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Antimicrobial activity of southern African medicinal plants with dermatological relevance

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A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Pharmacy

Johannesburg, 2013

Declaration

I, Unathi Mabona, declare that this dissertation is my own work. It is being submitted in fulfilment for the degree of Master of Pharmacy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

.....

Unathi Mabona

.....

Date

Dedication

I dedicate this dissertation to my loving parents, siblings and supportive partner.

Thank you for your support, encouragement and faith in me.

This work would not have been possible without you.

Publications submitted for review/ conference presentations

Publications arising from this study

U. Mabona, S.F. Van Vuuren. Southern African medicinal plants used to treat skin diseases. South African Journal of Botany, 2013, 87, 175-193.

Unathi Mabona, Alvaro Viljoen, Emmanual Shikanga, Andrew Marston , Sandy Van Vuuren. Antimicrobial activity of South African medicinal plants with dermatological relevance: From a screening approach, to combination studies and bioactive isolation. Journal of Ethnopharmacology, 2013, 148, 45-55.

Conference/presentation*

Mabona, U., van Vuuren, S.F., Shikanga, E.A., Viljoen, A.M., Marston, A. Indigenous South African medicinal plants with dermatological relevance: Antimicrobial activity and bioactivity-guided isolation. Indigenous Plant Use Forum, University of Venda, Thohoyandou, Limpopo, 2-5 July, 2012. [podium presentation].

*Appendix A

Abstract

Over 100 southern African medicinal plants with dermatological relevance have been identified, yet very limited scientific research to support claims for their effectiveness have been undertaken. With this in mind, a study was designed to investigate the antimicrobial properties of southern African medicinal plants used to treat skin inflictions, with specific emphasis on dermatologically relevant pathogens. Organic and aqueous extracts (132) were prepared from 47 plant species and screened for antimicrobial properties using the micro-titre plate dilution method. Most of the plant extracts demonstrated pathogen specific antimicrobial effects with a few exhibiting broad-spectrum activities. Plants demonstrating notable (MIC values ≤ 1.00 mg/ml) broad-spectrum activities against the tested pathogens include the organic extracts of *Aristea ecklonii, Chenopodium ambrosioides, Diospyros mespiliformis, Elephantorrhiza elephantina, Eucalyptus camaldulensis, Gunnera perpensa, Harpephyllum caffrum, Hypericum perforatum, Melianthus comosus, Terminalia sericea* and *Warburgia salutaris*. The organic extract of *E. elephantina*, a plant reportedly used to treat acne vulgaris, demonstrated noteworthy antimicrobial activity against *Propionibacterium acnes* (MIC value of 0.05 mg/ml). *Diospyros mespiliformis* reported for its traditional use to treat ringworm, also displayed noteworthy antimicrobial activity against *Trichophyton mentagrophytes* (MIC 0.10 mg/ml) and *Microsporum canis* (MIC 0.50 mg/ml).

The study also focused on finding a scientific rationale for the traditional use of plant combinations to treat skin diseases. Five different plant combinations (1:1) were investigated for potential interactive properties, which were identified through ΣFIC calculations. Since the 1:1 combination of *Pentanisia prunelloides* and *Elephantorrhiza*

elephantina demonstrated mostly synergistic antimicrobial interactions, the interactive properties of the combination at varied ratios were further investigated. The most synergistic interactions were noted for the aqueous root extracts of *P. prunelloides* combined with *E. elephantina* (root and rhizome), presenting with a mean ΣFIC value of 0.39.

In addition, a bio-active compound was isolated from *Aristea ecklonii*, a plant selected from the screening process which showed broad-spectrum activity against pathogens associated with skin diseases. A bioactivity guided fractionation process (including column chromatography and high speed counter-current chromatography) was adopted to fractionate the organic leaf extract of *A. ecklonii*. Fractionation of *A. ecklonii* resulted in the isolation of a bio-active compound, plumbagin, displaying noteworthy antimicrobial activity (MIC range between 2.00-16.00 μg/ml) against the tested skin pathogens.

The antimicrobial effects noted for the investigated plant extracts and respective plant combinations give some validation to the traditional use of medicinal plants to treat a variety of skin infections.

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List of acronyms and symbols

AIDS- Acquired immune deficiency syndrome

ATCC- American type culture collection

CDCL3- Chloroform

CFU- Colony forming units

CLSI- Clinical and Laboratory Standards Institute

COSY- Correlation spectroscopy

d- Doublet

dd- Double of doublets

DEPT- Distortionless enhanced polarisation transfer

DMSO- Dimethyl sulfoxide

FIC- Fractional inhibitory concentration

g- Gram

GMRSA- Gentamycin-methicillin resistant *Staphylococcus aureus*

H- Proton

HMBC- Hetero nuclear multiple bond connectivity

HMQC- Hetero nuclear multiple quantum coherence

TLC- Thin layer chromatography

HIV- Human immunodeficiency virus

HSCCC- High speed counter current chromatography

HPTLC- High performance thin layer chromatography

INT- *p*-Iodonitrotetrazolium violet

***J*-** Coupling constant

MHz- Megahertz

MIC- Minimum inhibitory concentration

mg- Milligram

ml- Millilitres

MRSA- Methicillin resistant *Staphylococcus aureus*

ND- Not determined

NMR- Nuclear magnetic resonance

PDA- Photodiode array

PTFE- Poly tetra-fluoro ethylene

Rf- Retention factor

r - Distance

R- Revolution radius

s- Singlet

TSA- Tryptone Soya agar

TSB- Tryptone Soya broth

UHPLC- Ultra high performance liquid chromatography

UV- Ultra violet

UV/VIS- Ultraviolet-visible

v/v- Volume per volume

w/v- Weight per volume

WHO- World Health Organisation

µg- Micrograms

µl- Microlitre

%- Percent

°C- Degrees celsius

Chapter 1: General introduction

1.1 Overview of skin diseases

The skin, as the largest organ of the body, plays a number of vital roles such as in protection, thermoregulation, percutaneous absorption, secretory and sensory activities (Njoroge and Bussmann, 2007). The acidic sebaceous secretions and surface structure of the skin are aggressive to many pathogens. The rich blood and lymphatic supply of the dermis (which is the inner-middle layer of the skin between the epidermal and endodermal layer of the skin) ensures that both specific and non-specific immune responses can be quickly recruited against pathogens that invade the skin. The skin's defence system may, however, be compromised if the surface is penetrated via injury, or thinned from the use of corticosteroids or excoriated by inflammatory processes (Bannister *et al*., 2000). Consequently, this makes the skin less resistant to infections and can also be exacerbated in immunocompromised patients who suffer from diabetes, HIV/AIDS, patients being treated with chemotherapy or corticosteroids which may promote skin fungal infections.

The need for treatment options is gradually becoming an important aspect of basic healthcare among various communities (Njoroge and Bussmann, 2007; Naidoo and Coopoosamy, 2011). The resilient nature of skin diseases and the social standard, where there is a burden of poverty, overcrowded living conditions, limited access to clean water and co-habitation with pets often play a vital role in the high prevalence and difficulty in treating skin diseases. Also, as a result of age related anatomical, physiological, behavioural and environmental factors, both the very young and elderly are considered to be more prone to skin diseases (Laube, 2004; Tomson and Sterling, 2007).

Another factor of concern with respect to dermatological infections is the impact from occupational sources. The prevalence of skin ailments is estimated to be as high as 34% of all occupational diseases worldwide (Njoroge and Bussmann, 2007; Abbasi *et al*., 2010). Occupational skin diseases are generally related to the long periods of exposure to chemicals, water and sun. Workers may present with skin diseases or conditions such as eczema, urticaria, sunburn or skin cancer (Fowler, 1998). Many of the lower income workers, such as miners or farming labourers in southern Africa are thus prone to skin diseases of occupational origin. Eczema is estimated to be the most common skin disorder diagnosed in the South African population (Hartshorne, 2003).

Skin infections may be attributed to a variety of micro-organisms, either viral (e.g. measles and herpes simplex), parasitic (e.g. scabies and lice), bacterial (e.g. impetigo and acne vulgaris) or fungal (e.g. candidiasis and tinea) in nature. Some important pathogens associated with infections of the skin are described in Sections 1.1.1 to 1.1.3. In addition to this, when the integrity of the skin is compromised (by injury or any other external or internal factors), many characteristic diseases either inflammatory or non-inflammatory may result, and these may range from mild skin rashes, dermatitis (eczema), psoriasis, acute erythema, vitiligo to burns and deep wounds (Bannister *et al*., 2000; van Hees and Naafs, 2001).

1.1.1 Bacterial pathogens commonly responsible for skin infections

Impetigo is a very common skin bacterial infection mainly caused by *Staphylococci* or *Streptococci* and is most common but not exclusive to children. Both *Staphylococci* and *Streptococci* form part of the natural physiological skin flora and are usually not pyogenic in non-immunocompromised patients (Vuong and Otto, 2002), but are the key causative

pathogens of impetigo. Impetigo is characterised by an intense inflammatory response and pus production, often occurring around the mouth, nose or superficial skin lesions (Bannister *et al*., 2000; van Hees and Naafs, 2001; Tadeg, 2004). Antibiotic misuse and lack of patient compliance has resulted in antibiotic resistance and the development of resistant strains such as methicillin resistant *Staphylococcus aureus* (MRSA) and gentamycin-methicillin resistant *Staphylococcus aureus* (GMRSA). These strains have become a major problem in many hospitals worldwide as they spread readily amongst patients and are commonly resistant to several antibiotics (Bannister *et al*., 2000). *Staphylococcus epidermidis*, similar to *S. aureus*, colonizes the skin and mucous membranes, but has also been regarded as the most important cause of skin nosocomial infections. Open wounds are susceptible to infections by most bacteria, with the Staphylococcal and Streptococcal resistant strain posing major difficulties in the treatment of these wounds (Weideman, 2005).

Furunculosis (commonly known as boils) is an infection of the sebaceous glands or sweat glands which when spread and coalesced together form carbuncles. The infection is mainly caused by *S. aureus* and the non-Staphylococcal infections such as the Gram-negative, *Pseudomonas aeruginosa* (Weckesser *et al.*, 2007). Similar to impetigo, it is characterised by an intense inflammatory response and pus production (Bannister *et al*., 2000; Tadeg, 2004). *Pseudomonas aeruginosa* is a pathogen frequently responsible for wound infections. Consequently, the inappropriate use of antiseptics and lack of patient compliance to antibiotics poses a challenge as wound healing time becomes prolonged (Weideman, 2005).

Acne vulgaris, a skin condition mainly common but not exclusive to teenagers, has considerable psychological impacts such as impaired self-image, low self-esteem,

self-consciousness and embarrassment (Magin *et al*., 2006). It is caused by *Propionibacterium acnes*, an anaerobic bacterium which is part of the natural skin flora. The bacterium causes skin infections in immunocompromised states and also where there is increased sebaceous gland activity.

The feet provide a breeding ground for bacteria and fungi due to excessive perspiration and warm environment around the feet, this results into foot odour, which is a common problem (http://www.betterhealth.vic.gov.au). The gas produced by *Brevibacterium* species around the feet normally plays a key role in foot odour production (Abramson, 1983).

1.1.2 Less common but relevant bacterial skin infections

Vibriosis is a condition in which severe, sometimes necrotizing, lesions form as a result of *Actinobacteria* species infections. *Actinobacteria* infections commonly occur in coastal environments. The bacterium is resistant to a wide range of antibiotics and is mainly acquired in areas such as Spain and Portugal (Bannister *et al*., 2000).

Erysipelas is an intra-dermal infection caused by opportunistic skin flora such as *Streptococcus pyogenes*. This infection is said to be extremely uncommon and often confused with cellulitis, but is an important marker for Streptococcal infections. While cellulitis is often as a result of complications from indwelling cannulation of veins, as this allows the ingress of pathogenic bacteria such as *Streptococcus pyogenes*, *S. aureus* and *Pasteurella multocida* or *Bartonella henselae* (typical of dog or cat bites) (Bannister *et al*., 2000).

Erythema chronicum migrans (rash) is a cutaneous manifestation of early Lyme disease, caused by the tick borne *Borrelia burgdorferi* infection. A superficial infection of the skin,

usually on the flexures, commonly known as erythrasma is a rare disease caused by *Corynebacterium minutissimum* or *Corynebacterium diphtheriae.* Most of these bacteria do not respond to a wide range of antibiotics as they are less common and not well investigated (Bannister *et al*., 2000; van Hees and Naafs, 2001).

Mycobacterium leprae which is a causative agent of leprosy, an indolent uncommon disease mainly affects the skin nerves and mucosa, but can also affect other parts of the body. Multi-drug treatment of this disease is recommended worldwide and includes anti-infective drugs such as rifampicin, dapsone and clofazimine (Bannister *et al*., 2000; van Hees and Naafs, 2001).

1.1.3 Fungal infections of the skin

As a result of the HIV/AIDS pandemic, many immunocompromised patients are prone to opportunistic fungal infections (van Hees and Naafs, 2001; Shai *et al.,* 2008). Yeasts and dermatophytes are typical fungal infection associated with the skin. Dermatophytosis is an infection of the keratinized tissues, i.e. hair, nails and the skin, and is commonly known as ringworm or tinia. It is referred to as onychomycosis when the finger nails are infected. Dermatophyte causing infections are mainly typical of the three genera: *Microsporum*, *Trichophyton* and *Epidermophyton* (Beneke *et al*., 1984; van Hees and Naafs, 2001; Tadeg, 2004).

Candidiasis caused by *Candida albicans*, is a yeast infection of the mucous membrane often occurring in adipose, immunocompromised and diabetic subjects (Weckesser *et al.*, 2007). The *Candida* yeast as well as other dermatophytes usually occur in low frequencies on the skin and mucous membrane without significant symptoms. As opportunistic pathogens, these may overgrow causing skin diseases such as intertrigo (body-yeast

infection), onychomycosis and dermato-mycosis (tinia or ringworm) (Bannister *et al*., 2000; van Hees and Naafs, 2001). When burns occur the skin losses its protective epithelial layer and since *Candida albicans* forms part of the skin flora, the wounds become prone to the opportunistic infection of by the yeast (Naidoo and Coopoosamy, 2011).

1.2 The global perspective on dermatological medicinal plant use

According to the World Health Organisation (WHO) (2011), about 70-95% of the world's population in developing countries relies mainly on plants for their primary health care. It is believed that this is due to poverty and lack of access to conventional medicines (Ayyanar and Ignacimuthu, 2011). Based on a survey done in 1993, the WHO has estimated that traditional practitioners treat about 80% of patients in India, 85% in Burma and 90% in Bangladesh for various disease conditions such as bronchial asthma, colds, cough, chronic fever, malaria, dysentery, convulsions, arthritis, diseases and emetic syndrome as well as skin diseases, along with many others (Prakash and Gupta, 2005). The use of traditional medicine by the patients in India, is still equally or more significant as the developing countries highlighted by WHO (2011) for being most dependent to traditional treatments, include the Middle East and Asia. The earliest reference on the use of plants as medicines was found in India and was said to be written between 3500 and 1600 B.C. Thus, it is not surprising to note that India who relies heavily on traditional medicines has also progressed well in terms of phytochemistry with more than 13,400 publications with relevance to traditional medicines. India also has more than 3,200 publications related to medicinal plants used specifically for skin diseases (Science Direct database, Access Date: 27/11/2012).

From a global perspective, ethnopharmacognostic evaluations and *in vitro* antimicrobial assessment of plants used to treat skin diseases have been widely addressed. In order to gain insight into the level of scientific and ethnobotanical surveys done on medicinal plants with dermatological relevance, a literature review was conducted using databases such as Science Direct and Scopus. Using the search words "skin and medicinal plants'' over 3,100 relevant articles were found on the Scopus database, with more than 1,500 related to toxicology, pharmacology and pharmaceutics and more than 1,400 references specific to medicinal use. When using search words such as "medicinal plants and skin diseases" on the Science Direct database there are over 9,500 reference articles with just over 220 related to medicinal plants and 205 relevant to essential oils (Access date: 27/11/2012).

With the aim of finding an alternative to conventional treatments with regard to skin diseases, an evidence-based review has addressed the traditional use of plants for wound treatment (Reuter *et al*., 2010). The review shows the advantages of using combined therapy compared to monotherapy, as well as the anti-inflammatory, antiseptic and bactericidal effects of various plants studied. The review also highlights that most of the plants are applied topically rather than administered orally and that the formulations can either be in the form of ointments, aqueous preparations or gels (Reuter *et al*., 2010).

1.3 Southern African medicinal plants used to treat skin diseases

Traditional medicine has not only gained popularity and approval, but it is sometimes the only system available in many African rural areas. Medicinal plant usage usually forms the backbone in numerous southern African rural communities for treating ailments with varying severity, this is due to limited access to conventional medicines in many remote areas (Weideman, 2005; Naidoo and Coopoosamy, 2011). The reliance of a large portion

of the population of southern Africa can also be attributed to a number of factors such as good accessibility to the medicinal plants from the markets and mostly the wild, affordability and extensive knowledge and expertise amongst the local communities (Street *et al.,* 2008). Medicinal plants are highly sought after to treat dermatological ailments due to their (perceived?) ability to stop bleeding, speed up wound healing and alleviate skin affected by burns amongst other biological properties (Naidoo and Coopoosamy, 2011). The validation of plants used to treat skin infections is therefore of vital importance if one considers that often the first line of therapy for approximately 60% of southern Africans is of botanical origin.

A review (van Vuuren, 2008), on South African plants investigated for antimicrobial activity, highlights that in spite of the numerous research activities where traditional medicinal practices are used to treat a plethora of inflictions including skin ailments, very little research has been done on dermatophytic pathogens and their response to medicinal plants. Southern Africa is said to contain approximately 10% of the world's plant diversity (George *et al*., 2001), boasting a unique and diverse botanical heritage with the number of plant species over 30, 000. Approximately 3000 plants are used for medicinal purposes for a variety of health needs (van Wyk *et al*., 1997). While examining the ethnobotanical literature (Watt and Breyer-Brandwijk, 1962; Pujol, 1990; Rood, 1994; Hutchings, 1996; Smith, 1996; von Koenen, 1996; Felhaber, 1997; van Wyk *et al*., 2000; van Wyk *et al.*, 2009; van Wyk *et al.,* 2011) over 100 southern African medicinal plants were reported to have dermatological relevance (Table 1.1).

Table 1.1 gives an overview of the different plants species used to treat skin ailments, their healing properties, parts of the plants used and the respective modes of administration.

Table 1.1

Plants used in southern Africa for the treatment of skin disorders.

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Acacia erioloba Edgew., Fabaceae	Giraffe thorn	Wood ash	Topical	Wound healing	Smith, 1996; von Koenen, 1996
Acacia mellifera[a] Benth., Fabaceae	Hookthorn or black thorn	Roots	Poultice	Wound healing	Smith, 1996; von Koenen, 1996; Mutai *et al*., 2009; van Wyk *et al*., 2011
Achyranthes aspera L., Amarantheceae	Devil's horsewhip	Roots	Topical ointment	Boils, abscesses	Perumal Samy *et al*., 1998; Hutchings, 1996
Acokanthera oblongifolia Benth. & Hook.f., Apocynaceae	Poison bush	Roots	Topical	Relieve itching	Palmer and Pitman, 1972; Hutchings, 1996; McGaw *et al*., 2000
Acokanthera oppositifolia (Lam.) Codd., Apocynaceae	Bushmans arrow poison	Powdered leaf	Topical	Anti-inflammatory	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Nielsen *et al*., 2012
Adansonia digitata L., Bombaceae	Baobab	Leaves	Topical	Wound healing	von Koenen, 1996; Lagnika *et al*., 2012
Agathosma betulina (P.J.Bergius) Pillans, Rutanaceae	Buchu	Bachu vinegar with leaf	Infusion/ tincture	Wash wounds and bruises	Watt and Breyer-Brandwijk, 1962; Smith, 1996; van Wyk *et al.*, 2000; Moolla, 2005
Albizia adianthifolia W.Wight, Fabaceae	Flat crown	Bark and roots	Lotion	Eczema and skin complaints	Boily and van Puyvelde, 1986; Bryant, 1996; Hutchings, 1996; van Wyk *et al.*, 2011
Aloe arborescens Mill., Xanthorrhoeaceae	Candelabra aloe	Leaves	Topical	Wounds, burns and various skin ailments	van Wyk *et al.*, 2000; Jia *at al*., 2008; Ghuman and Coopoosamy, 2011
Aloe ferox Mill., Xanthorrhoeaceae	Bitter aloe	Leaf sap, leaves and roots	Sap applied directly	Skin irritation, bruises, burns, psoriasis, skin cancer and eczema	Watt and Breyer-Brandwijk, 1962; Bruce, 1975; van Wyk *et al.*, 2000; Jia *at al.*, 2008; van Vuuren and Naidoo, 2010
Amygdalus persica L., Rosaceae	Peach tree	Leaves	Decoction	Sores	Smith, 1895

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Athrixia phylicoides DC., Asteraceae	Bush tea	Whole plant	Plant infusion	Sores and boils	Hutchings, 1996; Padayachee, 2011
Arctopus echinatus L., Apiaceae	Bear's foot.	Roots	Infusion	Skin irritation	Watt and Breyer-Brandwijk, 1962; van Wyk *et al.*, 2000; Magee *et al*., 2007
Aristea ecklonii Baker., Iridaceae	Blue stars	Whole plant	Topical	Shingles	Ngwenya *et al*., 2003
Artemisia afra Jacq. ex Willd., Asteraceae	Wormwood	Leaves	Decoction	Acne and boils	Smith, 1895; Hutchings, 1996; Rabe and van Staden, 1997
Aspalathus linearis (Burm.f.) R.Dahlgren, Fabaceae	Rooibos tea	Leaves	Directly applied	Eczema	van Wyk *et al.*, 2000; Joubert *et al*., 2008
Aspilia natalensis (Sond.) Wild, Asteraceae	Ingcolozi	Leaves	Leaf paste and infusion	Wounds and sores	Hutchings, 1996
Aster bakeranus Burtt Davy ex C. A. Sm., Asteraceae	Uhloshana	Roots	Lotion	Sores	Hutchings, 1996; Shale *et al*., 1999
Barleria rigida Spreng., Acanthaceae	Scorpion thistle	Roots or leaves	Decoction and powder	Wounds	von Koenen, 1996
Bauhinia petersiana Bolle, Fabaceae	Camel's foot	Leaves	Leaf extract	Wounds	von Koenen, 1996; Ahmed *et al*., 2012
Boophane disticha L.F., Amaryllidaceae	Tumbleweed, veld fan or windball	Bulbs	Topical	Septic wounds, boils, external sores and rheumatism	Watt and Breyer-Brandwijk, 1962; Bruce, 1975; Rabe and van Staden, 1997; Shale *et al*., 1999; van Wyk *et al.*, 2000
Bridelia micrantha Baill., Euphorbiaceae	Coastal golden leaf	Bark	Decoction	Burns and wounds	Mabogo, 1990; Hutchings, 1996; Samie *et al*., 2005; Adefuye *et al*., 2011; van Wyk *et al.*, 2011
Bulbine frutescens Willd., Xanthorrhoeaceae	Burn jelly plant	Slimy leaves	Topical	Wounds, burns, skin rash, itchiness and ringworm	Rabe and van Staden, 1997; van Wyk *et al.*, 2000; Ghuman and Coopoosamy, 2011
Bulbine natalensis Baker, Xanthorrhoeaceae	Ibhucu	Leaves, roots and leave sap	Applied directly to skin	Wounds and burns	Watt and Breyer-Brandwijk, 1962; Pujol, 1990; Rood, 1994; van Wyk *et al.*, 2000; Ghuman and Coopoosamy, 2011
Capparis tomentose Lam., Capparaceae	Woolly caper bush	Roots	Paste applied Topically	Wounds and leprosy	Hutchings, 1996; Buwa and van Staden, 2006

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Cardiospermum halicacabum L., Sapindaceae	Balloon vine	Leaves	Warm water leaf infusion	Body sores	Gerstner, 1938; Hutchings, 1996; Girish *et al*., 2008; Viji and Murugesan, 2010; Deepan *et al*., 2012
Carpobrotus edulis (L.) N.E.Br., Aizoeceae	Sour fig	Leaf juice and pulp	Juice directly applied to skin	Eczema, wounds and burns	Watt and Breyer-Brandwijk, 1962; Rood, 1994; van Wyk *et al.*, 2000; van der Watt and Pretorius, 2001
Cassine transvaalensis **Celastraceae**	Saffronwood	Bark	Infusion	Skin rashes, infections and inflammation	von Koenen, 1996; van Wyk and Gericke, 2000; Steenkamp *et al*., 2007
Catharanthus roseus G.Don, Apocynaceae	Madagascar periwinkle	Flowers milky sap	Topical	Insect bites and warts	Roberts, 1990; Hutchings, 1996; van Vuuren and Naidoo, 2010; Govindasamy and Srinivasan, 2012
Celosia trigyna Willd. ex Wall., Amaranthaceae	Woolflower	Leaves	Paste	Boils and skin complaints	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996
Centaurea benedicta (L.) L., Asteraceae	Holy thistle	Whole plant	Topical	Wounds and ulcers	van Wyk *et al.*, 2000
Centella asiatica (L.) Urb., Apiaceae	Pennywort	Leaves	Tinctures	Leprosy, wounds and acne	Boiteau, *et al.*, 1949; Smith, 1996; von Koenen, 1996; van Wyk *et al.*, 2000; Jagtap *et al*., 2009; Ullah *et al.*, 2009; Dash *et al*., 2011
Chenopodium ambrosioides Bert. ex Steud, Chenopodiaceas	Worm salt	Whole plant	Decoction	Eczema	Boily and van Puyvelde, 1986; Hutchings, 1996
Chironia baccifera L., Gentianaceae	Christmas berry	Whole plant	Topical	Leprosy, boils, acne and sores	Laidler, 1928; Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Thring, 2007
Chrysocoma ciliata L., Asteraceae	Beesbossie	Whole plant	Topical	Wounds	von Koenen, 1996; Ashafa and Afolayan 2009
Cinnamomum camphora (L.) J.Presl, Lauraceae	Camphor tree	Essential oil	Topical	Antiseptic, anti-inflammatory, anti-infective	van Wyk *et al.*, 2000
Cissampelos capensis Thunb. Menispermaceae	Davidjies	Rhizomes, roots and leaves	Paste	Boils, snakebite wounds, ulcers and syphilis sores	van Wyk *et al.*, 2000; Babajide *et al*., 2010

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Cissus quadrangularis L., Vitaceae	Veldt grape or devil's backbone	Shoots	Crushed shoots applied directly to wounds	Wounds	Murthy *et al*., 2003; Kashikar and George, 2006; Luseba *et al*., 2007; Mishra *et al*., 2009
Cnicus benedictus L., Asteraceae	Holy thistle	Whole plant	Paste	Wounds and ulcers	Bruneton, 1995; van Wyk *et al.*, 2000; Szabó *et al*., 2009
Combretum kraussii Hochst., Combretaceae	Forest bushwillow	Root	Topical	Dressing for wounds	Masoko *et al.*, 2007; van Wyk *et al.*, 2009
Combretum molle R.Br. ex G.Don, Combretaceae	Velvet bushwillow	Fresh or dry leaves	Topical	Dressing for wounds	Hutchings, 1996; van Wyk *et al.*, 2011; Masoko *et al*., 2007
Cotyledon orbiculata Forssk, Crassulaceae	Pig's ear	Leaf and leaf juice	Apply juice topically for warts removal, or place the hot leaf directly to the swollen part of the body.	Corns, warts, boils and anti-inflammatory	Watt and Breyer-Brandwijk, 1962; Rood, 1994; Bhat and Jacobs, 1995; Felhaber, 1997; van Wyk *et al.*, 2000
Crinum macowanii Baker, Amaryllidaceae	River lily	Bulbs and leaves	Topical	Sores, boils and acne	Smith, 1996; Rabe and van Staden, 1997; van Wyk *et al.*, 2000
Cucumis myriocarpus Naudin, Cucurbitaceae	Paddy melon	Raw fruit	Topical	Boils	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996
Datura stramonium L., Solanaceae	Jimson weed	Leaves	Skin patch	Boils, abscesses and wounds	Watt and Breyer-Brandwijk, 1962; Bruneton, 1995; Rabe and van Staden, 1997; van Wyk *et al.*, 2000; Priya *et al*., 2002; Saadabi and Moglad, 2011
Dichrostachys cinerea (L.) Wight & Arn., Fabaceae	Sickle bush	Bark	Topical	Abscesses and other skin conditions	Hutchings, 1996; Eisa *et al*., 2000
Dicoma anomala Sond. Asteraceae	Fever bush or stomach bush	Charred root, stems and leaves	Paste	Wounds, ulcers, ringworm and head sores	Boily and van Puyvelde, 1986; Hutchings, 1996
Dioscorea dregeana T.Durand & Schinz., Dioscoreaceae	Wild yam	Large fresh tubers	Decoction applied topically	Cuts and sores	Watt and Breyer-Brandwijk, 1962; Pujol, 1990; Kelmanson *et al*., 2000; van Wyk *et al*., 2000

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Diospyros mespiliformis Hochst. ex A.DC., Ebenaceae	African ebony	Roots and leaves	Decoction	Scars, skin rash, bruises, wounds and ringworm	von Koenen, 1996; van Wyk *et al*., 2011; Dangoggo *et al*., 2012; Shagal *et al*., 2012
Dodonaea angustifolia L.f., Sapindaceae	Sand olive	Leaves and tips of twigs	Decoction applied topically	Antipruritic, boils, and dressing for skin diseases of the head and face	Watt and Breyer-Brandwijk, 1962; Rood, 1994; Smith, 1996; van Wyk *et al.*, 2000; Teffo *et al*., 2010
Ekebergia capensis Sparrm., Meliaceae	Cape ash	Bark	Infusion	Abscesses, boils and acne	Pujol, 1990; Rabe and van Staden, 1997; van Wyk *et al.*, 2000; van Wyk *et al.*, 2011
Elephantorrhiza elephantina (Burch.) Skeels, Fabaceae	Eland's bean or elephant's root	Roots and rhizomes	Infusion applied topically, the root powder is sprinkled onto wounds and burns.	Acne, wounds, burns and other skin diseases	Pujol, 1990; van Wyk *et al.*, 2009; Mathabe *et al*., 2006
Embelia ruminata (E.Mey. ex A.Dc.) Mez, Myrsinaceae	Vidanga	Leaves	Tender leaf paste	Open wounds and leprosy	Kumara Swamy *et al.*, 2007; van Wyk *et al.*, 2009
Eriospermum abyssinicum Baker, Eriospermaceae	Cotton-seed lily	Leaves	Ointment	Wounds, ulcers, abscesses and boils	von Koenen, 1996
Erythrina lysistemon Hutch., Fabaceae	Common coral tree or lucky bean tree	Bark	Applied as poultice or powdered burnt bark for open wounds.	Sores, abscesses and open wounds	Coates Palgrave, 1977; Pujol, 1990; Hutchings, 1996; Rabe and van Staden, 1997; van Wyk *et al.*, 2000; Takahashi *et al*., 2004; More *et al*., 2008; van Wyk *et al*., 2011
Eucalyptus camaldulensis Dehnh., Myrtaceae	River red gum	Bark	Wash	Pimples	Hutchings, 1996; Babayi *et al*., 2004; Ayepola and Adeniyi, 2008; Musa *et al*., 2011
Euclea divinorum Hiern, Ebenaceae	Magic guarri	Roots and leaves	Topical	Skin rash and fresh bleeding wounds	Smith, 1996; von Koenen, 1996; Geyid *et al*., 2005; More *et al*., 2008
Ficus natalensis Hochst., Moraceae	Natal fig	Leaves	Hot compress	Wounds, boils, warts and growths	Hutchings, 1996; Rabe and van Staden, 1997; van Wyk *et al*., 2011
Ficus sur Forssk., Moraceae	Broom cluster fig	Bark	Compress	Boils	Palmer and Pitman, 1972; Hutchings, 1996
Galenia africana L., Aizoaceae	Yellowbush	Whole plant	Decoction	Wounds	Watt and Breyer-Brandwijk, 1962

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Glycyrrhiza glabra L., Fabaceae	Liquorice root	Rhizomes and roots	Topical	Anti-inflammatory, antipruritic and insect bites	Bruneton, 1995; van Wyk *et al.*, 2000; Motsei *et al*., 2003
Gnidia kraussiana Meisn., Thymelaeaceae	Yellow heads	Roots	Paste	Burns, small pox rash and boils	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Saadabi and Moglad, 2011
Graderia scabra Benth., Orobanchaceae	Pink ground-bells	Roots	Paste	Sores on the face	Hutchings, 1996
Grewia occidentalis L., Malvaceae	Crossberry	Bark	Bark soaked in hot water	Dress wounds	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Grierson and Afolayan, 1999
Guibourtia coleosperma (Benth.) J.Léonard, Fabaceae	African rosewood	Roots	Topical	Superficial skin scratches	von Koenen, 1996
Gunnera perpensa L., Gunneraceae	River pumpkin	Roots	Infusion	Dressing for wounds and psoriasis	Hutchings, 1996; Felhaber, 1997; Buwa and van Staden, 2006
Haemanthus coccineus L., Amaryllidaceae	Paintbrush lily or blood Flower	Leaves	Topical	Antiseptic for wounds and ulcers	von Koenen, 1996
Halleria lucida L., Scrophulariaceae	White olive	Unspecified parts	Topical	Skin complaints	Pooley, 1993; Hutchings, 1996; Adedapo *et al*., 2008
Harpagophytum procumbens DC. ex Meisn., Pedaliaceae	Devil's claw	Roots	Ointment	Sores, ulcers and boils	Watt and Breyer-Brandwijk, 1962; van Wyk *et al.*, 2000
Harpephyllum caffrum Bernh. ex Krauss Anacardiaceae	Wild plum	Bark	Topical	Acne and eczema	Pujol, 1990; van Wyk *et al.*, 2000; Buwa and van Staden, 2006; van Wyk *et al*., 2011
Helichrysum foetidum Moench, Asteraceae	Yellow everlasting	Leaves	Topical	Septic sores from circumcision wounds	Gerstner, 1938; Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Lourens *et al.*, 2004
Helichrysum odoratissimum Sweet, Asteraceae	Imphepho	Leaves	Ointment	Pimples	Hutchings and Johnson, 1986; Hutchings, 1996; Lourens *et al*., 2004
Hibiscus surattensis L., Malvaceae	Wild sour	Pounded leaf and stalk	Ointment	Inflammation, sores and skin irritation	Hutchings, 1996
Hoffmannseggia burchellii (DC.) Oliv., Fabaceae	Rush peas	Roots	Scrapings of fresh roots applied topically	Wounds	von Koenen, 1996

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Hypericum perforatum L., Hypericaceae	St. John's wort	Above ground parts	Topical	Wounds and first degree burns	Bruneton, 1995; van Wyk *et al.*, 2000; Saddiqe *et al*., 2010
Ilex mitis Radlk, Aquifoliaceae	Cape holly, African holly or waterboom	Ground bark	Paste or decoction	Skin rash and sores on the face	Hutchings, 1996; van Wyk *et al*., 2011
Ipomoea crassipes Hook., Convolvulaceae	One-day flower	Ground plant parts	Paste applied topically	Sores	Hutchings, 1996
Jasminum fluminense Vell., Oleaceae	Wild jasmine	Leaves and young shoots	Topical	Ulcers and boils	von Koenen, 1996
***Jatropha* curcas** L., Euphorbiaceae	Purging nut tree	Rhizomes	Topical	Wounds and boils	Hutchings, 1996; Perumal Samy *et al*., 1998; van Wyk *et al.*, 2000
Jatropha zeyheri Sond., Euphorbiaceae	Verfbol	Rhizomes or sap	Topical	Wounds, boils, open sores and burns	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Luseba *et al.*, 2007; van Wyk *et al.*, 2009
Kigelia africana (Lam.) Benth., Bignoniaceae	Sausage tree	Fruit	Topical	Ulcers, sores, abscesses and rheumatism	Watt and Breyer-Brandwijk, 1962; Coates Palgrave, 1977; Hutchings, 1996; van Wyk *et al.*, 2000; Shai *et al*., 2008; van Wyk *et al*., 2011
Lannea discolor Engl., Anacardiaceae	Live-long	Plant fibre	Fibre used as bandage	Wounds	Gelfand *et al.*, 1895; van Wyk *et al*., 2011
Lannea edulis Engl., Anacardiaceae	Wild grape	Bark	Applied topically	Boils and abscesses	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; van Wyk *et al.*, 2000
Lantana rugosa Thunb., Verbenaceae	Bird's brandy	Leaf, stem and ripe fruits	Paste	Festering sores and cuts	Smith, 1895; Roberts, 1990; Hutchings, 1996; Kelmanson *et al*., 2000; Suliman, 2010
Leonotis leonurus (L.) R.Br., Lamiaceae	Wild dagga or lion's tail	Leaves and stems	Decoction applied topically	Boils, eczema, itching and other skin diseases	Mabogo, 1990; Roberts, 1990; Pooley, 1993
Leontonyx angustifolius DC., Asteraceae	Beetbossie	Ointment	Topical	Ulcers	Watt and Breyer-Brandwijk, 1962; Lourens *et al.*, 2008
Lippia javanica Spreng., Verbenaceae	Lemon bush or fever tea	Leaves and roots	Paste	Skin diseases	Gelfand *et al.*, 1895; Hutchings, 1996; Samie *at al.*, 2005

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Lobostemon fruticosus H.Buek, Boraginaceae	Pajama bush	Leaves and twigs	Ointment	Wounds and other skin diseases	Smith, 1895; Watt and Breyer-Brandwijk, 1962; Rood, 1994; van Wyk *et al.*, 2000
Malva parviflora L. Malvaceae	Small mallow	Leaf	Hot leaf poultice	Inflammation and sceptic wounds	Watt and Breyer-Brandwijk, 1962; Grierson and Afolayan, 1999; von Koenen, 1996; Tadeg *et al.*, 2005
Matricaria nigellifolia DC., Asteraceae	Staggers weed	Leaves	Leaf infusion	Skin rash	Hutchings, 1996
Melia azedarach L., Meliaceae	China berry tree, bead-tree or cape lilac	Leaf, flower, bark and root	Ointment	Eczema and various skin	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Khan *et al.*, 2001; Sen and Batra, 2012
Melianthus comosus Vahl., Melianthaceae	Honey Flower	Leaves, leaf juice	Leaf poultice and leaf decoction. The leaf juice or paste is applied frequently for the treatment of wounds.	Bad sores, sceptic wounds, reduce swellings	Smith, 1895; Gerstner, 1938; Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Kelmanson *et al*., 2000
Melianthus major L., Melianthaceae	Giant honey flower	Leaves	Leaf poultice and leaf decoction	Sceptic wounds, sores and bruises	van Wyk *et al.*, 2009; Srividya and Sumithra, 2010
Mentha longifolia Huds., Lamiaceae	Wild mint	Leaves	Topical	Wounds	van Wyk *et al.*, 2000; Gulluce *et al.*, 2007
Momordica balsamina L., Cucurbitaceae	Balsam apple or african cucumber	Mashed fruit	Poultice and seeds in oil	Burns	Gerstner, 1938; Hutchings, 1996; Saadabi and Moglad, 2011
Myrothamnus flabellifolius Welw., Myrothamnaceae	Resurrection plant	Leaves and twigs	Dried powdered leaves applied topically	Burns and wounds	von Koenen, 1996; van Wyk *et al.*, 2000
Nymania capensis Lindb., Meliaceae	Chinese lanterns	Roots	Powder mixed with fat into an ointment	Wounds	von Koenen, 1996
Nymphaea caerulea Savigny, Nymphaeaceae	Blue water lily	Leaves and stems	Poultice	Skin rash and inflamed wounds	von Koenen, 1996
Ochna serrulata Walp., Ochnaceae	Mall-leaved plane or carnival ochna	Roots	Decoction applied topically	Gangrene infection	Bryant, 1996; Hutchings, 1996

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Opuntia ficus-indica Mill. Cactaceae	Prickly Pear	Leaves	Topical	Skin rash, ulcers, furuncles, fresh wounds and warts	Smith, 1996; von Koenen, 1996
Opuntia vulgaris Mill., Cactaceae	Drooping prickly pear	Plant juice	Topical	Warts	Smith, 1996; von Koenen, 1996
Osmitopsis asteriscoides Cass., Asteraceae	Mountain daisy or bellis	Leaves	Topical	Inflammation and cuts	van Wyk *et al.*, 2000; Viljoen *et al.*, 2003
Ozoroa engleri R.Fern. & A.Fern., Anacardiaceae	White resin tree	Bark, roots and leaves	Topical	Acute inflammation	Pooley, 1993; Hutchings, 1996
Pelargonium alchemilloides (L.) L'Hér., Geraniaceae	Wilde malva	Leaves	Leaf paste	Wounds and abscesses	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Shale *et al.*, 1999
Pellaea calomelanos Link, Adiantaceae	Hard fern	Leaves and rhizomes	Decoction or infusions applied topically	Boils and abscesses	Watt and Breyer-Brandwijk, 1962; Pujol, 1990; Hutchings, 1996; van Wyk *et al.*, 2000; Braithwaite *et al.*, 2008
Pentanisia prunelloides Walp., Rubiaceae	Wild verbena	Roots	Applied topically	Burns and swellings	van Wyk *et al.*, 2000; Yff *et al.*, 2002
Phyllanthus reticulatus Lodd., Euphorbiaceae	Potato bush or roast potato plant	Leaves	Powered leaf applied topically	Sores, burns and skin irritations	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Ram *et al.*, 2004
Phytolacca americana L., Phytolaccaceae	Ink berry or pigeon berry	Leaves	Paste applied topically	Wounds and swellings	Hutchings, 1996
Phytolacca octandra L., Phytolaccaceae	Red inkplant or pokeweed	Leaves	Paste applied topically	Septic wounds	Hutchings, 1996
Plantago afra L., Plantaginaceae	Ribwort plantain, black psyllium or flea-seed plant	Leaves	Ointment	Suppurating wounds, pustules, eczema, furuncles and itching	Hutchings, 1996
Priva cordifolia Druce, Verbenaceae	Heart-leaf velvet bur or heart-leaf priva	Ground seeds	Topical	Sores and wounds	Hutchings, 1996

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Psidium guajava L., Myrtaceae	Guava	Leaves	Infusions applied topically	Boils, ulcers and wounds	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; van Wyk *et al.*, 2000; Gutiérrez *et al.*, 2008; Abubakar, 2009; van Vuuren and Naidoo, 2010; van Wyk *et al.*, 2011
Rauvolfia caffra Sond. Apocynaceae	Kinaboom or quinine Tree	Bark	Topical	Apply to measles, urticaria and other skin rashes	Gerstner, 1938; Bryant, 1996; Hutchings, 1996; McGaw *et al*., 2000
Ricinus communis L., Euphorbiaceae	Caster bean tree	Leaf, burnt-pulverized seeds and bark	Applied as poultice	Wounds, sores and boils	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; van Wyk *et al.*, 2000; Luseba *et al*, 2007; Malik *et al*., 2011
Rothmannia capensis Thunb., Rubiaceae	Candlewood	Sap from fruit	Topical	Burns and wounds	Arnold and Gulumian, 1984; Hutchings, 1996; Steenkamp *et al*., 2007
Rumex lanceolatus Thunb., Polygonaceae	Common dock	Leaves	Topical	Abscesses, boils, bruises and tumours	Watt and Breyer-Brandwijk, 1962; Pujol, 1990; Hutchings, 1996; van Wyk *et al.*, 2000
Sarcostemma viminale (L.) R.Br., Asclepiadaceae	Caustic bush or rapunzel plant	Whole plant	Latex	Skin lesions, cuts, ulcers and septic wounds	von Koenen, 1996; Luseba *et al*, 2007
Scabiosa columbaria L., Dipsacaceae	Wild scabious or butterfly Blue	Leaves and roots	Ointment	Wounds	von Koenen, 1996; van Wyk *et al.*, 2000; van Vuuren and Naidoo, 2010
Scadoxus puniceus (L.) Friis & Nordal, Amaryllidaceae	Red paintbrush or paintbrush Lily	Bulbs and roots	Decoction applied topically	Wounds, ulcers, sores and allergies	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; van Wyk *et al.*, 2000
Scilla natalensis Planch., Hyacinthaceae	Blue squill or wild squill	Bulb	Applied topically	Boils and sores	Roberts, 1990; Hutchings, 1996; Rabe and van Staden, 1997; van Wyk *et al.*, 2000; Ghuman and Coopoosamy, 2011
Securidaca longepedunculata Fresen., Polygalaceae	Violet tree	Leaves and bark	Ointment	Wounds and sores	Hutchings, 1996; van Wyk *et al.*, 2000
Senecio concolor DC., Asteraceae	Idambiso or ibohlololo	Leaves	Paste	Cuts and wounds	Smith, 1895
Senecio latifolius DC., Asteraceae	Dan's cabbage, groundsel or ragwort	Leaves	Paste	Burns and wounds	Smith, 1895

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Senecio serratuloides DC., Asteraceae	Two day cure	Leaves and stems	Topical	Cuts, swelling, burns and sores	Pujol, 1990; Bhat and Jacobs, 1995; Hutchings, 1996; Kelmanson *et al.*, 2000
Senna italica Mill., Fabaceae	Wild senna	Roots	Topical	Wounds, burns and furuncles	Hutchings, 1996; Dabai *et al.*, 2012
Sida dregei Gand., Malvaceae	Spider leg	Leaves	Leaf paste	Sores	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Kelmanson *et al*., 2000
Sonchus oleraceus L., Asteraceae	Milk thistle sowthistle or smooth sow thistle	Whole plant	Ointment	Wounds and ulcers	Watt and Breyer-Brandwijk, 1962; Jimoh *et al.*, 2011
Solanum capense L., Solanaceae	Nightshade	Squashed berries	Topical	Warts and ringworm infected skin	von Koenen, 1996
Solanum giganteum Jacq., Solanaceae	Healing-leaf tree, red bitter-apple, red bitter-berry, thorny bug-tree	Leaves	Ointment	Festering sores	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996
Solanum hermannii Dunal, Solanaceae	Umthuma	Fruit sap, leaf paste and roots	Leaf paste or ointment	Wounds, boils and non-specific skin infections	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996
Solanum incanum Ruiz & Pav., Solanaceae	Bark weed, bitter apple	Leaves and roots	Topical	Wounds, furuncles and ringworm	Gerstner, 1938; Hutchings, 1996; von Koenen, 1996; Hamza *et al.*, 2006
Solanum nigrum L., Solanaceae	Black nightshade	Whole plant	Topical	Wounds, ulcers, septic pimples, furuncles, and ringworm	von Koenen, 1996; Malik *et al.*, 2011
Solanum panduriforme Drège ex Dunal, Solanaceae	Bitter apple	Sap	Topical	Non-specific skin infections	Hutchings, 1996; More *et al.*, 2008
Solanum tomentosum L., Solanaceae	Slang apple	Fruit	Topical	Non-specific skin infections	Batten and Bokelmann, 1966; Hutchings, 1996; Aliero and Afolayan, 2006

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Spermacoce natalensis Hochst., Rubiaceae	Insulansala	Roots	Topical	Febrile rash	Bryant, 1996; Hutchings, 1996
Stephania abyssinica Walp., Menispermaceae	Umbamba	Powdered roots	Decoction	Boils	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Geyid *et al.*, 2005
Terminalia sericea Burch. ex DC., Combretaceae	Silver cluster-leaf or silver *Terminalia*	Root sap or bark	Topical	Antiseptic for wounds, leprosy and snakebites	Watt and Breyer-Brandwijk, 1962; Pujol, 1990; Hutchings, 1996; Rabe and van Staden, 1997; van Wyk *et al.*, 2000; Moshi and Mbwambo, 2005; van Vuuren and Naidoo, 2010
Thespesia acutiloba (Baker f.) Exell & Mendonça, Malvaceae	Wild tulip tree	Bark	Topical	Skin ailments	Jenkins, 1987; Hutchings, 1996
Trichilia emetica Vahl, Meliaceae	Natal Mahogany	Leaves or fruits	Poultice	Bruises, eczema and wounds	Adeniji *et al*., 1998; Germanò *et al*., 2005; Geyid *et al*., 2005; Shai *et al.*, 2008; Komane *et al*., 2011; van Wyk *et al*., 2011
Turbina oblongata (E.Mey. ex Choisy) A.Meeuse, Convolvulaceae	Ubhoqo	Leaves	Topical	Sores and abscesses	Pujol, 1990; Hutchings, 1996
Venidium arctotoides Less., Asteraceae	Bitter gousblom or Ubushwa	Leaves	Leaf paste	Wounds	Smith, 1895
Vernonia adoensis Sch.Bip. ex Walp., Asteraceae	Inyathelo	Flowers	Topical	Scabies and other skin diseases	Pujol, 1990; Hutchings, 1996; Chitemerere and Mukanganyama, 2011
Viscum capense L.f., Santalaceae	Cape mistletoe	Whole plant	Topical	Warts and other skin complaints	Hutchings, 1996; Amabeoku *et al.*, 1998
Waltheria indica L., Malvaceae	Sleepy Morning, velvet leaf or marsh-mallow	Roots	Topical	Cleaning wounds	von Koenen, 1996; Olajuyigbe *et al*., 2011
Warburgia salutaris (Berto.f.) Chiov., Canellacea	Pepper-bark tree or fever tree	Bark	Topical	Skin complaints	Hutchings, 1996; Rabe and van Staden, 1997
Withania somnifera (L.) Dunal, Solanaceae	Poison gooseberry or winter cherry	Leaves and berries	Ointment	Open cuts, wounds, abscesses and inflammation	Watt and Breyer-Brandwijk, 1962; Boily and van Puyvelde, 1986; Pujol, 1990; Hutchings, 1996; van Wyk *et al.*, 2000; Malik *et al*., 2011; Saadabi and Moglad, 2011

Botanical name/ family	Common name	Parts used	Modes of administration	Healing properties	References
Ximenia caffra Sond., Olacaceae	Large sour plum	Roots	Topical	Wound that are difficult to heal, septic sores	von Koenen, 1996; Fabry *et al*., 1998; van Wyk *et al.,* 2011
Xysmalobium undulatum R.Br. Apocynaceae	Milk bush, milkwort, uzura or wild cotton,	Roots	Powder applied topically	Sores, wounds and abscesses	Watt and Breyer-Brandwijk, 1962; Pujol, 1990; Hutchings, 1996; Rabe and van Staden, 1997; van Wyk *et al.*, 2000; Buwa and van Staden, 2006
Zantedeschia aethiopica Spreng., Araceae	Arum lily or calla lily	Leaves	Leaf applied directly	Wounds, boils and sores	Watt and Breyer-Brandwijk, 1962; Rood, 1994; van Wyk *et al.*, 2000; Nielsen *et al*., 2012
Zanthoxylum capense Harv. Rutaceae	Small knobwood	Leaves	Topical	Sores	Bryant, 1996; Hutchings, 1996; Buwa and van Staden, 2006
Ziziphus mucronata Willd., Rhamnaceae	Buffalo-thorn	Leaves, roots and bark	Decoction applied topically	Boils, sores and swellings	Watt and Breyer-Brandwijk, 1962; Rood, 1994; Hutchings, 1996; Rabe and van Staden, 1997; Luseba *et al*, 2007; van Wyk *et al.*, 2000; van Wyk *et al*., 2011;

An in-depth examination of these medicinal plants used to treat various skin ailments shows that wound healing was the most prevalent (39%) treatment regimen followed by infectious diseases (30%) (Figure 1.1). These may comprise of bacterial (e.g. acne, boils, abscesses and leprosy), fungal (e.g. ringworm) or viral (e.g. shingles and measles) to a lesser extent. Some medicinal plants (25%) are indicated for the treatment of necrotising skin ailments such as sores and ulcers. Abnormalities of the epidermis (rashes, eczema, skin irritation, psoriasis, cancer and tumours) account for 17% of southern African medicinal plants, while other skin diseases (either unspecified or are as a result of indirect ailments caused by either insect or snake bites) are treated by 9% of the plant species. Less attention has been given to plants used for the treatment of burns and anti-inflammatory conditions (9%), with about 6% indicated for surface abrasions (bruises, lesions, cuts and scars). Only a minority (4%) of the plants were used to treat growths such as warts or corns on the skin (Figure 1.1). Upon further examination of the southern African plants used to treat skin diseases (Table 1.1), an overview of the plant parts used (Figure 1.2) and modes of administration (Figure 1. 3) are hereafter discussed.

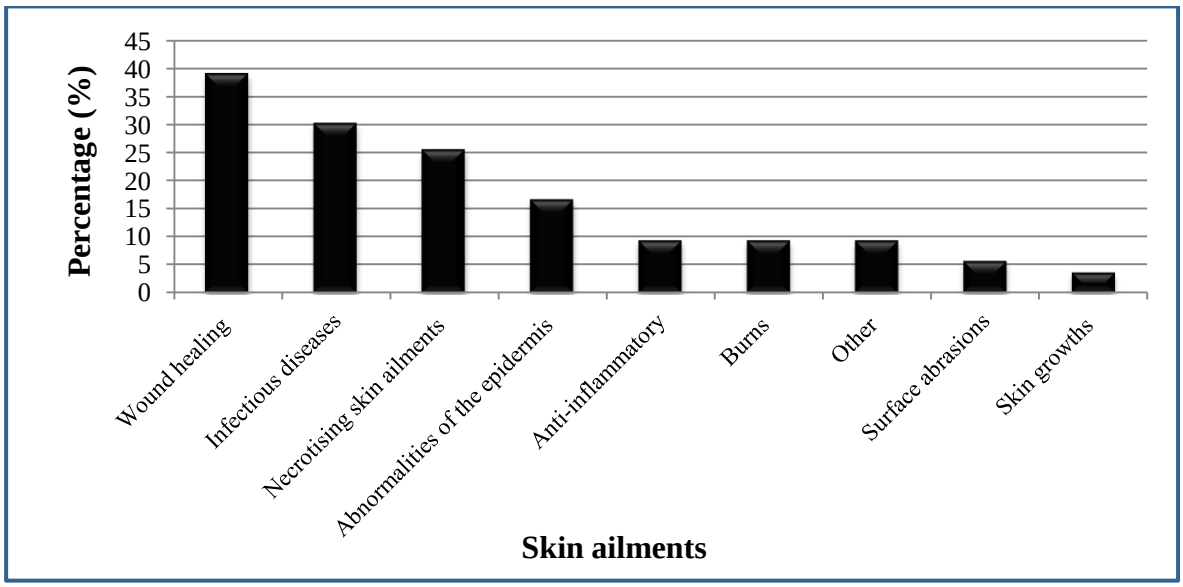


Figure 1.1: Percentage use of southern African medicinal plant against various skin ailments.

1.3.1 Plant parts used to treat skin diseases

Not surprisingly, it was found that the leaves are the most frequently used part of the plant, accounting for 43% (Figure 1.2). Many other indigenous communities worldwide, utilize mostly leaves for the preparation of traditional medicines. This was congruent with results obtained from other countries such as India, whereby leaves account for 50% of the plant parts used by Kani tribes in the Tirunelveli hills of Western Ghats (Ayyanar and Ignacimuthu, 2011). This is mainly due to the ease of harvesting of leaves compared to the underground parts or fruits and flowers (which only grow seasonally). Leaves are mainly active in photosynthesis and the production of metabolites which may be responsible for the synthesis of pharmacologically active compounds (Ayyanar and Ignacimuthu, 2011) hence they are mostly utilized. Roots are the second most frequently used part of the plant (22%) used for the treatment of skin diseases. This choice is less surprising considering difficulties encountered with unsustainable harvesting and plant destruction. Following this category is bark (11%), whole plant (7%), unspecified parts (5%) and fruits, rhizomes, bulb and flowers, which all account for less than 5%.

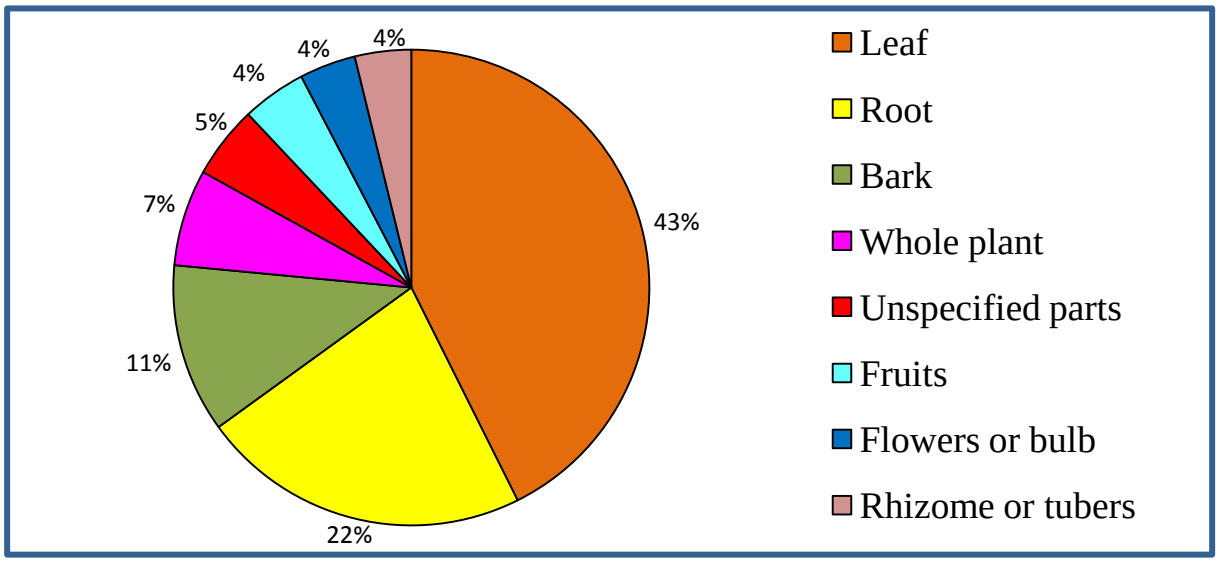


Figure 1.2: Plant parts used to treat skin inflictions in southern Africa.

1.3.2 Method of preparation and mode of administration

According to the recorded ethnobotanical literature (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; von Koenen, 1996; Felhaber, 1997; Rabe and van Staden, 1997; van Wyk *et al*., 2000; van Wyk *et al*., 2009), medicinal plants used for the treatment of skin ailments may be administered as a powder (leaves, root, bark or wood) sprinkled directly to the skin, used as a paste, saturated as a sap, ointment, poultice/compress, made into a leaf extract, decoction or infusion. Mostly the plants are prepared in an aqueous solution, as the traditional healers or lay people do not usually have access to lipophilic solvents. When organic solvents are required for preparation, alcohols such as ethanol are usually sought for extraction processes, as these are relatively inexpensive and freely available (Louw *et al*., 2002). Many preparations are poorly described (unspecified, 47%), whereas other preparations such as pastes (12%) and decoctions or infusions (19%) have been described in detail within the readily available ethnobotanical literature (Table 1, Figure 1.3). A decoction refers to the process of boiling any plant material in water or any other solvent, with the aim of extracting active substances. The liquid can then be used to cleanse wounds, and act as an antiseptic or applied to skin rashes. The preparation of infusions involves submerging the plant material in boiled or cold water for a specified period, which is then strained before use (von Koenen, 1996). This formulation is relatively simple and easy to prepare, hence it is the most frequently used.

The use of ointments and plant poultices account for 8% of the preparations. Usually, a heated mass of plant material is used in the form of a dressing, as either a cold or hot compress and applied directly to the affected area (Hutchings, 1996; van Wyk *et al.*, 2000). Plant powder (4%) and leaf sap or juice (2%), are less frequently used preparations adopted for skin disease management.

These preparations are applied topically as a poultice, ointments or decoctions. The ethnobotanical literature (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; von Koenen, 1996; Felhaber, 1997; Rabe and van Staden, 1997; van Wyk *et al*., 2000; van Wyk *et al*., 2009) have reported that topical application to the skin is the most common route used for treating skin infections, as this ensures quick and direct contact of the specific plant compounds to the site of action.

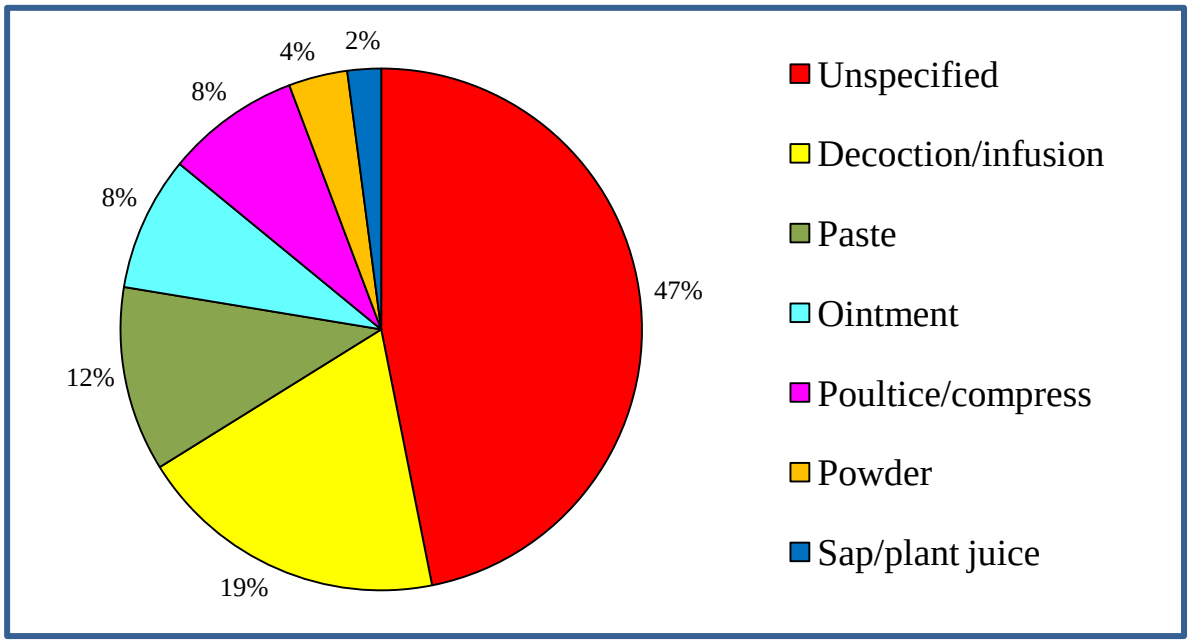


Figure 1.3: Modes of administration of medicinal plants used to treat skin ailments in southern Africa.

1.3.3 Plant combinations used to treat skin ailments

Another aspect sorely neglected in the antimicrobial investigations of plants used to treat skin infections is the investigation of pharmacological interactions in plant combinations. The therapeutic value of synergistic interactions has been known since antiquity and the African cultural healing system still relies on this principle in the belief that combination therapy may enhance efficacy. Without adequate validation, the ethnopharmacological information obtained will remain unchallenged. A number of plant combinations used to

treat various skin diseases (Table 1.2) have been reported (Smith, 1895; Hutchings, 1996; Felhaber, 1997), yet very few studies have been conducted to validate these claims. Some of the plants used in combination are also used individually to treat skin ailments (Table 1.1). However, some plant species such *Acorus calamus, Cyathula natalensis, Cyanella lutea, Hypoxis latifolia, Momordica foetida, Pittosporum viridiflorum* and *Vernonia natalensis* which are reportedly used in the combinations do not have any known dermatological relevance when used independently. Some plant species for e.g. *Pittosporum viridiflorum* and *Vernonia natalensis* are traditionally used to alleviate fever (Hutchings, 1996; van Wyk *et al*., 2009), a symptom very often present in bacterial infections. Hence, inclusion of these plants in a combination may be for the alleviation of other additional symptoms.

Some antimicrobial combination studies focusing on medicinal plants of South African origin such as, *Salvia chamelaeagnea* combined with *Leonotis leonurus*, *Artemisia afra* with *Eucalyptus globulus* and *Hypoxis hemerocallidea* with *Merwilla plumbea* (Kamatou *et al*., 2006; Suliman *et al*., 2010; Ncube *et al*., 2012), have been undertaken. However, specific attention has not been given to skin relevant pathogens such as *Propionibacterium acnes*, *Microsporum canis*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*, which have dermatological importance.

1.4.1 Antimicrobial properties of medicinal plants against skin relevant pathogens

While numerous studies have investigated the antimicrobial properties of many southern African medicinal plants used for a variety of ailments, relatively few have addressed the antimicrobial efficacies of plant species against dermatologically relevant pathogens.

Table 1.2
Plant combinations used in southern Africa for the treatment of skin ailments.

Combination	Parts used	Medicinal uses	Administration	Reference
Pelargonium alchemilloides + Malva parviflora	Leaves	Wounds and abscesses	Paste	Smith, 1895
Cyanella lutea + Leontonyx angustifolius	Unspecified parts	Boils, carbuncles and abscesses	Ointment	Smith, 1895; Watt and Breyer-Brandwijk, 1962
Cyanella lutea + Leontonyx angustifolius + Galenia africana + Lobostemon fruticosus + Melianthus comosus + Melianthus major	Unspecifies	Dressing to wounds	Ointment	Smith, 1895; Watt and Breyer-Brandwijk, 1962
Momordica foetida + Pittosporum viridiflorum + Vernonia natalensis	Roots or leaves	Boils	Decoctions	Watt and Breyer-Brandwijk, 1962; Hutchings, 1996
Combretum kraussii + Terminalia sericea	Roots	Wounds	Mixed and applied topically	Hutchings, 1996
Trichilia emetica + Cyathula natalensis	Stem fruit, seeds	Leprosy	Ointment	
Warburgia salutaris + Hibiscus surattensis	Leaves and stalk	Anti-inflammatory, sores and skin irritation	Lotion	
Elephantorrhiza elephantina + Dicoma anomala	Tubers	Acne	Externally	Felhaber, 1997
Elephantorrhiza elephantina + Pentanisia prunelloides	Tubers	Eczema	Externally	
Pentanisia prunelloides + Dicoma anomala	Tubers	Insect and sting bites	Externally	
Pentanisia prunelloides + Dicoma anomala + Hypoxis latifolia	Tubers, bulbs	Insect and sting bites	Taken orally	
Gunnera perpensa + Cassine transvaalensis	Rhizomes, bark	Psoriasis	Taken orally	
Pentanisia prunelloides + Jatropha zeyheri + Warburgia salutaris	Tubers, roots, bark	Cuts, bruises, blisters and burns	Taken orally	
Warburgia salutaris + Cassine transvaalensis + Acorus calamus	Bark, rhizomes	Cold sores and shingles	Taken orally	

Table 1.1 highlights (in bold) certain plant species traditionally used for dermatological purposes which have been included in various southern African antimicrobial studies incorporating limited skin relevant pathogens such as, *Staphylococci* species, *P. aeruginosa* and *C. albicans* using either disc diffusion and/or minimum inhibitory concentration assays (references given in Table 1.1).

The correlation between skin ailments and pathogens such as *Microsporum canis*, *Trichophyton mentagrophytes, Epidermophyton floccosum,* species have seldom been addressed with only a few antimicrobial studies related to South African plant extracts, such as Masoko *et al*. (2005); Masoko *et al*. (2007); Shai *et al.* (2008); Ghuman and Coopoosamy (2011). The treatment of dermatophytes have been addressed in other medicinal plant studies further afield than southern Africa, where plant extracts were observed to possess antifungal effects (Ali-Shtayeh and Ghdeib, 1999; Webster *et al*., 2008; Mutai *et al.*, 2009; Sule *et al*., 2010; Bhadauria and Kumar, 2011; Beatriz *et al*., 2012).

Propionibacterium acnes is an important bacterial pathogen responsible for the chronic inflammatory disease of the sebaceous glands and hair follicles of the skin. Infections usually result in acne vulgaris, a skin condition common but not exclusive to teenagers and has considerable psychological impacts (Magin *et al.,* 2006). Similar to the dermatophytes, it has been rarely addressed in southern Africa with respect to medicinal plant studies. The *in vitro* antimicrobial and anti-inflammatory properties of medicinal plants against *P. acnes* have been investigated in a number of studies abroad (Chomnawang *et al*., 2005; Kim *et al*., 2007; Kim *et al*., 2008; Tsai *et al*., 2010; Balakrishnan *et al*., 2011). The relevance of *P. acnes* has also been detailed in a review on the traditional uses, phytochemistry and

pharmacology of *Psidium guajava* (Gutiérrez *et al*., 2008), yet little attention has been given to this pathogen when investigating southern African plants.

Some *Brevibacterium* spp. are implicated in producing the odour associated with foul-smelling feet. These micro-organisms have been rarely addressed in correlation with the antimicrobial properties of medicinal plants. One study was found, where the *in vitro* investigation of *Brevibacterium* spp was undertaken on the antibacterial activities for the essential oil and methanol extracts of *Ziziphora persica*, a Turkish folk medicinal plant used for various ailments including wound healing (Ozturk and Ercisli, 2006). The discovery of medicinal plants that have antimicrobial properties against this pathogen may pilot future more natural treatment alternatives for foot odour.

1.4.2 Wound healing effects of medicinal plants

Wound healing properties of medicinal plants have a strong correlation to infections of the skin. Addressing this, are various South African studies that have investigated wound healing remedies, taking into account the possible impact of bacterial infection (Grierson and Afolayan, 1999; Steenkamp *et al*., 2004; Fennell *et al.*, 2004; Luseba *et al*., 2007; Reuter *et al.*, 2010). A review by Dahanukar *et al*. (2000) on the pharmacology of medicinal plants and natural products details the wound healing efficacies of aqueous extracts of latex from *Euphorbia neriifolia* (Nivadung) topically applied to surgical wounds on guinea pigs. In addition, it details the wound healing effects of organic extracts (alcoholic, petroleum ether, chloroform, propylene glycol and glycosidal) of *Centella asiatica* used topically in various formulations (ointments, creams and gels) to treat open wounds on rat models where the gel formulation showed activity. Furthermore, the wound healing effects of four other plant extracts on both immunocompromised and healthy rats

i.e. *Aloe vera* (leaves); *Aegle marmelos* and *Moringa oleifera* (root and root bark) and leaves of *Tridax procumbens* have been identified (Dahanukar *et al*., 2000).

1.4.3 Anti-inflammatory effects of medicinal plants

While looking at the wound healing effects of the medicinal plants it is also important to consider the inflammatory processes involved in wound formation and many other skin conditions (urticaria, skin allergies, acne vulgaris, eczema and psoriasis). To validate the efficacy of southern African plants to treat inflammatory skin diseases, the anti-inflammatory properties have been addressed in some studies. A study by Pillay *et al*. (2001) identified the cyclo-oxygenase inhibiting and antibacterial activities of South African *Erythrina* species. Cyclo-oxygenase is an enzyme responsible for inflammatory processes expressed as two isomers COX-1 and COX-2, with COX-2 induced in inflamed tissue. *Erythrina* is a genus with approximately 120 species used across South African rural areas for a variety of ailments including the disinfection of wounds. The antibacterial, anti-inflammatory and antimutagenic effects of some medicinal plants used in South Africa for the treatment of wounds and retained placenta in livestock have been investigated by Luseba *et al*. (2007). The study by Luseba *et al*. (2007) identified the anti-inflammatory (cyclo-oxygenase-1 and-2 inhibition) effects of some of the South African medicinal plants used to treat skin diseases such as; *Cissus quadrangularis*, *Ricinus communis* and *Ziziphus mucronata*. Marnewick *et al.* (2005) identified the anti-oxidant, anti-inflammatory and antitumour properties of *Aspalathus linearis* and the respective chemical compounds. In addition, Frum (2006) investigated the *in vitro* inhibitory effects against 5-lipoxygenase and anti-oxidant activities of South African medicinal plants commonly used topically to treat skin diseases. The study included plants such as; *Aloe ferox*, *Artemisia afra*, *Bulbine* species, *Carpobrotus edulis*, *Cotyledon orbiculata*, *Datura stramonium*, *Halleria lucida*,

Harpagophytum procumbens, Helichrysum odoratissimum, Kigelia africana, Leonotis leonurus, Lippia javanica, Melianthus comosus, Pentanisia prunelloides, Rauvolfia cafra, Rothmannia capensis, Scilla natalensis, Trichilia emetica, Warburgia salutaris and *Ziziphus mucronata*. *Melianthus comosus* was noted as the most active with an IC_{50} value of 13.84 ± 1.18 ppm displaying the strongest 5-lipoxygenase inhibitory effects.

1.4.4 Toxicity effects of medicinal plants

Plants used for therapeutic purposes are normally assumed to be safe and free of toxicity. This is mainly due to the long term use of medicinal plants for the treatment of diseases based on basic knowledge accumulated and shared from generation to generation over many centuries. However, recent scientific studies have highlighted the toxic, mutagenic and carcinogenic effects of many plants used as traditional medicine (Fennell *et al*., 2004). Medicinal plants used to treat skin ailments are known to produce adverse effects such as allergic reactions, phytodermatitis, and a high risk of photosensitization. The evidence based review on botanicals in dermatology by Reuter *et al*. (2010) identifies certain medicinal plants which have been used for dermatological purposes, which have also been reported for their toxic effects. These include *Euphorbia helioscopia, Citrus bergamia, Matricaria recutita, Inula helenium* and *Tanacetum parthenium.* An ethnopharmacological study by Fennell *et al*. (2004) identified over 50 African medicinal plants which were screened for their safety and efficacy through analysing their pharmacological and toxicology effects. The toxic effects of the plants were investigated using the Ames test (*in vitro* bacterial and mammalian cells assay), micronucleus test (white blood cell chromosomes) and comet test (DNA damage). Amongst these, are medicinal plants used in South Africa for the treatment of skin infections such as; *Boophane disticha, Catharanthus roseus, Crinum macowanii, Kigelia africana*, *Ochna serrulata, Scilla natalensis, Trichilia*

emetica and *Ziziphus mucronata*, which were found to have some level of toxicity. Skin irritation tests done on mice models with *Aloe ferox* and *Aloe arborescens* showed no irritation on both damaged and healthy skin (Jia *et al*., 2008). In a study by Steenkamp and Gouws (2006) which investigated the cytotoxicity effects of South African medicinal plants it was identified that *Centella asiatica* and *Cnicus benedictus* did not exhibit any cytotoxic effects against cancer cell lines. Mapunya *et al*. (2012), highlighted the toxicity effects of *Harpephyllum caffrum* and *Aloe arborescens* against melanocytes, when examining these plants as skin-lightners. *Sideroxylon inerme,* another plant used for skin-lightening purposes has been reported to have toxicity effects against melanocytes (Momtaz *et al.,* 2008). Studies such as these provide some insight into plants used to treat hyperpigmentation of the skin. The use of plants as topical agents for cosmetic, skin-lightning potential and other traditional applications have not been discussed in detail in this study. Since the toxicity of some medicinal plants may result in the mutagenicity of cells, it is essential that the effects of medicinal plants be investigated for these side effects. A study by Verschaeve and van Staden (2008) investigated the mutagenic and antimutagenic effects of South African plants, including species with dermatological relevance such as *Boophane disticha, Crinum macowanii, Harpephyllum caffrum, Acokanthera oblongifolia, Catharanthus roseus, Xysmalobium undulatum, Artemisia afra, Senecio serratuloides, Kigelia africana, Warburgia salutaris, Dioscorea dregeana, Euclea divinorum, Ricinus communis, Ekebergia capensis, Trichilia emetica, Ochna serrulata, Ziziphus mucronata,* and *Datura stramonium*.

1.4.5 Phytochemical investigations

Due to the increasing resistance of pathogens to conventional antimicrobials, plant compounds are of interest as antiseptics and alternative microbial substances (Gibbons,

2005; Weckesser *et al*., 2007; Ayyanar and Ignacimuthu, 2011). The development of modern medicine relies greatly on plant bio-active compounds. According to the WHO (2011), it is estimated that at least 25% of the prescribed conventional medicines used worldwide are derived from medicinal plants.

To fully comprehend the pharmacological properties of medicinal plants it is important to understand the phytochemistry. This involves the investigation of secondary metabolites of plants as they are considered to be responsible for many biological activities including antibacterial and antifungal effects (Lall *et al*., 2006; Aremu, 2009). A review by George *et al.* (2001) revealed that by 2001 about 350 of 3000 South African plant species used for medicinal purposes, had been investigated for their phytochemical properties. Interest in bio-prospecting and development of new treatment alternatives has inspired research in this field, however, the elucidation of new phytochemicals is timely and expensive (George *et al.*, 2001). A number of phytochemical studies have since been extensively dedicated to investigating medicinal plants and these also highlight plants used for dermatological purposes. Some of these studies include, van der Watt and Pretorius, 2001 (*Carpobrotus edulis*); Louw *et al*., 2002 (*Boophane disticha*); Fennell *et al*., 2004 (*Warburgia salutaris, Erythrina lysistemon, Kigelia africana*); Gutiérrez *et al*., 2008 (*Psidium guajava*); Joubert *et al*., 2008 (*Aspalathus linearis*); McGaw *et al*., 2008 (*Scadoxus puniceus, Rauvolfia caffra, Artemisia afra, Athrixia phylicoides, Dicoma anomala, Mentha longifolia, Zizipus mucronata, Lippia javanica, Dodonaea angustifolia, Solanum incanum*); Moolla and Viljoen, 2008 (*Agathosma betulina*); Aremu, 2009 (*Acokanthera oppositifolia, Cotyledon orbiculata*) and Abbasi *et al.*, 2010 (*Achyranthes aspera, Datura stramonium, Ricinus communis*). A review by van Vuuren (2008) also highlighted some phytochemistry studies where plants traditionally used to treat skin ailments. *Aloe ferox, Helichrysum* spp., *Gunnera perpensa* and *Terminalia sericea* were found to possess antimicrobially active

chemical compounds. While the phytochemistry of some medicinal plants has been explored, numerous plants still lack comprehensive scientific data to validate the pharmacological effects of the medicinal plants and their respective bio-active compounds. Thus, it is important to couple the phytochemical studies with antimicrobial investigations.

1.5 Pharmaceutical applications

There are a number of pharmaceutical products (mainly topical) used to treat skin diseases. The use of petroleum jelly and mineral oil as moisturisers are widely sought after and relied on by many communities in Africa. However, the use of petroleum jelly and mineral oil plays a vital role in the precipitation of skin ailments. Skin irritation and aggravation of existing inflammatory conditions, are common side effects, as these formulations tend to occlude the pores of sweat ducts and also precipitate any trapped microbial infections due to the favourable humid and warm conditions. As an alternative to petroleum jelly or mineral oil, ointments or creams are usually the formulations of choice. While creams are suitable for wet or acutely inflamed lesions, ointments are usually preferred for chronic, dry or lichenified lesions (van Hees and Naafs, 2001). Furthermore, ointments are said to be the ideal emollients with greater penetration and adherence to the skin, suitable for skin conditions such as chronic eczema, psoriasis and severe cases of fungal infections (van Hees and Naafs, 2001; Goswami *et al*., 2008).

Mupirocin (Bactroban®) and fusidic acid (Fucidin®) are pharmaceutical preparations indicated in the treatment of Staphylococcal infections and are available as cream or ointment preparations. Skin irritation is a common side effect of these agents (Gibbon, 2008). These are old topical pharmaceutical agents to which antimicrobial resistance is readily acquired (Gibbons, 2008). Metronidazole (Rozex®) (which is available in a gel

formulation), is a broad-spectrum antiprotozoal and antibacterial agent, indicated for the treatment of rosacea, and presents with skin irritation as a common side effect (Gibbon, 2008).

Pharmaceutical products used to treat fungal skin or yeast infections, are usually directed at skin infections such as candidiasis (*C. albicans*), tinea versicolor or pityriasis versicolor (*Malassezia furfur*) and dermatophytosis caused by micro-organisms such as *Trichophyton*, *Epidermatophyton* and *Microsporum* species. The pharmaceutical products available to treat these conditions include nystatin (e.g. Mycostatin®) creams and ointments used for candidiasis; a broad-spectrum antifungal clotrimazole (Canesten® cream) used for both candidiasis and dermatophytosis, and ketaconazole (Nizshampoo®) indicated for tinea capitis (van Hees and Naafs, 2001; Gibbon, 2008; Jamaloodien *et al*., 2012). While hypersensitivity reaction towards the antifungals is a rare side effect, local skin irritation may occur.

Common superficial inflammatory dermatoses such as atopic and seborrhoeic eczema, photosensitive reactions, psoriasis chronic discoid lupus, lichen lupus and alopecia areate are frequently managed by corticosteroids. These include diluted hydrocortisone agents (Dilucort® and Mylocort® creams or ointments) and high strength agents indicated for severe cases. Typical examples include, betamethasone (Betnovate® cream) and mometasone (Elocon® cream, ointment or lotion) (van Hees and Naafs, 2001; Gibbon, 2008; Jamaloodien *et al*., 2012).

Silver sulfadiazine (Silbecor®) is a chemotherapeutic topical cream used to treat infected leg ulcers, pressure sores and burns, with common side effects such as local skin discomfort and hypersensitivity reactions (Gibbon, 2008). In cases such as the clinical treatment of warts, the keratolytic agents used for removing hyperkeratinised skin and

warts such as high concentrated urea, salicylic acid or podophyllin are used with caution, as to avoid contact with healthy tissue. These preparations are also not used for facial or oral mucosal warts, due to the level of toxicity to normal skin cells (van Hees and Naafs, 2001; Gibbon, 2008).

Acne vulgaris is treated with topical preparations as the first line of treatment. Commonly used topical preparations include benzoyl peroxide (Benzac® AC5 gel or Clearasil® cream) which acts as a desquamating agent, while Azelaic acid (Skinoren® cream or gel) is used as a topical antimicrobial agent effective against *Propionibacterium acnes*. Clindamycin (Dalacin-T® lotion) and Erythromycin (Ilotycin TS® solution) are also topical antibiotics especially useful in inflammatory acne. The second line of treatment involves the use of Isotretinoin (Roaccutane®) and systemic antibiotics such as tetracyclines, erythromycin and co-trimoxazole which are usually sought for the management of intractable acne where the topical preparations are said to be ineffective (van Hees and Naafs, 2001; Gibbon, 2008; Jamaloodien *et al*., 2012). Isotretinoin or any other topical retinoid products are generally used as last options due to their long list of side effects ranging from photosensitivity, skin irritation to behavioural changes. The most detrimental side effects are congenital deformities or fatalities consequently to the teratogenic nature of the drug when given to pregnant subjects (van Hees and Naafs, 2001; Gibbon, 2008).

The use of conventional drugs in combination for the treatment of skin diseases is usually not recommended but used only in intractable or severe cases. For example, combination preparations containing corticosteroids and an antimicrobial agent are not recommended. Reason being, that corticosteroids lower resistance of the skin to bacterial and fungal infections and consequently counteract the effects of antimicrobials. However, certain conditions such as inflammatory dermatophyte infections (imidazole plus corticosteroids)

and infected eczema (quinoline antiseptic plus corticosteroid) may respond better to the combination therapy than the recommended monotherapy (van Hees and Naafs, 2001; Gibbon, 2008).

While looking at the numerous available pharmaceutical skin formulations it can be observed that there is increased prevalence of multi-drug resistance of pathologic micro-organisms as well as, undesired adverse effects, such as burning sensations, stinging, photo-hypersensitivity, skin irritation, and more severe, anaphylactic shock (van Hees and Naafs, 2001; Gibbon, 2008; Alviano and Alviano, 2009). Also the persistence of skin infections which leads to ongoing expensive conventional treatments as well as limited access to primary health care facilities (clinics) all are important factors that contribute to difficulties in treating skin diseases. The development of new products (where special attention has been given to natural products with dermatological relevance) can lead to potentially effective agents, which may additionally be less expensive and therefore more affordable to the majority of the economically underprivileged communities (Alviano and Alviano, 2009).

1.5.1 Topical versus orally administered formulations

The topical route of administration for plant derived skin formulations over the systemic administration (mainly oral) is a considerable advantage, taking into account the speed to market and smaller amount of data required (Gibbons, 2008). When developing a systemic drug, the pharmacokinetics profile needs to be analysed, hence there is a difference in cost and speed to market. Pharmacokinetics studies include, understanding the metabolism, absorption, distribution to the target site and excretion of the waste or drug metabolites from the body, with regard to systemic formulations. In contrast to the systemic

formulations, topical formulations bypass metabolism, are usually not intended for absorption and are directly applied to the site of action and since there is sometimes very minimal absorption, the excretion of the drug is usually not a major concern (Makoid *et al*., 1996; Gunaratna, 2001). However, analyses for therapeutic efficacy, as well as safety of topical formulation need to be addressed, as they may also present product-related side effects which are also notable for systemic formulation.

1.6 Commercial products containing botanical ingredients for skin ailments

A review by Semkina (2005) done in Russia on drug synthesis methods and manufacturing technology, highlights phyto-preparations that are used worldwide for the treatment and prophylaxis of versatile dermatological and cosmotological disorders. Mentioned in the review are ointment based chamomile extracts containing oil, sterol, flavonoids, tannins and polysaccharide compounds which exhibit prominent anti-inflammatory and antimicrobial properties effective in the treatment of infectious and non-infectious eczema and dermatitis.

The anmarin (1%) isolated from the *Ammi mayus* seeds; produces antimycotic activity against dermatophytes and yeast infections (*Trichophyton* species, *Microsporum canis* and *C. albicans*) and also has some bacteriostatic properties against Gram-positive bacteria. In addition, the anmarin drug is considered to have cosmetic benefits as it stimulates hair growth and produces keratolytic and epithelializing actions. It is also considered tolerable based on a clinical investigation conducted with 652 patients who confirmed high efficacy and good tolerance of the formulation, comparable to clotrimazole (Semkina, 2005).

In a review done by van Wyk (2008), it is highlighted that despite South Africa's rich ethnobotanical heritage few medicinal plants have been fully commercialised. However,

other plant species are still in the process of commercialisation (George *et al*., 2001, Weideman, 2005). A broad review of commercially important southern African medicinal plants identifies about 38 indigenous species which have been commercialised. The review incorporated plants which have dermatological importance, such as: *Agathosma betulina*, *Aloe ferox*, *Artemisia afra*, *Aspalathus linearis*, *Athrixia phylicoides*, *Bulbine frutescens*, *Carpobrotus edulis*, *Centella asiatica*, *Gunnera perpensa*, *Harpagophytum procumbens*, *Hypericum perforatum*, *Kigelia africana*, *Leonotis leonurus*, *Lippia javanica*, *Lobostemon fruticosus*, *Trichilia emetica*, *Warburgia salutaris*, *Withania somnifera* and *Xysmalobium undulatum* (van Wyk, 2008) some of these plants were also highlighted by George *et al*. (2001) and Vermaak *et al.* (2011).

Medicinal plants used for dermatological purposes both, traditionally and in the cosmetic industry are gaining more value, as many skincare products are now being supplemented with plant extracts. Examples of cosmetic products containing plant extracts include, the African Extracts™ Rooibos (*Agathosma betulina*) body care products (http://www.africanextracts.com/) and Alcare aloe® skin care products which contain *Aloe ferox* (http://www.aloe.co.za/). Furthermore, some of the commercialised medicinal plants are now developed for consumer use to treat skin ailments. These include, the Sausage Tree Cream® (*Kigelia africana*) marketed for its use to treat eczema, psoriasis and other skin ailments (http://www.faithful-to-nature.co.za/Sausage-Tree-Cream-Africana-Kigelia-p-92.html), as well as Puremedy® (containing *Hypericum perforatum*) which is used to treat a variety of skin ailments including wounds and skin infections (http://www.puremedy.com/calendula_stjohns.html). Figure 1.4 depicts some of the developed products which contain southern African medicinal plants used to treat skin ailments, as well as supplemented cosmetic items.



Figure 1.4: Commercial products containing southern African medicinal plants; A) Alcare aloe®; B) Sausage Tree Cream®; C) Puremedy®; D) African Extracts™ Rooibos.
http://www.aloe.co.za/
http://www.africanextracts.com/
http://www.faithful-to-nature.co.za/Sausage-Tree-Cream-Africana-Kigelia-p-92.html
http://www.puremedy.com/calendula_stjohns.html

1.7 Overview of this study

Despite the available scientific data, more research is still required to validate the antimicrobial effectiveness of numerous medicinal plants used traditionally to treat dermatological ailments. The antimicrobial effects of these plants need to be directed towards more fastidious skin pathogens, as these have been excluded from many screening studies. In addition to this, the therapeutic advantage of using plant combinations needs to be investigated in order to ascertain any potential interactive properties. The scientific

investigation findings of medicinal plant use for the treatment of various types of infections may therefore, enhance utilization of efficacious medicinal plant remedies and give rational validation for the use of these plants for therapeutic purposes (Weideman, 2005; Rukangira, 2012).

While ongoing research has uncovered the phytochemistry of numerous medicinal plants, it is still pivotal to establish an in-depth scientific evaluation of plants demonstrating noteworthy antimicrobial properties. This may provide insight for potential future compound synthesis directives.

1.7.1 Aims and objectives

The overall aim of the study was to investigate the antimicrobial effectiveness of southern African medicinal plants used for the treatment of skin diseases against pathogens of dermatological relevance. The following objectives were thus proposed;

- To complete a thorough literature review and compile an inventory of medicinal plants used to treat skin disorders.
- To collect relevant plant material and thereafter prepare aqueous and dichloromethane: methanol extracts.
- To implement the micro-titre plate dilution method in order to determine the minimum inhibitory concentration (MIC) of plant extracts against pathogens responsible for skin infections.
- To identify possible antimicrobial interactions of plant species by determining the fractional inhibitory concentrations index (ΣFIC) of 1:1 combinations. Also, to further investigate the interactive properties of plant extracts combined at various ratios.

- To implement a bio-activity guided isolation process (including column chromatography and high speed counter-current chromatography) in order to isolate the bio-active compound from a plant extract showing good antimicrobial activity and thereafter evaluate the antimicrobial effects of the pure compound using the micro-titre plate dilution method.

Chapter 2: Screening of antimicrobial efficacy of southern African medicinal plants against dermatologically relevant pathogens

2.1 Introduction

A review on the ethnobotanical literature revealed that more than 100 southern African medicinal plants are used to treat a variety of dermatological ailments, including boils, sores, bruises, septic wounds, acne vulgaris and ringworm infections (Table 1.1, Chapter 1). It was also noted that even though various studies have addressed the antimicrobial effects of medicinal plants, there is still limited scientific data to support claims made for the effectiveness of medicinal plants against some skin relevant pathogens. These findings incited an investigation of the *in vitro* antimicrobial properties of southern African medicinal plants against skin relevant pathogens. Therefore the aim of this part of the study was to investigate the antimicrobial effects of the organic (dichloromethane: methanol, 1:1) and aqueous extracts of the southern African medicinal plants against pathogens with dermatological relevance.

2.2 Materials and methods

2.2.1 Plant collection and identification

Plants were collected according to the current bio-prospecting legislation of the Biodiversity Act 10 of 2004, which aims at protecting threatened species and ecosystems, by ensuring sustainability of biological and indigenous resources and equity. None of the target species are included on the list of threatened or endangered species and hence a threatened or protected species permit was not required. The selection of the plants was

based on their reported traditional use for dermatological treatments and availability from various locations. Various plant parts such as leaf, fruit, stem, bark, rhizome, root, root bark and whole plant as reported in literature (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; von Koenen, 1996; Felhaber, 1997; Rabe and van Staden, 1997; van Wyk *et al*., 2000; van Wyk *et al*., 2009) to be used for skin ailments, were harvested. A total of 47 different plant species were collected from designated botanical gardens (Table 2.1). Plants available at Walter Sisulu Botanical Garden were harvested in collaboration with Andrew Hankey Asst. Curator South African National Biodiversity Institute, where conservative harvesting was permitted. Professor Sandy van Vuuren assisted with plant collection for plants obtainable from Zululand and those readily available from Wits Medical School Medicinal Gardens. Professor Alvaro Viljoen assisted with the identification of the plant species obtainable from Pretoria-Villieria, Pretoria National Botanical Garden, Magaliesburg, Haenertsburg, Rayton and Swaziland. Table 2.1 depicts all the collected plant species including the parts collected and the location from which they were harvested (Figure 2.1). Voucher specimens were prepared for each species and are housed in the Department of Pharmacy and Pharmacology, University of the Witwatersrand.

2.2.3 Preparation of organic (dichloromethane: methanol) solvent extracts

To prepare extracts, the collected plants were separated according to the part that is traditionally used to treat skin inflictions (leaf, root, bulb, bark or rhizome) and were left to dry at room temperature for a minimum of two weeks. Plants with succulent leaves, fruits and tubers were dried in the oven (Memmert) at 37 °C for 24 hours. They were ground using a pestle and mortar and then into finer powder using the Variable Speed Rotor Mill Pulverisette (Fritsch). Extracts were prepared by submerging (±20 g) the dried, crushed

plant material in a 1:1 mixture of dichloromethane and methanol (DCM: MeOH) (Merck) and left in the platform shaker incubator (Labcon) at 37 °C for 24 hours (Figure 2.1). The samples were re-extracted with a fresh solvent for another 24 hours and thereafter filtered and left in a fume hood for the solvent to evaporate.

2.2.4 Preparation of aqueous extracts

The use of volatile solvents by phytochemists is far removed from the traditional application of these medicinal plants where indigenous people mainly utilize water for extraction procedures (Frum, 2006). To eliminate the discrepancies between the traditional and scientific perspective, it was pivotal to include aqueous extracts in this study which would allow for comparison between the two types of extracts for their antimicrobial efficacies. Aqueous extracts were prepared by submerging the macerated plant material in sterile distilled water, and these were then left in the platform shaker incubator, overnight at ambient temperature. Thereafter the liquid extracts were filtered and stored at -80 °C before lyophilisation. Aqueous extracts were lyophilised using a freeze dryer (Virtis) for a minimum of 72 hours to seven days. Before use, the aqueous extracts were left under UV light overnight to eliminate possible microbial contaminants. All plant samples were stored in sealed bottles at room temperature until further use.

2.3 Screening of medicinal plants for antimicrobial properties

2.3.1 Preparation of plant samples for antimicrobial testing

Samples used for the minimum inhibitory concentration (MIC) assays were prepared by weighing out the crude extracts and calculating the volume of solvent to be added in order to create a sample concentration of 64 mg/ml. Based on recommendations by Eloff (1998a)

acetone was used as the solvent of choice as it has minimal antimicrobial effects. In cases where the plants were insoluble in acetone, 50% DMSO (diluted with water) were used.



Collection of plant material



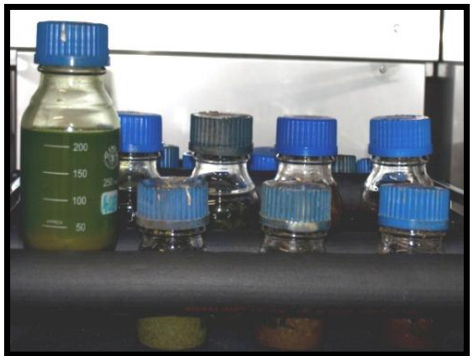
Plant grinding with pestle and mortar



Plant grinding to finer powder



Weighing of plant sample for extraction



Liquid plant extracts in platform shaker

Figure 2.1: Collection of plant material and preparation of extracts from various plant parts using organic and aqueous solvents.

Table 2.1

Plant name, plant part, locality and percentage yield of South African medicinal plants commonly used for dermatological purposes.

Plant species	Collected plant part	Collection site and voucher number	% Yield for organic extracts[a]	% Yield for aqueous extracts[a]
Acacia erioloba Edgew.	Bark	WSBG[b] UM173	17.7	4.2
	Leaf	WSBG[b] UM160	26.8	6.0
Acokanthera oppositifolia (Lam.) Codd.	Leaf	WSBG[b] UM156	20.9	12.9
Aloe arborescens Mill.	Leaf	WSBG[b] UM152	19.7	16.5
Athrixia phylicoides DC.	Leaf	Haenertsburg AV999	9.3	4.6
Aristea ecklonii Baker.	Leaf	Random Harvest Indigenous Nursery UM163	12.3	5.9
	Roots	Random Harvest Indigenous Nursery UM164	64.0	11.4 0
Bauhinia macranthera Benth. ex Hemsl.	Leaf	WSBG[b] UM155	25.4	20.5
Boophane disticha L.f.	Leaf	WSBG[b] UM165	55.9	6.5
Bridelia micrantha Baill.	Bark	WSBG[b] UM149	0.3	1.0
	Leaf	WSBG[b] UM150	33.4	3.6
Chenopodium ambrosioides Bert. ex Steud.	Leaf	Near Rayton (Gauteng) B & F 14	3.7	7.1
Cissampelos capensis Thunb.	Leaf	Sun Valley (Western Cape) UM128	10.6	4.1
Cotyledon orbiculata Forssk.	Leaf	WSBG[b] UM135	14.4	18.2
Dicoma anomala Sond.	Tuber	WSBG[b] UM167	13.0	17.6
Dioscorea dregeana T.Durand & Schinz.	Tuber	WSBG[b] UM174	6.1	8.8
Diospyros mespiliformis Hochst. ex A.DC.	Leaf	WSBG[b] UM151	25.6	9.4
Dodonaea angustifolia L.f.	Leaf	Pretoria-Villieria UM125	22.6	7.1
Ekebergia capensis Sparrm.	Bark	WSBG[b] UM139	12.6	0.6
	Leaf	WSBG[b] UM138	9.3	11.1
Elephantorrhiza elephantina (Burch.) Skeels	Leaf	WSBG[b] UM171	50.1	11.3
	Roots and rhizomes	WSBG[b] UM172	5.0	8.9
Embelia ruminate (E.Mey. ex A.Dc.) Mez	Leaf	WSBG[b] UM175	31.7	5.3
Erythrina lysistemon Hutch.	Leaf	Pretoria-Villieria UM132	18.7	4.4

Plant species	Collected plant part	Collection site and voucher number	% Yield for organic extracts[a]	% Yield for aqueous extracts[a]
Eucalyptus camaldulensis Dehnh.	Bark	Pretoria-Villieria UM122	14.0	7.2
Ficus natalensis Hochst.	Leaf	Pretoria-Villieria UM131	3.6	8.2
Ficus sur Forssk.	Bark	WSBG[b] UM140	1.3	1.3
	Leaf	WSBG[b] UM141	8.9	6.3
Gunnera perpensa L.	Leaf	WSBG[b] UM168	7.0	13.1
	Rhizomes	WSBG[b] UM176	18.5	10.5
Halleria lucida L.	Leaf	WSBG[b] UM177	11.9	7.3
	Stem	WSBG[b] UM178	4.6	3.2
Harpephyllum caffrum Bernh. ex Krauss	Bark	Pretoria-Villieria UM128	10.4	9.7
Hypericum perforatum L.	Leaf	UW[c] UM162	40.6	6.2
Ilex mitis Radlk.	Bark	WSBG[b] UM144	0.1	6.3
	Leaf	WSBG[b] UM145	28.7	6.1
Kigelia africana (Lam.) Benth.	Fruit	Zululand UM161	5.4	11.0
Lannea discolor Engl.	Leaf	Pretoria-Villieria UM121	17.0	9.7
Lantana rugosa Thunb.	Leaf	Rayton (Gauteng) B & F10	7.7	4.1
Malva parviflora L.	Leaf	WSBG[b] UM166	17.2	18.7
Melianthus comosus Vahl.	Leaf	WSBG[b] UM147	21.7	9.8
Melianthus major L.	Leaf	WSBG[b] UM142	15.7	17.8
Mentha longifolia Huds.	Leaf	WSBG[b] UM148	8.4	10.4
Opuntia ficus-indica Mill.	Leaf	Pretoria VillieriaUM120	5.9	12.0
Pellaea calomelanos Link.	Leaf	WSBG[b] UM146	16.1	9.4
	Rhizomes	WSBG[b] UM179	30.6	3.8
Pentanisia prunelloides Walp.	Root bark	PNBG[d] UM182	10.6	10.7
	Roots stripped	PNBG[d] UM183	5.8	4.8
Pittosporum viridiflorum Sims.	Leaf	WSBG[b] UM159	16.0	7.7
Rauvolfia caffra Sond.	Leaf	WSBG[b] UM137	11.6	6.6
Rothmannia capensis Thunb.	Leaf	WSBG[b] UM157	13.3	2.5
Scadoxus puniceus (L.) Friis & Nordal	Roots and rhizomes	WSBG[b] UM143	7.4	10.3
Solanum incanum L.	Leaf	WSBG[b] UM158	4.3	6.6
Terminalia sericea Burch. ex DC.	Roots	Swaziland AdCAV21	33.1	15.2
Trichilia emetica Vahl.	Leaf	WSBG[b] UM169	13.6	14.2

Plant species	Collected plant part	Collection site and voucher number	% Yield for organic extracts[a]	% Yield for aqueous extracts[a]
Vernonia natalensis Sch.Bip. ex Walp.	Leaf	WSBG[b] UM170	10.5	4.0
	Roots	WSBG[b] UM180	10.2	6.6
Viscum capense L.f.	Whole plant	Magaliesburg UM119	15.4	9.1
Warburgia salutaris (G.Bertol.) Chiov.	Bark	WSBG[b] UM181	7.9	5.1
	Leaf	WSBG[b] UM154	15.8	7.6
Zantedeschia aethiopica Spreng.	Leaf	WSBG[b] UM136	20.4	7.6
Ziziphus mucronata Willd.	Bark	Pretoria-Villieria UM126	10.0	8.6
	Leaf	Pretoria-Villieria UM127	10.6	21.9

[a] percentage yield expressed for organic (dichloromethane: methanol, 1:1 v/v) and aqueous extracts per dry weight of grounded plant material weighed; [b] Walter Sisulu Botanical Garden, Johannesburg, South Africa; [c] University of the Witwatersrand Medicinal Garden, Johannesburg, South Africa; [d] Pretoria National Botanical Garden, Pretoria, South Africa.

2.3.2 Preparation of cultures

Culture and media preparation were performed as detailed in the National Committee for Clinical Laboratory Services (NCCLS) guidelines (2003). The micro-organisms chosen for analysis were selected based on their pathogenesis and dermatological relevance. Micro-organism strains that were used are the American Type Culture Collection (ATCC) strains. Five aerobic Gram-positive bacteria with dermatological importance were selected for analysis and include; *Staphylococcus aureus* ATCC 25923, methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 43300, gentamycin-methicillin resistant *Staphylococcus aureus* (GMRSA) ATCC 33592, *Staphylococcus epidermidis* ATCC 2223, *Brevibacillus agri* ATCC 51663, and one anaerobic bacterium *Propionibacterium acnes* ATCC 11827 was selected. *Pseudomonas aeruginosa* ATCC 27858 was selected to represent the class of Gram-negative bacteria, while dermatophytes such as *Trichophyton*

mentagrophytes ATCC 9533, *Microsporum canis* ATCC 36299 and the yeast *Candida albicans* ATCC 10231 were also tested. Each bacterial culture was grown in Tryptone Soya broth (TSB) (Oxoid), for 18-24 hours at 37 °C. To prepare the *Propionibacterium acnes* culture, an inoculum from the frozen stock was added to Thioglycolate broth. As *P. acnes* is a fastidious anaerobe, the culture was incubated under anaerobic conditions using a candle gas jar for seven days at 37 °C. Dermatophytes *Trichophyton mentagrophytes* and *Microsporum canis* were grown and maintained in Sabouraud's Dextrose agar (Oxoid) incubated at 35 °C for approximately seven days at 100% relative humidity, *Candida albicans* was grown in TSB and incubated at 37 °C for 48 hours. Cultures were streaked on Tryptone Soya agar plates (TSA) (Oxoid), while the dermatophytes were streaked Sabouraud's Dextrose agar plates, which were then incubated at suitable conditions to confirm the purity, with exception of *P. acnes*.

2.3.3 Minimum inhibitory concentration assay

2.3.3.1 General screening of pathogens

A serial micro-dilution assay was used to quantify the minimum inhibitory concentration (MIC) values for plant extracts using tetrazolium violet reduction as an indicator of growth (Eloff *et al*., 1998b). Using aseptic manipulation, 100 µl of distilled sterile water was instilled in each well of a 96 well micro-titre plate (Figure 2.2). A volume of 100 µl of plant extracts at starting concentrations of 64 mg/ml in acetone or 50% DMSO (for plants insoluble in acetone) (Table 2.2) were individually transferred into the first row of the micro-titre plate. Serial dilutions were performed on the plant extracts diluting by 50% in water (Figure 2.2). Before addition to the micro-titre plates, all the cultures were sub-cultured in suitable broth. The culture was diluted until just turbid (0.5 McFarland

standard) and then adjusted to 1:100 ratio to ensure a density of approximately 1×10^6 colony forming units/ml (CFU/ml). A volume of 100 µl of the sub-culture was added to all the wells. Each plate was subsequently sealed with a sterile adhesive sealing film (AEC Amersham) to prevent evaporation of the test sample. The micro-titre plates were incubated at 37 °C for 24 hours and at 25 °C for 48 hours for bacteria and yeast, respectively. After incubation, as an indicator of growth 40 µl of *p*-iodonitrotetrazolium (INT) chloride (0.04% w/v) (Sigma Aldrich®) dissolved in sterile water was added to each well of the micro-titre plates. The minimum inhibitory concentration was defined as the lowest concentration of the test sample where there is no visible microbial growth. Tests were performed at least in duplicate.

2.3.3.2 Antimicrobial screening of fastidious pathogens

When testing for antimicrobial properties of the plant extracts towards the more fastidious pathogens such as *Propionibacterium acnes* bacteria and dermatophytes *Trichophyton mentagrophytes* and *Microsporum canis* modifications to the standard MIC method were employed. *Propionibacterium acnes* micro-titre plates were incubated under anaerobic conditions using a candle gas jar at 37 °C for seven days without the sterile adhesive seal film on the micro-titre plates. This allows for the exposure of the micro-organism to the CO_2 environment. A growth indicator (INT) (40 µl) was added after incubation.

When testing the dermatophytes the aseptic manipulation of the micro-titre plates as outlined in the method previously stated in Section 2.3.3.1 was adhered to. Thereafter the plates were transferred to the biohazard safety cabinet number II (BSC No. II) (Labotec) where the culture was added to the micro-titre plates. Latex gloves were worn during this procedure to ensure personnel safety. As an indicator of growth, 40 µl of INT was added to

each of the micro-titre plate wells before incubation (Masoko *et al*., 2007). The micro-titre plates were subsequently covered with a sterile adhesive film and incubated for seven days at 35 °C and 100% relative humidity.

2.3.3.3 Positive and negative controls

Allopathic antimicrobials displaying sensitivities were used as reference antimicrobial agents and positive controls. These include ciprofloxacin (Sigma Aldrich®) and amphotericin B (Sigma Aldrich®) due to their broad-spectrum activity against a wide range of bacteria and fungi, respectively. Positive controls were aseptically prepared with sterile water to achieve stock concentrations of 0.01 mg/ml (ciprofloxacin) and 0.1 mg/ml (amphotericin B). These positive controls were included in each assay to confirm antimicrobial susceptibility. Negative controls included the solvent control and culture control. Acetone and DMSO solvent controls with a final starting concentration of 25% and 12.5% v/v, respectively, were included to ascertain if the solvent exhibited any inhibitory effects towards the pathogens. Water was used as a solvent control when screening aqueous extracts. Sterile media and the pathogen growing independently (culture control) was included in all assays. The sterile media was included to verify support of growth towards the specific pathogens. The culture growing independently was used as a standard to read the results. When INT was added to the culture control column, colour change was monitored (from colourless to pink) and only when developed were the results read. This could take anything from two (e.g. *S. aureus*) to 24 hours (e.g. *C. albicans*) depending on the organism's growth pattern.

2.4 Results and discussion

2.4.1 Plant extracts yields

A total of 132 plant extracts belonging to 47 plant species were prepared and investigated for their antimicrobial properties. Extract yield, expressed as a percentage per dry weight of grounded plant material weighed, was calculated for both types of extracts; aqueous and organic (dichloromethane: methanol) (Table 2.1).

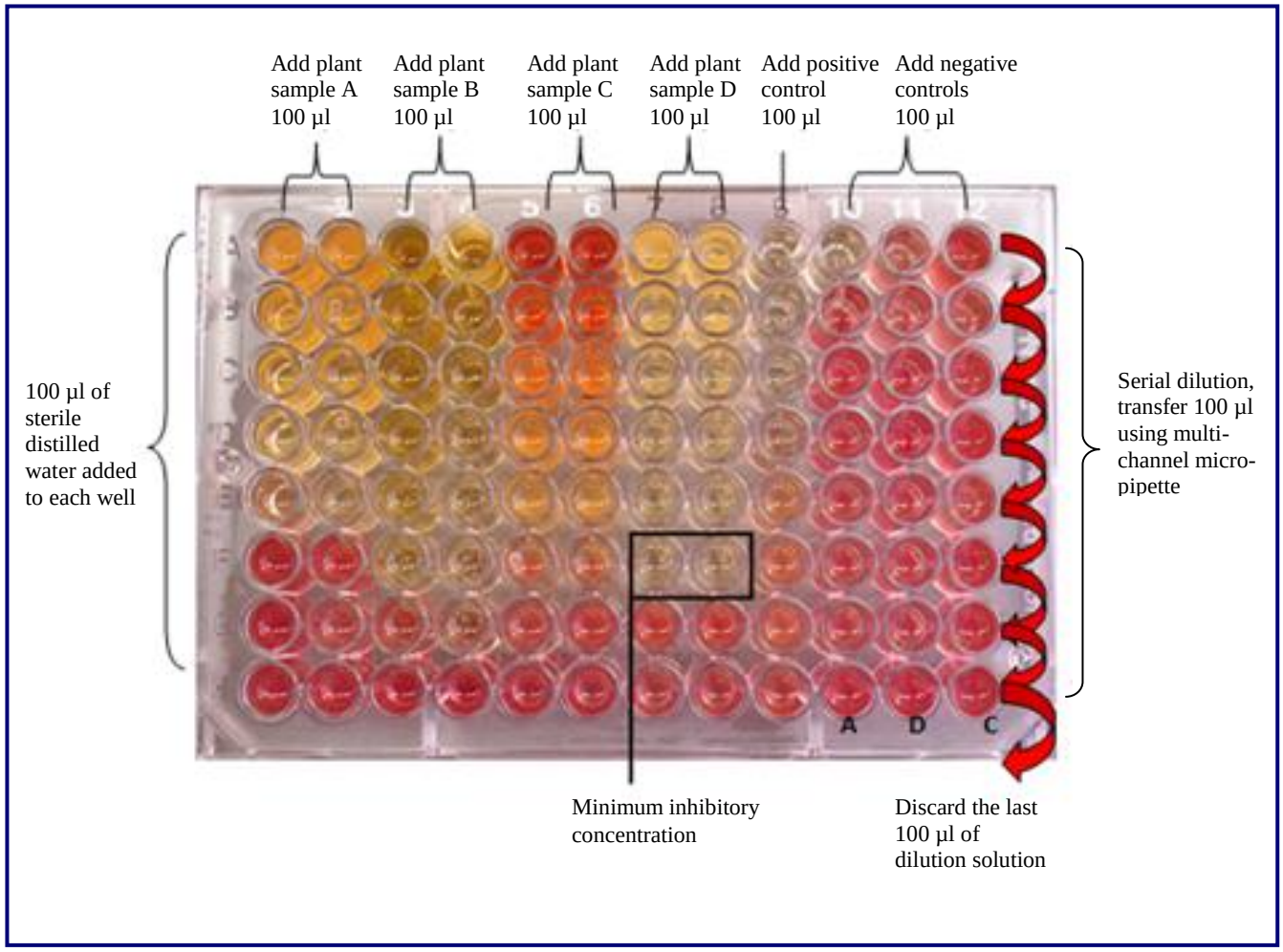


Figure 2.2: Representation of the micro-titre plate dilution method (A: acetone, D: DMSO and C: culture control). Water was added as a negative control when screening aqueous extracts.

2.4.2 Antimicrobial screening

2.4.2.1 General screening

The antimicrobial effects of the 132 plant extracts were tested against pathogens (*S. aureus*, MRSA, GMRSA, *S. epidermidis* and *C. albicans*) and are shown in Table 2.2. Generally, activity varied greatly depending on the pathogen studied, but a few plant species (as detailed hereafter) showed interesting results specifically as the positive antimicrobial efficacy had a direct correlation to the traditional use. As a means of interpretation, plant extracts that displayed MIC values of 1.00 mg/ml or lower were considered noteworthy (Gibbons, 2004; Rios and Recio, 2005; van Vuuren, 2008; Ncube *et al*., 2012). A number of organic plant extracts displayed noteworthy antimicrobial activity against the evaluated skin pathogens. Some of the notable plants included; *Aristea ecklonii, Chenopodium ambrosioides, Diospyros mespiliformis, Elephantorrhiza elephantina, Erythrina lysistemon, Eucalyptus camaldulensis, Gunnera perpensa, Halleria lucida, Melianthus comosus, Melianthus major* and *Terminalia sericea*.

Aristea ecklonii is used traditionally to treat shingles (Ngwenya *et al*., 2003) (Table 1.1, Chapter 1). Leaf infusions of this plant are also administered as enemas and have been reported to treat sicknesses accompanied by fever and cough as well as syphilis (Hutchings, 1996). The current findings demonstrate noteworthy antimicrobial properties for *A. ecklonii,* with leaf and root organic extracts displaying the greatest antimicrobial effect against tested skin pathogens (lowest MIC value of 0.01 mg/ml for the root extract against *S. aureus*). The antifungal properties of *Aristea ecklonii* against plant pathogens have been reported by Pretorius *et al*. (2002), where 100% antifungal inhibition was observed against all strains tested. This is the first report for efficacy against skin pathogens.

A decoction of *Chenopodium ambrosioides* is traditionally used to treat eczema, erysipelas, wounds and skin infections (Hutchings, 1996; Pesewu *et al*., 2008). The organic extract of *C. ambrosioides* demonstrated noteworthy antimicrobial activity against both the Gram-positive and Gram-negative bacteria (MIC values between 0.25-0.80 mg/ml). Antimicrobial properties of *C. ambrosioides* against *S. aureus* and *C. albicans* (MIC values ≤1.00 mg/ml) have been previously identified by Suliman (2010).

The root or leaf decoctions of *Diospyros mespiliformis* have been reported for use to treat a variety of skin ailments such as scars, skin rashes, bruises, wounds and ringworm (von Koenen, 1996; van Wyk *et al*., 2011) (Table 1.1, Chapter 1) The organic leaf extract showed the same level of antimicrobial inhibition (MIC 1.00 mg/ml), regardless of the pathogen tested. The antimicrobial effects of *D. mespiliformis* leaf extracts (aqueous and organic) have been reported by Dangoggo *et al.* (2012), where zones of inhibition were observed against the *S. aureus* and *P. aeruginosa* using an agar well diffusion assay. Similar findings were reported by Shagal *et al.* (2012), as both the organic and aqueous extracts of the leaves showed zones of inhibition against *S. aureus*.

The roots and rhizomes of *Elephantorrhiza elephantina* are traditionally used to treat acne and other skin diseases. The crushed roots are submerged in water over night. The infusion is then used topically and when acne is being treated, the face is held over vapour arising from a boiled warm infusion (Pujol, 1990; van Wyk, 2009). It has also been reported that the root powder is sprinkled topically onto wounds and burns (von Koenen, 1996). Variations and certain similarities were noted between the leaf, root and rhizome of *E. elephantina* with respect to antimicrobial efficacy. The organic extracts of the different parts all showed noteworthy activity against the tested pathogens (MIC values between 0.38-1.00 mg/ml), with the exception of the root extract against *P. aeruginosa* (MIC value

2.00 mg/ml). The antimicrobial properties of *E. elephantina* against *S. aureus* have been highlighted in a study conducted by Mathabe *et al.* (2006) which focused on medicinal plants used to treat diarrhoea. Noteworthy antimicrobial effects for the different organic and aqueous extracts were reported in the previous study, where extracts presented with MIC values between 0.16-0.31 mg/ml. The organic extract investigated here demonstrated comparably higher MIC values (0.38-1.00 mg/ml) to those obtained by Mathabe *et al.* (2006). The incongruent results can be attributed to the different plant part (stem rhizome) used in the previous study and the use of different solvents for extraction (Mathabe *et al.*, 2006).

The bark of *Erythrina lysistemon* is used traditionally as a poultice to treat sores, swelling and abscesses. The powdered burnt bark is also used to treat open wounds (Table 1.1, Chapter 1). The organic extract of *E. lysistemon* was noted to possess antibacterial properties against bacterial pathogens demonstrating an average MIC value of 0.20 mg/ml, hence giving some validation to its traditional use for the treatment of sores, abscesses and open wounds (Table 1.1, Chapter 1). The antimicrobial effects of *E. lysistemon* leaf have been addressed in previous studies (Rabe and van Staden, 1997; Fennell *et al*., 2004) where the screening was carried out using the diffusion method. The antimicrobial properties of *E. lysistemon* organic leaf extract have been previously reported in a study by Mukandiwa *et al*. (2012). Previous results showed antimicrobial activities of the organic leaf extract (MIC values between 0.078-0.31 mg/ml) against Gram-positive and Gram-negative bacteria, including *S. aureus* and *P. aeruginosa*.

The bark of *Eucalyptus camaldulensis* is used traditionally as a wash for the treatment of pimples (Table 1.1, Chapter 1). The organic and aqueous extracts of *E. camaldulensis* bark exhibited noteworthy antimicrobial activities against *S. aureus* and its respective resistant

strains (MIC values between 0.25-1.00 mg/ml). *Staphylococcus epidermidis* (MIC value 0.50 mg/ml) and *C. albicans* (MIC value 0.50 mg/ml) also showed antimicrobial susceptibilities towards *E. camaldulensis* organic extract. The ability of *E. camaldulensis* organic crude extract to inhibit microbial growth is in agreement with previous reports which detail its effect against skin related pathogens such as *S. aureus* and *P. aeruginosa* (Adeniyi and Ayepola, 2008; Ayepola and Adeniyi, 2008). Ayepola and Adeniyi (2008) highlighted antimicrobial effects of the dichloromethane extract (MIC value 0.63 mg/ml) against *S. aureus*. Adeniyi and Ayepola (2008) demonstrated antimicrobial effects of the methanol extract (MIC value 0.31 mg/ml) against *P. aeruginosa*.

The organic extract of *Gunnera perpensa* leaf exhibited noteworthy antimicrobial activity (MIC values between 0.20-1.00 mg/ml) against the tested pathogens, with similarities in antimicrobial efficacy noted for the aqueous extract against *S. aureus* and respective resistant strains (MIC values between 0.50-1.00 mg/ml). The organic and aqueous extract of *G. perpensa* rhizome demonstrated antimicrobial activity (MIC value 0.50 mg/ml) towards *S. aureus* and *C. albicans,* respectively. Decoctions or infusions of root or rhizome and leaf of *Gunnera perpensa* are traditionally used as a dressing for wounds and for psoriasis (Table 1.1, Chapter 1). Therefore, the findings give some validation for the traditional use of leaves as an antiseptic and dressing for wounds. Findings were similar to those reported in literature (Steenkamp *et al.,* 2004; Drewes *et al*., 2005; Buwa and van Staden, 2006; Nkomo and Kambizi, 2009). Steenkamp *et al.* (2004) addressed the antimicrobial properties of *G. perpensa* root where the methanol and aqueous extracts of the plant were screened against various bacterial pathogens. It was reported that the methanol extract demonstrated noteworthy antimicrobial activity against *S. aureus* (1.00 mg/ml). Buwa and van Staden (2006) examined the antimicrobial efficacy of *G. perpensa* root, where noteworthy activity (MIC value 0.78 mg/ml) was observed for the aqueous

extract against *S. aureus*. However, the variations (such as different bacterial strains, part of the plant screened, and different extraction solvents) between the current and previous studies do not allow for any further comparison.

Halleria lucida is used traditionally for the treatment of various skin ailments and has also been reported for its use to treat earache (Hutchings, 1996). The findings in this study demonstrate that the plant possesses some antimicrobial effects since the leaf showed predominantly antibacterial (MIC value 0.25-1.00 mg/ml) activity for the organic extracts. The aqueous extract also demonstrated noteworthy antimicrobial effects against *S. aureus* (MIC value 0.50 mg/ml) and MRSA (MIC value 1.00 mg/ml). In a study by Adedapo *et al*. (2008), it was demonstrated that the leaf did not possess any antimicrobial activity, with the stem extract displaying noteworthy activity (MIC value 1.00 mg/ml) against *S. epidermidis*. The different extraction solvent used in the previous study and the collection site, may account for the disparity observed in the current study.

The leaf juice of *Melianthus comosus* and *Melianthus major*, as well as leaf prepared as a poultice or decoction have traditionally been used to treat sores, septic wounds, ringworms and to reduce swelling (Table 1.1, Chapter 1). *Melianthus comosus* and *M. major* (organic extracts) both showed predominantly noteworthy antimicrobial activity. The aqueous extracts showed similar antimicrobial effects against tested pathogens with an average MIC value of 0.77 mg/ml. Weideman (2005) has previously reported the antimicrobial activity of *M. major* aqueous, organic (methanol and acetone) extracts, where the plant extracts were noted to poses noteworthy activities (MIC values <1.00 mg/ml) against *S. aureus*, MRSA and *P. aeruginosa*. The sensitivity of these pathogens to the organic extracts, give some validation to the traditional use of *Melianthus comosus* and *Melianthus major* as these pathogens play an important role in wound infections.

Leaves and/or rhizomes of *Pellaea calomelanos* applied topically as a decoction or infusion have been reported to be traditionally used to treat boils and abscesses (Table 1.1, Chapter 1). The current study highlights the antimicrobial effects of the leaf organic extract, which displayed noteworthy antimicrobial effects (with exception to MRSA) with MIC values between 0.02-0.75 mg/ml against the tested skin pathogens. The root and rhizome organic extract of *P. calomelanos* demonstrated noteworthy activities against MRSA (0.25 mg/ml) and *P. aeruginosa* (1.00 mg/ml). The antimicrobial effects of the plant, which focused on pathogens specific to respiratory disease, have been previously addressed by Braithwaite *et al.* (2008). However, limited studies have been directed to the antimicrobial effects of the plant towards dermatologically relevant pathogens.

Terminalia sericea is used for a variety of ailments, and has been reported for its dermatological relevance by Watt and Breyer-Brandwijk, 1962; Pujol, 1990; Hutchings, 1996 and van Wyk *et al.*, 2000. The root sap or bark is applied externally as an antiseptic for wounds and treatment of leprosy and snakebites (Table 1.1, Chapter 1). In the current study, *Terminalia sericea* exhibited mostly noteworthy broad-spectrum antimicrobial effects against skin relevant pathogens, hence supporting its use for dermatologically related ailments. Similarly, results obtained by Steenkamp *et al*. (2004) displayed noteworthy activity of *T. sericea* (aqueous and methanol) extracts against *S. aureus* (MIC values 1.00 mg/ml). The antimicrobial effects of *T. sericea* can also be corroborated by the finding observed by Suliman (2010) where the plant was identified as having noteworthy antimicrobial activity against *S. aureus* and *C. albicans* (MIC value 1.00 mg/ml). The antimicrobial activity of *T. sericea* has also been extensively studied especially for conditions associated with diarrhoea and respiratory ailments (Eloff, 1999; Fyhrquist *et al*., 2002; Eldeen *et al*., 2006; Masoko *et al*., 2005; Moshi and Mbwambo, 2005; Tshikalange *et al*., 2005; Eldeen and van Staden, 2007).

The leaves or fruits of *Trichilia emetica* are traditionally used as a poultice for the treatment of bruises, eczema and wounds (Table 1.1, Chapter 1). A previous review on this plant has highlighted some antimicrobial assays, amongst other extensive biological studies (Komane *et al.,* 2011). The current study highlights noteworthy antimicrobial effects of the leaf organic extract, which showed MIC values between 0.03-0.40 mg/ml against the tested skin pathogens (Table, 2.2), with the exception of activities found against *C. albicans*. Even though *T. emetica* did not exhibit noteworthy activity against *C. albicans*, positive antimicrobial effects (MIC value between 0.30-0.60 mg/ml for hexane, dichloromethane and acetone) on the pathogen have been previously reported by Shai *et al*. (2008). The antimicrobial effects of *T. emetica* on *P. aeruginosa* have also been reported (Shai *et al*. 2008) where the organic extract (acetone) exhibited noteworthy antimicrobial effects (MIC value of 0.40 mg/ml). A previous study by Germanò *et al.* (2005) confirmed the antimicrobial effects of *T. emetica* against *S. aureus* where the organic (ethyl ether fraction) root extract demonstrated noteworthy activity against eight strains of *S. aureus* (MIC values between 15.60–31.25 µg/ml), while the aqueous extract had an average MIC value of >500 µg/ml.

Warburgia salutaris is traditionally used for a variety of ailments (van Wyk, 2008) and has been known to be combined with *Hibiscus surattensis* to treat sores and skin irritations (Table 1.1, Chapter 1). The organic bark extract displayed noteworthy antimicrobial activity presenting with MIC values between 0.10-0.50 mg/ml against tested pathogens. Similar noteworthy activity for *Warburgia salutaris* methanol extract against *Staphylococcus aureus* has been reported by Rabe and van Staden (1997).

Table 2.2

Screening of South African plant extracts for antimicrobial activity against selected skin pathogens (MIC recorded in mg/ml).

Plant samples	*S. aureus* ATCC 25923		[a]MRSA ATCC 43300		[b]GMRSA ATCC 33592		*S. epidermidis* ATCC 2223		*P. aeruginosa* ATCC 27858		*C. albicans* ATCC 10231	
	[c]D:M	Aqueous	[c]D:M	Aqueous	[c]D:M	Aqueous	[c]D:M	Aqueous	[c]D:M	Aqueous	[c]D:M	Aqueous
Acacia erioloba bark[d]	**0.50**	4.00	**1.00**	2.00	**1.00**	4.00	**1.00**	4.00	2.00	8.00	**1.00**	2.00
Acacia erioloba leaf	**1.00**	4.00	2.00	4.00	2.00	4.00	2.00	8.00	2.00	16.00	2.00	2.00
Acokanthera oppositifolia leaf	**0.75**	16.00	4.00	8.00	4.00	4.00	4.00	>16.00	1.50	>16.00	2.00	16.00
Aloe arborescens leaf	2.00	4.00	2.00	4.00	**1.00**	4.00	**1.00**	4.00	**1.00**	>16.00	**1.00**	>16.00
Athrixia phylicoides leaf[d]	**1.00**	4.00	2.00	4.00	2.00	4.00	2.00	8.00	2.00	4.00	2.00	4.00
Aristea ecklonii leaf	**0.20**	2.00	**0.20**	4.00	**0.20**	4.00	**0.10**	2.00	**0.20**	4.00	**0.30**	8.00
Aristea ecklonii roots	**0.01**	2.00	**0.05**	**1.00**	**0.05**	**1.00**	**0.05**	**1.00**	**0.20**	**1.00**	**0.05**	4.00
Bauhinia macranthera leaf	2.00	16.00	2.00	>16.00	2.00	>16.00	2.00	>16.00	**0.50**	>16.00	2.00	>16.00
Boophane disticha leaf	4.00	>16.00	2.00	>16.00	2.00	>16.00	**0.50**	>16.00	**1.00**	>16.00	**0.50**	16.00
Bridelia micrantha bark	2.00	8.00	2.00	6.00	2.00	4.00	2.00	8.00	2.00	>16.00	4.00	4.00
Bridelia micrantha leaf	2.00	8.00	**1.00**	4.00	**1.00**	16.00	2.00	16.00	2.00	>16.00	2.00	16.00
Chenopodium ambrosioides leaf	**0.80**	4.00	**0.25**	8.00	**0.50**	8.00	**0.50**	16.00	**0.25**	>16.00	2.00	8.00
Cissampelos capensis leaf	2.00	>16.00	4.00	>16.00	2.00	>16.00	2.00	>16.00	2.00	>16.00	2.00	8.00
Cotyledon orbiculata leaf	1.50	>16.00	4.00	>16.00	**1.00**	>16.00	**0.38**	>16.00	**0.50**	>16.00	**0.25**	>16.00
Dicoma anomala tuber[d]	**0.50**	4.00	**0.50**	8.00	**0.50**	8.00	**0.50**	8.00	8.00	8.00	2.00	8.00
Dioscorea dregeana tuber[d]	2.00	>16.00	>16.00	>16.00	**1.00**	>16.00	1.25	>16.00	2.00	>16.00	2.00	>16.00
Diospyros mespiliformis leaf	**1.00**	1.75	**1.00**	4.00	**1.00**	2.00	**1.00**	4.00	**1.00**	2.00	**1.00**	8.00
Dodonaea angustifolia leaf[d]	1.60	**0.50**	**0.50**	**1.00**	1.60	**1.00**	4.00	4.00	2.00	>16.00	4.00	4.00
Ekerbergia capensis bark	**1.00**	4.00	**1.00**	2.00	2.00	4.00	**0.38**	2.00	**0.75**	16.00	1.00	2.00
Ekebergia capensis leaf	**0.50**	>16.00	8.00	6.00	**0.50**	8.00	**0.50**	8.00	**1.00**	16.00	**1.00**	16.00
Elephantorrhiza elephantina leaf	**0.50**	16.00	**1.00**	8.00	**0.50**	8.00	**0.38**	16.00	**1.00**	12.00	**1.00**	16.00

Plant samples	*S. aureus* ATCC 25923		[a]MRSA ATCC 43300		[b]GMRSA ATCC 33592		*S. epidermidis* ATCC 2223		*P. aeruginosa* ATCC 27858		*C. albicans* ATCC 10231	
	[c]D:M	Aqueous	[c]D:M	Aqueous	[c]D:M	Aqueous	[c]D:M	Aqueous	[c]D:M	Aqueous	[c]D:M	Aqueous
Elephantorrhiza elephantina roots + rhizomes[d]	**0.50**	2.00	**0.50**	**1.00**	**0.50**	2.00	**1.00**	4.00	2.00	4.00	**1.00**	4.00
Embelia ruminate leaf	2.00	3.00	1.50	4.00	**1.00**	**0.25**	**0.38**	>16.00	**0.75**	8.00	**1.00**	**0.40**
Erythrina lysistemon leaf	**0.20**	8.00	**0.20**	8.00	**0.20**	8.00	**0.20**	8.00	**0.20**	>16.00	2.00	16.00
Eucalyptus camaldulensis bark[d]	**0.50**	**0.63**	**0.50**	**0.50**	**0.25**	**1.00**	**0.50**	2.00	2.00	4.00	**0.50**	2.00
Ficus natalensis leaf	**0.25**	4.00	**0.25**	2.00	**0.50**	4.00	4.00	4.00	4.00	>16.00	2.00	8.00
Ficus sur bark	**0.75**	>16.00	**1.00**	>16.00	1.25	>16.00	2.00	>16.00	2.00	>16.00	8.00	>16.00
Ficus sur leaf	4.00	>16.00	2.00	>16.00	4.00	>16.00	4.00	>16.00	**1.00**	>16.00	2.00	4.00
Gunnera perpensa leaf	**0.40**	**0.50**	**0.25**	**1.00**	**0.20**	**1.00**	**0.25**	2.00	**1.00**	8.00	**0.50**	1.60
Gunnera perpensa rhizomes[d]	**0.50**	4.00	8.00	4.00	2.00	4.00	2.00	8.00	2.00	8.00	2.00	**0.50**
Halleria lucida leaf	**0.40**	**0.50**	**0.25**	**1.00**	**0.40**	2.00	**1.00**	4.00	**0.50**	8.00	4.00	4.00
Halleria lucida stem	**0.25**	2.00	**1.00**	8.00	**0.50**	4.00	2.00	16.00	2.00	8.00	2.00	8.00
Harpephyllum caffrum bark	**0.40**	**1.00**	**0.50**	**0.25**	**0.50**	**0.25**	**0.50**	**1.00**	**0.25**	2.00	**1.00**	**0.25**
Hypericum perforatum leaf	**0.50**	**1.00**	6.00	**0.50**	4.00	**1.00**	**0.13**	**1.00**	**0.50**	4.00	**1.00**	**0.40**
Ilex mitis bark	4.00	>16.00	4.00	6.00	2.00	6.00	3.00	8.00	1.50	8.00	6.00	8.00
Ilex mitis leaf	4.00	8.00	8.00	8.00	4.00	8.00	2.00	8.00	2.00	16.00	4.00	8.00
Kigelia africana fruit	4.00	16.00	4.00	>16.00	4.00	>16.00	1.50	>16.00	2.00	16.00	**1.00**	>16.00
Lantana rugosa leaf	2.00	4.00	2.00	4.00	2.00	8.00	1.50	8.00	2.00	>16.00	3.00	8.00
Lannea discolor leaf	2.00	16.00	**1.00**	16.00	2.00	4.00	2.00	16.00	**1.00**	12.00	2.00	8.00
Malva parviflora leaf	**0.50**	8.00	2.00	4.00	**0.50**	4.00	2.00	>16.00	**1.00**	>16.00	2.00	16.00
Melianthus comosus leaf	**0.40**	1.60	**0.50**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.10**	2.00	**0.50**	**0.25**
Melianthus major leaf	**1.00**	**0.50**	2.00	**0.50**	**1.00**	**0.50**	2.00	**1.00**	1.25	2.00	**0.50**	4.00
Mentha longifolia leaf	**1.00**	2.00	**1.00**	4.00	2.00	4.00	**1.00**	8.00	2.00	4.00	2.00	8.00
*Opuntia ficus-indica*leaf	16.00	>16.00	16.00	>16.00	8.00	>16.00	4.00	>16.00	4.00	>16.00	4.00	>16.00
Pellaea calomelanos leaf	**0.75**	4.00	2.00	8.00	**0.50**	4.00	**0.02**	4.00	**0.75**	8.00	**0.50**	12.00
Pellaea calomelanos rhizomes	3.00	>16.00	**0.25**	>16.00	2.00	>16.00	2.50	>16.00	**1.00**	>16.00	4.00	>16.00
Pentanisia prunelloides root	4.00	8.00	4.00	16.00	8.00	>16.00	4.00	>16.00	8.00	>16.00	8.00	8.00

Plant samples	*S. aureus* ATCC 25923		[a]MRSA ATCC 43300		[b]GMRSA ATCC 33592		*S. epidermidis* ATCC 2223		*P. aeruginosa* ATCC 27858		*C. albicans* ATCC 10231	
	[c]D:M	Aqueous	[c]D:M	Aqueous	[c]D:M	Aqueous	[c]D:M	Aqueous	[c]D:M	Aqueous	[c]D:M	Aqueous
bark[d]												
Pentanisia prunelloides roots stripped[d]	4.00	4.00	4.00	8.00	8.00	4.00	8.00	16.00	8.00	>16.00	8.00	16.00
Pittosporum viridiflorum leaf[d]	4.00	4.00	8.00	8.00	8.00	4.00	8.00	4.00	8.00	>16.00	2.00	2.00
Rauvolfia caffra leaf	2.00	8.00	4.00	8.00	4.00	4.00	4.00	16.00	2.00	>16.00	4.00	>16.00
Rothmannia capensis leaf	**0.50**	4.00	4.00	4.00	2.00	4.00	4.00	8.00	4.00	>16.00	2.00	4.00
Scadoxus puniceus rhizomes + roots[d]	8.00	>16.00	8.00	12.00	4.00	>16.00	1.50	>16.00	2.00	>16.00	8.00	3.00
Solanum incanum leaf	**0.50**	>16.00	**1.00**	>16.00	**1.00**	>16.00	0.50	>16.00	0.50	>16.00	2.00	>16.00
Terminalia sericea roots[d]	1.60	1.60	**0.50**	**0.25**	**0.80**	**0.25**	**0.25**	**0.25**	**0.25**	4.00	**0.50**	**0.80**
Trichilia emetica leaf	**0.40**	>16.00	**0.40**	>16.00	**0.40**	>16.00	**0.20**	>16.00	**0.03**	>16.00	2.00	>16.00
Vernonia natalensis leaf[d]	4.00	>16.00	8.00	>16.00	4.00	>16.00	8.00	>16.00	4.00	>16.00	4.00	>16.00
Vernonia natalensis roots	4.00	4.00	6.00	8.00	8.00	6.00	6.00	8.00	8.00	>16.00	16.00	8.00
Viscum capense whole plant	2.00	4.00	4.00	>16.00	4.00	8.00	4.00	>16.00	4.00	>16.00	4.00	8.00
Warburgia salutaris bark	**0.40**	4.00	**0.50**	4.00	**0.50**	4.00	**0.50**	8.00	**0.10**	>16.00	**0.50**	>16.00
Warburgia salutaris leaf	**0.80**	4.00	2.00	4.00	**0.50**	8.00	**1.00**	16.00	**1.00**	>16.00	2.00	16.00
Zantedeschia aethiopica leaf	4.00	8.00	4.00	4.00	4.00	>16.00	1.00	4.00	**0.50**	4.00	**1.00**	2.00
Ziziphus mucronata bark[d]	**1.00**	**0.25**	2.00	**0.25**	2.00	**0.25**	4.00	2.00	**1.00**	16.00	4.00	4.00
Ziziphus mucronata leaf	**0.50**	4.00	2.00	8.00	**1.00**	8.00	2.00	>16.00	**0.50**	>16.00	4.00	>16.00
Ciprofloxacin/ amphotericin B positive control (µg/ml)	0.69	1.25	0.83	1.25	0.55	0.63	0.47	1.25	0.31	1.25	1.25	1.25
Acetone[e]/ DMSO[f]/ water negative control	8.00[e]/ 16.00[f]	>16.00	16.00[e]/ >16.00[f]	>16.00	>16.00[e]/ >16.00[f]	>16.00	8.00[e]/ 8.00[f]	>16.00	4.00e/ 8.00[f]	>16.00	8.00[e]/ 16.00[f]	>16.00

[a] MRSA methicillin resistant *Staphylococcus aureus*; [b] gentamycin-methicillin resistant *Staphylococcus aureus*; [c] D: M dichloromethane: methanol; [d] plant sample dissolved in DMSO; written in bold are noteworthy antimicrobial activities [e] MIC value for acetone negative control; [f] MIC value for DMSO negative control.

Even though some of these antimicrobially active plants (e.g. *E. elephantina*, *E. lysistemon, E. camaldulensis, G. perpensa* and *H. lucida*) have been addressed in previous studies, it is worth noting that these reports have not screened this selection of plants against a wide range of dermatological relevant pathogens. While antimicrobial properties were noted from the current findings, most of the results were not comparable to previous reports. Such disparity could be accounted for, by differences such as the antimicrobial screening methods used, pathogen strains, geographical source (wild or cultivated plant material), plant part used and extraction solvents.

2.4.2.2 Fastidious pathogens

This section of the study addresses the antimicrobial properties noted against fastidious pathogens (*P. acnes, B. agri, T. mentagrophytes* and *M. canis*) which have dermatological relevance and have been neglected in most previous antimicrobial screening studies.

Represented in Table 2.3 are the antimicrobial results obtained from the screening of medicinal plants against fastidious skin pathogens such as *P. acnes, B. agri, T. mentagrophytes* and *M. canis.*

Propionibacterium acnes and *T. mentagrophytes* showed interesting sensitivities towards most of the organic extracts evaluated with average MIC values of 1.90 and 1.23 mg/ml determined against these pathogens, respectively.

Medicinal plants reported to be traditionally used to treat acne vulgaris and pimples include *E. elephantina, E. capensis, E. camaldulensis* and *H. caffrum* (Hutchings, 1996; van Wyk *et al.*, 2000). The organic extracts of these plants displayed noteworthy activity against *P. acnes* with MIC values between 0.05-1.00 mg/ml. This finding gives some validation for the traditional use of these plants for treating acne vulgaris and pimples.

Charred root, stems and/or leaf of *Dicoma anomala* are traditionally used as a paste to treat wounds, ulcers, ringworm and for head sores. The current findings demonstrate selective noteworthy antimicrobial effects against *T. mentagrophytes* (MIC value of 0.03 mg/ml), hence giving some validation for its traditional use to treat ringworm infections. The antimicrobial effects of *D. anomala* have been addressed by Boily and van Puyvelde (1986) and Steenkamp *et al.* (2004) to determine its effect against a number of pathogens. However, none of the previous studies have addressed the antimicrobial effects of *D. anomala* against *T. mentagrophytes* and *M. canis.*

A decoction from squashed roots of *Diospyros mespiliformis* is used traditionally in the treatment of ringworm infections (von Koenen, 1996). The antimicrobial effects of this plant against the dermatophytes give some validation to the traditional use especially for treating ringworm as the organic extract demonstrated noteworthy antimicrobial effects against *Trichophyton mentagrophytes* (MIC 0.10 mg/ml) and *Microsporum canis* (MIC 0.50 mg/ml).

Leaf decoctions of *M. comosus* and *M. major* are applied topically for the treatment of ringworm (van Wyk, 2009). The organic extracts of these plants demonstrated noteworthy activity against *T. mentagrophytes* and *M. canis* with MIC values of 0.05 mg/ml and 0.50 mg/ml, respectively. The aqueous extracts of *M. comosus* displayed noteworthy antimicrobial effects against the dermatophytes (MIC values between 0.50-1.00 mg/ml), with *T. mentagrophytes* also showing antimicrobial susceptibility to the aqueous extract of *M. major* (1.00 mg/ml). This gives some validation to traditional use of these plants as antifungals.

Aqueous extracts of *Aristea ecklonii* leaf showed notable results towards *B. agri* (MIC value 0.75 mg/ml) and *T. mentagrophytes* (MIC value 1.00 mg/ml), with the aqueous root

extracts showing similar activity against the two dermatophytes. The organic root extract of the plant displayed noteworthy antimicrobial effects against *P. acnes* and *T. mentagrophytes* with an MIC value of 0.03 mg/ml.

The organic extracts of *G. perpensa* leaf showed noteworthy antimicrobial effects against the fastidious pathogens with MIC values between 0.03-1.00 mg/ml. The leaf aqueous extracts also demonstrated noteworthy antimicrobial effects (0.10-1.00 mg/ml) against the fastidious pathogens (with exception of *P. acnes*). This report provides new evidence for efficacy against *P. acnes*, *B. agri*, *T. mentagrophytes* and *M. canis*.

Table 2.3

Screening of South African plant extracts for antimicrobial activity against fastidious skin pathogens (MIC recorded in mg/ml).

Plant samples	***B. agri* ATCC 51663**		***P. acnes* ATCC 11827**		***T. mentagrophytes* ATCC 9533**		***M. canis* ATCC 36299**	
	[a]D:M	**Aqueous**	**[a]D:M**	**Aqueous**	**[a]D:M**	**Aqueous**	**[a]D:M**	**Aqueous**
A. erioloba leaf	**0.50**	8.00	**0.20**	**0.50**	**0.50**	8.00	2.00	8.00
A. erioloba bark[b]	**0.50**	2.00	**0.20**	**0.25**	**1.00**	8.00	**1.00**	**1.00**
A. oppositifolia leaf	**0.50**	16.00	4.00	2.00	2.00	>16.00	2.00	**1.00**
A. arborescens leaf	2.00	16.00	**0.50**	4.00	**0.25**	8.00	16.00	8.00
A. phylicoides leaf[b]	3.00	8.00	2.00	2.00	**1.00**	>16.00	**1.00**	>16.00
A. ecklonii leaf	8.00	**0.75**	**0.05**	1.50	**0.05**	**1.00**	16.00	2.00
A. ecklonii roots	2.00	2.00	**0.03**	4.00	**0.03**	**0.50**	4.00	**1.00**
B. macranthera leaf	4.00	4.00	**0.50**	**1.00**	**1.00**	4.00	2.00	2.00
B. disticha leaf	8.00	>16.00	**0.50**	**1.00**	2.00	16.00	2.00	>16.00
B. micrantha bark	4.00	2.00	**1.00**	**0.25**	2.00	>16.00	2.00	2.00
B. micrantha leaf	2.00	**0.50**	**1.00**	**1.00**	**1.00**	8.00	**1.00**	4.00
C. ambrosioides leaf	**0.50**	8.00	**0.40**	2.00	**0.25**	2.00	4.00	4.00
C. capensis leaf	**1.00**	>16.00	**0.25**	**0.50**	**1.00**	2.00	**1.00**	8.00
C. orbiculata leaf	4.00	12.00	**0.25**	16.00	2.00	>16.00	**1.00**	8.00
D. anomala tuber[b]	4.00	8.00	4.00	16.00	**0.03**	4.00	4.00	8.00
D. dregeana tuber[b]	**0.25**	>16.00	2.00	2.00	2.00	>16.00	4.00	>16.00
D. mespiliformis leaf	**0.50**	0.50	**0.05**	2.00	**0.10**	4.00	**0.50**	4.00
D. angustifolia leaf[b]	**1.00**	3.00	2.00	4.00	**0.50**	2.00	2.00	4.00
E. capensis leaf	4.00	2.00	**1.00**	4.00	2.00	4.00	2.00	4.00
E. capensis bark	2.00	16.00	**1.00**	4.00	**1.00**	8.00	**1.00**	8.00

Plant samples	*B. agri* ATCC 51663		*P. acnes* ATCC 11827		*T. mentagrophytes* ATCC 9533		*M. canis* ATCC 36299	
	[a]D:M	Aqueous	[a]D:M	Aqueous	[a]D:M	Aqueous	[a]D:M	Aqueous
E. elephantina leaf	2.00	>16.00	**1.00**	**0.25**	2.00	>16.00	16.00	8.00
E. elephantina roots + rhizomes[b]	**0.50**	**0.50**	**0.05**	2.00	**1.00**	4.00	**0.50**	4.00
E. ruminate leaf	4.00	>16.00	**1.00**	>16.00	4.00	>16.00	2.00	8.00
E. lysistemon leaf	8.00	16.00	**0.08**	**0.25**	**1.00**	8.00	2.00	16.00
E. camaldulensis bark[b]	**0.25**	**0.20**	**0.10**	2.00	**1.00**	**1.00**	4.00	2.00
F. natalensis leaf	2.00	4.00	8.00	**1.00**	**0.50**	4.00	4.00	4.00
F. sur bark	8.00	>16.00	2.00	**0.25**	**1.00**	8.00	>16.00	>16.00
F. sur leaf	2.00	>16.00	4.00	**0.25**	**0.25**	16.00	**1.00**	4.00
G. perpensa leaf	**0.38**	**0.10**	**0.03**	2.00	**0.03**	**0.25**	**1.00**	**1.00**
G. perpensa rhizomes[b]	4.00	4.00	**0.25**	**1.00**	**1.00**	8.00	4.00	16.00
H. lucida leaf	**1.00**	**1.00**	**0.38**	**1.00**	**1.00**	16.00	2.00	2.00
H. lucida stem	**1.00**	8.00	2.00	**0.25**	2.00	>16.00	2.00	8.00
H. caffrum bark	**0.50**	**0.50**	**0.18**	**0.50**	**0.50**	2.00	**1.00**	4.00
H. perforatum leaf	**1.00**	**1.00**	**1.00**	**0.50**	**1.00**	8.00	**1.00**	4.00
I. mitis bark	4.00	4.00	4.00	2.00	4.00	2.00	4.00	16.00
I. mitis leaf	3.00	4.00	3.00	**1.00**	2.00	8.00	**1.00**	>16.00
K. africana fruit	**1.00**	>16.00	**1.00**	2.00	4.00	16.00	8.00	>16.00
L. rugosa leaf	**0.50**	4.00	**0.50**	**1.00**	**0.05**	4.00	2.00	4.00
L. discolor leaf	**1.00**	4.00	**1.00**	**1.00**	**0.05**	16.00	4.00	2.00
M. parviflora leaf	4.00	>16.00	8.00	**0.25**	**0.05**	4.00	>16.00	>16.00
M. comosus leaf	**0.25**	2.00	**0.10**	**0.25**	**0.05**	**0.50**	**0.50**	**1.00**
M. major leaf	**0.25**	2.00	**0.10**	**1.00**	**0.05**	**1.00**	**0.50**	4.00
M. longifolia leaf	2.00	4.00	**0.50**	**1.00**	**0.80**	2.00	**1.00**	2.00
O. ficus-indica leaf	8.00	>16.00	4.00	2.00	2.00	16.00	8.00	16.00
P. calomelanos leaf	4.00	2.00	**0.50**	4.00	**1.00**	8.00	2.00	4.00
P. calomelanos rhizomes	4.00	>16.00	4.00	2.00	2.00	8.00	8.00	16.00
P. prunelloides root bark[b]	4.00	8.00	**1.00**	4.00	2.00	>16.00	2.00	16.00
P. prunelloides roots stripped[b]	2.00	4.00	**0.50**	4.00	4.00	4.00	**1.00**	8.00
P. viridiflorum leaf[b]	2.00	8.00	8.00	**0.25**	**0.50**	**1.00**	**1.00**	**1.00**
R. caffra leaf	**0.50**	4.00	4.00	2.00	2.00	8.00	**1.00**	**1.00**
R. capensis leaf	**1.00**	2.00	8.00	4.00	2.00	8.00	4.00	8.00
S. puniceus rhizomes + roots[b]	16.00	>16.00	8.00	4.00	4.00	4.00	≥16. 00	8.00

Plant samples	*B. agri* ATCC 51663		*P. acnes* ATCC 11827		*T. mentagrophytes* ATCC 9533		*M. canis* ATCC 36299	
	[a]D:M	Aqueous	[a]D:M	Aqueous	[a]D:M	Aqueous	[a]D:M	Aqueous
S. incanum leaf	**1.00**	16.00	4.00	2.00	**1.00**	2.00	2.00	4.00
T. sericea roots[b]	**0.50**	8.00	**0.03**	**0.25**	**0.03**	**0.03**	**0.50**	**1.00**
T. emetica leaf	4.00	>16.00	**0.04**	2.00	**1.00**	16.00	2.00	8.00
V. natalensis leaf[b]	2.00	>16.00	4.00	**0.50**	**1.00**	16.00	4.00	8.00
V. natalensis roots	8.00	8.00	**1.00**	**1.00**	**1.00**	16.00	4.00	16.00
V. capense whole plant	**1.00**	8.00	2.00	4.00	**1.00**	8.00	**1.00**	2.00
W. salutaris leaf	2.00	16.00	4.00	**1.00**	**0.50**	16.00	4.00	8.00
W. salutaris bark	2.00	8.00	**1.00**	2.00	**0.03**	8.00	2.00	4.00
Z. aethiopica leaf	8.00	8.00	**1.00**	**0.50**	1.50	4.00	**1.00**	2.00
Z. mucronata leaf	2.00	>16.00	8.00	**0.50**	2.00	>16.00	4.00	>16.00
Z. mucronata bark[b]	**1.00**	2.00	**0.20**	**1.00**	2.00	4.00	2.00	4.00
Ciprofloxacin/ amphotericin B positive control (µg/ml)	0.16	0.16	1.25	1.25	25.00	25.00	12.50	12.50
Acetone[c]/ DMSO[d]/ water negative control	>16.00[c]/ 16.00[d]	>16.00	>16.00[c]/ 16.00[d]	>16.00	>16.00[c]/ 16.00[d]	≥ 16.00	>16.00[c]/ 16.00[d]	>16.00

[a] D:M dichloromethane: methanol; [b] plant sample dissolved in DMSO; written in bold are noteworthy antimicrobial activities; [c] MIC value for acetone negative control; [d] MIC value for DMSO negative control.

Antimicrobial efficacy of *W. salutaris* organic bark extract was noted against *P. acnes* (MIC value of 1.00 mg/ml) and *T. mentagrophytes* (MIC value of 0.03 mg/ml). While the leaf organic extract showed noteworthy antimicrobial effects against *T. mentagrophytes* (MIC value of 0.50 mg/ml). No previous studies have reported on the antimicrobial effects of *A. ecklonii, G. perpensa* and *W. salutaris* against any of the fastidious pathogens studied here.

It was worth noting that the extracts of *A. erioloba* (bark), *D. mespiliformis* (leaf), *H. perforatum* (leaf), *M. comosus* (leaf), *M. major* (leaf) and *T. sericea* (roots) showed mostly noteworthy activities towards the tested fastidious skin pathogens. These findings

provide some validation for the traditional uses of these plants for wound treatment as antiseptics and also look favourable as potential antifungals for ringworm infections.

Even though *B. agri* was noted as the least susceptible pathogen (especially towards the aqueous extracts) together with *M. canis*, plants such as *E. elephantina* (roots + rhizomes), *E. camaldulensis* (bark), *G. perpensa* (leaf), *H. lucida* (leaf), *H. caffrum* (bark), and *H. perforatum* (leaf) displayed MIC values between 0.10-100 mg/ml, thus exhibiting noteworthy antimicrobial activities for both extracts. Similar noteworthy activities against *M. canis* were noted for medicinal plants such as, *A. erioloba* (bark), *G. perpensa* (leaf), *M. comosus* (leaf), *P. viridiflorum* (leaf), *R. caffra* (leaf) and *T. sericea* (roots) demonstrating MIC values between 0.50-1.00 mg/ml for both extracts.

2.4.3 Antimicrobial effects of the different plant extracts against the skin pathogens

The tested skin pathogens showed varying sensitivities towards the examined plant extracts. Among the Gram-positive bacteria, *S. aureus* was noted as the most sensitive bacteria towards the organic plant extracts. *Trichophton mentagrophytes* was noted as the most sensitive dermatophyte towards the antimicrobial effects of the organic extracts. The aqueous extracts showed the most activity against *P. acnes*. When comparing the antimicrobial effects of the plant extracts, similar efficacies were observed against the Gram-negative bacteria and the yeast/dermatophytes (Figure 2.3). The Gram-negative bacteria possess an outer membrane, which contains a lipopolysaccharide layer which make it more hydrophobic (Hancock, 1997), thus it was not surprising to note that *P. aeruginosa* did not demonstrate major sensitivity towards the aqueous extracts. The

aqueous extracts showed better antimicrobial effects against the Gram-positive bacteria, while there were not notable variations for the organic extracts.

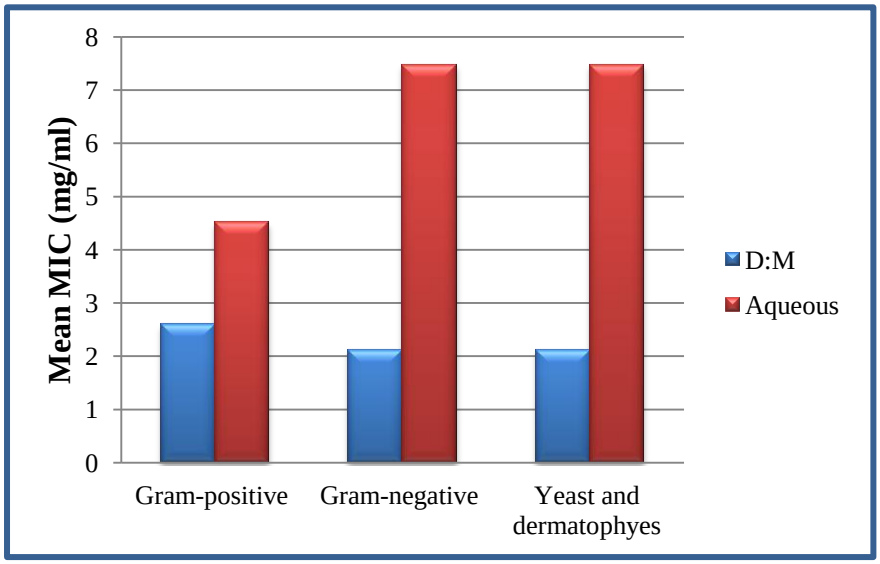


Figure 2.3: Representation of the antimicrobial efficacies of the plant extracts against the different pathogen groups; D:M (dichloromethane: methanol, 1:1).

2.4.4 Comparative analysis of the antimicrobial efficacies of different plant parts

Even though the leaf is the most frequently used plant part used traditionally to treat skin ailments, there are incidences where bark or roots are recommended (Section 1.3.1, Chapter 1). When comparing the antimicrobial activities of leaf extract to bark, it was noted that where bark was recommended for traditional use, the extract exerted better antimicrobial effects compared to the leaf, however, the extracts of *F. sur*, *I. mitis* and *Z. mucronata* showed no major differences (Figure 2.4). Certain variations were noted where both root and leaf extracts of the same species were examined. With root extracts of plants such as *A. ecklonii* and *E. elephantina* showing more prominent antimicrobial effects compared to the leaf extract, however the same could not be said for *V. natalensis*.

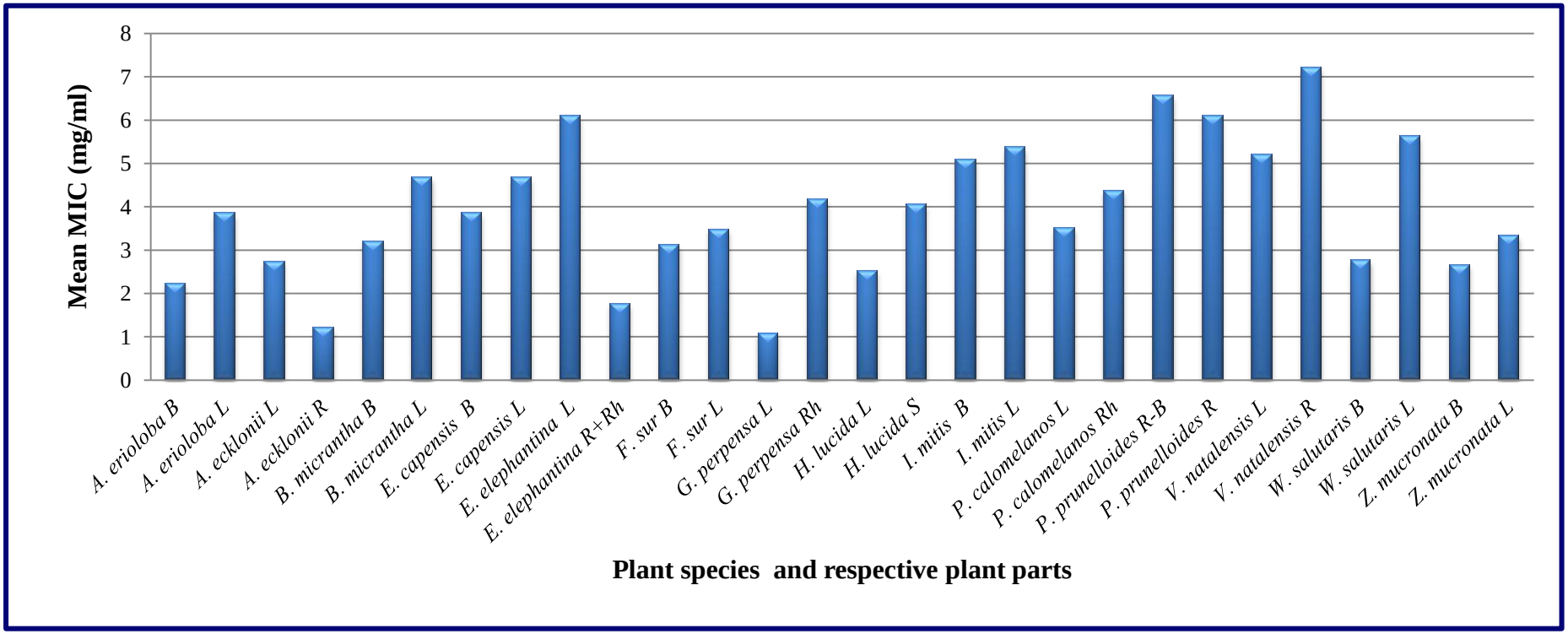


Figure 2.4: Representation of average antimicrobial activity of plant parts against dermatological relevant pathogens (B-bark; L-leaf, R-roots, Rh-rhizome).

It has previously been recommended that leaves be used to substitute the traditionally used bark or root in order to promote sustainable harvesting (Lewu *et al*., 2006).

Based on the current findings which highlight better activity for root and bark, it cannot be recommended that all traditional formulations which utilize roots or bark to treat skin ailments be substituted with leaf. Only in some instances, as in the case of the rhizomes of *G. perpensa* as well as *P. calomelanos* and roots of *V. natalensis* which can be substituted with the leaves of these plants since the leaf extracts displayed better antimicrobial efficacy (Figure 2.4). It is worth noting that the bark of *W. salutaris* only had a better overall effect for the organic extracts when compared to the leaf samples. As the traditional use is usually aqueous by nature, the possibility of substitution of leaf material for bark may be warranted. This could possibly protect this plant species, which is rapidly dwindling in numbers in the wild.

2.5 Conclusions

- The aqueous extracts displayed less efficacy compared to the organic extracts for most of the plant samples investigated against the tested pathogens including the fastidious pathogens.
- Exceptions were noted for the aqueous extracts of *D. angustifolia*, *E. camaldulensis* and *G. perpensa* which showed antimicrobial efficacies (MIC range 0.50-1.00 mg/ml) against *S. aureus* and resistant strains. Also, noteworthy antimicrobial activity (MIC value of 0.25 mg/ml) of the aqueous extract of *E. ruminate* was noted against GMRSA.
- Plants demonstrating notable broad-spectrum activities included organic extracts from *A. ecklonii*, *C. ambrosioides*, *D. mespiliformis*, *E. elephantina*,

E. camaldulensis, G. perpensa, H. caffrum, H. perforatum, M. comosus, T. sericea and *W. salutaris.*

- Notable antimicrobial properties of the organic extracts from *E. elephantina* (roots and rhizomes; MIC value of 0.05 mg/ml), *E. capensis* (MIC value of 1.00 mg/ml), *E. camaldulensis* (MIC value of 0.10 mg/ml) and *H. caffrum* (MIC value of 0.18 mg/ml) against the pathogen *Propionibacterium acnes* give some validation to the reported traditional use of the plants to treat acne vulgaris.
- The organic extracts of *D. anomala* as well as *D. mespiliformis* both showed noteworthy antimicrobial activity towards *T. mentagrophytes* having MIC values of 0.03 and 0.10 mg/ml, respectively. The aqueous and organic extracts of *M. comosus* showed antimicrobial effects (MIC range 0.05-1.00 mg/ml) against *T. mentagrophytes* and *M. canis*. Since *D. anomala, D. mespiliformis* and *M. comosus* are traditionally used for ringworm infections, the noteworthy antimicrobial effects against the dermatophytes gives some validation for their reported traditional uses.
- The Gram-positive bacteria demonstrated overall better sensitivity towards the antimicrobial effects of the aqueous extracts, while the organic and aqueous extracts showed similar efficacies towards the Gram-negative bacteria and yeast/dermatophytes.
- When comparing the antimicrobial effects of leaf compared to the bark, roots or rhizomes which are traditionally used to treat skin ailments, it was noted that the bark, root and rhizome extracts mostly demonstrated better activity with the exception of *G. perpensa, P. calomelanos* and *V. natalensis.*

Chapter 3: Interactive studies on medicinal plants used in combination to treat skin ailments

3.1 Introduction

Although the antimicrobial activity of the plants selected for this study have been examined for their antimicrobial efficacy in a general screening (Chapter 2), there are some incidences where various plants are traditionally combined (Table 1.2, Chapter 1). For this part of the study an in depth examination was undertaken on select combinations to identify a possible scientific rational in using plant combinations to treat skin ailments. The synergistic, additive, non-interactive or antagonistic interactions between plants reported to be used in combination was thus investigated.

3.2 Materials and methods

3.2.1 Collection, identification and preparation of extracts

The collection, identification of plant species and the preparation of extracts for each plant species is described in Section 2.2, Chapter 2. Selection of plants for combination studies was based on availability at the time of collecting. Five double combinations comprising of different plants parts from three plant species (*E. elephantina D. anomala*, *P. prunelloides*) were the focus of this study.

3.2.2 Antimicrobial activity assay

3.2.2.1 Preparation of microbial cultures

Skin related pathogens, Gram-positive *S. aureus* ATCC 25923, MRSA ATCC 43300, GMRSA ATCC 33592 and *S. epidermidis* ATCC 2223, Gram-negative *P. aeruginosa* ATCC 27858 and yeast *C. albicans* ATCC 10231 were selected and prepared according to specifications detailed in Section 2.3.2, Chapter 2. Pathogen selection was based on a representation of Gram-positive, Gram-negative and yeast strains. It was not practical to include all dermatologically relevant strains in this part of the study.

3.2.3 Interactive combination studies

3.2.3.1 Plant extracts combined in a 1:1 ratio: Determination of fractional inhibitory concentrations (ΣFIC)

Plant extracts with a starting concentration of 64 mg/ml were mixed in 1:1 ratios. Following the micro-titre plate dilution technique detailed in Section 2.3.3 (Chapter 2), 100 µl of the mixture (containing 50 µl of each plant sample used in the combination) was transferred to the first row of the 96-well micro-titre plate containing 100 µl of sterile water and the MIC values determined against tested pathogens. The MIC values were determined for 1:1 combinations to establish any interactions affecting antimicrobial activity (van Vuuren and Viljoen, 2008). The plant samples used in combinations were also examined for antimicrobial properties independently (Chapter 2). Positive and negative controls were included in each assay as detailed in Section 2.3.3.3 (Chapter 2). Tests were performed at least in duplicate. The fractional inhibitory concentration (FIC) was calculated for each combination. Antimicrobial analysis was carried out by

determining the fractional concentration index (ΣFIC) as defined by van Vuuren and Viljoen (2011) using the following equation;

Equation 3.1

$$\text{FIC (i)} = \frac{\text{MIC (a) in combination with (b)}}{\text{MIC (a) independently}} \qquad \text{FIC (ii)} = \frac{\text{MIC (b) in combination with (a)}}{\text{MIC (b) independently}}$$

FIC (i) and FIC (ii) in the equations represent the different plants in combination, while MIC (a) and MIC (b) represent the individual plant extracts. The sum of the FIC, known as the FIC index was thus calculated as ΣFIC= FIC (i) + FIC (ii), and was used to determine the interaction between the two plants. This may be classified as either synergistic (<0.50), additive (>0.50-1.00), indifferent (>1.00-4.00) or antagonistic (>4.00) (Suliman *et al*., 2010; van Vuuren and Viljoen, 2011).

3.2.3.2 Interactive properties of plant extracts at various ratio combinations (isobologram construction)

Once the ΣFIC's for the (1:1) combinations were identified, combinations with synergistic interactions were further investigated at various ratios. Nine combinations of the plant extracts at a starting concentration of 64 mg/ml were prepared at various ratios. The MIC assay was conducted on the nine ratios i.e. 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9 of the plants in combination. A volume of 100 µl of each ratio combination was then transferred to the prepared micro-titre plate and analysed for microbial growth inhibition as detailed in Section 2.3.3, Chapter 2. The results were then plotted on an isobologram using GraphPad Prism® software (Version 5), allowing for a figurative representation of the interaction of the various ratio combinations (van Vuuren and Viljoen, 2008; Suliman *et al.*, 2010; van Vuuren and Viljoen, 2011).

The isobolograms were interpreted by examining the data points of the ratios where the MIC for each concentration is determined in relation to the independent MIC value. Following isobologram interpretation recommended by van Vuuren and Viljoen (2011), data points falling below or on the 0.50 line were interpreted as synergistic. Points between 0.50 and or on the 1.00 line were interpreted as additive and points above the >1.00 but ≤4.00 line were defined as either non-interactive or antagonistic (>4.00) (Figure 3.1). For all assays, conventional antimicrobials (ciprofloxacin for the bacteria and amphotericin B for the yeast) were included as positive controls. Negative controls, where the solvent is analysed without plant samples, were also included in all repetitions. Assays were undertaken at least in duplicate and the mean values noted.

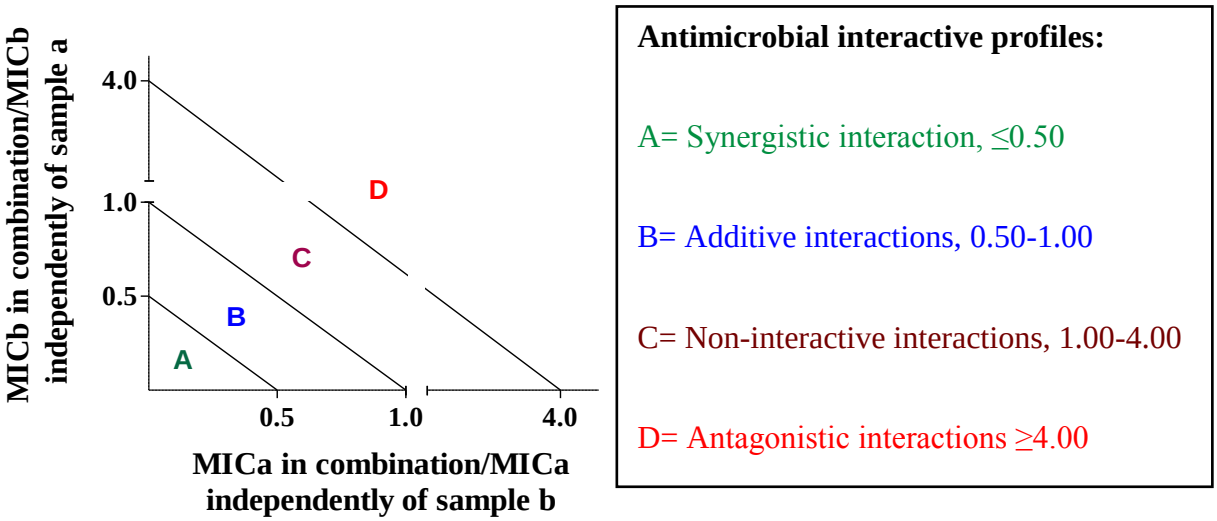


Figure 3.1: Interpretation of the isobologram as defined by van Vuuren and Viljoen (2011).

3.3 Results and discussion

Five different plant combinations were assayed for interactive properties. The mean MIC's and ΣFIC's of these combinations against the six pathogens are presented in Table 3.1.

Table 3.1

Average MIC (expressed in mg/ml) and ΣFIC values for plant combinations in a 1:1 ratio.

Combinations		***S. aureus* ATCC 25923**		**[a]MRSA ATCC 43300**		**[b]GMRSA ATCC 33592**		***S. epidermidis* ATCC 2223**		***P. aeruginosa* ATCC 27858**		***C. albicans* ATCC 10231**		**MIC and ∑FIC mean values**	
		[c]D:M	**[d]Aq**	**[c]D:M**	**[d]Aq**	**[c]D:M**	**[d]Aq**	**[c]D:M**	**[d]Aq**	**[c]D:M**	**[d]Aq**	**[c]D:M**	**[d]Aq**	**[c]D:M**	**[d]Aq**
Elephantorrhiza elephantina + *Dicoma anomala* tuber	MIC	1.00	4.00	2.00	4.00	1.00	4.00	1.00	8.00	2.00	8.00	2.00	8.00	1.50	6.00
	ΣFIC	2.00	2.50	4.00	1.25	2.00	0.75	1.50	1.50	**0.63**	1.50	2.13	1.00	2.04	1.42
Elephantorrhiza elephantina + *Pentanisia prunelloides* root bark	MIC	1.00	1.00	2.00	4.00	0.50	4.00	1.00	4.00	2.00	>16.00	2.00	2.00	1.41	3.00
	ΣFIC	1.13	0.56	2.25	1.13	0.56	0.63	0.63	0.63	0.63	ND	1.25	**0.25**	1.07	0.64
Elephantorrhiza elephantina + *Pentanisia prunelloides* root	MIC	1.00	1.00	2.00	1.00	0.50	1.00	1.00	2.00	2.00	>16.00	2.00	2.00	1.42	1.40
	ΣFIC	1.13	**0.38**	2.25	0.56	0.53	**0.38**	0.56	**0.31**	0.63	ND	1.13	**0.31**	1.04	**0.39**
Pentanisia prunelloides root bark + *Dicoma anomala* tuber	MIC	1.00	4.00	1.00	8.00	1.50	>16.00	1.00	16.00	8.00	>16.00	4.00	8.00	2.75	9.00
	ΣFIC	1.13	0.75	1.13	0.75	1.69	ND	1.13	1.50	1.00	ND	2.13	1.00	1.37	1.00
Pentanisia prunelloides roots stripped + *Dicoma anomala* tuber	MIC	1.00	8.00	1.00	2.00	1.00	8.00	1.00	4.00	4.00	8.00	4.00	16.00	2.00	7.67
	ΣFIC	1.13	2.00	1.13	**0.38**	1.06	1.50	1.06	**0.38**	**0.50**	0.75	1.56	3.00	1.07	1.34

[a] MRSA methicillin resistant *Staphylococcus aureus*; [b] GMRSA gentamycin-methicillin resistant *Staphylococcus aureus*; [c] D:M dichloromethane: methanol (1:1); [d] Aq aqueous extracts; ND ΣFIC index not determined as MIC values >16.00 mg/ml.

Plant species incorporated in the combinations were *Dicoma anomala*, *Elephantorrhiza elephantina* and *Pentanisia prunelloides*. These plants have also been reported to be used independently and in combination for a variety of skin ailments (Table 1.1, Chapter 1). Mostly, non-interactive effects were noted. However, some interactions worth highlighting are the combination of *Dicoma anomala* with *Elephantorrhiza elephantina* (organic extracts) where the ΣFIC value was 4.0, which was bordering on an antagonistic effect. The combinations of *Pentanisia prunelloides* (root) with either *Elephantorrhiza elephantina* or *Dicoma anomala* demonstrated selective synergistic interactions. One interesting combination was that of *Pentanisia prunelloides* with *Elephantorrhiza elephantina* (roots). Even though *Pentanisia prunelloides* (root) did not exert any noteworthy antimicrobial effects when screened independently, synergistic interactions were noted when the aqueous extract of the plant was combined with *Elephantorrhiza elephantina* (root and rhizome), presenting with a mean ΣFIC value of 0.39.

Considering that the traditional use of plants in combination is not an exact science (where formulations are accurately measured to the exact mg or µg quantity), this combination was combined in various ratios to determine if different concentrations of the two plants in the mixture may result in alternate interactions (Figure 3.2 and 3.3).

From the isobolograms synergistic interactions were observed for the aqueous extracts combination (*P. prunelloides*: *E. elephantina*) against *S. aureus* with the majority of the ratio points falling below the 0.5 line. Synergistic interactions were noted for the combination (*P. prunelloides*: *E. elephantina*) ratios where equal or larger (5:5-1:9) quantities of *E. elephantina* were used in the mixture (Figure 3.2). Additive interactions were observed for the combination with two ratio combinations 6:4 and 7:3 (*P. prunelloides*: *E. elephantina*) falling between 0.50-1.00 region. Non-interactive

antimicrobial interactions were also noted for two ratio combinations (8:2 and 9:1) where *P. prunelloides* was in the majority. Additive interactions were observed for most of the organic extract combinations. Similarly to the aqueous extract, non-interactive antimicrobial interactions were noted for two combinations 8:2 and 9:1 where *P. prunelloides* was found to be in higher concentrations (Figure 3.2).

The aqueous extracts combination against GMRSA exhibited antimicrobial interaction profiles similar to those of *S. aureus*. However, some variations were noted for the organic extracts combination where three combinations (*P. prunelloides*: *E. elephantina*; 8:2, 6:4 and 5:5) showed non-interactive antimicrobial interactions (Figure 3.2).

The *P. prunelloides: E. elephantina* combination displayed mainly synergistic interactions when examined against *S. epidermidis*, with most of the ratios distributed in the isobologram region below 0.5. Additive interactions were noted for two ratio combinations (8:2 and 6:4) where the concentration of *P. prunelloides* is in majority, while non-interactive interactions were observed for the (*P. prunelloides*: *E. elephantina*) 9:1 ratio (Figure 3.2). Additive interactions were predominant for the organic extracts with all the ratio points falling in the region between 0.50-1.00, with exception of one ratio (*P. prunelloides*: *E. elephantina*; 8:2) showing non-interactive properties (Figure 3.2). No antagonistic interactions were observed and synergistic interactions were predominant for the aqueous extracts at varying ratios against *S. aureus*, GMRSA and *S. epidermidis.*

When examining the antimicrobial interactive profiles of the *P. prunelloides* and *E. elephantina* combination (aqueous extract) against MRSA and *C. albicans*, it was noted that the combination mainly exhibited additive effects (Figure 3.3).

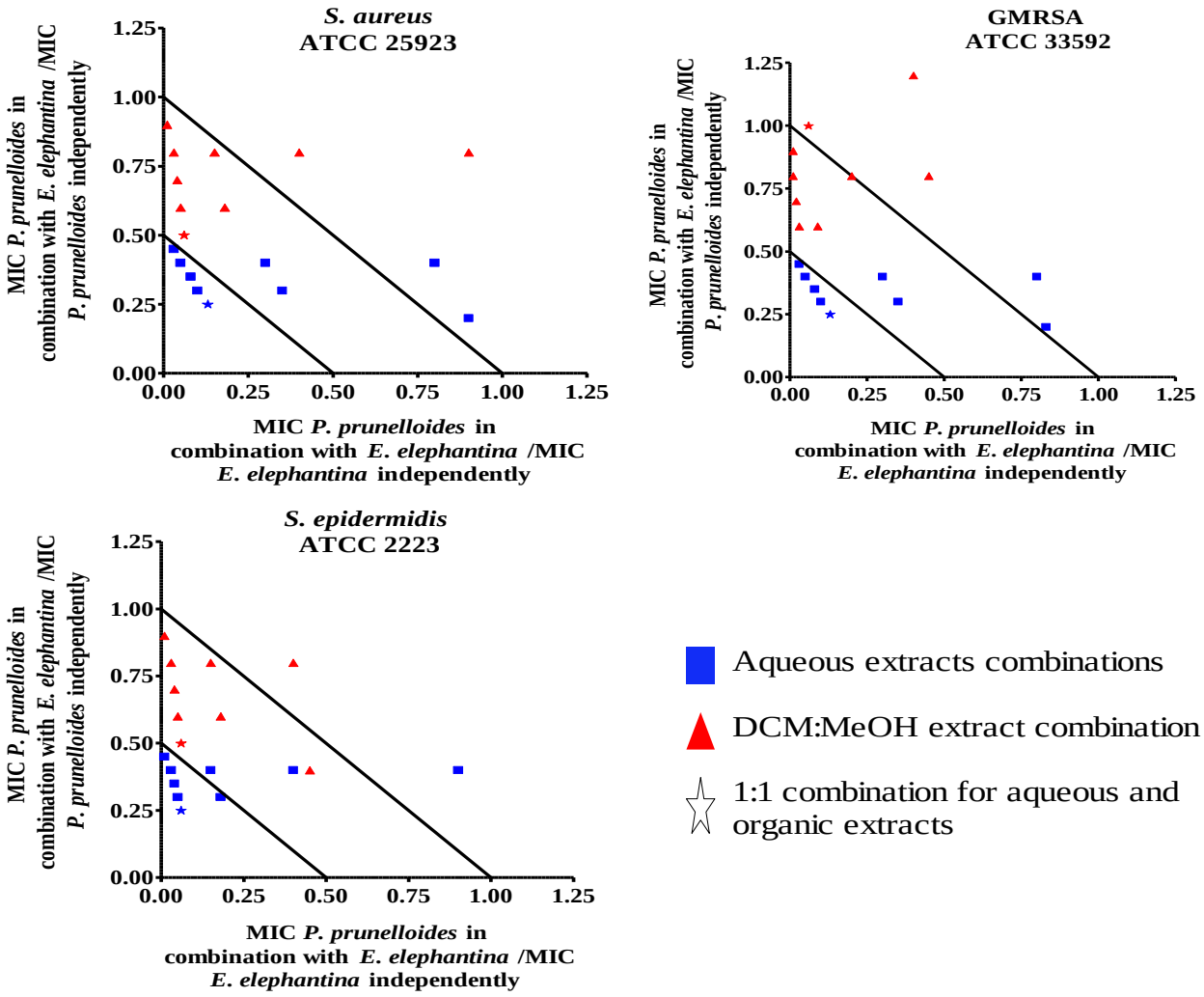


Figure 3.2: Isobologram representation of *Pentanisia prunelloides* and *Elephantorrhiza elephantina* combination against *S. aureus*, GMRSA and *S. epidermidis*.

It was observed that all the ratio combinations plotted for the aqueous extract against MRSA fell in the additive vicinity between 0.50-1.00, with the exception of one ratio (*P. prunelloides*: *E. elephantina*) 6:4 which fell in the region >1.00 hence displaying a non-interactive antimicrobial interaction (Figure 3.3). A similar profile was noted for the organic extracts with the combination displaying mostly additive interactions, however, it was noted that three combinations (*P. prunelloides*: *E. elephantina*; 9:1, 7:3 and 5:5) showed non-interactive antimicrobial interactions (Figure 3.3). The combination exhibited

predominantly additive interactions against *C. albicans* for both aqueous and organic extracts. Non-interactive interactions were also evident for four aqueous extract combinations (*P. prunelloides*: *E. elephantina*; 9:1, 8:2, 7:3 and 6:4). Five combinations of the organic extracts also displayed a non-interactive antimicrobial interaction (9:1; 8:2; 7:3; 5:5 and 4:6) (Figure 3.3).

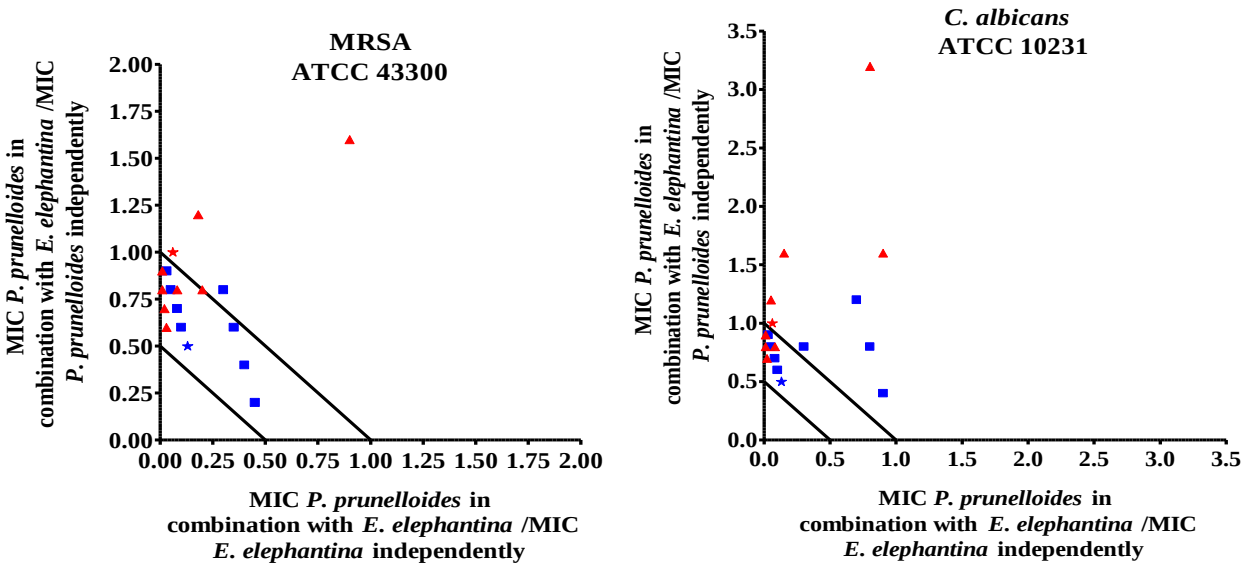


Figure 3.3: Isobologram representation of *Pentanisia prunelloides* and *Elephantorrhiza elephantina* combination against MRSA and *C. albicans*.

The most favourable interactions were mainly observed for the aqueous extracts. It was also worth noting that the 1:1 combinations of the aqueous extracts demonstrated the most synergistic interactions, lending some credibility to the traditional use of water preparations for medicinal purposes. From the isobolograms (Figure 3.2 and 3.3), it can be determined that as the concentration of *P. prunelloides* decreases and that of *E. elephantina* increases, the greater the antimicrobial effect for both organic and aqueous extract combinations. From this finding, we can surmise that the enhanced efficacy of the

combination was mainly attributed to the antimicrobial effects of *Elephantorrhiza elephantina*.

While the individual plant species (*P. prunelloides* and *E. elephantina*) are indicated for various dermatological effects, the combination is indicated for eczema. Individually the plant species are prepared for topical application. Surprisingly the combination preparation is administered orally (Felhaber, 1997). Even though this plant combination is not traditionally targeted for its antimicrobial properties, based on these findings, the aqueous combination looks favourable as an antiseptic or antimicrobial.

3.4 Conclusions

- Synergistic interactions were noted when the aqueous extract of *P. prunelloides* was combined with *E. elephantina* (root and rhizome) in a 1:1 combination, presenting with a mean ΣFIC value of 0.39.
- No antagonistic interactions were observed.
- Synergistic interactions were predominant for the aqueous extracts at varying ratios against *Staphylococcus aureus*, GMRSA and *Staphylococcus epidermidis*, with most ratio combinations plotting in the region below the 0.50 line of the isobologram.
- The 1:1 combination of *P. prunelloides* and *E. elephantina* aqueous extracts demonstrated the most synergistic interactions, lending some credibility to the traditional use of water preparations for medicinal purposes.

Chapter 4: *Aristea ecklonii*: Bioactivity-guided isolation of an antimicrobially active compound

4.1 Introduction

The antimicrobial investigation of the dichloromethane: methanol (1:1) extract of the *Aristea ecklonii* leaf indicated noteworthy antimicrobial effects (Section 2.4.2, Chapter 2), against most of the tested skin pathogens. The plant species demonstrated broad-spectrum activity with MIC values between 0.05-0.30 mg/ml, with exception of *B. agri* and *M. canis* which showed less microbial inhibition. Given these results and considering that limited research that has been done on this plant, a decision was taken to subject the dichloromethane: methanol leaf extract of *A. ecklonii* to bioactivity-guided fractionation in order to isolate compound/s of possible antimicrobial interest.

4.1.1 Botanical description

Aristea ecklonii is a spreading, evergreen, rhizomatous perennial plant with stiff, upright and grass-like leaves. It grows to between 30 and 70 cm tall in a tight clump. The flowers are bright blue, blooming in spring or early summer and the flowering stalks become covered with dozens of small, blue saucer-shaped flowers standing above the leaves (Figure 4.1). Each flower lasts for one day and only opens in bright light (Csurhes, 2008).

4.1.2 Distribution

The genus *Aristea* constituting of more than 40 species is known to be native to sub-Saharan and central Africa and Madagascar occurring in lower elevations. This plant

can be found along the Drakensburg, Cape Province, Transvaal, Natal and grows in many gardens (Csurhes, 2008).



(a) **(b)**

Figure 4.1: *Aristea ecklonii* plant (a); blue flowers of the plant in its flowering stage (b).
http://sydneyweeds.org.au/weed/blue-star/
http://www.prioryplants.co.uk/SouthAfricanplants/81/display.aspx

4.1.3 Medicinal uses

The leaves of *Aristea* species have been reported to be traditionally used for various ailments such as colds, flu, malaria, toothache and skin bruises (Theunissen, 2002). Ngwenya *et al*. (2003), has reported that Zulu traditional healers use the whole *Aristea ecklonii* plant for the treatment of shingles. It has also been detailed that the leaf infusion of this plant is administered as enemas for the treatment of sicknesses accompanied by fever, cough as well as syphilis (Hutchings, 1996).

4.2 Materials and methods

4.2.1 Preparation of the crude extract

A bulk sample of *Aristea ecklonii* plant material was purchased from the Random Harvest Indigenous Nursery located north of Johannesburg. A voucher specimen (voucher number

UM163) was prepared and housed at the Department of Pharmacy and Pharmacology, University of the Witwatersrand. Aerial parts of the plant were separated from the roots and left to air dry at room temperature for two weeks. They were then ground into coarse powder using a Variable Speed Rotor Mill Pulverisette (Fritsch). The plant material was ground into a finer powder using a Salton Elite high speed grinder. The powder was sieved using a 600 microns sieve (Protea Holdings Limited) to ensure a uniform particle size. A mass of 60 g dried ground leaf material was extracted using 600 ml of the 1:1 mixture of dichloromethane (DCM) and methanol. The leaf and solvent mixture was then sonicated using Sonorex™ Digital 10 P ultrasonic bath (Bandelin) for 20 min at 25 °C. Thereafter, the leaf and solvent mixture was filtered using a Whatman® folded filter paper. This procedure was repeated using fresh solvent (10x) until the resulting extract was clear. The ten filtrates were then combined and evaporated to dryness using a Rotavapor® R-215 (Buchi) to concentrate the extract. Concentrated extract was then re-suspended in 200 ml of 1:1 mixture of dichloromethane and methanol. In order to determine the yield an aliquot of the extract (1 ml) was transferred into a glass vial and dried in a vacuum oven (Vismara, Ltd) at 40 °C for 12 hours. The yield was then calculated by extrapolating the mass of the extract in 200 ml.

4.2.2 High performance thin layer chromatography (HPTLC) and thin layer chromatography (TLC)

The screening of the extract, the fractions from liquid-liquid partitioning step and the combined fractions from column chromatography (CC) were analysed using HPTLC as well as those obtained from high speed counter-current chromatography (HSCCC). The fractions collected from the column were analysed by TLC before pooling those with similar composition. Screening was performed using a semi-automated Camag HPTLC

system consisting of a TLC 4 Sampler, with an automated ADC2 development chamber, a Chromatogram Immersion Device III, a Digistore Reprostar 3 and a TLC Scanner 3 (Camag) (Figure 4.2). The HPTLC system was controlled using Wincat® (version 1.4.4.6337) planar chromatography software. The TLC auto-sampler, fitted with a 25 µl syringe and connected to nitrogen gas, was used for sample application.

Aliquots of the extract representing 2.0 µl per band (15 mm wide and 10 mm apart) were spray applied onto a strip (11 x 10 cm) cut from an Alugram™ Sil G/UV_{254} aluminium-backed TLC plate (2 mm layer; Macherey-Nage; 20 × 20 cm). The plate was developed in the automated development chamber using a twin trough chamber, by the ascending technique, to a migration distance of 85 mm. A solvent combination of chloroform: methanol: water: glacial acetic acid (100: 50: 50:1 v/v/v/v) was selected as the most appropriate mobile solvent. The chamber was saturated with the mobile phase for 20 minutes at 25 ºC and conditioned to 47% relative humidity using KSCN. Ultraviolet/visible (UV/Vis) wavelengths of 254 and 366 nm were used to visualise developed plates. Thereafter the compounds were derivatized with *p*-anisaldehyde reagent in the chromatogram immersion device.

The TLC plate technique is considerably simple, rapid and cost effective for the screening of bioactive constituents in natural products (Frum, 2006; Liu, 2011). Derivatized plates were then heated on a hot plate at 100 °C (Fried electric). This ensured enhancement of the colours representing the different compounds in the extract. The TLC plate was subjected to bio-autography assay and the non-polar portion was selected for further evaluation.

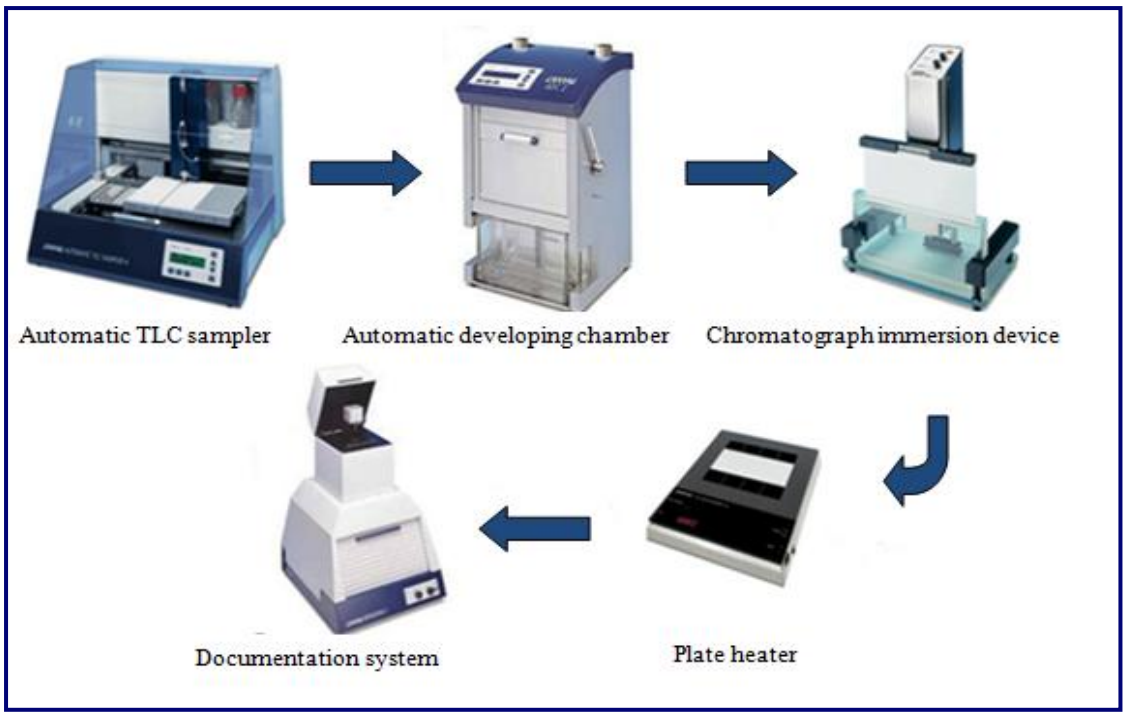


Figure 4.2: HPTLC process using automatic TLC sampler, automatic developing chamber, chromatograph immersion device, plate heater and TLC scanner (documentation system) (Camag).
http://www.hptlc.us/v/products/application/ats4.html
http://www.camag.com/v/products/development/adc2.html

4.2.3 Solvent (liquid-liquid) partitioning

As depicted in the HPTLC plates (Figure 4.5 and 4.6), crude extract are usually complex mixtures of various metabolites characterised by varying chemical and physical properties. The liquid extract was partitioned by adding distilled water into 200 ml of extract to obtain a total volume of 400 ml. Equal volume of a non-polar dichloromethane solvent was added and the mixture was swirled gently before being left to settle in a separating funnel. The resulting two phases were then separated after equilibration by collecting the lower phase (non-polar dichloromethane). The partitioning was repeated by adding fresh dichloromethane until a clear lower phase was obtained (Figure 4.3). Separated fractions were concentrated by evaporation in Buchi rotavapor R-215. Portions of these fractions

were analysed using TLC and subjected to bio-autography assay. Based on the bio-autography results the dichloromethane portion was selected for further analysis.

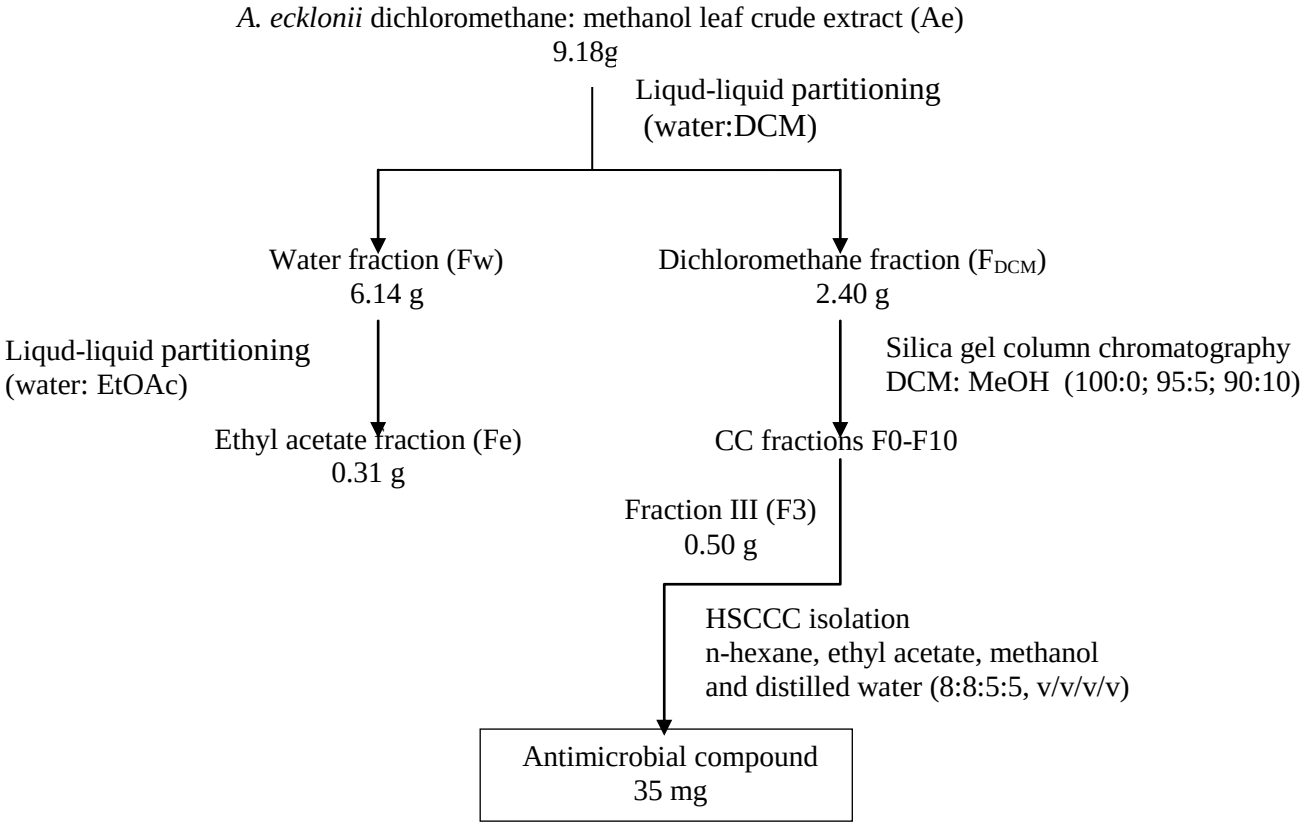


Figure 4.3: Bio-activity guided fractionation process (fractions and sub-fractions which showed promising antimicrobial activities on the bio-autography assays were considered for further purification).

4.2.4 Silica gel column chromatography (CC)

Separation of chemical compounds in CC is mainly dependent on the partitioning differences of the constituents between the stationary (sorbent) and mobile phase (eluent). Column chromatography reduces the number of compounds present per fraction and therefore allows for easy separation of compounds when subjected to HSCCC. A glass column (60 cm long and 40 mm i.d.) clamped on an upright position was wet packed and used for fractionation. A mass of 30 g of silica gel (Kieselgel 60, Macherey-Nagel) was packed onto the column and DCM was used as the mobile phase. The dry dichloromethane

fraction, from liquid-liquid partitioning step, was dissolved in the initial mobile phase (DCM) solvent and applied onto the column. Anhydrous sodium sulphate (Saarchem, Merck) was applied on top of the extract to prevent moisture absorption into the column. Separation was achieved by gradient elution, which involved eluting the column with varying polarities of a DCM and methanol mixture (100:0; 95:5; 90:10) (Figure 4.3). Fractions (100 ml) were collected from the column and analysed using TLC with a DCM and methanol (90:10) mixture used as the development solvent. *p*-Anisaldehyde was used as the derivatization reagent. Fractions presenting with similar chemical composition were combined and evaporated to dryness. A total of eleven fractions were obtained (F0-F10) and after autobiographic assay, Fraction F3 was selected for HSCCC separation (Figure 4.3).

4.2.5 High speed counter-current chromatography

Counter-current chromatography (CCC), is a liquid-liquid chromatographic method, which makes use of a support-free liquid stationary phase that is held in place by a simple or complex centrifugal force field (Ito, 2005). The choice of this chromatography method is encouraged by a number of factors mainly being, no irreversible absorption, total recovery of the injected sample, reduction in tailing of fractions, low solvent consumption and low risk of sample degradation among other advantages (Ito, 2005; Marston and Hostettmann, 2006). The success of this method mainly depends on the suitable choice of a two-phase solvent system. For the purpose of this study, a two phase solvent system was prepared containing n-hexane, ethyl acetate, methanol and distilled water (8:8:5:5, v/v/v/v). The two phase solvent mixture was vigorously shaken and allowed to settle. Once thoroughly equilibrated in a separating funnel at room temperature, the two phases were separated and filtered (0.45 µm Millipore nylon filters) before use. The sample solution was prepared by

dissolving approximately 120 mg of the dry extract of Fraction, F3 from CC separation into 2 ml of the solvent mixture consisting of equal volumes of the two phases (Figure 4.3).

The hydrodynamic HSCCC instrument, De Spectrum Centrifuge (multilayer coil-planet J-type), (Dynamic Extractions Ltd.) (Figure 4.4) was used. It is equipped with two preparative coils which are connected in series (wrapped with poly tetra-fluoro ethylene; PTFE tubing, 1.6 mm i.d., 140 ml total volume for semi-preparative column). The inner br-value was measured (0.52) at the internal end of the coil and outer br-value was 0.86 (br = r/R, where r is the distance from the coil to the holder shaft, and R, the revolution radius or the distance between the holder axis and central axis of the centrifuge). Solvent was pumped with a Q-Grad Quaternary Gradient pump (Scientific Systems Inc.) and monitoring of the effluent was achieved with a Sapphire UV/Vis variable wavelength detector (Ecom) at 270 nm. Fractions were collected with a model FC 204 fraction collector (Gilson, Middleton, WI). A manual sample injection valve with a 6.0 ml loop from Rheodyne (Rohnert Park, CA) was used to introduce the sample into the column. The resulting fractions were analysed using HPTLC and subjected to autobiographic assay.

4.2.6 Determination of the purity of the isolated compound

The purity of the isolated compound was determined by UHPLC. The UHPLC system used was comprised of a Waters Acquity ultra high performance liquid chromatography sample manager (Waters™), a binary solvent manager and a photodiode array (PDA) detector (210 – 400 nm). Separation was achieved using a Waters Acquity reversed phase UHPLC BEH C18 column (2.1 x 100 mm, 1.7 µm particle size), fitted with a Van Guard pre column (2.1 x 5 mm, 1.7 µm). An injection volume of 1 µl was applied and the column temperature was adjusted to 40 ºC. The mobile phase consisted of (A) 0.5% acetic acid and

(B) acetonitrile at a flow rate of 0.3 ml/minute. Gradient elution was employed, starting with 90% A and 10% B, changing to 50% B in 15 minutes, then changing to 100% B in 1 minute, with a post-run time of 1 minute. The purity of the isolated compound was calculated from the integrated peak area.



Figure 4.4: High speed counter-current chromatography (Dynamic Extractions Ltd.) used to isolate active compounds from the CC fraction (F3) of *A. ecklonii*.

4.2.7 Structure elucidation

The structure of the isolated compound was determined by using nuclear magnetic resonance **(**NMR) in collaboration with Prof. A. Marston (Department of Chemistry, University of the Free State, Bloemfontein, South Africa). The structure was also confirmed by comparison of the spectral data with that from literature. The NMR spectra were recorded on a Bruker 600 Avance II NMR at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C and distortionless enhancement through polarisation transfer (DEPT) NMR. Comprehensive structure determination involved 2D NMR spectroscopic analyses, including correlated spectroscopy (COSY), hetero nuclear multiple quantum coherence (HMQC) and hetero nuclear multiple bond connectivity (HMBC) were performed using

standard Bruker micro programs. Measurements were made in $CDCl_3$, using solvent signals for calibration.

4.2.8 Antimicrobial activity

4.2.8.1 Bio-autography agar diffusion assay

To identify the bio-active compounds, the dichloromethane: methanol (1:1) *Aristea ecklonii* leaf extract was subjected to bio-activity guided fractionation using thin layer chromatography (TLC). Developed TLC plates were used for bio-autographic assays at various stages of isolation. The bio-autographic assay is considered a quick method to detect and separate active constituents from complex plant extracts since the assay is practically simple and requires no special equipment (Gu *et al*., 2004). Silica gel TLC plates spotted with the crude extract and isolated fractions were placed on an upright position on a sterile petri dish layered with TSA. Pathogen selection was based on a selection of organisms to represent Gram-positive, Gram-negative and yeast strains (*S. aureus* ATCC 25923, MRSA ATCC 43300, GMRSA ATCC 33592, *S. epidermidis* ATCC 2223, *P. aeruginosa* ATCC 27858 and *C. albicans* ATCC 1023). Not all dermatologically relevant strains were selected for the sake of brevity. Cultures were prepared and inoculated into TSA in a 1:100 ratio to obtain an approximate inoculum size of 1×10^6 CFU/ml (van Vuuren *et al*., 2006). The inoculated TSA was then layered on top of the TLC plate. Discs submerged into test sample (antimicrobial compound) solution were placed on top of the agar. The agar plates were then incubated at 37 °C for 24 hours and 48 hours for bacteria and yeast, respectively. The microbial growth indicator, INT was then sprayed onto the plates for visualisation and the plates compared to the reference TLC plates. Bioactive fractions were determined as having a zone of inhibition around the active

band clearly demarcated by the colour change, from colourless to pink where there is microbial growth.

4.2.8.2 Micro-titre plate dilution technique: Minimum inhibitory concentration (MIC)

A serial micro-dilution technique as detailed in Section 2.3.3, Chapter 2 was used to quantify the antimicrobial activity for the crude extract, isolated bioactive fractions and compound. The crude extract and the isolated fractions were each dissolved in acetone at a starting concentration of 5 mg/ml. Where necessary, the starting concentration was diluted to 0.5 mg/ml.

4.3 Results and discussion

4.3.1 Bio-assay guided isolation of Compound 1

The yield of the extract was 9.18 g (18.4% of dry weight). For structural elucidation or bio-assay guided isolation, it is considered the first and necessary step to do solvent partitioning (Liu, 2011). This is intended to divide the chemical compounds into several groups based on chemical and physical compatibilities, where compounds with varying polarities can be partitioned into various fractions. This is achieved by the principle that "like dissolves like" (Liu, 2011). Analysis of the crude extract of *A. ecklonii* using HPTLC revealed a number of compounds. Figure 4.5, A-C shows HPTLC chromatograms of the crude DCM: MeOH (1:1; v:v) observed under 254 and 366 nm as well as after derivatization using *p*-anisaldehyde reagent. The best activity on the TLC bio-autography was observed for the compound with retardation factor (R_F) 0.72. The compound was visibly clear as a red band under 366 nm (Figure 4.5, chromatograph A). After liquid-liquid partitioning using ethyl acetate (EtOAc) and DCM, and analysis of the resulting

fraction using HPTLC, the compound of interest was observed in the EtOAc and DCM fractions (Figure 4.6). The aqueous fractions from the EtOAc and DCM partitioning steps consisted of mainly polar compounds which showed poor activity on TLC bio-autography, hence they were not targeted for isolation. Even though the ethyl acetate fraction comprised of Compound 1 it was not an obvious choice for further analysis, the fraction of choice was supported by results obtained from MIC and bio-autographic assays. The DCM fraction exhibited noteworthy antimicrobial effects, making a suitable choice for bio-activity guided fractionation. Bio-autography assays showed that the DCM portion displayed growth inhibition hence it was selected for further isolation.

Analysis of fractions obtained from CC by TLC and pooling those with similar compositions, resulted in 11 major combinations (F0-10) (Figure 4.7). Due to the low polarity of the compounds constituting this fraction, DCM: MeOH (90: 10; v:v), was selected as the most appropriate development solvent for both TLC analysis of the collected fractions and for HPTLC analysis of the combined fractions. Fraction F3 was noted to possess the bio-active compound, which appeared as a blue band under 254 nm, a red fluorescent band under 366 nm and a yellow band after derivatization with *p*-anisaldehyde reagent.

When Fraction F3 was subjected to further separation using HSCCC, a pure compound was obtained. The HSCCC chromatogram obtained from separation of fraction F3 is depicted in Figure 4.8. This separation yielded 35 mg of the isolated Compound 1, which appeared as orange needle-like crystals at room temperature (25 °C). The purity of Compound 1 was determined both by HPTLC and UHPLC (99%). The HPTLC chromatogram of Compound 1 is shown in Figure 4.9, while the UHPLC fingerprint of the

DCM: MeOH extract of *A. ecklonii* and the chromatograms of the isolated compound are depicted in Figure 4.10, A and B respectively.

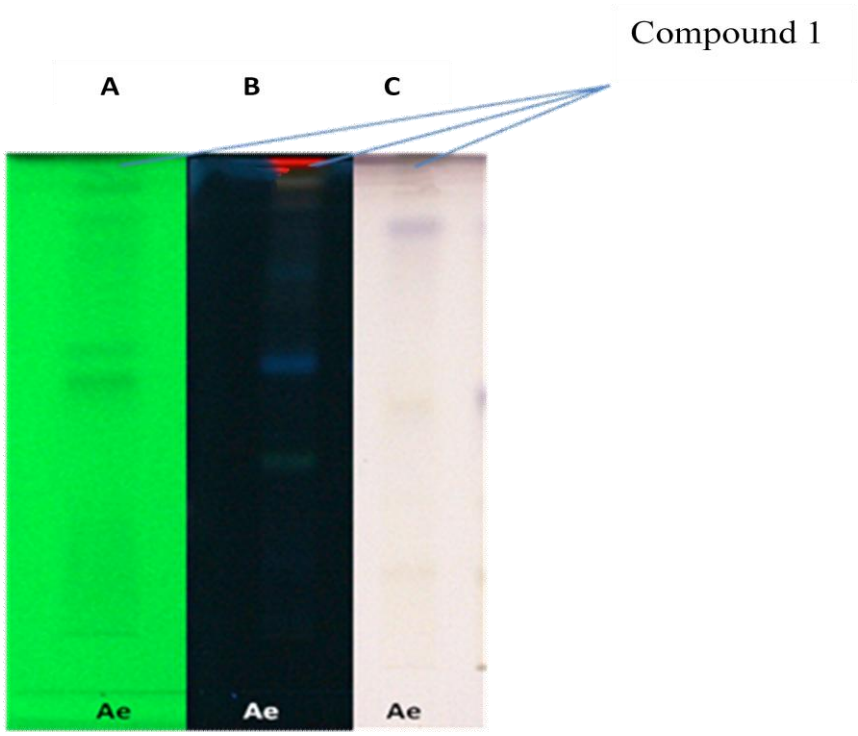


Figure 4.5: HPTLC chromatograms of the crude DCM: MeOH extract of *Aristea ecklonii*, chromatographs at varying wavelengths A) 254 nm, B) 366 nm and C) after derivatization with *p*-anisaldehyde reagent.

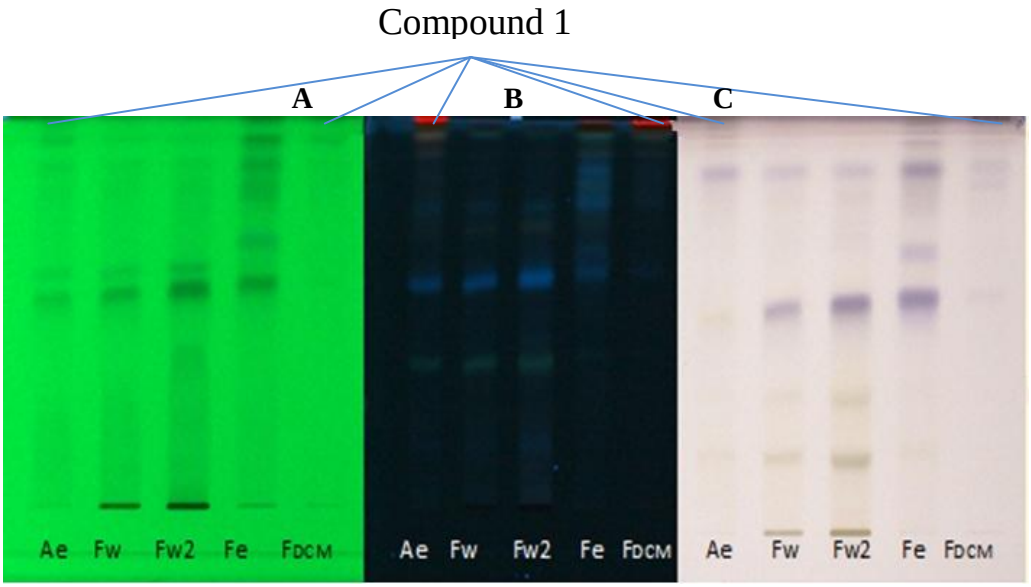


Figure 4.6: HPTLC chromatograms showing fingerprints of the crude (dichloromethane: methanol, 1:1) extract (Ae) and the fractions (Fw, Fw2, Fe, F_{DCM}) from liquid-liquid chromatography of *Aristea ecklonii*. A) 254 nm, B) 366 nm, and C) after derivatization with *p*-anisaldehyde reagent.

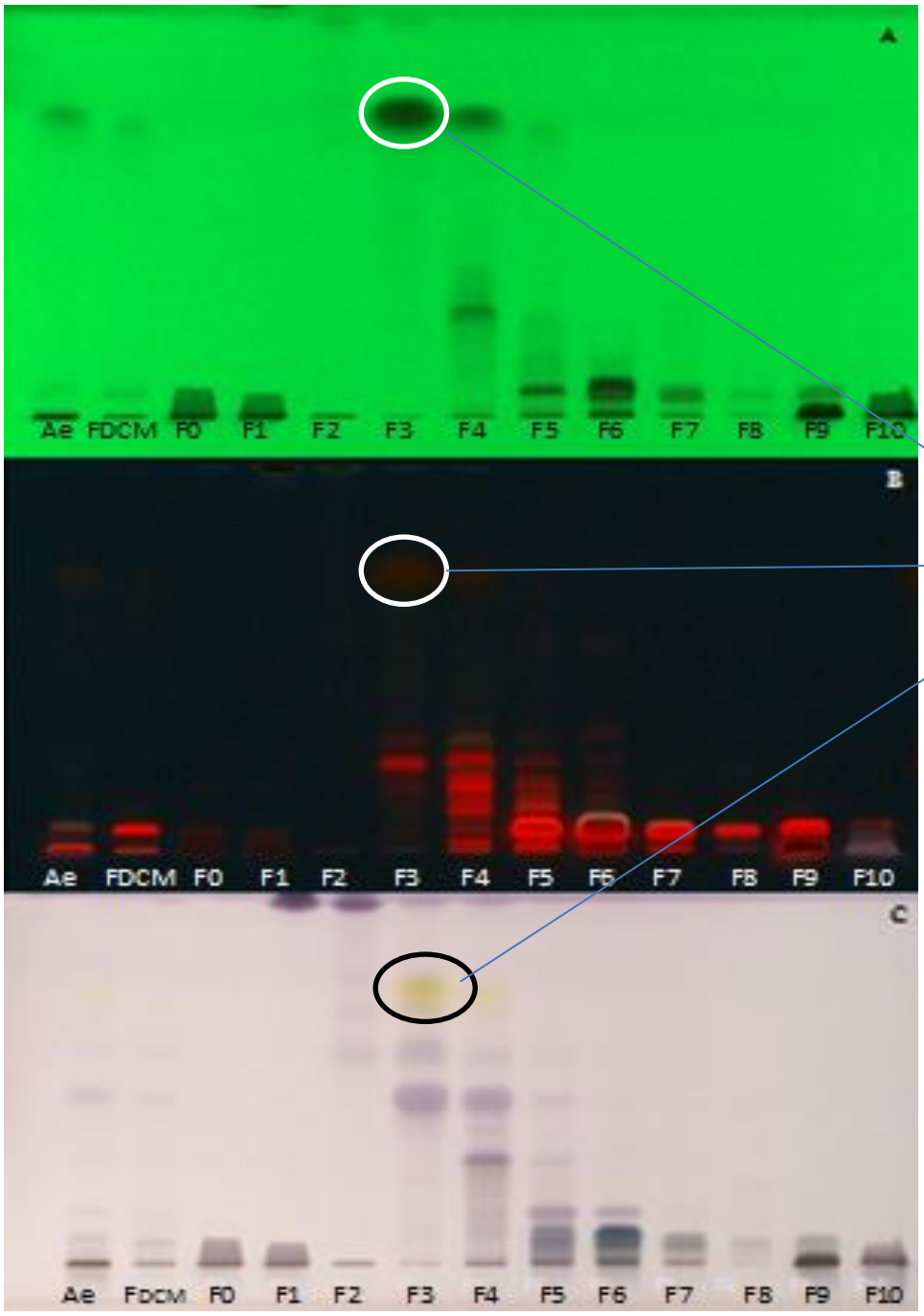


Figure 4.7: HPTLC chromatograms showing fingerprints of the crude (dichloromethane: methanol, 1:1) extract (Ae), F_{DCM} and eleven fractions obtained from column chromatography A) 254 nm, B) 366 nm, and C) after derivatization with *p*-anisaldehyde reagent.

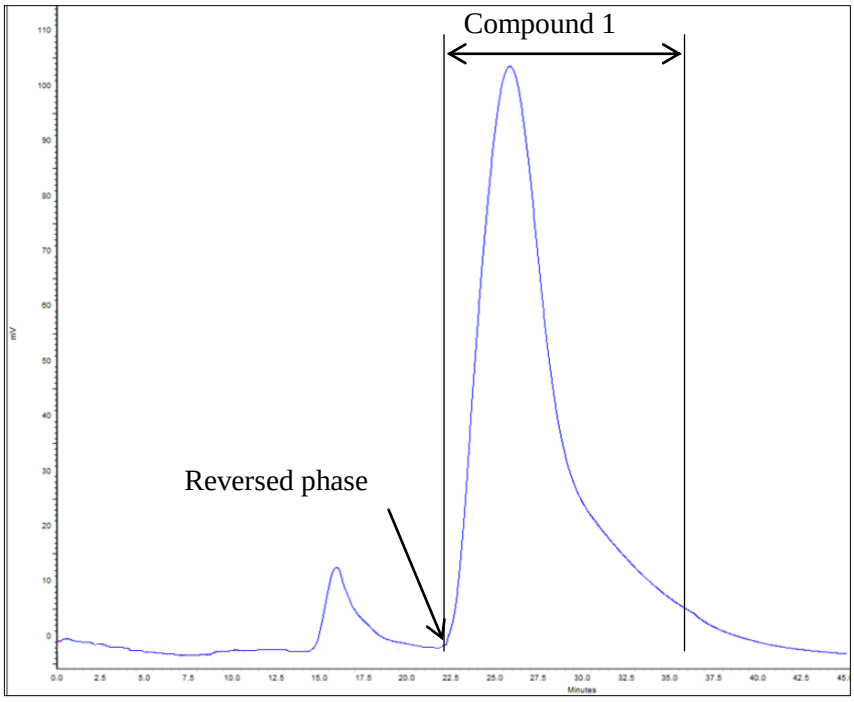


Figure 4.8: HSCCC chromatogram of Fraction 3 obtained from silica gel column chromatography. Solvent system: n-hexane: ethyl acetate: methanol: distilled water (8:8:5:5, v/v/v/v) instrument run for 45 minutes. Isolated compound (Compound 1).

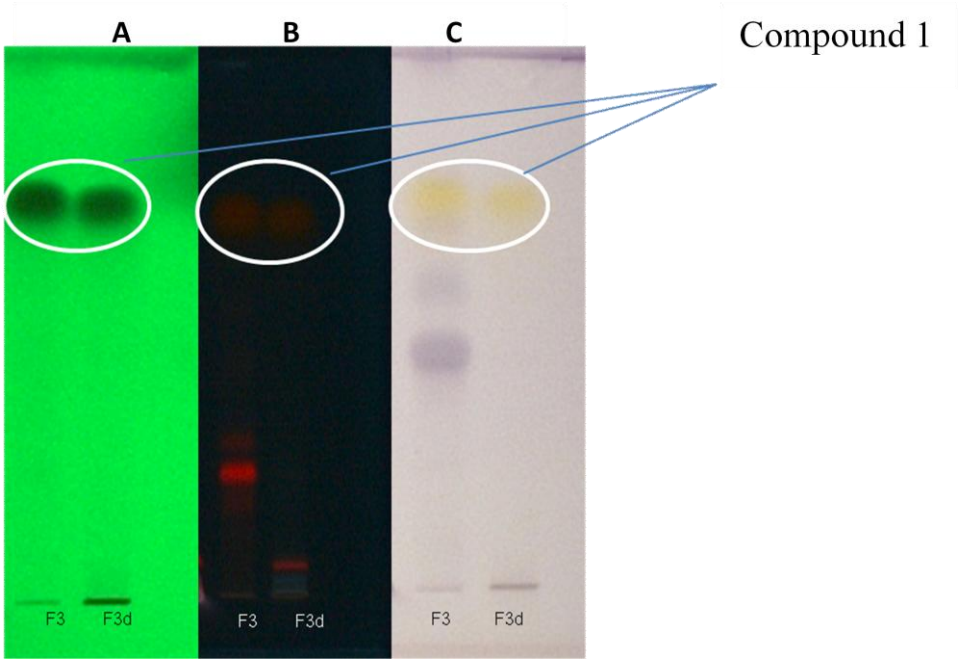


Figure 4.9: HPTLC chromatogram of Fraction F3 from column chromatography separation and Compound 1 (F3d) from HSCCC separation. HPTLC mobile phase DCM: MeOH (90:10).

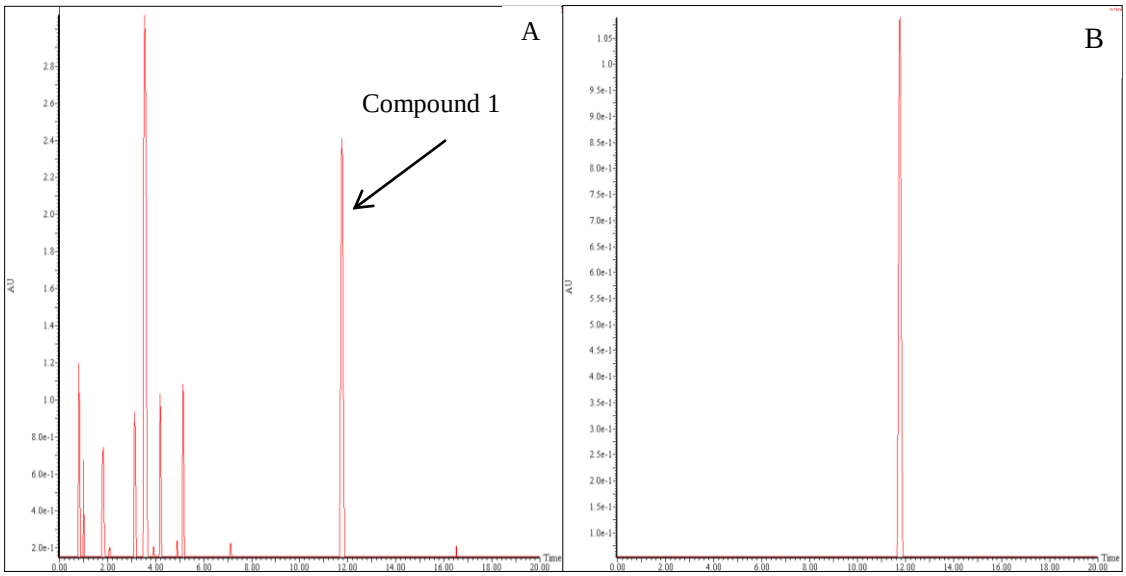


Figure 4.10: UHPLC fingerprint of the crude DCM: MeOH extract and the chromatogram of Compound 1 obtained from HSCCC separation.

4.3.2 Structure elucidation of Compound 1

Compound 1 appeared as orange needle-like crystals at room temperature with a UV/Vis absorbance wavelength of 267 nm (Figure 4.11). The structure of Compound 1 was determined by comprehensive 1 dimension (1D) and 2D ^{1}H and ^{13}C NMR and by comparison of the spectral data with that from literature. The 2D NMR experiments used for structure elucidation included DEPT, COSY, HSQC and HMQC. This compound consists of an aromatic ring fused to a cyclohaxenedione ring. The ^{1}H and ^{13}C DEPT NMR spectra for Compound 1 are given in Appendix A. The isolated compound showed the following NMR signals; ^{1}H NMR ($CDCl_3$) δc: 6.85 (1H, s, H-3), 7.24 (1H, d, j=7.5, 8.4 Hz, H-6), 7.66 (1H, dd, J=7.5, 8.4 Hz, H-7), 7.57 (1H, d, J=7.5 Hz, H-8), 2.15 (3H, s, Me-2), 11.97 (–OH); ^{13}CNMR($CDCl_3$), δ: 184.6 (C-1), 149.6 (C-2), 135.1 (C-3), 190.3 (C-4); 160.3 (C-5), 123.5 (C-6), 136.0 (C-7), 118.6 (C-8), 132.1 (C-8a), 114.9 (C-4a), 14.9 (2-CH_3). The presence of an aromatic ring was indicated by the presence of six carbon signals at ∂_C:160.3, 136.0, 132.1, 123.5, 118.6 and 114.98 ppm in the aromatic region, corresponding to the benzene ring. Three aromatic proton signals at ∂_H:7.24, 7.66 and 7.57

ppm, in the ^{1}H spectrum, confirmed the presence of the tri-substituted aromatic ring. Compound 1 was identified as plumbagin (Figure 4.12) and the identity was confirmed by comparing the spectral data (NMR and UV/Vis) obtained to literature data (Kumar *et al*., 1985; Bothiraja *et al*., 2011). The presence of the two carbonyl carbons on the cyclohaxenedione ring in the ^{13}C spectrum is confirmed by the presence of carbon signals at ∂_C*:*190.3 and 184.6 ppm. The singlet proton signal in the ^{1}H spectrum at ∂_H*:* 2.15 ppm and the chemical shift at ∂_C*:* 135.1ppm in the ^{13}C spectrum confirmed the presence of the methyl substituent on the cyclohexendione ring. The olefenic singlet proton signal at ∂_H*:* 6.85 ppm and the carbon signals at ∂_C*:* 135.1 and 149.6 ppm on the ^{13}C spectrum indicated the presence of the double bond in the cyclohexendione ring.

A study by Bothiraja *et al*. (2011) reported that plumbagin has been isolated from the roots of *Plumbago zeylanica* L. (Plumbaginaceae), which is a semi-climbing sub herb which is traditionally used for treatment of rheumatic pain, scabies, sprains, skin diseases and wounds.

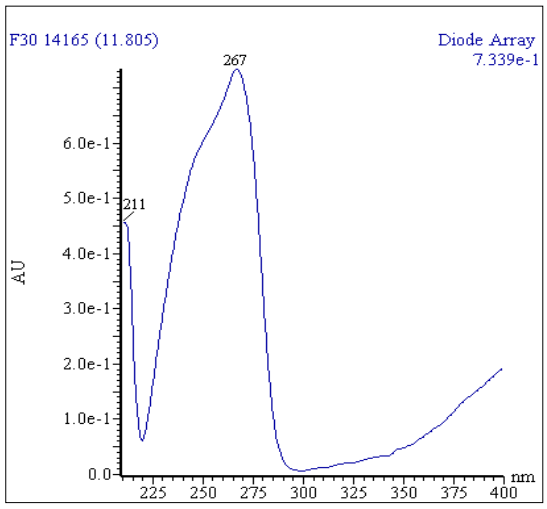


Figure 4.11: UV/Vis absorbance of Compound 1 (plumbagin) isolated from *Aristea ecklonii* leaves.

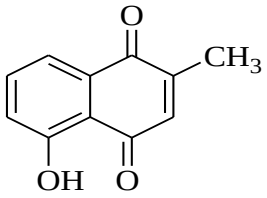


Figure 4.12: Structure of the napthoquinone plambagin, isolated from *Aristea ecklonii*.

4.3.3 Antimicrobial activity

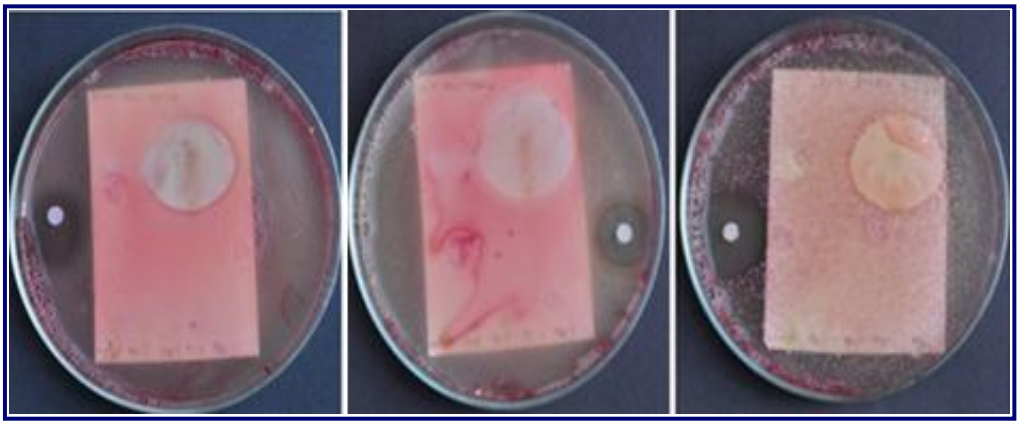
4.3.3.1 Bio-autography assay

The dichloromethane: methanol (1:1) *Aristea ecklonii* crude extract subjected to bio-activity guided fractionation yielded a bio-active compound observed from the non-polar fraction. Similar results were noted for all tested pathogens. Microbial growth inhibition against Gram-positive *Staphylococcal* spp. Gram-negative *P. aeruginosa* and the yeast *C. albicans* was observed. The clear zones observed on the TLC plates inoculated with culture suspension and sprayed with INT indicated growth inhibition attributed to the antimicrobial activity of the compound present in *A. ecklonii* leaf (Figure 4.13). The discs submerged in the compound solution also showed a zone of inhibition against tested pathogens. This technique gives qualitative analysis, hence it was important to include micro-titre plate assays for a more quantitative analysis.

4.3.3.2 Micro-titre plate dilution technique: minimum inhibitory concentration (MIC)

The antimicrobial properties of the compound were further investigated using the micro-titre plate dilution technique for a more quantitative approach. Plumbagin together with the crude (dichloromethane: methanol) extract, dichloromethane fraction and fraction F3

(fraction with isolated compound) displayed noteworthy antimicrobial activity against all tested pathogens (Table 4.1).



(a) **(b)** **(c)**

Figure 4.13: Bio-autography agar plates sprayed with INT; TLC plates spotted with crude extract and fraction F3 (DCM fraction with the compound), diffusion discs soaked in the compound solution. Bio-autography assay for a) *S. aureus*, b) MRSA, c) *C. albicans*.

Minimum inhibitory concentrations against a selection of pathogens for *A. ecklonii* (bulk) crude extract, fractions and the isolated compound are shown in Table 4.1. Isolated compounds which demonstrated MIC values below 100 µg/ml are considered to have clinical relevance (Gibbons, 2004). Furthermore, according to Rìos and Recio (2005), an isolated compound is considered to have very interesting antimicrobial activity when MIC values below 10 µg/ml are observed. Thus, the bio-active compound, plumbagin, displayed noteworthy antimicrobial activity (MIC range between 2.00-16.00 µg/ml) against all the tested pathogens, with particularly good activity against *S. epidermidis* (4.00 µg/ml) and *C. albicans* (2.00 µg/ml). The crude extract of *A. ecklonii*, fractions and isolated fractions yielded the lowest MIC values against *S. epidermidis* (Table 4.1), which is considered the most important cause of nosocomial skin infections (Section 1.1.1, Chapter 1). The antimicrobial effects of the compound against *C. albicans* were comparable to the

commercial antifungal, amphotericin B as they had the same MIC value (2.00 µg/ml). This was worth noting as it correlated with the susceptibility patterns for clinical yeast isolates, where amphotericin B was identified as having an MIC range of 0.06-2.00 µg/ml (Fothergill, 2012).

Table 4.1

Average minimum inhibitory concentration (µg/ml) of *Aristea ecklonii*, fractions and plumbagin

Plant fractions	***S. aureus* ATCC 25923**	**MRSA ATCC 43300**	**GMRSA ATCC 33592**	***S. epidermidis* ATCC 2223**	***P. aeruginosa* ATCC 27858**	***C. albicans* ATCC 10231**
Crude extract	156.00	156.00	156.00	78.00	156.00	313.00
DCM[a] fraction	39.00	78.00	39.00	20.00	78.00	78.00
Fraction F3	20.00	39.00	20.00	8.00	20.00	39.00
Plumbagin	**8.00**	**16.00**	**16.00**	**4.00**	**8.00**	**2.00**
Ciprofloxacin/ amphotericin B positive control	0.31	0.63	0.31	0.31	0.31	2.00
Acetone negative control (mg/ml)	16.00	>16.00	>16.00	>16.00	16.00	>16.00

[a] DCM: dichloromethane

The antimicrobial properties of *Aristea ecklonii* together with its chemical constituents against skin relevant pathogens, have not been investigated previously, however, the presence of plumbagin in both the roots and leaves of the plant was first reported by Kumar *et al*. (1985). Plumbagin isolated from *Plumbago* species such as *P. zeylanica* and *P. scandens* in a previous study also demonstrated noteworthy antimicrobial activity exhibiting much lower MIC values 1.56 and 0.78 µg/ml against *S. aureus* and *C. albicans*, respectively (de Paiva *et al*., 2003). Contrary to the current antimicrobial results for

plumbagin, it was noted that the effects of the compound against *S. aureus* and *C. albicans* were incongruent to the previous findings. As noted in Chapter 2, various reasons can account for this disparity, such as by the different culture strains used for *S. aureus* (ATCC 6538), the solvent used to dissolve the compound (30% DMSO for previous assay), the plant (roots) part and species from which the compound was isolated from. A diffusion assay by Jeyachandran *et al*. (2009) on plumbagin isolated from the root extract of *P. zeylanica* also showed noteworthy activity against a variety of pathogens including *S. aureus* (MIC <1.00 µg/disc) and *P. aeruginosa* (MIC >3.00 µg/disc).

Plumbagin isolated from *Plumbago* species such as *Plumbago zeylanica* and *Plumbago scandens* has been reported to possess anti-carcinogenic as well as time and concentration dependant toxicity in human peripheral blood cells (de Paiva *et al*., 2003; Wang *et al*., 2008; Bothiraja *et al*., 2011; Seshadri *et al*., 2011), which may warrant caution when used in higher concentrations.

4.4 Conclusions

- The bio-autography assays revealed the presence of an active compound in the non-polar dichloromethane portion of *A. ecklonii* leaf crude extract.
- The separation of the non-polar dichloromethane portion using column chromatography resulted in the fractionation of eleven fractions (F0-F10).
- The follow-up bio-autography assays showed the presence of an antimicrobially active compound in fraction F3.
- Further purification of fraction F3 using HSCCC resulted in the isolation of an antimicrobial compound, plumbagin.

- Plumbagin demonstrated noteworthy antimicrobial effects against tested pathogens with an MIC range between 2.00-16.00 μg/ml.
- Antimicrobial effects of plumbagin against *C. albicans* were comparable to the commercial antifungal amphotericin B (2.00 μg/ml).

Chapter 5: General conclusions

5.1 Overview

An in-depth literature review was conducted and over 100 plants from southern Africa used for the treatment of skin ailments were identified (Table 1.1). It was noted that many of the plant species had limited scientific data to support claims for their antimicrobial effectiveness, especially against skin specific pathogens such as *P. acnes, B. agri,* as well as dermatophytes such as *T. mentagrophytes* and *M. canis*. It was also observed from the literature that the use of plant combinations to treat specific skin ailments had also been sorely neglected in many antimicrobial studies in spite of the reported traditional use of polyherbal plant remedies. This study was undertaken with the aim of validating the traditional use of medicinal plants from an antimicrobial perspective, as well as finding a scientific rational for the use of plant combinations to treat skin ailments.

5.1.1 Antimicrobial activity

A total of 132 plant extracts (organic and aqueous) were prepared and screened for antimicrobial effects against skin relevant pathogens including fastidious pathogens such as *P. acnes, B. agri,* dermatophytes *T. mentagrophytes* and *M. canis*. When investigating the antimicrobial activities of plants used for skin infections it was found that most of the plant extracts demonstrated pathogen specific antimicrobial effects, with about 8.3% exhibiting broad-spectrum activities against most of the tested pathogens. Plants demonstrating notable broad-spectrum activities against the tested pathogens included extracts from *A. ecklonii, C. ambrosioides, D. mespiliformis, E. elephantina, E. camaldulensis, G. perpensa, H. caffrum, H. perforatum, M. comosus, T. sericea* and

W. salutaris. Notable antimicrobial properties of extracts of *E. elephantina*, *E. capensis*, *E. camaldulensis* and *H. caffrum* against *P. acnes* give some validation to the reported traditional used to treat acne vulgaris. *Dicoma anomala*, *D. mespiliformis* and *M. comosus* traditionally used for ringworm infections, demonstrated noteworthy antimicrobial against the dermatophytes thus giving some validation for their reported traditional uses. The aqueous extracts, displayed less efficacy compared to the organic extracts for most of the plant samples.

5.1.2 Interactive antimicrobial activity

To identify possible antimicrobial interactions of plant species used in combination to treat skin ailments, five different (1:1) combinations were selected and investigated for potential interactive properties. Synergistic interactions noted for the aqueous extracts of *P. prunelloides* combined with *E. elephantina* (1:1) warranted further investigation, where the interactive properties of the extracts were evaluated at various ratio combinations. The various combinations of *P. prunelloides* and *E. elephantina* showed that the interactions between these plants may vary depending on the concentration in which they are combined. Synergistic interactions noted for *P. prunelloides* combined with *E. elephantina* provide some validation to the traditional use of the combinations, where water is mainly utilized to prepare medicinal plants for administration.

5.1.3 Isolation of an antimicrobial compound through a bio-autography guided assay

Enhanced antimicrobial activities were observed for the naphthoquinone plumbagin isolated from *Aristea ecklonii*. This supports the importance of coupling phytochemistry assays with antimicrobial studies in order to gain basic scientific insight on the traditional

use of medicinal plants. The antimicrobial properties noted for the isolated compound, plumbagin, also provide insight for potential future compound synthesis directives.

5.2 Recommendations for future studies

Indigenous use of medicinal plant preparation mainly utilizes water for extraction procedures. Researchers, however, often focus on organic solvents for laboratory testing. One needs to always consider the traditional mode of administration when validating efficacy. Where the plants are directly applied to the skin such as using a poultice (used as a compress), or the sap and/or juice poured directly, as well powder sprinkled on the affected skin, alternate methods should be considered for assaying efficacy. Agar diffusion assays where the poultice, sap or juice as well as powder of the plant species could be placed directly on the pathogen inoculated agar plate, could possibly mimic the typical conditions where plants are applied directly to the skin.

Some skin infections are found to be deep in the skin layers, as the infections mainly affect the hair follicles creating boils which are situated in the innermost layer of the skin. The benefits of the plants used can only be seen when the plant preparations are able to permeate the surface of the skin and treat the target site of infection (Goswami *et al*., 2008). The permeability of plant preparations is rather unnecessary when treating superficial skin conditions like urticaria, skin irritation or sunburn, in which case antipruritic, skin calming agents or protective skin barrier applications such as pastes may be sought to manage such conditions. Therefore, permeability and absorption studies such as those conducted by Röpke *et al*. (2002) and Cole and Heard (2007) are warranted for medicinal plants, specifically used to treat inflictions associated with the inner layers of the skin, including their suitability for the intended therapeutic effects.

Since the combinations were examined for antimicrobial efficacy against a limited selection of organisms, further investigations of the antimicrobial effects against other relevant pathogens such as *P. acnes, B. agri,* and dermatophytes such as *T. mentagrophytes* and *M. canis* are warranted.

As some of the plant combinations are traditionally used as a treatment for ailments such as eczema, psoriasis, insect and sting bites and inflammation, further investigations are warranted pertaining to anti-inflammatory and antioxidant effects of these plants and their combinations. Additionally, selected plants could be targeted for future study on wound healing proliferation, which plays an important curative role in the overall health of the skin.

The use of allopathic antimicrobial agents together with plant extracts has become a major concern for medical practitioners. With skin infections, typically being difficult to treat, the possible lack of compliance to allopathic drugs by patients may be problematic. As patients find that infections are not readily cured, more and more natural remedies are been sought and in some cases the co-administration may prove to have adverse effects. The use of traditional medicines together with conventional medicines is usually not recommended, as the interactions may prove to decrease or increase the pharmacological and toxicology effects of the respective components (Weideman, 2005). Van Vuuren and Viljoen (2011) highlighted a number of plants used in combination with conventional drugs for a variety of ailments. It is therefore important to understand and investigate the pharmacological effects of plants when combined with conventional drugs for the treatment of skin ailments in order to recognize favourable and unfavourable combinations.

5.3 Final conclusive comments

The positive findings from this study provide a scientific basis for the traditional use of plants and some of their combinations and could pave a way for future investigation in this area. In general, the outcome of this study is that a scientific basis for the use of medicinal plants as dermatological agents such as wound antiseptics and antifungals has been established. This may also provide directives for future studies in the treatment of skin specific infections.

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Appendix A: Abstract of conference presentation

Antimicrobial properties of South African medicinal plants with dermatological relevance: Isolating bio-active compounds from the most active plant.

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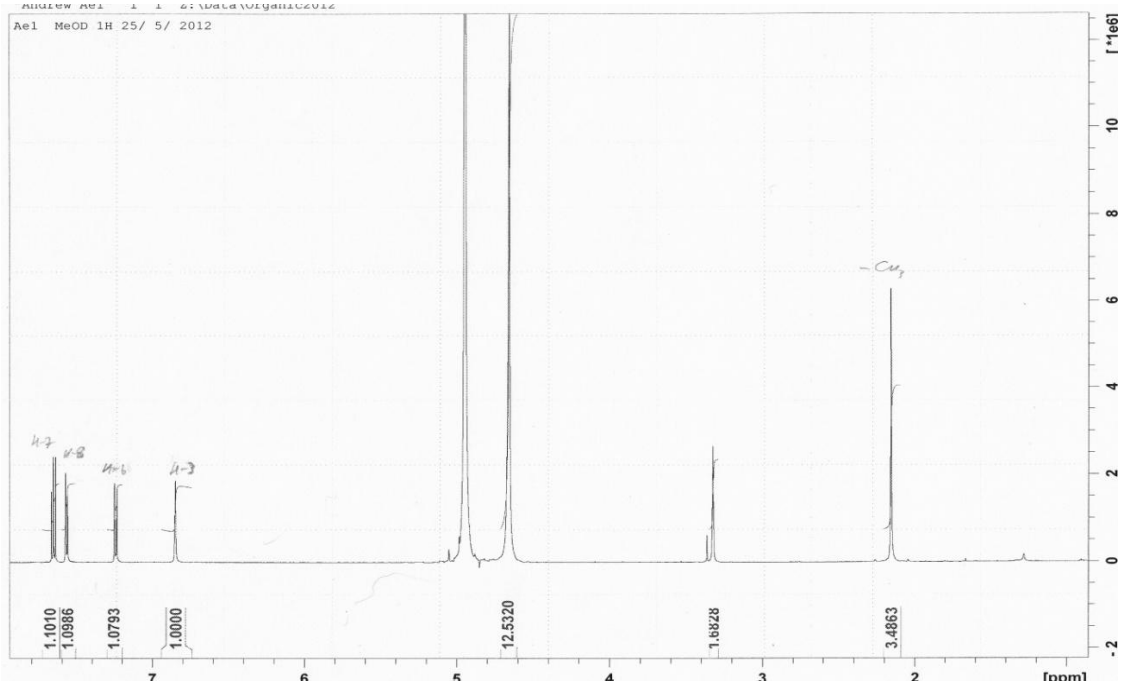
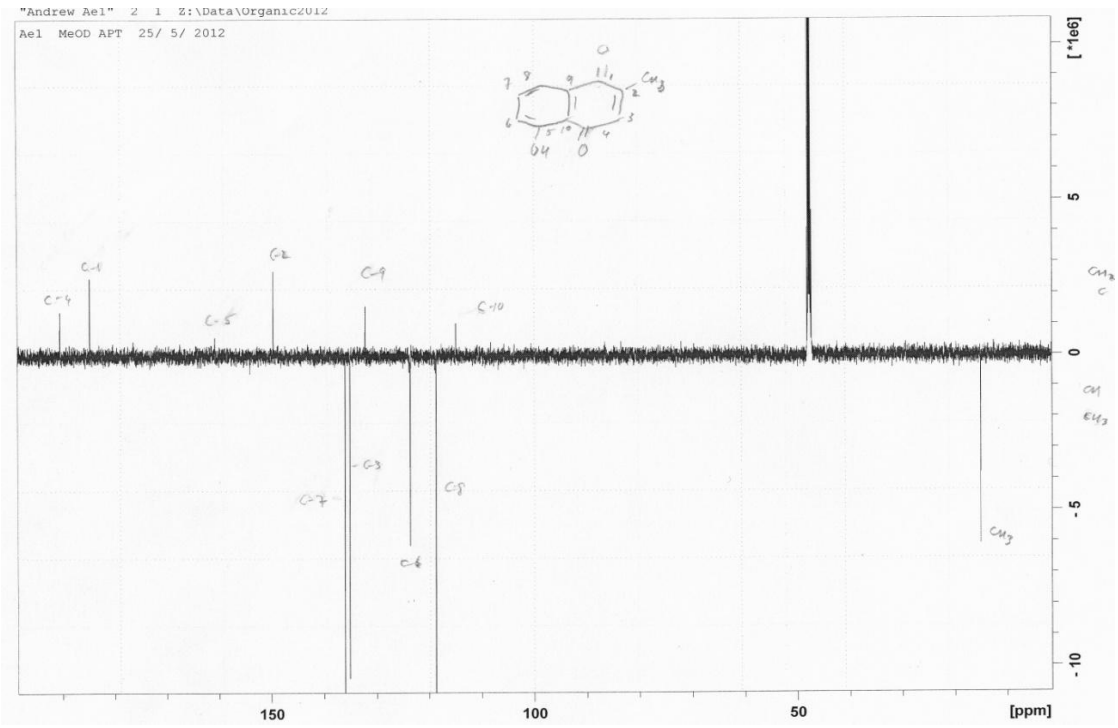
Many South African medicinal plants are reported to be used for dermatological conditions. However, not many studies have been dedicated to investigating the antimicrobial properties of the plants against dermatological relevant pathogens. To address this, a study has focused on investigating the antimicrobial effectiveness of 47 plant species used traditionally to treat skin inflictions, with special interest on fastidious skin pathogens such as anaerobic *Propionibacterium acnes*, and skin dermatophytes *Microsporum canis* and *Trichophyton mentagrophytes*. Dichloromethane: methanol (1:1) and aqueous extracts were prepared and investigated for their antimicrobial properties using the micro-titre plate dilution method. Plants traditionally used for the treatment of acne vulgaris (*Chenopodium ambrosioides, Ekebergia capensis, Harpephyllum caffrum* and *Elephantorrhiza elephantina*) showed potential with an MIC range between 0.05-1.00 mg/ml. Promising broad-spectrum antimicrobial activity was observed particularly for *Aristea ecklonii*, and hence selected for bioactivity-guided compound isolation. The most prominent antimicrobial activity for the isolated naphthoquinone plumbagin was observed against *Staphylococcus epidermidis* having an average MIC value of 3.91 µg/ml. The traditional dermatological use of the plants in this study is mostly validated, as was demonstrated by the noteworthy broad-spectrum activity against all tested skin pathogens. This is especially observed for *Aristea ecklonii* and the isolated compound having significant antimicrobial activity.

Appendix B: Nuclear magnetic resonance spectra of Compound 1



1H NMR spectrum of Compound 1



13CDEPT NMR spectrum of Compound 1