Reactive Oxygen Species (ROS) such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$), nitric oxide ($NO^{\cdot}$), peroxyl (ROO,) and hydrogen peroxide (H_2O_2) are generated and can increase the possibility of a wide range of common degenerative diseases by preventing or minimizing oxidation-related diseases (Schinella at al., 2002; Roy et al., 2011; Albano et al., 2012; Lall & Kishore, 2014). A number of diseases have been accounted due to the ROS, which are also known as free radicals. This occurs when oxygen is supplied in excess or its reduction is insufficient. ROS are associated with oxidative damage of protein, and degenerative diseases such as eye disease like cataracts, cancer, liver diseases, ulcerative colitis, cirrhosis, coronary heart failure, arteriosclerosis, diabetes mellitus and wounds (Trouillas et al., 2003; Marwah et al., 2007; Kamatou et al., 2008; Roy et al., 2011; Kowsalya, 2012; Paulpriya & Mohan, 2012; Aremu et al., 2013; Sakthidevi & Mohan, 2013). Furthermore, free radicals are reported to cause lipid peroxidation, aggregation of protein and degradation of DNA, protein and polysaccharides which eventually destroy cell membranes and kill cells (Roy et al., 2011; Sakthidevi & Mohan, 2013). A high radical scavenging capacity could be responsible for wound healing and ophthalmic uses of plants, through inhibition of ROS, which are often radicals (Marwah et al., 2007).

Wound healing processes could include cell proliferation, suppression of inflammation and contraction of the collagen tissue and could be delayed by ROS or microbial infection (Pattanayak & Sunita, 2008). This activity may be a solution in preventing a number of diseases through free radical scavenging (Sakthidevi &

Mohan, 2013). Hence, in humans it explains the useful capacity of antioxidants as contributing remedies for such ailments.

5.1.2. Antioxidant radical scavenging

Antioxidants are defined as scavengers which are able to protect the human body against free radicals that may cause pathological conditions such ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, ageing process, and dermantis (Diwan et al., 2012; Omotayo & Borokini, 2012; Saxena et al., 2013). They are also known as the free radical scavengers which are involved in delaying or preventing an oxidative reaction catalysed by free radicals. Such antioxidants terminate or suppress these chain reaction formation by removing free radical intermediates because of their redox properties (Marwah et al., 2007; Pattanayak & Sunita, 2008; Roy et al., 2011; Kowsalya, 2012) as they function as hydrogen donors and reducing agents (Marwah et al., 2007). Their mechanism can also act by inhibiting enzymes or by chelating trace elements involved in free radical production, scavenging reactive species and up-regulating or protecting antioxidant defences (Essop et al., 2008). It has been reported that low levels of antioxidants or the inhibition of the antioxidant enzymes can cause oxidative stress and may damage or kill cells (Roy et al., 2011). Hence, increasing the antioxidant intake can neutralize the free radicals and protect the body from cell damage (Kowsalya, 2012), especially increasing the dietary intake of fruits and vegetables, legumes, whole grains, nuts, seeds, edible fungi, herbs and spices (Saxena et al., 2013), many of which are rich sources of antioxidants (Marwah et al., 2007; Albano et al., 2013). Natural antioxidant defence system functions in protecting aerobic organisms from oxygen toxicity involving enzymatic and non-enzymatic mechanisms (Schinella et al., 2002). Recent research shows that plants produce these chemicals in order to protect themselves, but studies have shown that many phytochemicals can also protect humans against diseases (Saxena et al., 2013). Ever since then, there is high interest and focus on the beneficial health effects of phytochemicals.

Antioxidants are classified under three classes, namely, carotenoids, flavonoids and polyphenols (Lall & Kishore, 2014). Phytoconstituents such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavones, flavonols,

catechins, anthocyanins, proanthocyanins (Marwah et al., 2007), phenols, flavonoids, tannins, nitrogen compounds, vitamin C and E, terpenoids, diterpenoids, phytate and phyoestrogenes (Roy et al., 2011; Albano et al., 2013) and some endogenous metabolites in plants are an indication of the possible presence of antioxidants. The antioxidative effect of medicinal plants may be mainly due to the phytochemical components. Such antioxidants are produced in response to various stimuli in plants. They play protective roles including, free radical-scavenging, UV light-absorbing, and antiproliferative agents (Kennedy & Wightman, 2011) against various stress factors (Saxena et al., 2013).

Plant secondary metabolites, namely phenolic compounds have gained an increased and renowned interest due to their presence and use as antioxidants (Aremu et al., 2013; Saxena et al., 2013).

5.1.3. Role of phenolic acids and flavonoids as antioxidants

According to Murugan & Mohan (2012), the phenolic acids and flavonoids are the principal antioxidant constituents of natural plant products. Phenolic compounds are produced in plants to serve a diverse range of purposes including defence against pathogens and different forms of environmental stress, amongst them heat stress, moisture stress, and UV radiation (Ncube et al., 2011). They serve in the maintenance of the human body (Anitha, 2012; Sakthidevi & Mohan, 2013; Lall & Kishore, 2014) and in the prevention of a number of diseases (Kamatou et al., 2008). Phenolic compounds possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Yadav and Agarwala, 2011). This is due to the peptides found in the plants. The flavonoids are said to be non-toxic and interestingly reduce the risk of developing cancer, cardiovascular disorders, obesity, diabetes, aging-diseases, urinary tract infections and periodontal diseases (Fawole et al., 2010; Gnanaraja et al., 2014). In the crude extract form, the flavonoids are reported to have been utilized for their anti-inflammatory capacity in the cosmetic industry (Lall & Kishore, 2014). It is also reported that phenolic compounds such as

gallotannins, condensed tannins and flavonoids are known to inhibit some molecular targets of pro-inflammatory mediators in inflammatory responses, so they can be anti-inflammatory or alternate the inflammatory processes as they are antioxidants which reduce scavenging radicals (Luseba et al., 2007; Fawole et al., 2010; O'Doherty et al., 2010).

The activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Kamatou et al., 2008; Shabbir et al., 2013; Sakthidevi & Mohan, 2013). They may also have a metal chelating potential (Marwah et al., 2007). Hence, the synergistic effect of both antimicrobial and antioxidant activity accelerate the wound healing processes (Pattanayak & Sunita, 2008) such as anti-inflammatory and anti-tumor activities (Sakthidevi & Mohan, 2013).

5.1.4. Synthetic vs natural antioxidants

The effects of oxidation of the lipids that occur through the process of raw materials storage, processing, heat treatment and further storage of the final products has led to the deterioration of food products (Tepe et al., 2005). Hence, there are commonly and widely used antioxidants- Butylated hydroxyanisol (BHA) and Butylated hydroxytolune (BHT) which are known as synthetic chemicals. They are commonly used in food industries as preservatives for preventing or delaying the oxidation process, although there is an increasing interest in natural food additives, i.e., spice or spice extracts, which can have the dual role of natural antioxidants and seasoning (Tepe et al., 2005; Albano et al., 2013). Reports have stated that there are possible carcinogenic effects and toxic properties from these antioxidants which have resulted in their reduced usage (Tepe et al., 2005; Kamatou et al., 2008). Natural antioxidants have, therefore, been extensively employed in recent years (Kamatou et al., 2008; Sakthidevi & Mohan, 2013). Naturally, medicinal plants and herbs can help eliminate free radicals by acting as antioxidants in neutralizing and reducing decomposing peroxide (Shabbir et al., 2013). Many plant species are said to have antioxidants similar to the synthetic antioxidants without any potential side effects, and are used as alternatives in the food processing industry and in preventive medicine (Roy et al., 2011). Recent studies are focusing on replacing synthetic

antioxidants with naturally occurring antioxidants to avoid potential toxicity of synthetic antioxidants (Shabbir et al., 2013; Sakthidevi & Mohan, 2013). Hence, there is an increasing interest in the antioxidant effects of derived herbal compounds and their role in human health and diseases (Schinella et al., 2002).

5.1.5 Aim of this study

The aim of this chapter was to determine the total phenolic content from various plant extracts, as well as to examine antioxidant activity of those plant extracts. In this study, 1-1-diphenyl-2-picryl hydrazyl (DPPH) and total capacity of antioxidants were selected to determine the antioxidant activity of different plant species with different extracts. DPPH is a widely used method, and is said to be stable free radicals and is used to assess the radical scavenging activity of antioxidants (Nisa et al., 2013).

5.2. Materials and methods

5.2.1. Preparation of plant material

Air dried plant material was crushed using a blender and was extracted separately by shaking approximately 100 g of each plant species in 95% methanol for 24 to 36 hours. Then, the extracts were filtered by using Whatman No 1 filter paper. The extract solutions were concentrated to dryness using a rotary evaporator at 40°C under reduced pressure. The extracts were packed and closed in sterile bottles and refrigerated until use.

5.2.2. Determination of total phenolic compounds

Determination of total phenolic compounds was done following the method of Singleton et al. (1999) with modifications by Wolfe et al. (2003). A stock solution of 50 µl of aliquot (1 mg/ml) was prepared. Then, 150 µl of 7.5% Na_2CO_2 was added to neutralize the reaction, followed by 50 µl of Folin C reagent (10%). The aliquots were presented in duplicates. The tubes were vortexed for 15 seconds and allowed to stand for 30 minutes at 40ºC for colour development. The mixture was then

incubated for 40 minutes at 45ºC. After incubating, the absorbance was read at 765 nm using a spectrophotometer. The total phenolic content was expressed as mg/g gallic acid equivalent using the equation obtained from a calibration using varying concentration of the standards i.e. tannic or gallic acid). In this case gallic acid was used as a standard. The results of the total phenol content were extrapolated from gallic acid curve (R^2 =0.9236) and expressed as gallic acid equivalents (GAE mg/g). The use of Folin C reagent gives different responses to different phenolic compounds, depending on chemical structures (Marwah et al., 2007).

5.3. DPPH radical scavenging activity

The method of Ursini et al. (1994) and Liyana-Pathirana & Shahidi (2005), with slight modifications, was used. An aliquot was prepared from 150 µl extract with 150 µl 0.4 mM (0.004M) DPPH in methanol solution prepared in 1.0 ml of the solution and mixed with 1.0 ml of extract in methanol containing 0.02, 0.05, 0.1, 0.4, 0.8 mg of the extract. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H (Paulpriya & Mohan, 2012; Sakthidevi et al., 2013). The reaction mixture was vortexed thoroughly to dissolve the extract and left in the dark at room temperature for 30 minutes. The absorbance of 517 nm of the mixture was measured by a spectrophotometer. The changes in colour from deep violet to light yellow were measured at 517 nm. The color of the reaction mixture changed from purple to yellow with decreased absorbance at 517 nm (Roy et al., 2011) and the discolouration indicated the potential of the plant extract to scavenge free radical due to its ability to donate hydrogen (Paulpriya & Mohan, 2012). Ascorbic acid was used as a reference/ a positive control. The IC_{50} which is the concentration at which there is 50% decolourization of the DPPH by the test sample determined using GraphpadLower absorbance values of reaction mixture indicated higher free radical scavenging activity. All the tests were performed in duplicate/triplicate and the results were averaged. IC_{50} is the concentration value which scavenged 50% of the DPPH radicals. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration. Ascorbic acid was

used as reference compound. The capability to scavenging the DPPH radical was calculated using the following formula (Sakthidevi & Mohan, 2013):

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(Abs_{control} - Abs_{sample})]}{(Abs_{control})} \times 100$$

Where $Abs_{control}$ is the absorbance of DPPH radical + methanol;
Abs_{sample} is the absorbance of DPPH radical + sample extract /standard. Where A control= A control – A blank and A sample = A sample – A blank.

5.4. Total antioxidant capacity

The Phosphomolybdate method was done as described by Umamaheswari & Chatterjee (2008) with slight modifications. The phosphomolybdate assay system was used to determine the total antioxidant activity of the methanol, ethanol, acetone and aqueous extract and various fractions. An aliquot of 0.1 ml of sample solution containing a reducing species was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 mM sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate); 100 µl of each sample was added and incubated at 95ºC, in a water bath for 90 minutes. After cooling at room temperature, the absorbance was recorded at 765 nm against reagent blank. Total antioxidant capacity of the ascorbic acid was also determined for reference. Total antioxidant capacity was determined by using the following formula:

$$\text{Total antioxidant capacity (\%)} = \frac{[(Abs_{control} - Abs_{sample})]}{(Abs_{control})} \times 100$$

Where $Abs_{control}$ is the absorbance of DPPH radical + methanol;
Abs_{sample} is the absorbance of DPPH radical + sample extract /standard. Where A control= A control – A blank and A sample = A sample – A blank.

5.5. Statistical analysis

In vitro and other parametric assays were performed in triplicate and results are shown as mean ± SD. Antioxidant potential of the samples in different assays was determined as IC_{50} values by applying GraphPad Prism 5-software. Statistical significance was determined among various treatments with one way ANOVA test. A statistical significance of $P < 0.05$ was considered to be significant.

5.6. Results and discussions

5.6.1. Determination of total phenolic content

The results for total phenolic content present in plants used against skin ailments are presented in **Table 5.1**. The determination of total phenolic content was carried out based on the absorbance values of the various extract solutions reacted with Folin-C reagent and then compared with the standard solutions of gallic equivalents (Singleton et al., 1999; Hossain & Shah, 2015)

Table 5.1: Total phenolic content present in different plants used against skin ailments in the Free State Province.

Plant name	Extracts (values in mg GAE/g)			
	MeOH	**EtOH**	**Acetone**	**Aqueous**
P. prunelloides	0.46±0.07	0.50±0.07	0.47±0.07	1.21±0.07
H. depressa	2.09±0.07	0.53±0.07	1.01±0.07	0.56±0.07
***C. orbiculata* (stem)**	1.48±0.64	1.20±0.64	1.48±0.64	0.78±0.64
X. undalatum	0.58±0.67	1.08±0.67	0.67±0.67	0.86±0.67
D. sylvatica	0.56±0.13	0.75±0.13	1.48±0.13	1.00±0.13
L. clavatum	0.75±0.13	1.50±0.13	0.92±0.13	0.67±0.13
E. bicolar	0.56±0.09	0.93±0.09	1.05±0.09	0.47±0.09
M. plumbea	0.60±0.25	0.52±0.25	0.61±0.25	1.08±0.25
E. autumnalis	0.86±0.43	0.86±0.43	0.47±0.43	0.60±0.43
Gallic acid (standard reference)	0.87±0.07			

Total phenolics as gallic acid equivalent GAE mg/g of extracts using the calibration curve (using varying concentration of the standards-gallic acid. Results are given as mean standard deviation or mean of duplicate assays, ± SE (standard error). P values less than $P<0.05$ are considered significant.

Extracts that showed the highest total phenolic content were, *H. depressa, C. orbiculata*, *D. slyvatica*, *E. bicolar*, and *L. clavatum*. *H. depressa* methanolic extract had the highest phenolic content at 2.09±0.07 mg GAE/g, followed by *C. orbiculata* acetone extract at 1.48±0.64 mg GAE/g. In other studies, methanolic extracts were observed to show the highest total phenolic and flavonoid contents, due to the fact that methanol has a high polarity index than other solvents and is able to extract more phenolic and flavonoid compounds (Cowan, 1999; Namuli et al., 2011). According to Tepe et al. (2005), when polar extracts exhibit a stronger activity than the non-polar extracts, there is an indication that polyphenols or flavanones, and flavonoids play an important role in the activity of that particular extract. *H. depressa* had a coefficient correlation of R^2= 0.923 which is considered good or better.

A good phenolic content was displayed with the organic extracts of *C. orbiculata* with values ranging from 1.20±0.64-1.48±0.64 mg GAE/g. *D. sylvatica* acetone and aqueous extracts exhibited a good total phenolic content (1.00-1.48±0.13 mg GAE/g). Acetone and ethanol extracts of *E. bicolar* and *L. clavatum* displayed good total phenolic content with values ranging from 0.92±0.13 to 1.50±0.13 mg GAE/g. A minimum phenolic content was observed with *E. autumnalis* alcoholic extracts (0.86±0.43 mg GAE/g).

The *P. prunelloides* aqueous extract and *X. undulatum* ethanol extract also exhibited higher phenolic contents with values of 1.21±0.07 and 1.08±0.67 mg GAE/g, respectively. According to Marwah et al. (2007) aqueous alcohol is generally considered the best solvent for extracting phenolic compounds from plant materials. However, *X. undulatum, C. orbiculata, E. autumnalis, M. plumbea,* and *E. bicolar* had a lower coefficient correlation (R^2= 0.409 - 0.598).

5.6.2. *In vitro* DPPH radical scavenging antioxidant assay

Figure 5.1 and **Table 5.2** show the scavenging effects of plant extracts on DPPH radical activity. Most species portrayed promising antioxidant activity in the DPPH assay. A lower or reduced IC_{50} value suggests better antioxidant activity. The *in vitro* antioxidant activities of methanol extracts are presented in Table 5.2. Ascorbic acid was used as a frame of reference since it is a known antioxidant which can scavenge DPPH and other free radicals (Nisa et al., 2013). Plant species which showed a higher and stronger antioxidant than that of ascorbic acid were *C. orbiculata, L. clavatum, H. depressa,* and *D. sylvatica* (**Table 5.2**). The scavenging effects of methanolic extracts were in the following order: *C. orbiculata> H. depressa >L. clavatum>P. prunelloides>D. sylvatica*. The species had promising antioxidant activity with activity being dose-dependent; increasing doses produced greater antioxidant activity (**Figure 5.2**). This is due to the natural antioxidative substances which usually have a phenolic moiety in their molecular structure, and this can further suggest that such plants may have high polyphenolic content (Essop et al., 2008).

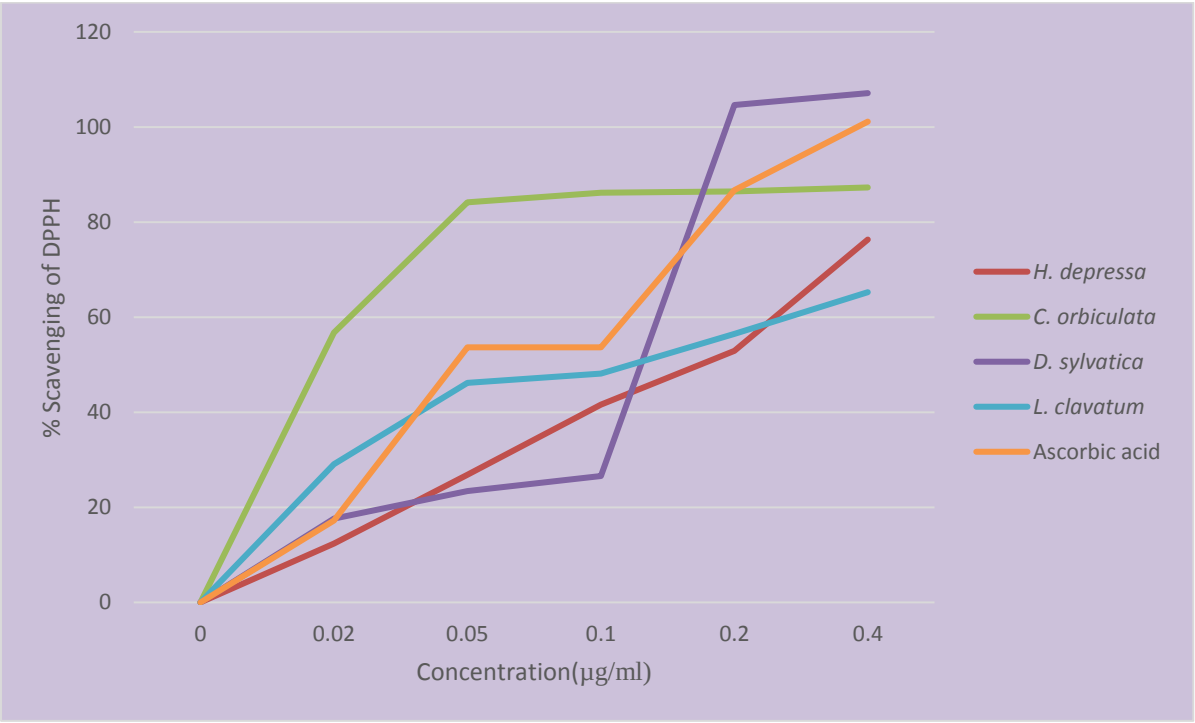

Figure 5.1: DPPH radicals scavenging activity of methanol extracts of medicinal plants used against skin ailments at various concentrations.

Table 5.2: DPPH radical scavenging activity of extracts from plants used against skin ailments.

Plant name		**DPPH assay** IC_{50} **values (µg/ml)**		
	Methanol	**Ethanol**	**Water**	**Acetone**
P. prunelloides	0.27±0.03	0.27±0.03	0.38±0.08	0.38±0.07
H. depressa	0.23±0.37	0.36±0.946	0.24±0.691	0.74±0.356
C. orbiculata	0.10±0.03	0.37±0.188	0.44±0.198	0.20±0.05
L. clavatum	0.25±0.06	0.24±0.04	0.18±0.02	0.57±0.33
D. sylvatica	0.40±0.04	0.28±0.01	0.12±0.03	0.28±0.02
Ascorbic acid	0.27±0.11			

IC_{50} values for methanol, ethanol, aqueous and acetone extracts. IC_{50}: mean of duplicate assays.

DPPH scavenging activity was found to be best in *C. orbiculata* methanol extract with an IC_{50} value of 0.10±0.03 µg/ml, followed by *D. sylvatica* aqueous extract (0.12±0.03 µg/ml). *C. orbiculata* displayed a weaker correlation coefficient at R^2= 0.589. The means among the different extracts it did not show any significant difference at P<0.05, except for methanol and acetone extracts. From other studies, *Dioscorea* species tuber displayed good activity (Roy et al., 2011; Murugan & Mohan, 2012; Paulpriya & Mohan, 2012; Sakthidevi & Mohan, 2013). The antioxidant activity may be due to the presence of total phenolic content in the plant extract (Pattanayak & Sunita, 2008).

Good DPPH activity was also observed in *L. clavatum* aqueous extract with an IC_{50} value of 0.18±0.02 µg/ml, followed by ethanol extract (0.24±0.04 µg/ml) and methanol extract (0.25±0.06 µg/ml). *L. clavatum* revealed a good correlation among the p-values at P<0.05 between methanol, ethanol and aqueous extracts. A study undertaken by Orhan et al. (2007) reported that the antioxidant activity of *L. clavatum* extracts has not been reported, however a compound known as huperzine A, a potent anticholiesterase alkaloid isolated from *L. serratum* (*Huperzia serrate*), was found to have *in vitro* antioxidant capacity.

H. depressa methanol and aqueous extracts displayed good activity with IC_{50} values of 0.23±0.37 µg/ml and 0.24±0.691 µg/ml, respectively. In a previous study, *Hermannia* species showed a promising antioxidant activity in both DPPH and $ABTS^{*+}$ assays, with *H. cuneifolia* being the most active (10.26 ± 0.29 and 10.32 ± 0.34 µg/ml) (Essop et al., 2008). Moreover, Lall & Kishore (2014) tested the plant methanol extract of aerial parts and observed a 50% inhibition at 0.125 mg/ml.

Low or weaker DPPH scavenging activity was observed with *P. prunelloides* aqueous and acetone extracts with IC_{50} values of 0.38±0.08 µg/ml. The selection or use of solvents such as water and acetone is reported to give low values for the extent of reduction (et al., 2001; Molyneux, 2004). This means that such extracts are more potent and have the ability to quench the DPPH radical, which indicates that the extract was a good antioxidant with radical scavenging activity (Murugan & Mohan, 2012).

There is also a positive relationship between antioxidant potential and amount of phenol antioxidant index (Hossain and Shah, 2015). The phenolic compounds are said to possess bioactive compounds which are responsible for the biological activities exhibited by plant extracts (Trouillas et al., 2003; Aremu et al., 2010 & 2011; Dzoyem et al., 2014; Baba and Malik, 2015; Hossain and Shah, 2015). Plants with high phenolic content have been popularly used as anticarcinogenic, antimicrobials, antihelminths, and anti-inflammatories (Tapiero et al., 2002; Tapas et al., 2008; Aremu et al., 2010; Roy et al., 2011; Ncube et al., 2011; Shabbir et al., 2013; Baba and Malik, 2015).

5.6.3. Total capacity of antioxidant using phosphomolybdenum assay

Figure 5.2 and Table 5.3 show the total capacity of antioxidant activity of plant extracts. A lower or reduced IC_{50} value suggests a better antioxidant activity. The total overall antioxidant capacity were in the following order: *L. clavatum>D. slyvatica> H. depressa> P. prunelloides> C. orbiculata.*

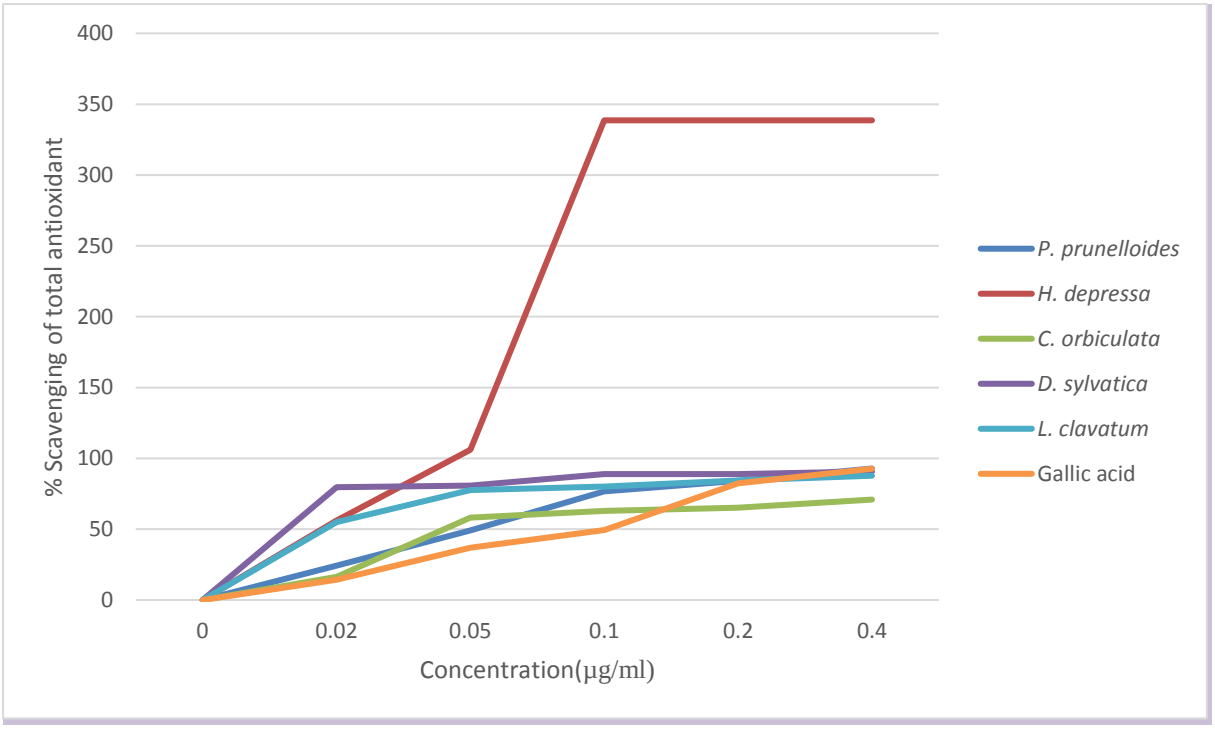

Figure 5.2: Total capacity of antioxidant activity of methanol extracts of medicinal plants used against skin ailments at various concentrations.

Table 5.3: Total capacity of antioxidant of extracts from plants used against skin ailments.

Plant name	**Extracts (IC_{50} values in µg/ml)**			
	Methanol	**Ethanol**	**Acetone**	**Water**
P. prunelloides	0.03±0.369	0.96±0.369	0.56±0.369	0.35±0.659
H. depressa	0.19±0.356	0.03±0.365	0.48±0.264	0.32±0.489
C. orbiculata	0.44±0.157	0.58±0.124	0.62±0.267	0.36±0.126
L. clavatum	0.11±0.04	0.29±0.08	0.08±0.04	0.03±0.09
D. sylvatica	0.08±0.01	0.04±0.03	0.14±0.061	0.77±0.434
Gallic acid	0.135±0.365			

IC_{50} values for methanol, ethanol, aqueous and acetone. IC_{50}: mean of duplicate assays, mean ±SEM. $P<0.05$ was considered to be significantly different.

The total capacity of antioxidant activity was found to be best in *D. sylvatica* ethanol extracts with an IC_{50} value of 0.04±0.03 µg/ml, followed by methanol extract (0.08±0.01 µg/ml). As discussed previously by Pattanayak & Sunita (2008) and Sakthidevi & Mohan (2013), the Dioscoreaceae family is known for its good antioxidative and good phenolic contents. Concerning *L. clavatum*, the best total antioxidant was observed in aqueous extracts (0.03±0.09 µg/ml), followed by acetone extract (0.08±0.04 µg/ml), and methanol extract (0.11±0.04 µg/ml). Analysing with One Way ANOVA Tests and correlation tests, the *D. sylvatica* and *L. clavatum* revealed a good significance at $P<0.05$ among the different extracts, with a good correlation coefficient at $R^2=0.768$. *H. depressa* ethanol extracts and *P. prunelloides* methanol extracts also displayed the best total antioxidants with IC_{50} values of 0.03±0.365 µg/ml and 0.03±0.369 µg/ml, respectively.

5.7. Conclusions

The plants tested for the total phenolic content, DPPH and total capacity of antioxidant assay displayed good *in vitro* antioxidant activity. Based on the results, it can be concluded that *H. depressa, C. orbiculata*, *D. slyvatica*, *E. bicolar, P. prunelloides* and *L. clavatum* can be potential sources of natural antioxidants. These plants displayed higher phenolic contents.

In all the assays, the reference compounds, ascorbic and gallic acid, were either less or higher than the extracts regarding their IC_{50} values. The screened plant species, therefore, exhibited a significant activity and it may also be speculated that due to their good antioxidant activity they can have healing properties. There is, therefore, a need to investigate the chemical constituents which are responsible for the antioxidant activity.

Chapter 6

Anti-inflammatory assay

6.1 Introduction

Inflammation is a pathophysiological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells (Sosa et al., 2002; Kim et al., 2004). Inflammation is defined by Muleya et al. (2015) as an important process involved in the defence mechanisms of an organism against infectious and other deleterious stimuli. It is the normal response to tissue injury caused by physical trauma, toxic chemicals, or microbiological agents. Inflammation is the body's effort to destroy invading organisms, remove irritants, and provide the stage for tissue repair (Vijayalakshmi et al., 2011). In this way the inflammatory responses are said to alter infectious agents and evade control by means of immune-regulatory mechanisms. The complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases (Tunon et al., 1999; Sosa et al., 2002). The state of being inflamed is accompanied with symptoms such as colour red, edema, fever, a sense of heat, loss of function and often pain (Sosa et al., 2002; Kim et al., 2004). Such responses are said to be potentially harmful and are commonly observed in diseases such as rheumatoid arthritis and asthma as they are involved in enzyme activation, mediator release, extravation of fluid (oedema), cell migration, tissue breakdown and repair (Taylor & van Staden, 2002; Matu and van Staden, 2003). Hence, inflammation is described as a complex process with a variety of mediators (Fennell et al., 2001; Taylor & van Staden; 2004).

The main function of the immune system is the defense of a host against pathogens and tumors. There are two major types of defense mechanism systems involved in a host defense against infectious agents and tumors. These are the innate and adaptive defence mechanism systems, they differ by the ways in which they recognize antigens (Hedi & Norbert, 2004). Inflammation is categorized under acute, immune response and chronic symptoms. These mechanisms are controlled by the presence of a group of substances called chemical mediators, each with a specific role at some definite stage of the inflammatory reaction (Essop, 2005).

6.1.1. The mechanism of arachidonic acid biosynthesis

The mechanism of inflammation involves a series of events in which the metabolism of arachidonic acid plays an important role (Bouriche et al., 2005; Akula & Odhav, 2013). The cell damage associated with inflammation acts on cell membrane to cause leukocytes to release lysosomal enzyme, which causes the liberation of arachidonic acid from precursor compounds (Hedi & Norbert, 2004; Essop, 2005). Eicosanoid precursors such as thromboxanes and leucotrines are transformed by one and two pathways in the body which are catalysed by either prostaglandin endoperoxide synthatase or the lipoxygenase (Smith, 1990; Taylor & van Staden, 2002; Burnett et al., 2007). The arachidonic acid is generated when the damaged cell membrane phospholipids by the action of phospholipase A_2 are in high levels (**Figure 6.1**). Subsequently, the metabolism of arachadonic acid by 5-lipoxygenase (5-LOX) produces the biologically active leukotrienes which are involved in the mediation of various inflammatory disorders (Trouillas et al., 2003; Charlier & Michaux, 2003). Stimulation of these inflammatory cells elevates intracellular Ca^{2+}, releases arachidonic acid and incorporates molecular oxygen by 5-lipoxygenase to yield the unstable epoxide leukotriene A4 that may then be converted to cystenyl leukotrienes or peptidoleukotrienes (Katzung, 2001). Hence, the process of inflammation is counteracted due to it being one of the manifestations of oxidative stress and the pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins, which are all induced by oxidative stress (Dzoyem et al., 2014). This includes active pro-inflammatory mediators namely prostaglandins, leukotrienes, histamine, serotonin, bradykinin, cytokines (Amoo et al., 2013; Essop, 2005), where leukotrienes are some of the mediators, which are also known as autocoids (Essop, 2005). Leukotrienes are produced in the body and are responsible for the sensation of inflammation and pain (Taylor & van Staden, 2001).

Leukotrienes are an important family of eicosanoid lipid mediators derived from the metabolism of arachidonic acid (AA) and associated with asthma and allergic reactions (Hedi & Norbert, 2004). They are also reported to consist of a larger group of highly potent molecules with diverse biological functions such as asthma, chronic

obstructive pulmonary disease, cancer, osteoporiosis and atherosclerosis (Iranshahi et al., 2009).

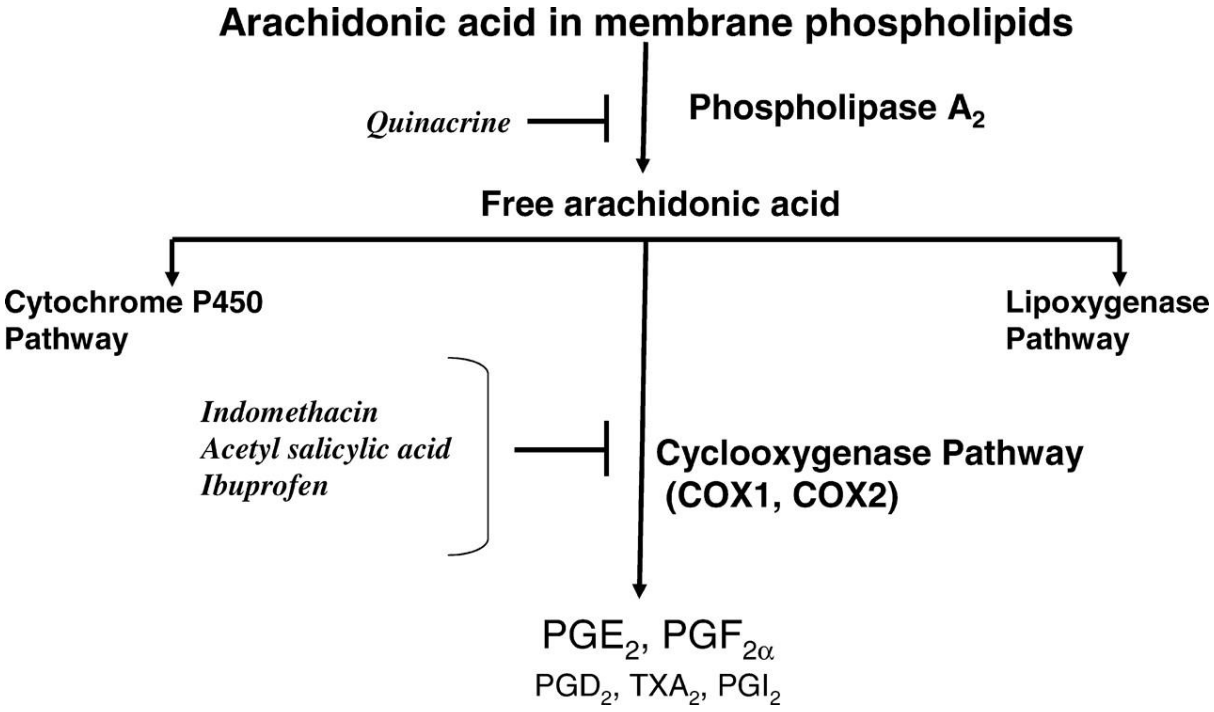

Figure 6.1: A schematic diagram showing the Arachidonic acid metabolism. The nonesterified form of arachidonic acid metabolized via 3 main metabolic pathways involving cyclooxygenases, lipoxygenases and cytochrome P450 enzymes (Exp Biol Med, 2006).

6.1.2. The lipoxygenase pathway

There are a variety of key enzymes that play an effective role in the inflammatory process and immune-regulatory responses. One of the key enzymes which has been recently reported and investigated is the lipoxygenase. Lipoxygenase is involved in the leukotriene pathway. Lipoxygenases are a family of iron- containing enzymes that catalyse the deoxygenation of polyunsaturated fatty acids in lipids containing a *cis*, *cis*-1-4-pentadiene structure. Lipoxygenases are defined as key enzymes in the biosynthesis of leukotrienes that play an important role in several inflammatory diseases, including asthma, glomerulonephritis, acute lung injury, inflammatory bowel disease, psoriasis, and eczema (Henderson, 1994; Trouillas at

el., 2003; Hedi & Norbert, 2004; Dzoyem et al., 2014). The lipoxygenase not only has a 5-lipoxygenase pathway, but also the 12- and 15-lipoxygenases. Their major role is to convert arachidonic acid, which is a component constituting of phospholipids membrane, into proinflammatory mediators called leukotriens (Iranshahi et al., 2009).

Lipoxygenases are found in plants, fungi, and animals. 5-LOX occurs in various mammalian species comprising of monomeric proteins of 75-80 kDa, containing about 673 amino acids (Charlier & Michaux, 2003). Higher plants contain multiple lipoxygenases with at least 8 identified in soybean. One of the most widely studied lipoxygenases in soybean are 1-LOX and 3- LOX, and a 15-LOX which represents one of the main proteins in rabbit (Brash, 1999). The action of lipoxygenases generates compounds that can regulate specific cellular responses (Katzung, 2001). According to Charlier & Michaux (2003), the direct approaches involve the redox inhibitors or antioxidants which interfere with the redox cycle of 5-LOX, non-chelator agents and non-redox competitive inhibitors, which compete with A to bind the enzymes active site. They also function in inflammatory cells, such as neutrophils and macrophages, leukotriens, which have important effects on dendritic cells (DC)-mediated adaptive immunity- inflammation, immune responses and host defense against infection (Hedi & Norbert, 2004). The inhibition of LOX may influence the inflammation processes and thus be of interest for modulation of the lipoxygenase pathway (Dzoyem et al., 2014).

Recently there has been trending interests in the development of lipoxygenase inhibitors for therapeutic indications (Iranshahi et al., 2009). Although anti-inflammatory drugs are widely used, the prolonged consumption of these medications is usually coupled with numerous side effects, and therefore there is a need to explore alternative strategies with the help of natural dietary products (Trouillis et al., 2003; Iranshahi et al., 2009).

6.1.3. Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are the most widely prescribed drugs worldwide. They include aspirin, indomethacin, diclofenac, naproxen and ibuprofen, and are known to possess anti-

inflammatory, anti-pyretic and analgesic properties (Taylor & van Staden, 2002; Charlier & Michaux, 2003). NSAIDs are reported to form a large chemical composition, where they assist in the relief of pain and inflammation (Taylor & van Staden, 2001). They act by inhibiting the activity of COX enzymes; they are more potent inhibitors of COX-1 and COX-2 in several model test systems (Taylor & van Staden, 2002). Although the currently used steroidal anti-inflammatory drugs (SAID) and NSAIDs treat acute inflammatory disorders, these conventional drugs have not been successful to cure chronic inflammatory disorders such as rheumatoid arthritis (RA) and atopic dermatitis (AD) (Charlier & Michaux, 2003; Kim et al., 2004; Umaru et al., 2009). In recent studies, the use of NSAIDs has showed a reduced toxicity in the gastrointestinal tract and kidneys (Charlier & Michaux, 2003). This has led to controversial effects as the NSAIDs are beneficial and yet have adverse effects, and these characters both relate to the ability of the drug to inhibit prostaglandin synthesis.

6.1.4. Medicinal plants as new strategies for anti-inflammatory activity

Medicinal plants are widely used, and have been used for decades, in folk medicine of many countries to treat different inflammatory conditions and skin inflammations and other infectious diseases due to their wide variety of biologically active compounds (Lin et al., 1999; Shale et al., 1999; Matu and van Staden, 2003; Fawole et al., 2010; Ravipati et al., 2012; Kumar et al., 2013; Murugesan & Deviponnuswamy, 2014). Previous studies have revealed that several plants have been used in traditional Ayurvedic medicine for the treatment and management of inflammatory disorders and wound healing activities (Shaikh et al., 2015). A plant is said to constitute multitudes of different molecules that act synergistically on targeted elements of the complex cellular pathway, unlike modern allopathic drugs which only contain single active components that target one specific pathway (Kumar et al., 2013). This has gained medicinal plants a renowned interest and importance because, as plant-based drugs or raw drugs used in traditional medicine, they have been recognised for their easy accessibility, safety, efficacy and cost effectiveness (Apu et al., 2012; Murugesan & Deviponnuswamy, 2014; Shaikh et al., 2015). Many studies have been undertaken in search for antimicrobial, anti-inflammatory and other therapeutic agents which have the ability to reduce both internal and external

swelling, and inflammation (Lin et al., 1999; Apu et al., 2012; Kumar et al., 2013). Such interests have been focused on medicinal plants which possess phytochemicals that have known to exhibit potent anti-inflammatory effects in the treatment of inflammation by using various models (Murugesan & Deviponnuswamy, 2014). Recent studies have also reported that many dietary phytochemicals such as polyphenols can inhibit arachidonic acid peroxidation, and COX inhibitors, especially COX-2 inhibitors with their properties or stimulatory effects (Shaihk et al., 2015).

6.1.5. Aim of this study

The aim of this chapter was to screen traditional medicinal plants used against skin ailments for the presence of anti-inflammatory properties.

6.2. Materials and methods

6.2.1. Lipoxygenase assay

The 5-LOX activity was determined using the method as reported by Evans (1987) and modified by Baylac & Racine (2003) and Trouillas et al. (2003). Linoleic acid was used as the substrate for the 5-lipoxygenase enzyme (Cayman). An anti-Arachidonate 5 lipoxygenase, antibody produced in rabbit used (Sigma-Aldrich). The plants that displayed higher activity in antibacterial and antifungal screening and antioxidant assays were subjected to anti-inflammatory. Plants were extracted with methanol, ethanol, acetone and water.

Standardization was first carried out using the reference sample made up of 10 µl of DMSO, 2.95 ml of phosphate buffer (pH 6.3) pre-warmed in a water bath at 25ºC. 50 µl of linoleate solution (100 µM final concentration) was added to 12 µl enzyme and 12 µl of phosphate buffer. The production of the conjugated dienes was measured over a period of 10 min at 234 nm. Two sets of controls were run with the reference samples. An aliquot of 50 µl of the stock solution containing DMSO (10 µl: 10 mg/ml) and Tween 20 mixture; 1: 29 v/v was placed in a 3 ml cuvette, followed by 2.95 ml of

pre-warmed 0.1 M potassium phosphate buffer (pH 6.3) and 48 µl of linoleic acid solution and pre-warmed in a waterbath. Thereafter, 12 µl of the ice-cold buffer (potassium phosphate) was mixed with 12 µl (100 U) of the thawed enzyme, after which each sample test with different plant extracts in five different concentrations (0.02, 0.05, 0.1, 0.2, and 0.4 mg/ml) was added. The mixture was then transferred to the cuvette and the contents of the cuvette were shaken and read in the spectrophotometer. Two samples were prepared and mixed with DMSO and Tween 20 mixture to serve as controls (no enzyme inhibition). The production of the conjugated dienes was measured over a period of 10 min at 234 nm. Nordihydroguaiaretic acid (NDGA) was used as a positive control (enzyme inhibition). The IC_{50} value (concentration at which 50% of the enzyme was inhibited) of each sample test was determined using Graphpad. The percentage inhibition of the enzyme activity was calculated by comparing with the controls (Tween® 20/DMSO mixture). The experiment was conducted in duplicate.

6.3. Results and discussions

Table 6.1 shows the inhibitory effects of plant extracts on the anti-inflammatory activity, and Figure 6.2 demonstrates the percentage inhibition of methanol extracts of different plants at different concentrations in comparison with NDGA. NDGA is used as reference compound for studies of 5-lipoxygenase inhibition due to its widely reported strong inhibitory activity on this enzyme (Safayhi et al., 1992; Abad et al., 1995). This also shows that an increase in concentration of the plant extracts tends to increase anti-inflammatory activity of the plant extracts exponentially.

Table 6.1: Table shows the 5-lipoxygenase inhibitory activity of different plant extracts, represented in IC_{50} values (µg/ml).

Plant name	**5-lipoxygenase assay**			
	Methanol	**Ethanol**	**Water**	**Acetone**
P. prunelloides	0.42±0.02	0.26±0.06	0.33±0.35	0.24±0.61
H. depressa	0.64±0.24	0.64±0.08	0.56±0.45	0.12±0.44
C. orbiculata	0.26±0.04	0.09±0.02	0.47±0.24	0.45±0.64
L. clavatum	0.61±0.02	0.02±0.08	0.26±0.56	0.64±0.32
D. sylvatica	0.49±0.364	0.65±0.02	0.25±0.54	0.24±0.44
NDGA	0.58±0.35			

IC_{50}: mean of duplicate assays, mean ±SEM.

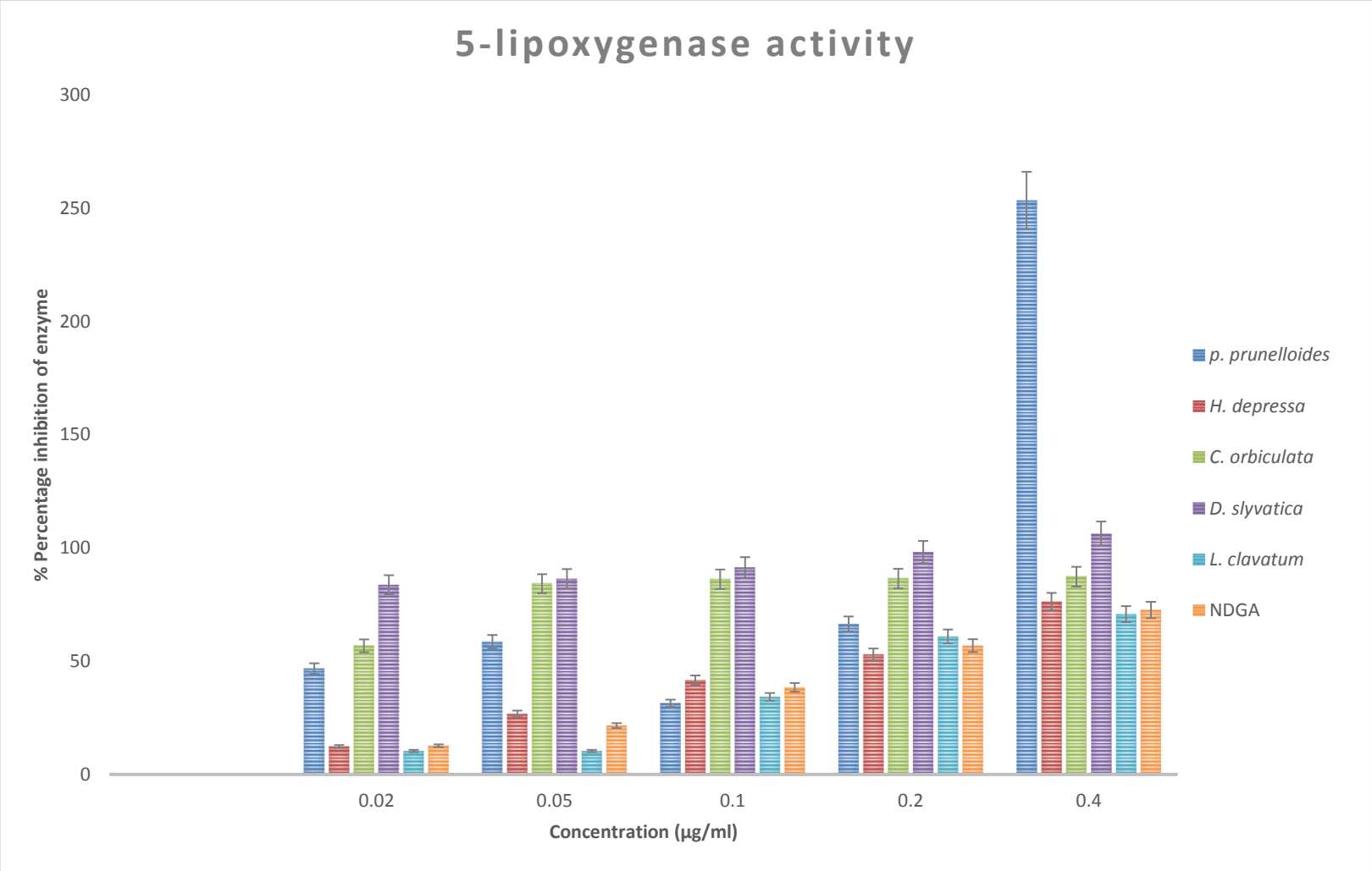

Figure 6.2: The percentage inhibition of 5-lipoxygenase by the methanol plant extracts in comparison to NDGA.

Most species portrayed promising anti-inflammatory activity in 5-LOX assay with plant extracts possessing the most significant inhibitory activity. A higher inhibitory activity was associated with a lower or reduced IC_{50} value (Kamatou et al., 2005; Lertsatthanakorn et al., 2006; Amoo et al., 2009). Plant species which showed a higher and stronger anti-inflammatory activity than that of NDGA were *P. prunelloides*, *C. orbiculata, D. sylvatica*, *L. clavatum,* and *H. depressa* (Table 6.1). The *C orbiculata* and *P. prunelloides* displayed the best activity where all extracts inhibited 5-LOX. The 5-LOX activity was found to be the best in all *P. prunelloides* extracts with acetone extract exhibiting the highest activity at 0.24±0.61 µg/ml, followed by ethanol extract at 0.26±0.06 µg/ml, aqueous extract (0.33±0.35 µg/ml) and methanol extract (0.42±0.02 µg/ml) (Table 6.1 and Figure 6.2). Studies undertaken by Yff et al. (2002) and Muleya et al. (2015) reported on inhibitory effects of *P. prunelloides* against 15-LOX. The inhibitory activity was at 79%. It is suggested that the difference in activity could be due to the difference in LOX enzymes and methods used in determining the anti-inflammatory property.

C. orbiculata ethanol extract (0.09±0.02 µg/ml) and methanol extract (0.26±0.04 µg/ml) displayed the best activity compared to water and acetone extracts. Amabeoku and Kabatende (2012) reported on anti-inflammatory activity of *C. orbiculata* using rats. The authors observed that the leaf methanol extract significantly reduced the carrageenan-induced oedema in the rats' paws.

Concerning *D. sylvatica*, good activity was detected with aqueous extract (0.24±0.44 µg/ml), and acetone extract (0.25±0.54 µg/ml). *D. slyvatica* methanol extract displayed good activity as the concentration increased (Figure 6.2).

The *L. clavatum* ethanol extract displayed the best activity with an IC_{50} value of 0.02±0.08 µg/ml. *H. depressa* acetone extract was the only active extract (0.12±0.44 µg/ml). Studies undertaken by Essop et al. (2008) reported that all the *Hermannia* species exhibited moderate anti-inflammatory activity in the 5-lipoxygenase assay with the exception of *H. cuneifolia*. The *H. cuneifolia* inhibited 50% of the 5-lipoxygenase activity at a concentration of 15.32±5.49 µg/ml in comparison to Trolox at 2.39±0.71 µg/ml. It is suggested that lower inhibitory values do not necessarily

mean that the plant does not possess anti-inflammatory properties, the active compounds could work at other sites in the complex process of inflammation (Taylor & van Staden, 2001; Akula & Odhav, 2013).

6.4. Conclusions

Our results confirm that *P. prunelloides*, *C. orbiculata*, and *D. sylvatica* had an effective anti-inflammatory potential or better inhibition of the 5-LOX enzyme as demonstrated by reduced IC_{50} values which were lesser than NDGA. All the plant extracts showed moderate and promising inhibition of the 5-LOX enzyme. All the plant extracts which did not portray good activity in this assay cannot be excluded from the possibility that the plants do have anti-inflammatory activity, which may be displayed at other events of other inflammatory routes. Hence, a combination of this assay with an evaluation of the radical scavenging activity by the DPPH method could constitute a good indication on the potential anti-inflammatory activity of a drug (Akula & Odhav, 2013; Essop et al., 2008). Oxygen radicals are well known to be produced during the inflammatory processes, hence such antioxidants may provide information about the potential activity of a drug on inflammatory processes (Akula & Odhav, 2013). Such compounds which are reported to possess anti-inflammatory activities are the major plant phenols, flavonoids and phenolic acids (Talhouk et al., 2007).

Chapter 7

General conclusions and recommendations

The skin is a complex organ that is able to resist infections based on its properties (Nestor et al., 2004; Dryden 2010; Mabona et al., 2013). Its most crucial function is playing a key immunity role in protecting the body by forming a part of a defence and mechanical barrier to the surrounding environment, and thereby preventing invasion by the pathogens. It also aids in sustaining microorganisms that influence human health and disease (Ong et al., 2002; Torok & Conlon, 2005; Nestor et al., 2004; Grice et al., 2009). Exposure of skin to infections provides a substratum that is favourable for a wide variety of microorganisms to contaminate and colonize. Examples of such infections include leg ulcers, burns and surgical or traumatic wounds which mostly allow entry and colonization of a wide range of bacteria (Dryden, 2010). Infections can be due to group A streptococci or *S. aureus* which are part of the normal skin flora, or secondary infections due to chronic conditions or complicated SSTIs such as eczema or atopic dermatitis (Robson, 1997; Dryden, 2000; DiNubile & Lipsky, 2004). Other factors implicated in skin infections are vascular insufficiency, disrupted venous or lymphatic drainage, sensory neuropathies, diabetes mellitus, previous cellulitis, the presence of a foreign body, accidental or surgical trauma, obesity, poor hygiene and certain immunodeficiencies (DiNubile & Lipsky, 2004). Due to an increased demand for cheaper medicines, high rates of unemployment and greater incidences of infection from skin disorders such as burns, inflamed wounds, psoriasis, etc., there is an interest in investigating and validating traditional medicine as primary healthcare and safety measures for human consumption (Ndhlala et al., et al., 2010).

Ethnobotanical information (Chapter 2) revealed that inflamed wounds, eczema, bites, ringworm, boils, septic sores and burns were common among the South Africans. This study documented 22 plant species used by the traditional healers and herbalists of the Free State Province for the treatment of wounds and skin infections.

Nine frequently used medicinal plants, namely *Pentanisia prunelloides, Cotyledon orbiculata, Hermannia depressa, Dioscorea sylvatica, Lycopodium clavatum, Merwilla plumbea, Eucomis bicolar, Eucomis autumnalis* and *Xysmalobium undulatum* were screened for the presence of phytochemicals such as saponins, alkaloids, tannins, anthraquinones, terpenoids, and steroids (Chapter 3). Components observed from the study were saponins, flavonoids, tannins, and terpenoids, whereas alkaloids, cardiac glycosides, steroids and anthraquinones were least present. Terpenoids were found to be active against bacteria (Mahmood et al., 1993). Saponins, phenolic compounds and tannins have already been documented to possess anti-inflammatory, anthelmintic, analgesic, anticancer and antidiabetic activities (Molefe, 2013).

Aqueous fractions are reported to be highly complex and include the majority of primary metabolites from the plant material (Taylor and van Staden, 2001). However, in this study, aqueous extracts revealed the least presence of phytochemicals from different plant species in comparison to methanol, ethanol and acetone. According to Molefe (2013), acetone has the ability to dissolve and extract numerous compounds and secondary metabolites with a wide range of polarity and can form a homogenous mixture with different solvents than water. Secondary metabolic production is responsible for the bioactivity in plants (Ncube et al., 2011; Molefe, 2013). The absence of phytochemicals could also be explained by seasonal variation, for instance, in spring, vegetative material starts to develop high phenolic concentrations which could be due to high fluctuating temperatures and dry conditions experienced in spring (Ncube et al., 2011).

Concerning antimicrobial studies (Chapter 4), *P. prunelloides*, *H. depressa*, *C. orbiculata*, *L. clavatum* and *M. plumbea* were the only plant species which exhibited high MIC values against test microorganisms. *P. prunelloides*, ethanolic extract showed the best activity against *B. pumilus* and *S. aureus* and minimal activity was observed in methanol extract against *P. aeruginosa*. *H. depressa* acetone and ethanol extracts displayed the best activity against *B. pumilus* and *P. aeruginosa.* It was noteworthy that aqueous extracts showed good activities against *E. coli*, *S. aureus* and *P. aeruginosa*. Concerning *C. orbiculata* acetone extract, good activity

was observed against *P. aeruginosa*. Good activities were observed with *L. clavatum* acetone extract inhibiting *S. aureus*, *P. aeruginosa* and *E. coli*. *M. plumbea* ethanolic extracts also exhibited good antibacterial activity against *S. aureus*. Ethanol and acetone extracts were observed to be the best solvents in screening plant extracts for antibacterial activity. The lower or poor activity observed from plant extracts can be due to the absence of certain phytochemicals in different plant extracts.

As for antifungal activity, *P. prunelloides*, *H. depressa, C. orbiculata* and *D. sylvatica* revealed the best inhibition against *C. albicans* and *T. mucoides*. All solvents of *P. prunelloides* were active against both fungal strains. All *H. depressa* organic solvents extracts displayed great MIC values against both fungi. Concerning *X. undulatum,* only acetone extracts showed good inhibition against both fungi. *C. orbiculata* stem and leaf alcoholic extracts exhibited good antifungal activity. Other good antifungal activities were observed with *E. autumnalis* acetone extract, *D. slyvatica* acetone and methanol extract and *M. plumbea* ethanolic extract. Water extracts displayed the least activity. Nonetheless, it is still used as a major extractant in the use of traditional herbal preparations. Most of the active constituents in these plant species are non-polar and water does not extracts all the active compounds that might be present in the plant (Kemalson et al., 2000; Ncube et al., 2011; Molefe, 2013). Hence, it displayed poor activity. Traditional healers do not have access to other more lipophilic solvents.

An antioxidant free radical scavenging assay was evaluated from the six medicinal plants based on total phenolic content. The ability of the plants extracts to scavenge a standard free radical (DPPH) (Chapter 5) confirms that the plants had a promising antioxidant potential. *H. depressa* methanol, *C. orbiculata* acetone, *D. slyvatica* acetone and aqueous extracts and *L. clavatum* acetone and aqueous extracts showed the highest total phenolic content. *P. prunelloides* aqueous extracts and *X. undulatum* ethanol extract also displayed a good total phenolic content. All the methanol plant extracts, had promising antioxidant properties when compared with ascorbic acid. *C. orbiculata* methanol extract, *D. slyvatica* aqueous extract, *L.*

clavatum aqueous, ethanol and methanol extracts exhibited a very good antioxidant activity. *H. depressa* methanol and aqueous extracts were also observed to possess good antioxidant activity.

The total capacity of antioxidant activity was used to test the overall antioxidant using the phosphomolybdenum assay. A higher activity was observed with *D. sylvatica* ethanol and methanol extracts, *L. clavatum* aqueous and acetone extracts, *H. depressa* ethanolic extract and *P. prunelloides* methanol extract. The DPPH free radical scavenging activity of the plant extracts increased with increasing concentration i.e. the free radical activity was dose dependent. Those that displayed low and limiting activity can be suggested to possess low antioxidant content within the plant. Methanol is the used and preferred solvent in antioxidant studies due to reduction of DPPH in methanol solution, in the presence of a hydrogen donating antioxidant as a result of the formation of the non-radical form DPPH-H (Paulpriya & Mohan, 2012; Sakthidevi & Mohan, 2013). The use of other solvents such as water and acetone, seem to give low values for the extent of reduction. This means that such extracts are more potent.

In chapter 6, an anti-inflammatory assay was employed using the 5-Lipoxygenase enzyme activity against four medicinal plants. All the tested plants, *L. clavatum*, *P. prunelloides*, *C. orbiculata*, and *H. depressa,* exhibited promising anti-inflammatory activities. *P. prunelloides*, *C. orbiculata*, *L. clavatum, D. slyvatica*, and *H. depressa* displayed a good anti-inflammatory activity with lower IC_{50} values than NDGA.

In conclusion, *in vitro* antimicrobial, antioxidant radical scavenging and anti-inflammatory assays provided us with new drug leads from the medical plants used by the healers of the Free State. In the antibacterial tests, some plant extracts managed to inhibit against Gram-negative strains, such as *P. aeruginosa*, which is responsible for a number of skin ailments.

Recommendations

Based on the results of ethnobotanical studies, there is a need to establish medicinal plant gardens and nurseries as a conservation measure for the cultivation of medicinal plants that are under pressure from the harvesters

Concerning anti-inflammatory assay, it is recommended that other anti-inflammatory routes are used in order to determine whether the solvent extracts do exert anti-inflammatory activity in other steps of the complex anti-inflammatory cascade.

Further investigation on isolation and purification of active compound responsible for the antimicrobial, antioxidant and anti-inflammatory activities is recommended.

Compounds with potential anti-inflammatory properties need to be tested *in vivo* or against biochemical models of inflammation.

Municipalities and institutions (e.g. Universities, National Parks) must also play a role of educating the community about conservation of these valuable medicinal plants and also establish botanical gardens for these plants.

References

ABAD, M.J., BERMEJO, P., VILLAR, A. 1995. The activity of flavonoids extracted from *Tanacetum microphyllum* DC. (Compositae) on soybean lipoxygenase and prostaglandin synthetase. *General Pharmacology: The Vascular System*, Vol 26, no 4, pp 815-819.

ABBASI, A.M., KHAN, M.A., AHMAD, M., ZAFAR, M., JAHAN, S., SULTANA, S. 2010. Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province, Pakistan. *Journal of Ethnopharmacology*, Vol 128, pp 322-335.

ABOABA, O.O., SMITH, S.I., OLUDE, F.O. 2006. Antibacterial Effect of Edible Plant Extract on *Escherichia coli* 0157:H7. *Pakistan Journal of Nutrition*, Vol 5 no.4, pp: 325-327.

ADVANCED TISSUE, 2015. www.**advancedtissue**.com/understanding-healing-stages-wounds (Accessed on 24 March 2016).

AKULA, U.S., & ODHAV, B. 2013. *In vitro* 5-lipoxygenase inhibition of polyphenolic antioxidants from undomesticated plants of South Africa. *Journal of Medicinal plants research*, Vol 2, no 9, pp 207-212.

ALAGESABOOPATHI, C. 2011. Ethnomedicinal plants used as medicine by the Kurumbatribals in Pennagaram region, Dharmapuri district of Tamil Nadu, India. *Asian Journal of experimental biologicall sciences*, Vol *2*. no 1, pp 140-142.

ALBANO, M.N., DA SILVEIRA, M.R., DANIELSKI, L.G., FLORENTINO, D., PETRONILHO, F., PIOVEZAN, A.P. 2013. Anti-inflammatory and antioxidant properties of hydroalcoholic crude extract from *Casearia sylvestris* Sw (Salicaceae).*Journal of ethnopharmacology*, *147*(3), 612-617.

ALBANO, S.M., LIMA, A.S., MIGUEL, M.G., PEDRO, L.G., BARROSO, J.G., FIGUEIREDO, A.C. 2012. Antioxidant, anti-5-lipoxygenase and antiacetylcholinesterase activities of essential oils and decoction waters of some aromatic plants. *Records of Natural Products Journal*, Vol 6, no (1), pp 35-48.

AMABEOKU, G.J., GREEN, I., KABATENDE, J. 2007. Anticonvulsant activity of *Cotyledon orbiculata* L. (Crassulaceae) leaf extract in mice. *Journal of ethnopharmacology*, Vol 112, no 1, pp 101-107.

AMOO, S.O., ADEYEMI, O. AREMU, M.M. VAN STADEN.J. 2013. Assessment of Long-Term Storage on Antimicrobial and Cyclooxygenase-Inhibitory Properties of South African Medicinal Plants. *Phytother. Res*, Vol. 27. pp. 1029–1035.

AMOO, S.O., FINNIE, J.F., VAN STADEN, J. 2009. *In vitro* pharmacological evaluation of three Barleria species. *Journal of ethnopharmacology*, Vol 121, no 2, pp 274-277.

AMUSAN, O.O.G., SUKATI, N.A., DLAMINI, P.S., SIBANDZE, F.G. 2007. Some Swazi phytomedicines and their constituents. *African Journal of Biotechnology*, Vol 6, no 3, pp. 267-272.

ANDERSON D.J. 2011. Surgical site infections. *Infectious diseases clinics of North America*, Vol 25, no 1, pp 135-153.

ANITHA, T. 2012. Medicinal plants used in skin protection. *Asian Journal of Pharmaceutical and Clinical Research*, Vol *5,* no 3, pp 35-38.

APU, A.S., BHUYAN, S.H., PROVA, S.S., & MUHIT, M.A. 2012. Anti-inflammatory activity of medicinal plants native to Bangladesh: A review. *Journal of Applied Pharmaceutical Sciences*, Vol *2*, no 2, pp 07-10.

AREMU, A.O., FAWOLE, O.A., CHUKWUJEKWU, J.C., LIGHT, M.E., FINNIE, J.F., & VAN STADEN, J. 2010. *In vitro* antimicrobial, anthelmintic and cyclooxygenase-

inhibitory activities and phytochemical analysis of Leucosidea sericea. *Journal of ethnopharmacology*, Vol *131*, no 1, pp 22-27.

AREMU, A.O., GRUZ, J., ŠUBRTOVÁ, M., SZÜČOVÁ, L., DOLEŽAL, K., BAIRU, M. W., & VAN STADEN, J. 2013. Antioxidant and phenolic acid profiles of tissue cultured and acclimatized Merwilla plumbea plantlets in relation to the applied cytokinins. *Journal of plant physiology*, Vol 170, no 15, pp 1303-1308.

ARMSTRONG, D., NEU, H., PETERSON, L. R., & TOMAS Z A. 1995. Editorial: The Prospects of Treatment Failure in the Chemotherapy of Infectious Diseases in the 1990s. *Microbial Drug Resistance*, Vol 1 no 1, pp. 1-4.

AWOYINKA, O.A., BALOGUN, I.O., OGUNNOWO, A.A. 2007. Phytochemical screening and in vitro bioactivity of *Cnidoscolus aconitifolius* (Euphorbiaceae). *Journal of Medicinal Plants Research*, Vol. 1, no 3, pp 063-065.

BABA, S.A., & MALIK, S. A. 2015. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University for Science*, *9*(4), 449-454.

BAGDONAS, R., TAMELIS, A., RIMDEIKA, R. 2003. *Staphylococcus aureus* infection in the surgery of burns. *Medicina (Kaunas)*, Vol *39, no* 11, pp 1078-81.

BALIK M.J., COX P.A. 1996. Plants, People and Culture. Scientific American Library, New York.

BANNISTER, K. P. 2010. Chemistry rooted in cultural knowledge: unearthing the links between antimicrobial properties and traditional knowledge in food and medicinal plant resources of the Secwepemc (Shuswap) Aboriginal Nation (Doctoral dissertation, University of British Columbia).

BAYLAC, S., & RACINE, P. 2003. Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. *International Journal of Aromatherapy*, Vol 13, no 2, pp 138-142.

BHANDARY S.K., KUMARI S.N., BHAT V.S., SHARMILA K.P., BEKAL M.P. 2012. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. NUJHS- Nitte University of Journal Of Health Science.

BHAT, R.B, JACOBS, T.V. 1996. Traditional herbal medicine in Transkei. *Journal of Ethnopharmacology*, Vol 48, pp 712.

BICKERS, D.R., LIM, H.W., MARGOLIS, D., WEINSTOCK, M.A., GOODMAN, C., FAULKNER, E., DALL, T. 2006. The burden of skin diseases: 2004: A joint project of the American Academy of Dermatology Association and the Society for Investigative Dermatology. *Journal of the American Academy of Dermatology*, Vol 55*,* no 3, pp 491-499.

BISNO, A.L., STEVENS, D.L. 1996. *Streptococcal* infections of skin and soft tissues. *New England Journal of Medicine*, Vol*334,* no 4, pp 240-246.

BOEHNCKE W.H., SCHON M.P., GIROMOLOMI G., BOS J.B., Thestrup-Pederson K., CAVAN A., NICKOLOFF B. 2005. Leukocyte extavasation as a target for anti-inflammatory therapy- Which molecule to choose? Experimental dermatology, Vol 14, no 1, pp 70.

BOHAM, B.A., KOCIPAI-ABYAZAN, R. 1974. Flavonoids and condensed tannis from leaves of *Hawallan vaccinium vaticultum* and *V. calycinium*. *Journal of Pacific Science*,Vol, 48, pp 458-463.

BOROKINI, T.I., OMOTAYO, F.O. 2012. Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. *Journal of Medicinal Plants Research*, Vol. 6, no 7, pp 1106-1118.

BOTHA, C.J., PENRITH, M.-L., 2008. Poisonous plants of veterinary and human importance in southern Africa. *Journal of Ethnopharmacology*, Vol 119*,* no 3, pp 549-558.

BOURICHE, H., MILES, E.A., SELLOUM, L., CALDER, P.C. 2005. Effect of *Cleome arabica* leaf extract, rutin and quercetin on soybean lipoxygenase activity and on generation of inflammatory eicosanoids by human neutrophils. *Prostaglandins, leukotrienes and essential fatty acids*, Vol 72, NO 3, PP 195-201.

BOWLER, P.G., DUERDEN, B.I., ARMSTRONG, D.G. 2001. Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews,* Vol 14, no 2, pp 244 69.

BRASH, A.R. 1999. Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. *Journal of Biological Chemistry*, Vol 274, no 34, pp 23679-23682.

BRIAN, F.H., THOMAS-BIGGER, J., GODMAN G. 1985. Basis of Therapeutics. Macmillan Publishing Company, New York. *The Pharmacoogy Journal*, 7 th edition pp. 716-718.

BROOK, I., FRAZIER, E.H., 1990. Aerobic and anaerobic bacteriology of perirectal abscesses. *Archives of Surgery Journal*, Vol 125, pp 1445-1451.

BROOKS, J.L., JEFFERSON, K.K. 2012. *Staphylococca*l Biofilms: Quest for the Magic Bullet. *Advances in applied microbiology*, Vol 81, no 63.

BRUNETON, J. 1995. Pharmacognosy, Phytochemistry, Medicinal Plants. Intercept Ltd., Andover. *Drug Invention Today*, Vol, 3, no 9, pp 214-215.

BUNIKOWSKI, R., MIELKE, M.E., SKARABIS, H., WORM, M., ANAGNOSTOPOULOS, I., KOLDE, G., RENZ, H. 2000. Evidence for a disease-promoting effect of Staphylococcus aureus–derived exotoxins in atopic dermatitis. *Journal of Allergy and Clinical Immunology*, Vol 105, no 4, pp 814-819.

BURNETT, B.P., JIA, Q., ZHAO, Y., & LEVY, R.M. 2007. A medicinal extract of Scutellaria baicalensis and Acacia catechu acts as a dual inhibitor of cyclooxygenase and 5-lipoxygenase to reduce inflammation. *Journal of medicinal food*, Vol 10, no 3, pp442-451.

BUWA, L.V., VAN STADEN, J. 2006. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. *Journal of Ethnopharmacology*, Vol 103, pp 139–142.

CASALI, R.E., TUCKER, W.E., PETRINO, R.A. 1980. Postoperative necrotizing fasciitis of the abdominal wall. The *American Journal of Surgery*, Vol 140, pp 787-790.

CHARLIER, C., & MICHAUX, C. 2003. Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. *European journal of medicinal chemistry*, Vo, 38, no 7, pp 645-659.

CHEN Y., XIE M.Y., GONG X.F. 2007. Microwave- assisted extraction used for the isolated of total triterpenoid saponins from *Ganoderma atrium*. *J. Food Eng*, Vol 81, pp 162-170.

CHEN, W. Y. & ROGERS, A. A. 2007. Recent insights into the causes of chronic leg ulceration in venous diseases and implications on other types of chronic wounds. *Wound repair and regeneration*, Vol 15, no 4, pp 434-449.

CHRISTOPHERS, E., & HENSELER, T. 1987. Contrasting disease patterns in psoriasis and atopic dermatitis. *Archives of dermatological research*, Vol 279, no 1, pp S48-S51.

COMPTON, J.S., MAAKE, L. 2007. Source of the suspended load of the upper Orange River, South Africa. *South African Journal of Geology*, Vol 110, no 2-3, pp 339-348.

COOPER R., LAWRENCE J.C. 1996. The prevalence of bacteria and implication for infection control. *J Wound Care*. Vol 5, no 6, pp 291-295.

COOPOOSAMY, R.M., NAIDOO, K.K., BUWA, L., MAYEKISO, B. 2010. Screening of *Siphonochilus aetiopicus* (Schweinf.) B.L Burtt for antibacterial and antifungal properties. *Journal of Medicinal Plant Research*, Vol 4, no 12, pp 1228-1231.

COOPOOSAMY, R.M., & NAIDOO, K.K. 2012. An ethnobotanical study of medicinal plants used by traditional healers in Durban, South Africa. *African Journal of Pharmacy and Pharmacology*, Vol 6, no 11, pp 818-23.

COWAN, M.M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Review*, pp 564-582.

CUTTING, K.F. 2001. “A dedicated follower of fashion? Topical medications and wounds." British journal of nursing-london-mark allen publishing limited, Vol 10, pp 9-18.

DATHAK, P., & IWU, M. 1991. Inhibition of xanthine oxidase activity by some flavonoid. *Fitoterapia*, Vol 63, pp 385.

DAVID, R., BICKERS, M.D., HENRY W.L., DAVID M., MARTIN A., WEINSTOCK C.G., FAULKNER E., GOULD C., GEMMEN E., DALL, MS. 2006. The burden of skin diseases: 2004. A joint project of the American Academy of Dermatology Association and the Society for Investigative Dermatology. *The American Academy of Dermatology*, Inc. Vol, 55, pp 490-500.

DAVIS, S. C., MERTZ, P. M., BILEVICH, E. D., CAZZANIGA, A. L., & EAGLSTEIN, W. H. 1996. Early Debridement of Second-Degree Burn Wounds Enhances the Rate of Epithelization-An Animal Model to Evaluate Burn Wound Therapies. *Journal of Burn Care & Research*, Vol 17, no 6, pp 558-561.

DAVIS, S.C., CAZZANIGA, A.L., EAGLSTEIN, W.H., MERTZ, P.M. 2005. Over-the-counter topical antimicrobials: Effective treatment?. *Archives of Dermatological Research*, Vol 297, pp 190-195.

DE LISSOVOY, G., FRAEMAN, K., HUTCHINS, V., MURPHY, D., SONG, D., & VAUGHN, B. B. 2009. Surgical site infection: incidence and impact on hospital utilization and treatment costs. *American journal of infection control*, Vol *37*, no 5, pp 387-397.

DE WET H., NCIKI S., VAN VUUREN S.F. 2013. Medicinal plants used for the treatment of various skin disorders by a rural community in northern Maputaland, *South Africa.Journal of Ethnobiology and Ethnomedicine*, Vol 9, pp51.

DIEGELMANN, R.F., EVANS, M.C. 2004. Wound healing: an overview of acute, fibrotic and delayed healing. *Frontiers in BioScience*, Vol 9, no 1, pp 283-289.

DINUBILE, M.J., LIPSKY B.A. 2004. Complicated infections of skin and skin structures: when the infection is more than skin deep. *Journal of Antimicrobial Chemotherapy,* Vol 53, *Suppl. S2*, ii37–ii50.

DIWAN, R., SHINDE, A., & MALPATHAK, N. 2012. Phytochemical composition and antioxidant potential of *Ruta graveolens* L. in vitro culture lines. *Journal of Botany.*

DOLD, A. P., & COCKS, M. L. 2002. The trade in medicinal plants in the Eastern Cape Province, South Africa. *South African Journal of Science*, *98*(11-12), 589-597.

DROSOU A., FALABELLA A., KIRSNER R.S. 2003. Antiseptics on Wounds: An Area of Controversy. *Wounds,* Vol 5, no 15, pp 149-166. Health management Public.

DRYDEN M.S., 2010. Complicated skin and soft tissue infection. *Journal of Antimicrobial Chemotheraphy*. Vol 65, no Suppl 3, pp iii35–44.

DUARTE, M.C.T., FIGUEIRA, G.M., SARTORATTO, A., REHDER, V.L.G., DELARMELINA, C. 2005. Anti*candida* activity of Brazilian medicinal plants. *Journal of Ethnopharmacology*, Vol 97, pp 305-311.

DZOYEM, J.P., KUETE, V., MCGAW, L.J., & ELOFF, J.N. 2014. The 15-lipoxygenase inhibitory, antioxidant, antimycobacterial activity and cytotoxicity of fourteen ethnomedicinally used African spices and culinary herbs. *Journal of ethnopharmacology*, Vol 156, pp 1-8.

DZOYEM, J.P., MCGAW, L.J., & ELOFF, J.N. 2014. *In vitro* antibacterial, antioxidant and cytotoxic activity of acetone leaf extracts of nine under-investigated Fabaceae tree species leads to potentially useful extracts in animal health and productivity. *BMC complementary and alternative medicine*, Vol 14, no 1, pp 1.

EDEOGA, H.O., OKWU, D.E., MBAEBLE, B.O. 2005. Phytochemical Constituents of some Nigerian Medicinal Plants. *African Journal of Biotechnology*, Vol 4, pp 685-688.

ELGORASHI, E.E., TAYLOR, J.L.S., MAES, A., DE KIMPE, N., VAN STADEN, J., VERSCHAEVE, L., JÄGER, A. K. 2002. The use of plants in traditional medicine: potential genotoxic risks. *South African journal of botany*, Vol 68, no 3, pp 408-410.

ELOFF, J.N. 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta medica*, Vol 64, no 8, pp 711-713.

ELOFF, J. N. 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants?. *Journal of ethnopharmacology*, Vol 60, no 1, pp 1-8.

ERON, L.J., LIPSKY, B.A., LOW DE et al. 2003. Managing skin and soft tissue infections: expert panel recommendations on key decision points. *Journal of Antimicrobial Chemotherapy*; Vol 52 Suppl 1: i3–17.

ESPINEL-INGROFF, A., & PFALLER, M.A., 1995. Antifungal agents and susceptibility

ESSOP, A. B. 2005. The biological activity and phytochemistry of selected Hermannia species (Doctoral dissertation, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg.

ESSOP, A.B., VAN ZYL, R.L., VAN VUUREN, S.F., MULHOLLAND D., VILJOEN A.M. 2008. The *in vitro* pharmacological activities of 12 South African *Hermannia* species. *Journal of Ethnopharmacology*, Vol 119, pp 615–619.

FAWOLE O.A., AMOO S.O., NDHLALA A.R., LIGHT M.E., FINNIE J.F., VAN STADEN, J. 2010. Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa. *Journal of Ethnopharmacology*, Vol 127, pp 235–241.

FENNELL, C.W., LINDSEY, K.L., MCGAW, L.J., SPARG, S.G G., STAFFORD I., ELGORASHIA E.E., GRACE O.M., VAN STADEN, J. 2004. Assessing African medicinal plants for efficacy and safety: Pharmacological screening and toxicology. *Journal of Ethnopharmacology*, Vol 94, pp 205–217.

FIGUEROA, J. L., FULLER, L. C., ABRAHA, A., & HAY, R. J. 1996. The prevalence of skin disease among school children in rural Ethiopia—a preliminary assessment of dermatologic needs. *Pediatric dermatology*, Vol *13, no* 5, pp 378-381.

FONACIER. L.S., DRESKIN, S.C., LEUNG, D.Y.M. 2010. Allergic skin diseases. *Journal of Allergy and Clinical Immunogy*, Vol 125, no 2, S138-S149.

GEORGE, J., LAING, M.D., & DREWES, S.E. 2001. Phytochemical research in South Africa. *South African Journal of Science*, Vol *97*.

GHAFUR, A., SHAREEK, P.S. 2012. Skin and Soft Tissue Infections. *MEDICINE*, *22*.

GHUMAN, S., & COOPOOSAMY, R.M. 2011. Crude sample preparation, extraction and in vitro screening for antimicrobial activity of selected wound healing medicinal plants in KwaZulu-Natal, South Africa: A review. *Journal of Medicinal Plant Research*, Vol 5, no 16, pp 3572-3576.

GIACOMETTI A., CIRIONI O., SCHIMIZZI A.M., DEL PRETE M.S., BARCHIESI F., DERRICO M.M., PETRELLI E., AND SCALISE G. 2000. Epidemiology and Microbiology of Surgical Wound Infections. *Journal of Clinical Microbiology*, Vol 38, no 2, pp 918-922.

GIORDANO, T. P., GIFFORD, A. L., WHITE, A.C., ALMAZOR, M.E.S., RABENECK, L., HARTMAN, C., MORGAN, R.O. 2007. Retention in care: a challenge to survival with HIV infection. *Clinical infectious diseases*, *44*(11), 1493-1499.

GNANARAJA R., PRAKASH V., PETER S., MAHENDRAVERMAN M. 2014.. Qualitative and quantitative phytochemicals analysis of selected fabaceae medicinal plants from Allahabad region. *The Pharma Innovation Journal*, Vol 3, no 7, pp 53-56.

GOLDENHEIM, P.D. 1993. An appraisal of povidone-iodine and wound healing. *Postgraduate Medicinal Journal*, Vol 69, no 53, S97-S105.

GUO, J.T., LEE, H.L., CHIANG, S.H., LIN, F.I., CHANG, C.Y. 2001. Antioxidant properties of the extracts from different parts of broccoli in Taiwan. *Journal of food and drug analysis*, *9*(2).

GOWRI R.N.S., & AGARWALA, M. 2011. Phytochemical analysis of some medicinal plants. *Journal of Phytology*, Vol 3, no 12, pp 10-14.

GOWRI, S., VASANTHA. K., 2010. Phytochemical Screening and Antibacterial Activity of *Syzygium cumini* (L.) (Myrtaceae) Leaves Extracts. *International Journal of PharmTech Research,* Vol.2, no.2, pp 1569-1573.

GRICE E.A., KONG H.H., CONLAN S., DEMING C.B., DAVIS J., YOUNG A.C., 2009. Topographical and Temporal Diversity of the Human Skin Microbe. NISC Comparative Sequencing Program, Bouffard G.G., Blakelesley R.W., Murray PR., Green E.D., Turner M.L., Segre J.A. *Science*, Vol 324, no 5931, pp 1190-1192.

HANEKE, E. 1997. Infections in dermatological surgery. *Diagnosis and treatment of skin infections. Blackwell Science, Oxford, United Kingdom*, 416-430.

HANSIS, M. 1996. Pathophysiology of infection-a theoretical approach. *Injury*, Vol 27, S-C5-5-C8.

HARBONE, J.B., 1973. Phytochemical methods, London, Chapman and Hall, Ltd. 188pp

HARBORNE, J.B. & BAXTER, H., 1993. Phytochemical dictionary. Taylor & Francis, London.

HARBORNE, J.B. 1973. Phenolic compounds. In *Phytochemical methods*. Springer Netherlands. Pg 33-38.

HAY, R. J., JOHNS, N.E., WILLIAMS, H.C., BOLLIGER, I.W., DELLAVALLE, R.P., MARGOLIS, D.J. MICHAUD, C. 2014. The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. *Journal of Investigative Dermatology*, Vol *134, no* 6, pp1527-1534.

HAY, R., BENDECK, S.E., CHEN, S., ESTRADA, R., HADDIX, A., MCLEOD, T., MAHÉ, A. 2006. Skin Diseases.. In: Jamison D.T, Breman J.G., Measham A.R et al (ed.). Disease Control Priorities in Developing countries. Washington (DC): World Bank. Chapter 37. 2nd edition.

HAY, R.J., & FULLER, L.C. 2011. The assessment of dermatological needs in resource-poor regions. *International Journal of Dermatology*, Vol 50, 552-557.

HEALTHCARE COST AND UTILIZATION PROJECT-HCUP. A federal-state-industry partnership in health data: 2004. 2005. Agency for Healthcare Research and Quality, Rockville.

HENDERSON, R., & COCKBURN, I. 1994. Measuring competence? Exploring firm effects in pharmaceutical research. *Strategic management journal*, Vol 15, S1, pp 63-84.

HODGES, N. 2002. Pharmaceutical applications of microbiological techniques. In Aulton M.E (Ed.), Pharmaceutics: The science of Dosage Form Design, second edition. Harcourt publishers Limited, London, pg 606.

HOLDSTOCK, T. L. 1979. Indigenous healing in South Africa: A neglected potential. *South African Journal of Psychology*, *9*(3-4), 118-124.

HOLZHEIMER, R.G. 2001. Antibiotic induced endotoxin release and clinical sepsis: a review. *Journal of Chemotherapy*, Vol 13 (sup2), pp 159-172.

HOSSAIN, M.A., & SHAH, M.D. 2015. A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant Merremia borneensis. *Arabian Journal of Chemistry*, Vol *8*, no 1, pp66-71.

HOSTETTMANN, K., MARSTON, A., NDJOKO, K., WOLFENDER, J.L. 2000. The potential of African plants as a source of drugs. *Current Organic Chemistry*, Vol 4, no 10, pp 973-1010.

http://www.lonelyplanet.com/South Africa/Free State (Accessed- Aprl, 2015).

HU, J., MCKOY, K., PAPIER, A., KLAUS, S., RYAN, T., GROSSMAN, H., CRAFT, N. 2011. Dermatology and HIV/AIDS in Africa. *Journal of global infectious diseases*, Vol 3, no 3, pp 275.

HUOVINEN, S., KOTILAINEN, P., JÄRVINEN, H., MALANIN, K., SARNA, S., HELANDER, I., & HUOVINEN, P. 1994. Comparison of ciprofloxacin or trimethoprim therapy for venous leg ulcers: results of a pilot study. *Journal of the American Academy of Dermatology*, *31*(2), 279-281.

HURINANTHAN, V. 2009. Immune modulatory effect of *D. cinrea, C dimidiatus, C*. *tomentosa and L leonurus*. Durban, South Africa.

HURINANTHAN, V. 2013. Anti-HIV activity of selected South African medicinal plants (Doctoral dissertation).

IGOLI, J.O., OGAJI, O.G., TOR-ANYIIN, T.A., IGOLI, N.P. 2005. Traditional Medicine Practice Amongst the Igede People of Nigeria. Part II. *African Journal of Traditonal, Complementary and alternative medicine,* Vol 2, no 2, pp 134–152.

IRANSHAHI, M.E.H.R.D.A.D., ASKARI, M., SAHEBKAR, A., ADJIPAVLOU-LITINA, D. 2015. Evaluation of antioxidant, anti-inflammatory and lipoxygenase inhibitory activities of the prenylated coumarin umbelliprenin.*DARU Journal of Pharmaceutical Sciences*, Vol *17*, no 2, pp99-103.

KAMATOU, G.P.P., VAN ZYL, R.L., VAN VUUREN, S.F., FIGUEIREDO, A.C., BARROSO, J.G., PEDRO, L.G., VILJOEN, A.M. 2008. Seasonal variation in essential oil composition, oil toxicity and the biological activity of solvent extracts of three South African *Salvia* species. *South African Journal of Botany*, Vol 74, pp 230–237.

KAMOTOU G.P.P., VILJOEN A.M., ERONO-BWALYA A.B., VAN ZYL R.L., VAN VUUREN S.F., LOURENS A.C.U. 2005. The *in vitro* pharmacological activities and a chemical investigation of three South Africa *Salvia* species. Journal Of Ethnopharmacology, Vol 102, no 3, pp 382-390.

KATZUNG, B.G., JULIUS, D.J. 2001. Histamine, serotonin, and the ergot alkaloids. *Basic and Clinical Pharmacology. 8th Ed. New York, NY: McGraw-Hill*, pp 265-88.

KAUR, G.J., & ARORA, D.S. 2009. Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. *BMC Complementary and Alternative Medicine,* Vol, 9, no 30,*pg 1-10.*

KEMALSON J.E., JAGER A.K., VAN STADEN J. 2000. Zulu medicinal plants with antibacterial activity. J Ethnopharmacol, Vol 69, pp241-246.

KENNEDY, D.O.,WIGHTMAN, E.L. 2011. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. *Advances in Nutrition: An International Review Journal*, Vol 2, no 1, pp 32-50.

KHAN, M. 2005. Antiseptics, iodine, povidineiodine and traumatic wound cleansing. Wound Cleansing, Vol 16, no 4, pp 8-10.

KIM, H.P., SON, K.H., CHANG, H.W., & KANG, S.S. 2004. Anti-inflammatory plant flavonoids and cellular action mechanisms. *Journal of pharmacological sciences*, Vol 96, no (3), pp229-245.

KINGSLEY A. 2001. Aproactive approach to wound infection. *Nurs Stand*, Vol 15, no 30, pp 50-8.

KLIMEK J.J. 1985. Treatment of wound infections. *Cutis*, Vol 15, pp 21-24.

KODURU, S., GRIERSON., D.S., AFOLAYAN, A.J. 2007. Ethnobotanical information of medicinal plants used for treatment of cancer in the Eastern Cape Province, South Africa. *Current Science*, Vol 92, no 7.

KONG, J. M., GOH, N. K., CHIA, L. S., & CHIA, T. F. 2003. Recent advances in traditional plant drugs and orchids. *Acta Pharmacologica Sinica*, *24*(1), 7-21.

KOWSALYA, V. 2012. Antibacterial Activity of Honey and *Erytlaria Acualis* against Bacteria Isolated From Burnt Wound Sepsis. *Journal of Pharmacy and Biological Sciences,* Vol 1, no 5, pp 01-20.

KUMAR S., BAJWA B.S., KULDEEP S., & KALIA A.N. 2013. Anti-inflammatory activity of herbal plants: A Review. *International Journal of Advances in Pharmacy, Biology and Chemistry*, Vol 2, no 2, pp 2277-4688.

LALL, N., & KISHORE, N. 2014. Are plants used for skin care in South Africa fully explored? *Journal of Ethnopharmacology*, Vol 153, no 1, pp 61-84.

LEKGANYANE, M.A., MATSEBATLELA T.M., HOWARD R.L., SHAI, L.J., MASOKO, P. 2012. The phytochemical, antibacterial and antioxidant activity of five medicinal plants against the wound infecting bacteria. *African Journal of Biotechnology*,Vol 11, no 68, pp13210-13219.

LEUNG, D.Y. 2000. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *Journal of Allergy and Clinical Immunology*, Vol 105, no 5, pp 860-876.

LI, J., CHEN, J.,& KIRSNER, R. 2007. Pathophysiology of acute wound healing. *Clinics in dermatology*, Vol *25*, no 1, pp9-18.

LIN, J., OPOKU, A.R., GEHEEB-KELLER, M., HUTCHINGS, A.D., TERBLANCHE, S.E., JÄGER, A.K., VAN STADEN, J. 1999. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. *Journal of Ethnopharmacology*, Vol *68, no* 1, pp 267-274.

LIYANA-PATHIRANA, C., & SHAHIDI, F. 2005. Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food chemistry*, Vol 93, no 1, pp 47-56.

LOURENS, A.C.U., Reddy, D., Başer, K.H.C., Viljoen, A. M., Van Vuuren, S.F. 2004. *In vitro* biological activity and essential oil composition of four indigenous South African Helichrysum species. *Journal of Ethnopharmacology*, Vol 95, no 2, pp 253-258.

LOUW, C.A.M., REGNIER, T.J.C., KORSTEN, L. 2002. Medicinal bulbous plants of South Africa and their traditional relevance in the control of infectious diseases. *Journal of Ethnophar*m ,Vol 82, pp 147-154.

LOWER, E.S. 1985. Activity of the saponins. Drug and Cosmetic Industry 135, 39-44.

LUSEBA, D., ELGORASHI, E.E., NTLOEDIBE, D.T., & VAN STADEN, J. 2007. Antibacterial, anti-inflammatory and mutagenic effects of some medicinal plants used in South Africa for the treatment of wounds and retained placenta in livestock. *South African Journal of Botany*, Vol 73, no 3, pp 378-383.

MABONA, U., VAN VUUREN, S.F. 2013. South African medicinal plants used to treat skin diseases. *South Afr J Botany*, Vol 87: 175-193.

MABONA, U., VILJOEN, A., SHIKANGA, E., MARSTON, A., VAN VUUREN, S. 2013. Antimicrobial activity of southern African medicinal plants with dermatological relevance: From an ethnopharmacological screening approach, to combination studies and the isolation of a bioactive compound.*Journal of ethnopharmacology*, *148*(1), 45-55.

MADIKIZELA, B., NDHLALA, A.R., FINNIE, J.F, VAN STADEN, J. 2013. *In Vitro* Antimicrobial Activity of extracts from plants used traditionally in Sato treat tuberculosis and related symptoms.. *Evidence-Based Complementary & Alternative Medicine* 8 pages.

MAHDI, J.G. 2010. Medicinal potential of willow: A chemical perspective of aspirin discovery. *Journal of Saudi Chemical Society*, Vol 14, no 3, pp 317-322.

MAHMOOD, A.D.E.E.L., RIFFAT, N.M., ZABTA, K.S., AQEEL, M. 2011. Ethnobotanical survey of plants from Neelum, Azad Jammu and Kashmir, Pakistan. *Pak J Bot*, Vol 43, pp 105-110.

MAHMOOD, N., PIZZA, C., AQUINO, R., DE TOMMASI, N., PIACENTE, S., COLMAN, S., HAY, A. J. 1993. Inhibition of HIV infection by flavanoids. *Antiviral research*, *22*(2-3), 189-199.

MAHMOUD I.I., MAROUK M.S.A., MORHARRAM F.A., EL-GINDI M.R., HASAN A.M.K. 2001. Acylated flavonoid glycosides from *Eugenia jambolana* leaves. Phytochemistry, Vol 58, pp 1239-1244.

MANDER, M. 1998. Marketing of indigenous medicinal plants in South Africa: A case study in KwaZulu-Natal.

MARINOVA, G., BATCHVAROV, V. 2011. Evaluation of the methods for determination of the free radical scavenging activity by DPPH. *Bulgarian Journal of Agricultural* Science, Vol 17, no 1, pp 11-24.

MARWAH, R.G., FATOPE, M.O., AL MAHROOQI, R., VARMA, G. B., AL ABADI, H., & AL-BURTAMANI, S. K. S. 2007. Antioxidant capacity of some edible and wound healing plants in Oman. *Food chemistry*, Vol 101, no 2, pp 465-470.

MASELENO, A., HASAN, M.M. 2012. Skin Diseases Expert System using Dempster-Shafer Theory. *International Journal of Intelligent Systems and Applications*, Vol 4, no 5, pp 38.

MATU, E.N., VAN STADEN, J. 2003. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *Journal of Ethnopharmacology*, Vol 87, no 1, pp 35-41.

MAYHALL, C.G. 1993. Surgical infections including burns. In Wenzel R.P (ed.), Prevention and control of nosocomial infections. The Williams Co., Baltimore MD. 2nd edition, pp 614-664.

MAZID, M., KHAN, T.A., MOHAMMAD, F. 2011. Role of secondary metabolites in defense mechanisms of plants. *Biology and medicine*, Vol 3, no 2, pp 232-249.

MCGUCKIN, M., GOLDMAN R., BOLTON, L., SALCIDO, R. 2003. Advances in Skin & Wound Care The Clinical Relevance of Microbiology in Acute and Chronic Wounds. *Clinical Management Extra: CME: CE*, Vol 16, no 1, pp 12-23.

MCKOY K. 2011. The importance of dermatology in global health. Burlington, MA, pp1-3.

MCKOY, K. 2013. The Importance of dermatology in Global Health. Lahey Clinic. Harvard Medical School, Department of Dermatology. Burlington, MA. 13.

MCNEIL, J.C., HULTEN, K.G., KAPLAN, S.L., MASON, E. O. 2014. Decreased susceptibilities to Retapamulin, Mupirocin, and Chlorhexidine among *Staphylococcus aureus* isolates causing skin and soft tissue infections in otherwise healthy children. *Antimicrobial agents and chemotherapy*, Vol 58, no 5, pp 2878-2883.

MIEAN, K.H., MOHAMED, S. 2001. Flavonoid (Myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry*, Vol 49, pp 3106-3112.

MOLEFE N, I., TSOTETSI A.M., ASHAFA A.O.T., THEKISOE O.M.M. 2013. In viro anthemilmintic activity of *Cotyledon orbiculata*, *Hermannia depressa* and *Nicotiana glauca* extracts against parasitic gastro-intestinal nemaodes of livestock. *Journal of Medicinal Plants Research*, Vol 7, no 9, pp 536-42.

MOLYNEUX, P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol*, *26*(2), 211-219.

MOTSEI, M.L.,. LINDSEY, K.L., VAN STADEN, J., JÄGER, A.K. 2003. Screening of traditionally used South African plants for antifungal activity against *Candida albicans. Journal of Ethnopharmacology*, Vol 86, pp 235–241.

MPOFU S.J., MSAGATI T.A.M., RUI,. KRAUSE, W.M., 2014. Cytotoxicity, phytochemical analysis and antioxidant activity of crude extracts from rhizomes of *Elephantorrhiza elephantina* and *Pentanisia prunelloides. Afr J Tradit Complement Altern Med*, Vol 11, no 1, pp 34-52.

MUCINA, L., RUTHERFORD, M.C., & POWRIE, L.W. 2005. Vegetation map of South Africa, Lesotho, and Swaziland 1: 1 000 000 scale sheet maps*. South African National Biodiversity Institute, Pretoria*. ISBN 1-919976-22-1.

MULAUDZI, R.B., NDHLALA, A.R., KULKARNI, M.G., FINNIE, J.F., & VAN STADEN, J. 2013. Anti-inflammatory and mutagenic evaluation of medicinal plants used by Venda people against venereal and related diseases. *Journal of ethnopharmacology*, Vol 146, no 1, pp 173-179.

MULEYA, E., AHMED, A.S., SIPAMLA, A.M., MTUNZI, F.M. MUTATU, W. 2015. Pharmacological properties of *Pomaria sandersonii, Pentanisia prunelloides* and *Alepidea amatymbica* extracts using *in vitro* assays. *Journal of Pharmaco gnosy and Phytotherapy,* Vol 7, no 1, pp 1-8.

MURUGAN, M., MOHAN, V.R. 2012. *In vitro* antioxidant studies of Dioscorea esculenta (Lour). Burkill. *Asian Pacific Journal of Tropical Biomedicine,* S1620-S1624.

MURUGESAN, D.E.E.P.A., & DEVIPONNUSWAMY, R.E.N.U.K.A. 2014. Potential anti-inflammatory medicinal plants-a review. *International Journal of Pharmacy and Pharmacology Sciences*, Vol 6, no 4, pp 43-49.

NAMULI, A., ABDULLAH, N., SIEO, C.C., ZUHAINIS, S.W., & OSKOUEIAN, E. 2011. Phytochemical compounds and antibacterial activity of Jatropha curcas Linn. extracts. *Journal of Medicinal Plants Research*, *5*(16), 3982-3990.

NCUBE, J.F. FINNIE, J., VAN STADEN J. 2011. Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. *South African Journal of Botany*, Vol 77, pp 387–396.

NDLOVU, T. 2009. Isolation and characterisation of some of the major compounds from *Pentanisia prunelloides* (Doctoral dissertation).

NESTER E.W., ANDERSON D.G., ROBERTS C.E., PEARSALL N.N., NESTER M.T. 2004. Microbiology: A human perspective. 4th Edition.

NESTOR, P.J., SCHELTENS, P., HODGES, J.R. 2004. Advances in the early detection of Alzheimer's disease.

NETHATHE, B.B., NDIP, R.N. 2011. Bioactivity of *Hydnora africana* on selected bacterial pathogens: Preliminary phytochemical screening. *African Journal of Microbiology Research*, Vol 5, no 18, pp 2820-6.

NICHOLS, B.R.L. 1999. Optimal treatment of complicated skin and skin structure infections. *Journal of Antimicrobial Chemotherapy*, Vol 44, no 1, pp 19-23.

NICHOLS, R.L., SMITH J.W. 1994. Anaerobes from a surgical perspective. *Clinical Infectious Diseases*, Vol 18, pp S280 – 286.

NISA, H., KAMILI, A.N., BANDH, S.A., LONE, B.A., PARRAY, J.A. 2013. Phytochemical screening, antimicrobial and antioxidant efficacy of different extracts of Rumex dentatus L.–A locally used medicinal herb of Kashmir Himalaya. *Asian Pacific Journal of Tropical Disease*, *3*(6), 434-440.

NOSTRO, A., GERMANEO, M.O., D'ANGELO, V., MARIANO, H., CANNALELLI, M.A. 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in Applied Microbiology*, Vol 30, pp 379- 384.

O'DOHERTY, M.G., GILCHRIST, S.E., YOUNG, I.S., MCKINLEY, M.C., YARNELL, J.W., GEY, K.F., & WOODSIDE, J.V. 2010. Effect of supplementation with B vitamins and antioxidants on levels of asymmetric dimethylarginine (ADMA) and C-reactive protein (CRP): a double-blind, randomised, factorial design, placebo-controlled trial. *European journal of nutrition*, Vol 49, no (8), pp483-492.

OBADONI, B.O., OCHUKO, P.O. 2001. Phytochemcial studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure Applied Science*, Vol 7, no 3, pp 455-459.

O'DELL, M. L. 1998. Skin and wound infections: an overview. *American family physician*, Vol 57, no 10, pp 2424-2432.

OMOTAYO, F.O., BOROKINI, T.I. 2012. Comparative phytochemical and ethnomedicinal survey of selected medicinal plants in Nigeria. *Scientific Research and Essays*, Vol *7*, no 9, pp 989-999.

ONG, P.Y., OHTAKE, T., BRANDT, C., STRICKLAND, I., BOGUNIEWICZ, M., GANZ, T., LEUNG, D.Y. 2002. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *New England Journal of Medicine*, Vol 347*,* no 15, pp 1151-1160.

ORHAN, I., ÖZÇELIK, B., ASLAN, S., KARTAL, M., KARAOGLU, T., ŞENER, B., CHOUDHARY, M. I. 2007. Antioxidant and antimicrobial actions of the clubmoss Lycopodium clavatum L. *Phytochemistry Reviews*, *6*(1), 189-196.

PAGE, G., & BEATTIE T. 1992. Infection in the accident and emergency department. In Taylor E.W (ed.), Oxford University Press. Oxford. United Kingdom. *Infection in surgical practice* pp 123-132.

PANDIT, D., & GORE, M. 1997. Nosocomial infections in patients with thermal injury and measures taken for prevention. *Bombay Hosp. Journal*, *39*(1).

PAPPA, K.A. 1990. The clinical development of mupirocin. *Journal of the American Academy of Dermatology,* Vol 22, pp 873-9.

PATTANAYAKA, S.P., & SUNITA, P. 2008. Wound healing, anti-microbial and antioxidant potential of *Dendrophthoe falcata* (L.f) Ettingsh. *Journal of Ethnopharmacology*, Vol 120, pp 241–247.

PAULPRIYA, K., & MOHAN, V. R. 2012. In vitro antioxidant potential of methanol extract of Dioscorea oppositifolia. *Science Research Reporter*, Vol 2, pp 239-245.

PRICE, K.R., JOHNSON, I.T., FENWICK, G.R., MALINOW, M.R. 1987. The chemistry and biological significance of saponins in foods and feedingstuffs.*Critical Reviews in Food Science & Nutrition*, Vol 26, no 1, pp 27-135.

PRIETO, P., PINEDA, M., AGUILAR, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemestry Journal*, Vol 269. no 2, pp 337-341.

PRIOR, R.L., CAO, G., MARTIN, A., SOFIC, E., MCEWEN, J., O'BRIEN, C., MAINLAND, C.M. 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of Vaccinium species.*Journal of Agricultural and Food Chemistry*, Vol 46, no 7, pp 2686-2693.

PRUITT, B.A., MCMANUS, A.T., KIN, S.H., GOODWIN, C.W. 1998. Burn Wounds Infections: Current Status. *World Journal of Surgery*, Vol 22, pp 135-145.

RAAHAVE, D., FRIIS-MODER, A., BJEWE-JEPSEN, THIIS-KNUDSEN J.J., RASMUSEN, L.B. 1986. The infective dose of aerobic and anaerobic bacteria in postoperative wound sepsis. *Archives of Surgery Journal*, Vol 121, pp 924-9.

RABE, T., & VAN STADEN, J. 1997. Antibacterial activity of South African plants used for medicinal purposes. *Journal of Ethnopharmacology*, Vol 56, no 1, pp 81-87.

RAINSFORD, K.D. (Ed.). 2004. *Aspirin and related drugs*. CRC Press.

RAO, V.R., & ARORA, R.K. 2004. Rationale for conservation of medicinal plants. Medicinal plants research in Asia, 1, 7-22.

REID, K.A., JAGER, A.K., LIGHT, M.E., MULHOLLAND, VAN STADEN, J. 2005.

ROBERT, C., & KUPPER, T.S. 1999**.** Mechanisms of diseases: Inflammatory skin diseases, T cells and Immune survellience. Epstein F.H (ed.). The New England *Journal of Medicine*, Vol 341, pp 24.

ROBSON, M.C, 1997. Wound Infection A Failure of Wound Healing Caused by an Imbalance of Bacteria. *Surgical Clinics of North America*, Vol 77, no 3 pp 630-650.

ROJAS, R., BUSTAMANTE, B., BAUER, J., FERNÁNDEZ, I., ALBÁN J., LOCKA*,* O. 2003. Antimicrobial activity of selected Peruvian medicinal plants. *Journal of Ethnopharmacology*, Vol 88, pp 199–204.

ROY, P., AMDEKAR, S., KUMAR, A., & SINGH, V. 2011. Preliminary study of the antioxidant properties of flowers and roots of Pyrostegia venusta (Ker Gawl) Miers. *BMC Complementary and Alternative Medicine*, Vol 11, no 1, pp 1.

RUTHERFORD, M.C., MUCINA, L., POWRIE, L. W. 2006. Biomes and bioregions of southern Africa. *The vegetation of South Africa, Lesotho and Swaziland. Strelitzia*, Vol 19, pp 30-51.

SAFAYHI, H., MACK, T.H.O.M.A.S., SABIERAJ, J.O.A.C.H.I.M., ANAZODO, M.I., SUBRAMANIAN, L.R., AMMON, H.P. 1992. Boswellic acids: novel, specific, nonredox inhibitors of 5-lipoxygenase. *Journal of Pharmacology and Experimental Therapeutics*, Vol 261, no 3, pp 1143-1146.

SAKTHIDEVI, G., MOHAN, V.R. 2013. Total Phenolic, Flavonoid Contents and *In vitro* Antioxidant Activity of *Dioscorea alata* l. Tuber. International Journal of *Pharmaceutical Sciences & Research*, Vol.5, no 5, pp 11 –119.

SAMY, R.P., & GOPALAKRISHNAKONE, P. 2010. Therapeutic Potential of plants as antimicrobials for drug discovery. *Evidence-Based of Complementary Alterntive Medicine*. pp 283-294.

SATHYA, V., BHARATHIDASAN, R., SELVI, .T.S., REBECCAL S.N., ILAKKIYA, R., PRABAKARAN, M. 2013. Quantitative, qualitative phytochemical analysis and *in vitro* antibacterial activity of *Bauhinia tomentosa* L. *Journal of Natural Products and Plant Resources*, Vol 3, no 2, pp 31-36.

SAUTOUR, M., MITAINE-OFFER, A.C., LACAILLE-DUBOIS, M.A. 2007. The Dioscorea genus: a review of bioactive steroid saponins. *Journal of natural medicines*, Vol 61, no 2, pp 91-101.

SAXENA, M., MIR, A. H., SHARMA, M., MALLA, M. Y., QURESHI, S., MIR, M. I., CHATURVEDI, Y. 2013. Phytochemical screening and in-vitro antioxidant activity isolated bioactive compounds from Tridax procumbens Linn.*Pakistan Journal of Biological Sciences*, Vol 16, no 24, pp 1971.

SAXENA, M., SAXENA, J., NEMA, R., SINGH, D., & GUPTA, A. 2013. Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry*, Vol 1, no 6.

SCHINELLA, G.R., TOURNIER, H.A., PRIETO, J.M., DE BUSCHIAZZO, P.M., & RIOS, J.L. 2002. Antioxidant activity of anti-inflammatory plant extracts. *Life sciences*, Vol 70, no 9, pp 1023-1033.

SCHIPPMANN, U., LEAMAN, D.J., CUNNINGHAM, A.B. 2002. Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. In Biodiversity and the Ecosystem Approach in Agriculture, Forestary and Fisheries. Ninth Regular session of the commission on Genetic Resources for Food and Agriculture. FAO, Rome, Italy, pp. 1-21.

SEN, C.K., GIRDILLO, G.M., ROY, S., KIRSNER, R., LAMBERT, L., HUNT, T.K., GOTTRUP, F., GURTNER, G.C., LANGAKER, M.T. 2009. Human skin wounds: A major and snowballing threat to public health and the economy. *Wound Repair and Regeneration,* Vol 17, pp 763-771.

SHABBIR, M., KHAN M.R., SAEED, N. 2013. Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of *Maytenus royleanus* leaves. *BMC Complementary and Alternative Medicine*, Vol 13, pp143.

SHAI, L.J., MCGAW, L.J., ELOFF, J.N. 2008. Characterization of compounds from *Curtusia dentata* (Cornaceae) active against *Candida albicans. South African Journal of Botany,* Vol 75, no 2, pp 363-366.

SHAIKH R.U., PUND M.M., GACCHE R.N. 2015. Evaluation of anti-inflammatory activity of selected medicinal plants used in Indian traditional medication system *in vitro* as well as *in vivo*. *Journal of Traditional and Complementary Medicine*, pp 1-7.

SHALE, T. L., STIRK, W. A., & VAN STADEN, J. 1999. Screening of medicinal plants used in Lesotho for anti-bacterial and anti-inflammatory activity.*Journal of Ethnopharmacology*, Vol *67, no* 3, pp 347-354.

SHEIKH, N., KUMAR, Y., MISRA, A. K., & PFOZE, L. 2013. Phytochemical screening to validate the ethnobotanical importance of root tubers of Dioscorea species of Meghalaya, North East India. *Journal of Medicinal Plants*, Vol 1, no 6.

SHENOY, C., PATIL M.B., KUMAR, R., PATIL, S. 2009. Preliminary phytochemical investigation and wound healing activity of *allium cepa* linn (liliaceae). *International Journal of Pharmacy and Pharmaceutical Sciences,* Vol. 2, Issue 2.

SINGH R.K., JOSHI V.K., & GAMBHIR S.S. 1998. Anti-inflammatory activity of some traditional medicinal plants. *Ancient Science of Life*, Vol 18, no 2, pp 160-164.

SINGLETON, V., R. ORTHOFER, R., LAMUELA-RAVENTOS, R. 1999. "Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent," in *Methods in Enzymology*, L. Packer, Ed.,. Academic Press, San Diego, Calif, USA. Vol. 299, pp. 152–315.

SOFOWORA, A. 1993. Medicinal plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria. pp 346.

SOSA, S., BALICK, M. J., ARVIGO, R., ESPOSITO, R. G., PIZZA, C., ALTINIER, G., & TUBARO, A. 2002. Screening of the topical anti-inflammatory activity of some Central American plants. *Journal of Ethnopharmacology*, Vol *81*, no 2, pp 211-215.

SPARG, S.G., VAN STADEN, J., JÄGER, A.K., 2002. Pharmacological and phytochemical screening of two Hyacinthaceae species: *Scilla natalensis* and *Ledebouria ovatifolia*. *Journal of Ethnopharmacology*, Vol 80, pp 95–101.

STEENKAMP, V., MATHIVHAA, E., GOUWS B.M.C., VAN RENSBURG, C.E.J. 2004. Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. 2004. *Journal of Ethnopharmacology*, Vol 95, pp 353–357.

STEVENS, D.L, BISNO, A.L, CHAMBERS, H.F., Everett, E. D., Dellinger, P., Goldstein, E. J., Wade, J. C.2005. Practice guidelines for the management of skin and soft-tissue infections. *Clinical Infectious Diseases*, Vol 41, no 10, pp 1373-1406.

SUMTHONG, P. 2007. Antimicrobial compounds as side products from the agricultural processing industry. Division of Pharmacognosy, Section of Metabolomics, Institute of Biology, Faculty of Science, Leiden University.

TADEG H., MOHAMMED, E., ASRES K., GEBRE-MARIAM, T. 2005. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *Journal of Ethnopharmacology*, Vol 100, pp, 168–175.

TALHOUK, R. S., KARAM, C., FOSTOK, S., EL-JOUNI, W., & BARBOUR, E. K. 2007. Anti-inflammatory bioactivities in plant extracts. *Journal of medicinal food*, Vol 10, *no* 1, pp1-10.

TAPAS, A.R., SAKARKAR, D.M., & KAKDE, R.B. 2008. Flavonoids as nutraceuticals: a review. *Tropical Journal of Pharmaceutical Research*, Vol 7, no 3, pp 1089-1099.

TAPIERO, H., TEW, K.D., BA, G.N., MATHE, G. 2002. Polyphenols: do they play a role in the prevention of human pathologies? *Biomedicine Pharmacotherapy*, Vol 56, no 4, pp 200-207.

TAYLOR, J.L.S., VAN STADEN, J. 2001. The effect of nitrogen and sucrose concentrations on the growth of *Eucomis autumnalis* (Mill.) Chitt. plantlets in vitro, and on subsequent anti-inflammatory activity in extracts prepared from the plantlets. *Plant growth regulation*, Vol 34, no 1, pp 49-56.

TAYLOR, J.L.S., VAN STADEN, J., JÄGER, A.K. 2002. COX-1 and COX-2 inhibitory activity in extracts prepared from Eucomis species, with further reference to extracts from E. autumnalis autumnalis. *South African journal of botany*, Vol 68 no 1, pp 80-85.

TEPE B., DAFERERA, D., SOKMEN, A., SOKMEN, M., POLISSIOU, M. 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chemistry*, Vol 90, pp 333–340.

THOMAS N., BROOK I. 2011. Animal bite-associated infections: microbiology and treatment. *Expert review of anti-infective therapy*, Vol 9, no 2, pp 215-226.

TÖRÖK, E., CONLON, C.P. 2005. Skin and soft tissue infections. *Medicine*, Vol 33, no 4, pp 84-88.

TORTORA, G.J., GRABOWSKI, S.R. 1996. Principles of anatomy and physiology. *Biological Sciences Textbook Inc.*

TREASE, G.E., & EVANS, W.C. 1989. Pharmacognosy. 13th. *ELBS/Bailliere Tindall, London*, 345-6.

TREASE, G.E., EVANS, W.C. 1978. Pharmacology 11th Ed. Bailliere Tindall Ltd, London. pp 60-75.

TROUILLAS, P., CALLISTE, C.A., ALLAIS, D.P., SIMON, A., MARFAK, A., DELAGE, C., DUROUX, J.L. 2003. Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas. *Food Chemistry*, Vol 80, no 3, pp 399-407.

TSCHACHLER, E., BERGSTRESSER, B.P., STINGL, G. 1996. HIV-related skin diseases. *Lancet*, Vol 348, pp 659-663.

TUNON, H., OLAVSDOTTER, C., & BOHLIN, L. 1995. Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin biosynthesis and PAF-induced exocytosis. *Journal of Ethnopharmacology*, Vol 48, no 2, pp 61-76.

UMAMAHESWARI, M., & CHATTERJEE, T.K. 2008. *In vitro* antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. *African Journal of Traditional, Complementary and Alternative Medicines*, Vol 5, no 1, pp 61-73.

UMARU, T., NWAMBA, C.O., KOLO, I., & NWODO, U.U. 2009. Antimicrobial activity of non-steroidal anti-inflammatory drugs with respect to immunological response: Diclofenac sodium as a case study. *African Journal of Biotechnology*, Vol 8, no 25.

URSINI, F., MAIORINO, M., MORAZZONI, P., ROVERI, A., & PIFFERI, G. 1994. A novel antioxidant flavonoid (IdB 1031) affecting molecular mechanisms of cellular activation. *Free Radical Biology and Medicine*, Vol 16, no 5, pp 547-553.

VAN HEMMEN, J.J. 2000. Essentials of Occupational Skin Management. A practical guide to the creation and maintenance of an effective skin management system. CL Packham. Limited Edition Press, Southport. ISBN 1-85988-045-2.

VAN VUUREN S.F. 2008. Antimicrobial activity of South African medicinal plants. *Journal of Ethnopharmacology*, Vol 119, pp 462–472.

VAN WYK B.E., VAN OUDSHOORN B., GERICKE N. 2009. Medicinal plants of South Africa. 2nd edition. Pretoria: Briza Publications.

VAN WYK B.E & VILJOEN A. 2011. Special issue on Economic Botany. South African Journal of Botany. 77: 809-1012.

VAN WYK, B.E. 2008. A broad review of commercially important southern African medicinal plants. *Journal of Ethnopharmacology*, Vol 119, no 3, pp 342-355.

Van-Burden, T.P & Robinson, T. 1981. The biochemistry of alkaloids.

VAN-BURDEN, T.P., ROBINSON, W.C. 1981. Formation of complexes between protein and Tannin acid. *Journal of Agriculture and Food Chemistry*.

VERMAAK, I., ENSLIN, G.M., IDOWU, T.O., VILJOEN, A.M. 2014. *Xysmalobium undulatum* (uzara)–review of an antidiarrhoeal traditional medicine. *Journal of ethnopharmacology*, Vol 156, pp 135-146.

VERPOORTE, R. 1998. Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development. *Drug Discovery Today*, Vol 3, no 5, pp 232-238.

VERPOORTE, R., VAN DER HEIJDEN, R., MEMELINK, J. 1998. Plant biotechnology and the production of alkaloids. Prospects of metabolic engineering. In: Cordell, G.A.

VIJAYALAKSHMI, A., RAVICHANDIRAN, V., VELRAJ, M., HEMALATHA, S., SUDHARANI, G., JAYAKUMARI, S. 2011. Anti–anaphylactic and anti–inflammatory activities of a bioactive alkaloid from the root bark of *Plumeria acutifolia* Poir.*Asian Pacific journal of tropical biomedicine*, Vol *1*, no 5, pp 401-405.

VON EIFF C., PETERS G., HEILMANN C. 2002. Pathogenesis of infections due to coagulase negative staphylococci. *The Lancet Infectious diseases*, Vol 2, no 11, pp 677-685.

WABE, N.T., MOHAMMED, M.A., RAJU, N.J. 2011. Ethnobotanical survey of medicinal plants in the Southeast Ethopia used in traditional medicine. *Spatula DD*, Vol 1, no 3, pp 153-158.

WANG, J.M & LIM, H.K. 2014. Necrotizing fasciitis: eight-year experience and literature review. *Brazilian Journal of Infectious Diseases*, Vol 18, no 2, pp 137-143.
WEBSTER, D., TASCHEREAU, P., BELLAND, R.J., SAND, C., RENNIE, R.P. 2008.

WEIDEMAN, L. 2007. An investigation into the antibacterial activities of medicinial plants traditionally used in the Eastern Cape to treat secondary skin infections associated with burn wounds (Doctoral dissertation).

WENFENG, HUANG, KUN Z. 2011. Cytotoxicity of a Plant Steroidal Saponin on Human Lung Cancer Cells. *Asian Pacific J Cancer Prev*, Vol 12, pp 513-517.

WOLFE, K., WU, X., LIU, R.H. 2003. Antioxidant activity of apple peels. *Journal of agricultural and food chemistry*, Vol 51, no 3, pp 609-614.

WORLD HEALTH ORGANIZATION (WHO). 2000. Traditional and Complementary medicines.

WORLD HEALTH ORGANISATION (WHO). 2005, Epidemiology and Management of Common Skin Diseases in Children in Developing Countries. pp 1-54.

WORLD HEALTH ORGANIZATION (WHO): Violence & injury prevention. WORLD HEALTH ORGANIZATION. 2012. (http://www.who.int/violence injury prevention/other-injury/burns/en/).

WORLD HEALTH ORGANIZATION (WHO). 2005. "Global Burden of Disease for the Year 2001 by World Bank Region." Disease Control Priorities Project.http://www.fic.nih.gov/dcpp.

WORLD HEALTH ORGANIZATION (WHO). 2005. Bulletin of the World Health Organization.

www.google.gallery.new-ecopsychology.org.2015. *Lycopodium clavatum*. Ekaterina Smirnova.

www.gov.za.2014. Government of South Africa. 2014. South Africa.info/about/geography/free-state.htm (Accessed-May, 2015).

www.South Africa.info/about/geography/free-state.htm (Accessed-May, 2015).

Yadav, R. N. S., & Agarwala, M. 2011. Phytochemical analysis of some medicinal plants. *Journal of phytology*, *3*(12).

YFF B.T.S., LINDSEY K.L., TAYLOR M.B., ERASMUS D.G., JAGER A.K. 2002. The pharmacological screening of *Pentanisia prunelloides* and the isolation of the antibacterial-compound palmitic acid. *Journal of Ethnopharmacology*, Vol 79, pp 101-107.