Table of Contents

LIST OF FIGURES

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

LIST OF TABLES

ACKNOWLEDGEMENT

I would like to thank the following people and institution:

Prof. Namrita Lall, my supervisor, for her valuable guidance, comments and suggestions during the course of the research, thesis writing, presentations and article publishing. Her passion for this project has been a great motivation.

Dr. Ahmed Hussein and Dr. Navneet Kishore for their imperative contribution and assistance with the isolation and chemical analysis of the compounds.

Prof. J.J.M. Meyer, Ms. Rene Swart, Mr. G. More, the research team and staff of the Department of Plant Science, for their support and assistance.

University of Pretoria for financial grant (UP Bursary).

My dear friends and colleagues- Marco, Carel, Smeetha, Danielle, CJ and Simin for their encouragement, understanding and support.

A special feeling of gratitude especially for my MUMMY-PAPA, in-laws, wonderful siblings and all my friends and family in India.

Pankaj and Yahvi for unconditional love, support and believing in me.

I thank God for giving me this opportunity and blessing me with all these wonderful people.

PREFACE

A section of the research presented in this thesis has been published as a book chapter and in peer-reviewed journals. A copy of the published manuscript has been attached for the ease of reference. Part of this research have also been presented at national and international conferences.

PUBLICATION

Book Chapter:

- **Sharma, R**., Lall, N. Acne, a review on epidemiology, pathogenesis and treatment options. In: Acne, etiology, treatment options and social effects. Nova Science Publishers, New York. P1-14.

Scientific publications in peer-reviewed journals:

- **Sharma, R.**, Kishore, N., Hussein, A., Lall, N., 2013. Antibacterial and anti-inflammatory effects of *Syzygium jambos* L. (Alston) and isolated compounds on acne vulgaris. BMC Complementary and Alternative Medicine 13, 292.
- **Sharma, R.**, Kishore, N., Hussein, A., Lall, N., 2014. Potential of plant *Leucosidea sericea* against *Propionibacterium acnes*. Phytochemistry Letters 7, 124-129.
- **Sharma, R.** and Lall, N., 2014. Antibacterial, antioxidant activities and cytotoxicity of plants against *Propionibacterium acnes*. South African Journal of Science (In press).

Patent:

- **Sharma, R.,** Lall, N., Kishore, N., & Hussein, A. International patent application no. PCT/IB2013/056343 in the name of University of Pretoria entitled Novel activity of South African plant for acne.

CONFRENCES

- **Sharma, R.**, Lall, N. Evaluation of antibacterial, antioxidant and mechanistics activities of a plant from Myrtaceae family addressing acne vulgaris. Fanie de Meillon Post-Graduate

Symposium. Faculty of Natural and Agricultural Science, Department of Plant Science, University of Pretoria, November 2011.

Awarded: Best PhD presentation.

- **Sharma, R.**, Lall, N. Evaluation of antibacterial, antioxidant and mechanistics activities of a plant from Myrtaceae family addressing acne vulgaris. 38th Annual Conference of the South African Association of Botanists (SAAB), University of Pretoria, 15 - 18 January 2012.
- **Sharma, R.,** Lall, N. Evaluation of antibacterial, antioxidant and mechanistics activities of a plant from Myrtaceae family addressing acne vulgaris. International Federation of Society of Cosmetic Chemists (IFSCC), Sandton Convention Centre, 15 - 19 October 2012 (International Conference).
- **Sharma, R.,** Lall, N. Evaluation of anti-inflammatory and antibacterial activity of two plants belonging to family Myrtaceae and Rosaceae to combat acne vulgaris**.** 39th Annual Conference of the South African Association of Botanists (SAAB), Drakensburg, 20 - 24 January 2013.

SUMMARY

Antibacterial and anti-inflammatory activity of *Syzygium jambos* and *Leucosidea sericea* in addressing acne vulgaris

by

Richa Sharma

Supervisor: **Prof. Namrita Lall**

Department: **Department of Plant Science**

Degree: **Ph.D. Medicinal Plant Science**

Acne is a common universal condition which affects all ages and can have a significant impact on psychosocial and physical aspects of a person's life. The conventional acne medication comes with various side effects. Also, bacterial resistance in an ongoing problem. These facts underscore an urgent need to search for alternative treatment of acne.

In this thesis, fifty one medicinal plants grown in South Africa were investigated for growth inhibitory properties against *Propionibacterium acnes*. The current study is the first scientific report of two plants namely, *Syzygium jambos* and *Leucosidea sericea* for their significant antibacterial activity against *P. acnes*. Both the aforesaid plant extracts exhibited noteworthy antioxidant and anti-inflammatory activity with no toxicity on mouse and human cell lines, very crucial for an anti-acne agent.

For purification of bioactive compounds, the ethanol extracts of *S. jambos* and *L. sericea* were subjected to bioassay guided fractionation. Three known compounds were purified for the first time from *S. jambos*, whereas, four known and one new compound were for the first time purified from *L. sericea* extract. An analogue of anacardic acid and alpha kosin were found to be active against *P. acnes* with significant antibacterial activity very comparable to positive drug control. The Transmission electron microscopy confirmed the lethal effects of plant extracts and bioactive compounds against the cells of *P. acnes*.

The combination study on the aqueous extract of *S. jambos* and *L. sericea* depicted additive and synergistic interactions. The aqueous extract of aforesaid plants in ratio 1:1 showed 24 h hydration effects in an *in vivo* study performed at a local industry in Pretoria.

The results gathered herein suggests that *S. jambos* and *L. sericea* could be possible alternative treatment for acne either alone or in combination with each other. The further clinical trials are underway.

ABSTRACT

Acne is a common universal condition which affects all ages and can have a significant impact on psychosocial and physical aspects of a person's life. Etiologically, it is a multifactorial skin disorder which is associated with pilosebaceous unit of the skin. Production of increased amount of sebum by the sebaceous gland is accompanied by the thickening of epidermis at the outlet to the pilosebaceous follicles. As a result, there is an obstruction to the flow of sebum outwards, and a comedo develops. *Propionibacterium acnes*, the causative agent plays a crucial role in the pathogenesis. Their colonization triggers the host's inflammatory response and leads to the production of inflammatory cytokines like interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α). Simultaneously, in anaerobic environment, the bacteria secrete various hydrolytic enzymes such as, nucleases, nuraminidases, hyaluronidases, acid phosphatases lecithinases and other lipases. Due to action of these enzymes, the sebum content changes and reactive oxygen species (ROS) may be released from the impacted damaged follicular walls. All these events result in the progression of inflammation and the pathogenesis of disease.

Ethnobotanical studies have documented the use of plants by local people for the treatment of various skin ailments. Also, plants contain numerous biological active compounds, many of which have been shown to possess antimicrobial activity. The current study focuses on the high potency of two plants namely, *Syzygium jambos* and *Leucosidea sericea* for their future use as an alternative treatment of acne.

From an *in vitro* antibacterial evaluation of 51 ethanol plant extracts against *P. acnes*, the aforesaid two plants were found to be the most active, with minimum inhibitory concentration (MIC) values of 31.25 (*S. jambos*) and 15.62 µg/ml (*L. sericea*).

Subsequent fractionation of *S. jambos* extract resulted in the isolation of squalene, an anacardic acid analogue and ursolic acid for the first time from this plant. Similarly, fractionation of *L. sericea* extract resulted in the isolation of phytol acetate, triacontanol, phytol, (*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol and alpha kosin for the first time form this plant. Of all the isolated compounds, anacardic acid analogue and alpha kosin were found to be the only active compounds against *P. acnes* (MIC 7.81 and 1.95 µg/ml, respectively). The transmission electron microscopy (TEM) confirmed the

lethality of plant extracts and bioactive compounds on the cells of *P. acnes*. The extract of *S. jambos* was found to be non-toxic to mouse melanoma B16-F10 cells and human macrophage U937 cell lines with EC_{50} (concentration at which 50% cells are viable) values of 450 and 60 µg/ml, respectively. Whereas, *L. sericea* extract exhibited moderate toxicity to B16-F10 cells (EC_{50} 55 µg/ml) and a comparatively higher toxicity to U937 cells (EC_{50} 26 µg/ml). Of all the isolated compounds, only alpha kosin and a commercially acquired compound, 'myricetin', were found to be toxic to both cell lines with EC_{50} values of <20 µg/ml. The significant antioxidant activity was shown by the extracts of *S. jambos*, *L. sericea*, isolated compound alpha kosin, commercially acquired compounds- myricetin, myricitrin and gallic acid with EC_{50} (concentration at which 50% free radical is scavenged) values varying from 0.9-5.1 µg/ml, comparable to vitamin C (EC_{50} 2 µg/ml), a known antioxidant agent. A significant inhibition of IL-8 and TNF-α was observed for *S. jambos*, *L. sericea*, ursolic acid and myricitrin.

The aqueous extract of *S. jambos* and *L. sericea* in 1:1 combination showed synergism and inhibited the growth of *P. acnes* at 0.7% (650 µg/ml), comparable to Cytobiol Iris A^2, a commercial anti-acne ingredient. The aqueous extract of *L. sericea* and *S. jambos*+*L. sericea* (1:1) showed hydrating potential for 24 h in an *in vivo* study performed at Future Cosmetics, Pretoria.

Chapter 1

General Introduction

CHAPTER 1

General Introduction

1.1. Background and motivation of the study

Acne vulgaris, one of the most common diseases of the skin is ascribed by the formation of comedones, papules, pustules, nodules and/or cysts as a result of obstruction to the flow of sebum outwards and inflammation of pilosebaceous units (hair follicles and their accompanying sebaceous gland) (Truter, 2009). It affects children and adolescents, most commonly in puberty. The acne lesions commonly appear on face, chest and back. In a research article by Nguyen and Su, (2011), a study on a 16-year-old shows that prevalence of acne was 94% for males and 92% for females, with 14% having moderate to severe acne. The appearance of acne after 25 years of age is 10% and after 40, 1% in men and 5% in women.

The Global Alliance is a group of physicians and researchers in the field of acne. In order to improve outcomes in acne, the Global Alliance in association with national dermatology societies has published recommendations and have formulated guidelines for the management of acne in 2003. Since then, there has been active participation of these organisations into acne management by considering the individual characteristics of the country and simultaneously harmonizing with international recommendations. In addition, the Global Alliance presented a written consensus opinion to the US Food and Drug Administration (FDA) Guidance for the Industry on Acne Vulgaris (Docket No. 2005D 0340) regarding development of drugs for acne and design of clinical trials in this arena. Considerable evidence shows that acne can be a psychologically damaging condition that can last for years. Many studies have reported persistent occurrence of acne at an adulthood age of 25 and older. The members of the Global Alliance therefore, believe that acne, one of the most common skin diseases treated in routine dermatologic care, should be recognized and investigated as a chronic disease (Thiboutot et al., 2009).

The healthy human skin is colonized by non-pathogenic microorganisms like *Staphylococci*, *Propionibacteria* and *Malassezia* yeasts. The locally dense or sparse populations, density and

distribution of these microbes may vary and depend on factors such as sebum secretion, occlusion, temperature, humidity and age of person (Elsner, 2006). *Propionibacterium acnes* is a facultative anaerobic bacterium that plays an important role in the pathogenesis of acne. The pathogenesis of acne is multifactorial, which begins due to increased sebum production at early puberty. *P. acnes* flourishes in the environment created by sebum and leads to primary or secondary skin infections. The abnormal follicular keratinisation occur as the flow of sebum is restricted and chemotactic factors are produced which act as pro-inflammatory mediators that lead to inflammation (Leyden, 1997).

The conventional treatment of acne vulgaris includes antibiotic therapy. The antibiotics are regarded as antibacterial whose primary mode of action is to decrease the number of *P. acnes* in the skin. However, many antibiotics have demonstrated inhibitory effects on the production of *P. acnes* associated inflammatory mediators and may affect acne by acting as anti-inflammatory agents, an action other than direct antibacterial. Each year, 5 million prescriptions for oral antibiotics and 1.4 million prescriptions for isotretinoin are dispensed for the treatment of acne in USA (Stern, 2000). Although antibiotic therapy has been used for more than 40 years to treat acne, changes in *P. acnes* antibiotic sensitivity did not become an issue until the 1970s (Leyden et al., 1973). Since then, the prevalence of resistant organisms has increased globally (Ross et al., 2001). As a result, clinicians have begun to re-evaluate therapeutic approaches with the goal of limiting the development of antibiotic-resistant organisms. Plants and plant extracts have been used for the treatment of skin disorders for centuries. Because of increasing resistance to antibiotics of many bacteria, plant extracts and plant compounds are of new interest for their usage as antiseptics and antimicrobial agents in dermatology (Augustin and Hoch, 2004).

1.2. Objectives of the study

The primary objectives of the present study were as follows:

- Evaluation of the antibacterial activity of the selected plant extracts and to isolate the active compound(s) from the potent plant extracts.
- Testing of cytotoxicity of samples on human macrophages (U937) and mouse melanoma (B-16-F10) cell lines.
- Determination of the inhibitory effects of plant extract on the pro-inflammatory mediator secretion in co-culture of U937 cell lines infected with *P. acnes*.

- Investigating the effect of Glutathione reductase on the potentially damaging radicals and reactive oxygen species in the presence of selected samples.
- Evaluation of the anti-inflammatory activity of plant extracts by the inhibition of nitric oxide (NO) production.
- Investigation of antioxidant activity of extracts.
- Confirming the antibacterial activity of potent extracts by means of Transmission Electron Microscopy.

The results that stem from these studies will provide valuable information with regard to further formulations of herbal products for acne and potential use of plant extracts for antimicrobial purposes.

1.3. Structure of thesis

Chapter 1 General Introduction

Chapter 2 A concise review on plants used as traditional medicine and as cosmeceuticals. Information about skin and acne, its pathogenesis as well as immunology.

Chapter 3 The detailed description of 51 plants including their medicinal uses and phytochemistry.

Chapter 4 The antibacterial activity of ethanolic extracts of fifty plant against *Propionibacterium acnes* using 96 well plated broth dilution methods along with their antioxidant activity and cytotoxicity.

Chapter 5 Isolation, purification and identification of active compound(s) from *Syzygium jambos* and the minimum inhibitory concentration (MIC) of isolated compound(s) against *P. acnes*. Further, cytotoxicity investigation of plant extract on mouse melanoma (B16-F10) and human leukemic monocyte lymphoma (U937) cells and their active principle for its effective therapeutic use; determination of inhibitory effects of sample on the pro-inflammatory mediator secretion in co-culture of U937 cells with *P. acnes*; investigation of

the effect of Glutathione reductase on the potentially damaging radicals and reactive oxygen species; evaluation of the anti-inflammatory activity of plant extract by the inhibition of nitric oxide (NO) production; investigation of antioxidant activity of samples and confirming the antibacterial activity of plant extract by means of Transmission Electron Microscopy.

Chapter 6 Isolation, purification and identification of active compound(s) from *Leucosidea sericea* and the MIC of isolated compound(s) against *P. acnes*. Further, cytotoxicity investigation of plant extract on B16-F10 and U937 cells and their active principle for its effective therapeutic use; determination of inhibitory effects of extract on the pro-inflammatory mediator secretion in co-culture of U937 cells with *P. acnes*; investigation of the effect of Glutathione reductase on the potentially damaging radicals and reactive oxygen species; evaluation of the anti-inflammatory activity of plant extract by the inhibition of nitric oxide (NO) production; investigation of antioxidant activity of samples and confirming the antibacterial activity of plant extract by means of Transmission Electron Microscopy.

Chapter 7 Synergistic activity of the aqueous extracts of *Syzygium jambos* and *Leucosidea sericea*

Chapter 8 Conclusions and Acknowledgements

Chapter 9 Appendices

1.4. References

Nguyen, R. and Su, J., 2011. Treatment of acne vulgaris. Paediatrics and Child Health, 21(3), 119-125.

Truter, I., 2009. Evidence based pharmacy practice: Acne vulgaris. South African Pharmaceutical Journal, 76(3), 12-19.

Thiboutot, D. et al., 2009. Management of acne: a report from Global Alliance to Improve Outcomes in Acne. Journal of American Academy of Dermatology, 60(5), 1-50.

Elsner, P., 2006. Antimicrobials and the skin physiological and pathological flora. Current Problems in Dermatology, 33, 35–41.

Leyden, J.J. 1997. Therapy for acne vulgaris. New England Journal of Medicine, 336: 1156–62.

Stern, R.S. 2000. Medication and medical service utilization for acne 1995–1998. Journal of American Academy of Dermatology, 43, 1042–8.

Leyden, J.J., Marples, R.R., Mills, O.H., et al., 1973. Gramnegative folliculitis- a complication of antibiotic therapy in acne vulgaris. British Journal of Dermatology, 88, 533–48.

Ross, J.I., Snelling, A.M., Eady, E.A., et al., 2001. Phenotypic and genotypic characterization of antibiotic-resistant Propionibacterium acnes isolated from acne patients attending dermatology clinics in Europe, the USA, Japan and Australia. British Journal of Dermatology, 144(2), 339–46.

Augustin, M. and Hoch, Y., 2004. Phytotherapie bei Hauterkrankungen. Urban und Fischer, München, 1–7.

Chapter 2

Acne: a review on epidemiology, pathogenesis and treatment options

CHAPTER 2

Acne: a review on epidemiology, pathogenesis and treatment options

A section of this chapter is published as a chapter in a book entitled “Acne, etiology, treatment options and social effects."

2.1 Introduction

Traditional medicine is almost as old as the existence of mankind. This statement is backed by evidence obtained from studies of human settlements of older civilizations. Paleontologists have found bunches of medicinal herbs among the fossilized remains (Normann and Snyman, 1996). Plants are the oldest source of medicine. People of almost all cultures have used them routinely. Before the modern pharmaceutical industry existed, people relied on folk knowledge and apothecaries (Spinella, 2005). To illustrate historical role of plant derived medicines, here are some well-known examples:

- Quinine- an alkaloid obtained from bark of *Cinchona pubescence*. Only effective remedy for malaria for more than 300 years.
- Atropine- alkaloid from *Atropa belladona*. Used as heart tonics, eye drops, and injected to treat Parkinsonism.
- Morphine, codine-alkaloid obtained from *Papaver sominiferum*. Morphine is powerful analgesic and codeine as headache remedy and ingredient of cough syrup.
- Taxol- diterpenoid from bark of *Taxus brevifolius*. Highly effective against cancer.
- Quassinoids- terpenoid from *Quassia amara,* is used to improve appetite and treat minor stomach ailments (Van Wyk et al., 1997).

Plants are of relevance to dermatology for both their adverse and beneficial effects on skin and skin disorders, respectively. Virtually all cultures worldwide have relied historically, or continue to rely on medicinal plants for primary health benefits. Approximately one-third of all traditional medicines are for treatment of wounds or skin disorders, compared to only 1-3% of modern drugs. The use of such medicinal plant extracts for the treatment of skin disorders arguably has been based largely on historical/anecdotal evidence. Beneficial aspects of medicinal plants on skin include: healing of wounds and burn injuries (especially *Aloe vera*); antifungal, antiviral and antibacterial activity

against skin infections such as acne, herpes and scabies (especially tea tree (*Melaleuca alternifolia*) oil); activity against inflammatory/immune disorders affecting skin (e.g. psoriasis); and anti-tumour promoting activity against skin cancer (Mantle et al., 2001).

2.2 Cosmeceuticals

Cosmetics based on herbs and other botanicals are as old as civilization itself. Egypt, the cradle of one of the earliest ancient cultures, pioneered natural perfumes and skin care preparations. Bath oils and rubs, moisturizing and cleansing lotions for skin, shampoos and conditioners for hair, all these products utilize the oils and other by-products of herbs both as main ingredients and subtle additives (Hoffmann and Manning, 2002). Plant based cosmetics were also common in ancient Greece and in Roman Empire (Burlando et al., 2010).

Plants have emerged as the best source of cosmetics ingredients that meet the consumer's growing demand of natural character, efficiency, safety, and are increasingly replacing synthetic ingredients. Nowadays, cosmetic ingredients are designed by producers, used by consumers and investigated by researchers to examine their potential for cosmeceutical development. In cosmetic lingo, there is a name given “cosmeceuticals” (Figure 2.1), that merges cosme(tic) and (pharma)ceutical implications. This term indicates cosmetic-pharmaceutical hybrids aimed at enhancing the beauty by means of ingredients that provide additional health-related function or benefit (Burlando et al., 2010).

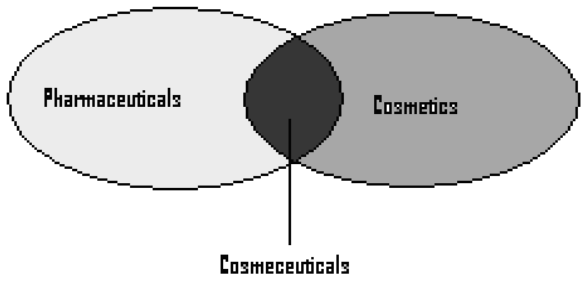


Figure 2.1: Cosmeceuticals

2.3 Skin and acne

Skin is the largest organ of the body. It serves many important functions, including protection, precutaneous absorption, temperature regulation, fluid maintenance, sensory and disease control

(Gebelein, 1997). Skin disorders affect all ages from neonatal to elderly and cause harm in number of ways. It has been estimated that skin diseases account to as high as 34% of all occupational disease (Spiewak, 2000).

Acne (Figure 2.2) is an altered or inflammatory state of hair follicle and associated sebaceous glands, involving the formation of a plug of keratin and sebum (a microcomedo) which can grow to form an open comedo (blackhead) or closed comedo (whitehead). It is further worsen by *Propionibacterium acnes*, thus producing inflammatory lesions such as papules, pustules, or nodule (Burlando et al., 2010). *P. acnes* are gram-positive anaerobic bacteria that are component of the normal microbiota of human skin.

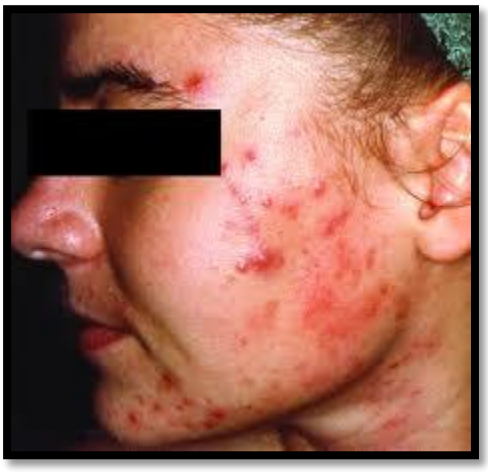


Figure 2.2: Appearance of acne on face (www.cosmesurge.com)

An increased secretion of sebum is accompanied by thickening of epidermis at the outlet to the pilosebaceous follicles. This creates an obstruction to flow, and a comedo develops. Colonization of the follicle with *P. acnes* and the host inflammatory response to this plays a pivotal role in the development of the typical inflammatory papulopustular lesion (Shaw and Kennedy, 2007).

It can range from occasional blemishes to a devastating, continuing episode leading to a permanent scaring. It develops due to genetic predisposition during puberty. The years of greatest severity are from 16 to 19. The location of acne lesions is generally face, neck, back and chest (Williams and Schmitt, 1996). Several rating scales are developed with the aim of trying to grade the severity of acne. Below is classification to describe three grades of acne.

• Mild acne

Patients with mild acne typically have predominantly open and closed comedones (blackheads and whiteheads) with a small number of active lesions normally confined to the face. Mild acne does not cause permanent scarring. Any or all of the following is present in case of mild acne: small, tender, red papules; pustules; and blackheads and/or whiteheads. Mild acne is therefore, characterized by the presence of a few to several papules and pustules, but no nodules.

• Moderate acne

Similar to mild acne, but more papules and pustules. Patients with moderate acne typically have a few to several nodules. Lesions are often painful and there is a real possibility of scarring.

• Severe acne

Similar to moderate acne but with nodular abscesses, leading to extensive scarring. Patients with severe acne have numerous or extensive lesions (Truter, 2009).

2.4 Epidemiology

Acne affects approximately 80% of people aged 11 to 30 years at some time, with about 60% of those sufficiently affected to seek treatment. Acne lesions typically develop at the onset of puberty. Girls therefore, tend to develop acne at an earlier age than boys. The peak incidence for girls is between 14 and 17 years, as compared with 15 to 19 years for boys. There may be a familial tendency to acne and it is slightly more common in boys, who also experience more severe involvement. Acne is more common in males than females during adolescence, but is more common in women than in men during adulthood. In addition, white patients are more likely to experience moderate to severe acne, although black skin is prone to worse scarring. Acne usually resolves within 10 years of onset, although up to five percent of women and one percent of men in their thirties can have mild persistent acne. The incidence of acne appears to have fallen in recent years, however, the reasons are unknown (Truter, 2009).

Community-based studies in the UK, Australia, New Zealand, and Singapore have found prevalence rates ranging from 27% in early adolescence to 93% in late adolescence. The proportions of acne vulgaris in hospital-based studies of skin disease in Africa have been reported to be 4.6% in Ghana, 6.7% in Nigeria, and up to 17.5% in South Africa. Although, in a preliminary study with regard to the dermatologic needs of a small rural community in Ethiopia, found that only three of 66 children (4.5%)

between the ages of 10 and 16 years attending a school had acne, the knowledge of the prevalence and severity of acne in the larger community in Africa is very poor (Yahya, 2009).

The first survey of dermatological disorders in South Africa was undertaken in 1957. In this study the relative frequency of skin disease in black patients was calculated as the percentage of dermatological outpatients. Out of 7029 dermatological outpatients, 1121 i.e. 16% were affected by acne from which 17.5% were black, 7.3% were white, 13.9% were coloured and 13.4% were Indian (Hartshorne, 2003).

2.5 *Propionibacterium acnes*, the causative agent

Propionibacterium acnes are pleomorphic, coryneform anaerobic Gram positive bacilli. They appear as small, opaque, enamel-white circular colonies. Cells can measure from 0.5 to 0.8 µm by 1.5 µm. Transmission electron micrograph of *P. acnes* showed the outer cell wall in lined with cytoplasmic membrane. In the centre is a nucleoid surrounded by ribosomes. Mesosomes are also present (Figure 2.3).

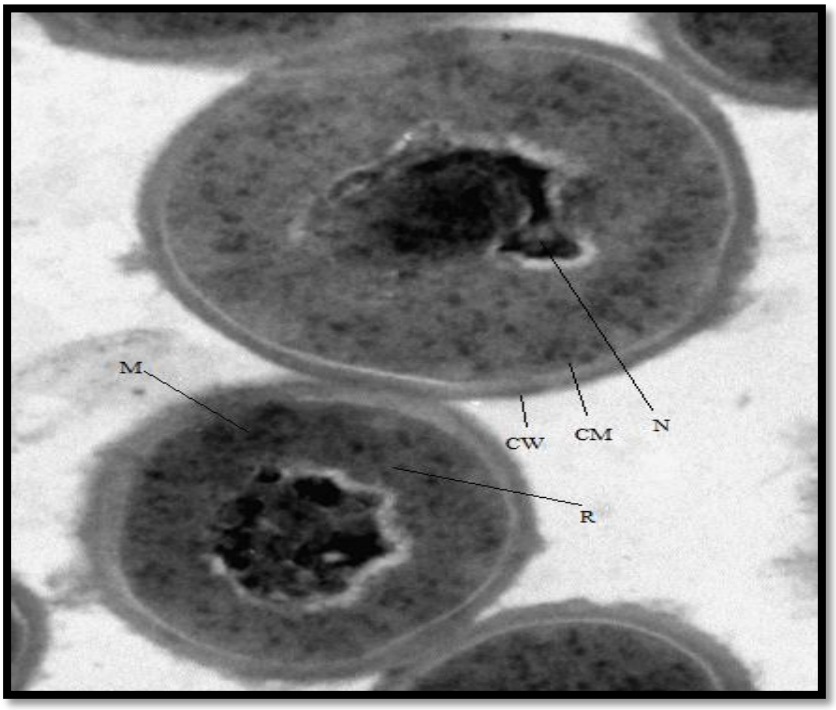


Figure 2.3: Transmission Electron micrographs of a thin section of *Propionibacterium acnes*. Labelled structures: cell wall (CW), cytoplasmic membrane (CM), nucleoid (N), ribosomes (R) and mesosomes (M).

Cutaneous *Propionibacteria* secrete nucleases, nuraminidases and hyaluronidases, acid phosphatases lecithinases and other lipases. It was suggested that hyaluronidase can split extracellular substance of cell wall of sebaceous ducts and thus increase the permeability of epithelial follicles. Nuraminidase can damage the cell and tissue membranes, affecting the sialic acid residues on surface of the cells. Under the action of proteases of *P. acnes*, which also possesses keratinolytic activity, small chemotactic peptides are produced that may have a role in the onset of inflammation (Vorobjeva, 1999).

2.6 Pathogenesis of *Propionibacterium acnes*

The pathogenesis of acne vulgaris is multifactorial, including increased sebum production, comedogenesis, *P. acnes* proliferation and inflammation (James, 2003). An increased secretion of sebum is accompanied by thickening of epidermis at the outlet to the pilosebaceous follicles (Figure 2.4 a). This creates an obstruction to flow, and a comedo develops (Figure 2.4 b). Colonization of the follicle with *P. acnes* and the host inflammatory response to this plays a pivotal role in the development of the typical inflammatory papulopustular lesion (Figure 2,4 c,d) (Shaw and Kennedy, 2007).

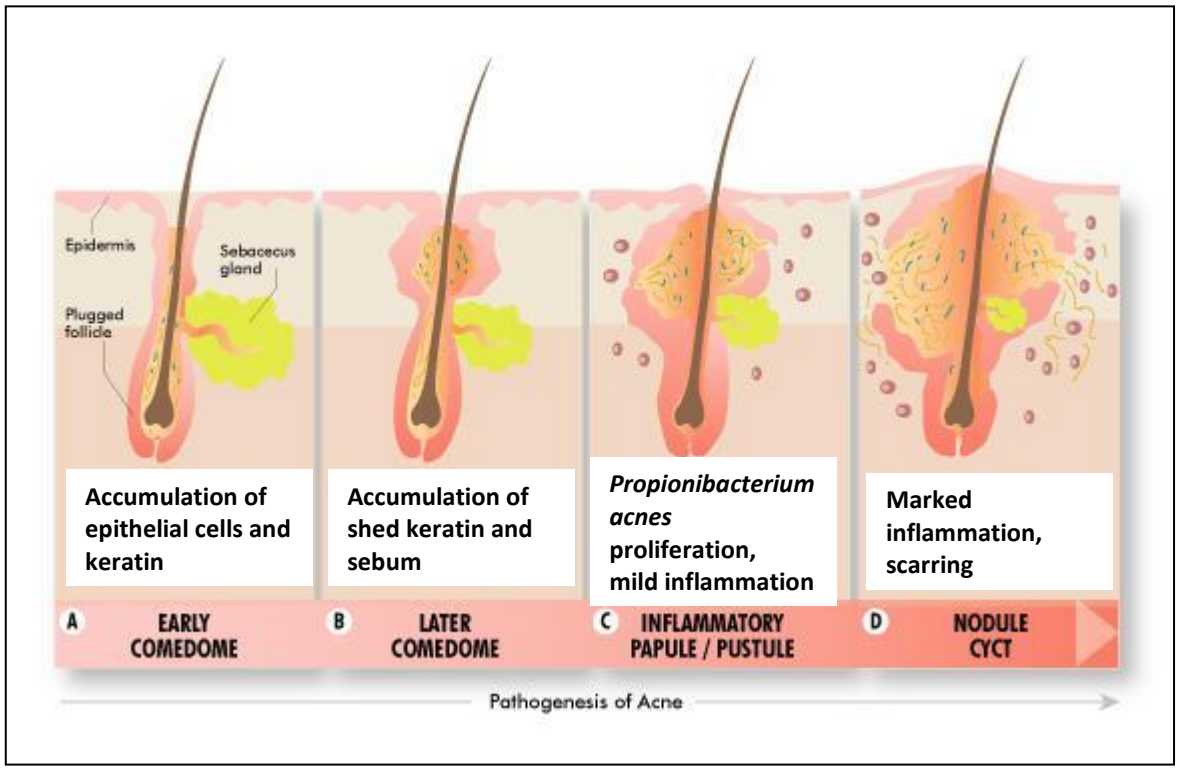


Figure 2.4: Pathogenesis of acne (www.deerfielddermatology.com)

The newest data concerning acne pathophysiology demonstrate that the relationships between the development of *P. acnes*, inflammation, and hyperkeratinization are more complex than previously recognized. Theories regarding the sequence of events in acne development have evolved in recent years. Firstly, it is known that *P. acnes* contribute to inflammation through activation of the innate immune system, including complement and toll-like receptors, and that oxidized lipids in sebum can stimulate inflammatory mediators, and it is clear that inflammatory proteins can mediate acne. These mediators include certain matrix metalloproteinases, which are present in sebum; it has been shown that the production of these matrix metalloproteinases decreases after the resolution of acne lesions with treatment. Fourth, and finally, it also has been shown that the sebaceous gland is part of a neuroendocrine organ; it is not yet known how sebaceous gland activity might be mediated using the neuroendocrine inflammatory apparatus, but this represents an area for potential research in the future (Friedlander et al., 2010).

2.7 Immunology of acne

Propionibacterium acnes is the predominant organism living on sebaceous region of skin. It metabolises the triglyceride of the sebum and triggers the inflammatory events associated with acne (Webster and Kim, 2008). *P. acnes* act as immunostimulator which produce a variety of enzymes and biologically active molecules like lipases, proteases, hyaluronidases and chemotactic factors involved in development of inflammatory acne. The main components of the pilosebaceous unit on the skin, such as keratinocytes and sebocytes, can be activated by *P. acnes*, leading to the production of pro-inflammatory cytokines (Leeming et al., 1985). *P. acnes* induce monocytes to secrete pro-inflammatory cytokines like interleukins (IL-8, IL-1β) and tumour necrosis factor-α (TNF-α) (Kim, 2005) and thus play an important role in pathogenesis of inflammatory acne (Figure 2.5).

P. acnes produce both high and low molecular weight chemotactic factors, one of which is lipase which attract human neutrophils. Once neutrophils arrive, enzymatic digestion of follicular wall occurs by neutrophil lysosomal hydrolytic enzymes. *P. acnes* itself also elaborates proteases and other degradative enzymes, which play some part in comedonal rupture and thus cause inflammation. They induce inflammatory response by activating innate immune cells, such as monocytes/macrophages to secrete proinflammatory cytokines, including IL-8, matrix metalloproteins (MMP) and TNF-α through toll like receptors (TLR) 2-dependent mechanism (Webster and Kim, 2008).

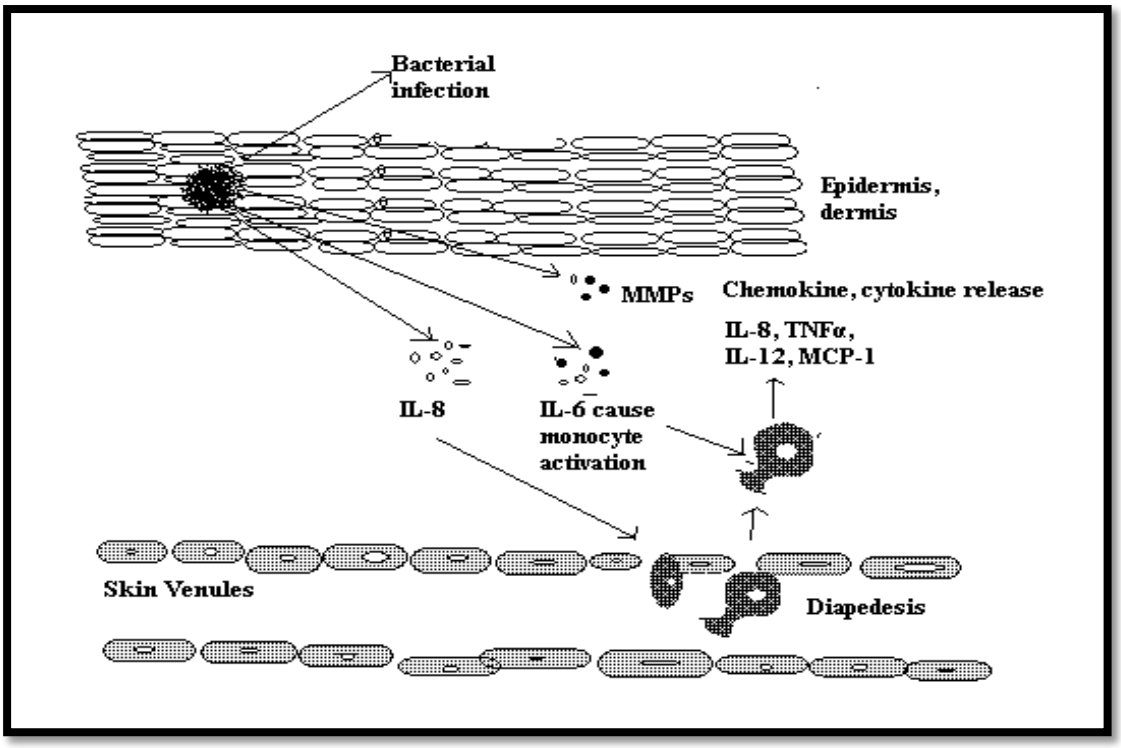


Figure 2.5: Inflammation of skin in response to the bacterial infection

The innate immune cells i.e. macrophages express pattern recognition receptors (PRRs), such as human TLRs, which are transmembrane proteins capable of mediating responses to pathogen-associated molecular patterns (PAMPs). When TLRs are activated by exposure to microbial ligands, various factors are activated which ultimately activate nuclear factor kappa B (NF-kB) that initiates TNF α, interleukin and MMP (Webster and Kim, 2008).

The TLR2-dependent production of IL-8 may be important in the pathogenesis of acne, as it is a known neutrophil chemo attractant, which contribute to the formation of inflammatory lesion. Matrix metalloproteins (MMP) play a role in inducing inflammation and scar formation in acne (Webster and Kim, 2008).

2.8 Conventional drugs available for treating acne

Since there is a correlation between the reduction in *P. acnes* number and clinical improvement in patients adequately treated with antimicrobial agents and/or antibiotic therapy, which reduces the population of *P. acnes*, has been a mainstay of treatment for acnes over the past 25 years.

The structured approach for treating acne is as follows:

For mild acne: Topical treatment indicated like use of a comedolytic (retinoid, benzoyl peroxide OR azelaic acid).

Possible side-effects: Benzoyl peroxide and retinoid cause dryness, redness and irritation of skin. Benzoyl peroxide also bleach clothes and hair whereas, azelaic acid leads to hypopigmentation.

For moderate acne: Oral plus topical treatment. Oral antibiotic like tetracycline if over 12 years of age, erythromycin, if younger.

Possible side-effects: Tetracyclines can be associated with photosensitivity, and patients should be cautious in terms of sun exposure. Erythromycin cause frequent gastrointestinal disorders.

For severe acne: Oral plus topical treatment with Dianette or Isotretinoin. A surgery may be recommended in extreme conditions.

Possible side effects: Dianette is associated with an increased risk of venous thromboembolism. Skin and mucous membrane dryness occur in almost all who are treated with Isotretinoin (Shaw and Kennedy, 2007).

Antibiotics have been implemented in the treatment of *P. acnes* due to their bacteriostatic nature, which reduces the pathogen numbers, lipase activity and chemotactic factors produced by the infection of the pathogen. Commonly used antibacterial- clindamycin, erythromycin and tetracycline are all from biological sources, more specifically bacteria. Erythromycin (Scaglione and Rossoni, 1998), retinoids and tretinoin (Wolf, 2002) are used as anti-inflammatory drugs for acne. Clindamycin originates from *Streptomyces lincolnensis*, Erythromycin - *Streptomyces erythreus;* and Tetracycline - *Streptomyces species*.

The crisis of newly emerging diseases and the resistance of many pathogens to currently used drugs, coupled with the adverse side-effects of many of these drugs have necessitated the continuous search for new drugs that are potent and efficacious with minimal or no adverse side-effects (Amoo, 2009). However, *P. acnes* strains with clinically significant antibiotic resistance are identified from acne patients with long antibiotic treatments (Ross et al., 1997) to both erythromycin and clindamycin due to their long-term viability as topical anti-acne therapies. Only through judicious use of combination

topical therapies (e.g., topical retinoid, benzoyl peroxide or azelaic acid plus clindamycin or erythromycin) can both clindamycin's and erythromycin's widespread utility be preserved in this disorder (Guay, 2007). The reported resistant strain of *P. acne* is P 37 which is erythromycin resistant (Ross et al., 1997). More recently it has been demonstrated that biofilm formation by *P. acnes* increases resistance against antimicrobial agents (Coenye et al., 2007). These problems may be the roots of clinical failure to treat the acne.

2.9 Possible potential of plants for treating acne

The plant kingdom is known to contain many novel biologically active compounds, many of which could potentially have a higher medicinal value when compared to some of the current medications. So there arises a need for search of new effective bioactive compounds to overcome this. A well-known plant extract studied for acne treatment, Tea tree oil or Melaleuca oil, originating from the Australian medicinal plant *Melaleuca alternifolia*, has been used in a clinical trial study to determine its effectiveness against acne (Carson et al., 2006). A crude drug extract called Kushen that is made from the fried roots of *Sophora flavescens* (Leguminosae) contained prenylflavanone derivatives were shown to have antibacterial activity against *P. acne* (Kuroyanagi et al., 1995). During previous studies *Hemidesmus indicus, Eclipta alba, Cucubito pepo, Euphorbia hirta* showed MIC value of 0.05, 0.66, 1.25 and 1.55 mg/ml, respectively (Kumar et al., 2007). Rhinacanthins-rich *Rhinacanthus nasutus* extract exhibited potent bacterioststic activity against *P. acnes* with MIC value of 8-16 µg/ml (Puttarak et al., 2010). The antibacterial activity of pomegranate rind extract containing 13% w/w ellagic acid exhibited a bacteriostatic activity against *P. acnes* at MIC of 15.6 µg/ml (Panichayupakaranant et al., 2010). Methanolic extracts of *Rosa damascene*, *Eucommia ulmoides* and *Ilex paraguariensis* were found to inhibit the growth of *P. acnes* with MICs of 2, 0.5 and 1 mg/ml, respectively (Tsai et al., 2010).

South Africa has remarkable biodiversity and a rich, extant herbal medicine tradition with origins that probably reach back to Paleolithic times. It is estimated that there are at least 2,00,000 indigenous healers in South Africa. Medicinal plants are widely used in traditional therapeutics, and it is likely that at least 2500 species of plant are commonly used as medicines. A South African pharmaceutical company, Noristan Ltd, investigated South African medicinal plants over a period of almost 20 years. Noristan found that 80% of the local medicinal plants that they had tested exhibited pharmacological

activity (Normann and Snyman, 1996).

There are many South African plants which are used in herbal cosmetics. Rooibos (*Aspalathus linearis*) is rich in flavanoids, polyphenols, phenolic acids, oligosaccharides and polysaccharides (Dos et al., 2005). Rooibos proved to exhibit anti-inflammatory and anti-microbial properties and is used for cosmetic applications. *Artemisia herba-alba* is also popular for skin ailments. A poultice of leaf is applied to any glandular or skin inflammation (Dweck, 1995). The leaves and roots of *Aloe ferox* are applied topically, sometimes mixed with animal fat, or taken internally to treat conditions such as eczema, dermatitis and acne (Van Wyk et al., 1997).

Therefore, there is a wide scope to obtain new drugs from plants that can have potential as antibacterial, anti-inflammatory and antioxidant agents.

2.10 References

Amoo, S.O., 2009. Micropropagation and medicinal properties of *Barleria greenii* and *Huernia hystrix.* Biological and Conservation Sciences Thesis, 413-653.

Burlando, B., Verotta, L, Comara, L., Bottini-Massa, E., 2010. Herbal Principles in Cosmetics: Properties and Mechanisms of Action CRC Press, USA, pp. xxi.

Carson, C.F., Hammer, K.A., Riley, T.V., 2006. *Melaleuca alternifolia* (Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties. Clinical microbiology reviews 19, 50-62.

Coenye, T., Peeters, E., Nelis, H.J., 2007. Biofilm formation by *Propionibacterium acnes* is associated with increased resistance to antimicrobial agents and increased production of putative virulence factors. Research in microbiology, 158, 386-392.

Cosmesurge, 2013. Online http://www.cosmesurge.com/non-surgical/acne-scar-treatment-dubai. Accessed on: 19 June 2013.

Deerfield Dermatology Associates, 2013. Online http://www.deerfielddermatology.com/adult-and-children-acne-treatments-deerfield-il.html. Accessed on: 19 June 2013.

Dos, A., Ayhan, Z., Sumnu, G., 2005. Effects of different factors on sensory attributes, overall acceptance and preference of rooibos (*Aspalathus linearis*) tea. Journal of Sensory Studies 20(3), 228–242.

Dweck, A.C., 1995. Cosmetics and toiletries advanced technology conference. In: Botanicals - Research of Actives.

Friedlander, S.F., Eichenfield, L.F., Fowler Jr, J.F., Fried, R.G., Levy, M.L., Webster, G.F. , 2010. Acne Epidemiology and Pathophysiology. Seminars in cutaneous medicine and surgery, 29, 2-4.

Gebelein, C.G., 1997. Colin, H., Wheatley (Eds.), Chemistry and Our Life. Graphic World Publishing Services, United States of America, 435–456.

Guay D.R., 2007. Topical clindamycin in the management of acne vulgaris. Expert Opinion Pharmacotherapy 8(15), 2625-64.

Hartshorne, S.T., 2003. Dermatological disorders in Johannesburg, South Africa. Clinical and experimental dermatology, 28, 661-665.

Hoffmann, F.W. and Manning, M., 2002. Herbal medicine and botanical medical fads. The Haworth Press, Binghamton.

James, J. L., 2003. A review of the use of combination therapies for the treatment of acne vulgaris. Journal of the American Academy of Dermatology, 49, S200-S210.

Kim, J., 2005. Review of the Innate Immune Response in Acne vulgaris: Activation of Toll-Like Receptor 2 in Acne Triggers Inflammatory Cytokine Responses. Dermatology, 211(3), 193-8.

Kumar, G. S., Jayaveera, K.N., Kumar, C.K., Sanjay, U.P., Swamy, B.M., Kumar, D.V., 2007. Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. Tropical Journal of Pharmaceutical Research 6(2), 717-723.

Kuroyanagi, M., Arakawa, T., Hirayama, Y., Hayashi, T., 1995. Antibacterial and Antiandrogen Flavonoids from *Sophora flavescens*. - Journal of Natural Products, 62(12), 1595.

Leeming, J.P., Holland, K.T., Cunliffe, W.J., 1985. The pathological and ecological significance of microorganisms colonising acne vulgaris comedones. Journal of medical microbiology, 20, 11-16.

Mantle, D., Gok M.A., Lennard, T.W., 2001. Adverse and beneficial effects of plant extracts on skin and skin disorders. Adverse drug reactions and toxicological reviews, 20(2), 89-103.

Normann, H. and Snyman, I., 1996. Relationship between the sources of traditional and western medicine. Indigenous knowledge and its uses in southern Africa, HSRC Publishers, Pretoria, P 45.

Panichayupakaranant, P., Tewtrakul, S., Yuenyongsawad, S., 2010. Antibacterial, anti-inflammatory and anti-allergic activities of standardised pomegranate rind extract. Food Chemistry, 123, 400-403.

Puttarak, P., Charoonratana, T., Panichayupakaranant, P., 2010. Antimicrobial activity and stability of rhinacanthins-rich *Rhinacanthus nasutus* extract. Phytomedicine, 17, 323-327.

Ross, J.I., Eady, E.A., Cove, J.H., Jones, C.E., Ratyal, A.H., Miller, Y.W., Vyakrnam, S., Cunliffe, W.J., 1997. Clinical resistance to erythromycin and clindamycin in cutaneous propionibacteria isolated from acne patients is associated with mutations in 23S rRNA. Antimicrobial Agents and Chemotherapy, 41, 1162-1165.

Scaglione, F. and Rossoni, G., 1998. Comparative anti-inflammatory effects of roxithromycin, azithromycin and clarithromycin. Journal of Antimicrobial Chemotherapy, 41, 47-50.

Shaw, L. and Kennedy, C., 2007. The treatment of acne. Paediatrics and Child Health, 17, 385-389.

Spiewak, R., 2000. Occupational skin diseases among formers. Occupational and Para-Occupational Diseases in Agriculture. Zagorski.

Spinella, M., 2005. Introduction. Concise handbook of psychoactive herbs. The Haworth Herbal Press, Binghamton, P 1.

Truter, I., 2009. Acne vulgaris. SA Pharmaceutical Journal.

Tsai, T., Tsai, T., Wu, W., Tseng, J.T., Tsai, P., 2010. In vitro antimicrobial and anti-inflammatory effects of herbs against *Propionibacterium acnes*. Food Chemistry, 119, 964-968.

Van Wyk, B.E., Oudtshoorn, B., Gericke, N., 1997. Medicinal plants of South Africa. Briza Publication, Pretoria.

Vorobjeva, L.I., 1999. The genus *Propionibacteria*. Propionibacteria, Kluwer Academic Publishers, Netherlands, P 34, 35.

Webster, G.F. and Kim, J., 2008. The Immunology of Acne. In: Anthony Gaspari, Stephen K. Tyring. (Eds.), Clinical and Basic Immunodermatology, Springer, London, P 217-222.

Williams, D. F., Schmitt, W. H., 1996. Skin care Products. Chemistry and technology of the cosmetics and toiletries industry, Blackie Academic and Proffessional, London.

Wolf, J., 2002. Potential anti-inflammatory effects of topical retinoids and retinoid analogues. Advances in Therapy 19, 109-118.

Yahya, H., 2009. Acne vulgaris in Nigerian adolescents, prevalence, severity, beliefs, perceptions, and practices. International Journal of Dermatology, 48, 498-505.

Chapter 3

Plants selected for the present study

CHAPTER 3

Plants selected for the present study

3.1 Introduction

According to the World Health Organisation, about 70-90% of the world's population relies mainly on plants for their primary health needs. Also, in recent years, medicinal plants have represented a primary health source for various pharmaceutical industries. It has been stated that "there is plant for every need on every continent" and remarkably it appears to be true (Ayyanar and Ignacimuthu, 2011). Based on a survey by WHO in 1993, it was estimated that traditional practioners provide 80-90% treatments in countries like India, Africa and Bangladesh for various ailments like malaria, colds, cough, fever, arthritis, skin disorders, oral diseases, tuberculosis and many others (Prakash and Gupta, 2005).

Herbal medicines are also an important part of the culture and traditions of African people (Mander, 1998). Southern Africa is one of the richest centres of plant diversity in the world (Arnold and De Wet, 1993). Many of these plants have been used for several centuries in traditional medicine for the prevention and treatment of ailments including microbial diseases (Iwu, 1993; Hutchings et al., 1996). The art of herbal healing has very deep roots in dermatological ailments as well. Today, most of the population in urban South Africa, as well as smaller rural communities, are reliant on herbal medicines for their health care needs (Mander, 1998).

Ethnobotanical studies have documented the use of plants by the local people for the treatment of various skin ailments (Hutchings et al., 1996). Different plant parts commonly used as cosmetics or face masks, known as *umemezis*, are widely used in southern Africa for skin problems like inflammation, wounds, burns, eczema and puberty acne (Van Wyk and Gerick, 2000).

Acne is one of the most common skin diseases where colonisation of *Propionibacterium acnes* plays an important role in pathogenesis and inflammation. For the present study, 51 plants were tested for their activity against *P. acnes*. A few plants were collected randomly whereas others were collected based on their ethnobotanical information. Next section contains description of the plants selected for this study. The biological activity and phytochemistry are summarised in table 3.1 at the end of this chapter.

3.2 Description of selected plants

3.2.1 *Acacia caffra* (Thunb.) Willd.

Description

Acacia caffra (Thunb.) Willd. belongs to the family Leguminosae and is commonly known as cat-thorn. It is a tree which can grow up to a height of 14 m and has an irregular spreading crown. The attractive foliage bears bright green, drooping and feathery leaves. The flower spikes are creamy white, large and conspicuous. The plant is indigenous to South Africa and is found in the coastal areas of KwaZulu-Natal and the Eastern Cape and in some areas of the Western Cape (Aubrey, 2001).

Medicinal use

The Zulus, an African tribe use the infusion of bark for blood disorders, various parts of the plant are administered by infants for abdominal problems (Hutchings et al., 1996).

Figure 3.2.1: *Acacia caffra* (www.plantzafrica.com)

Biological activity and phytochemistry

The previous studies have reported isolation of proteracacinidin *ent*-oritin-(4β → 5) epioritin-4β-ol, 8-*O*-methylepioritin-4α-ol, 3-*O*-methyl-7,8,4′-trihydroxy-flavone and other teracacidin analogues from the methanol extract of heartwood of *A. caffra* (Malan, 1995). However, based on literature search no antimicrobial activity of plant extract was found.

3.2.2 *Acacia galpinii* Burtt Davy

Description

Acacia galpinii Burtt Davy belongs to family Leguminosae and is commonly known as monkey-thorn. It is a large tree which grows up to a height of 30 m. It has luxuriant light green foliage with maroon to purple buds which open in cream catkin flowers. The bark is papery and flaky in patches. The young branches have smooth green bark. The plant is indigenous to Zambia, Zimbabwe, Malawi and South Africa (Mutshinyalo, 2003).

Medicinal Use

The plant is medicinally used as demulcent and mucilaginous (Van Wyk and Gerick, 2000).

Figure 3.2.2: *Acacia galpinii* (www.plantzafrica.com)

Biological activity and phytochemistry

It was found that the acetone and chloroform leaves extract of *A. galpinii* inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* (Eloff and Katerere, 2004). The previous researchers have isolated proanthocyanins, teracacidin and proteracacinidin epioritin from the methanol extract of the heartwood of *A. galpinii* (Bennie et al., 2002).

3.2.3 *Acacia mellifera* Benth.

Description

Acacia mellifera Benth. belongs to the family Leguminosae and is commonly known as Blackthorn. It is a tall round shrub or small tree which grows up to a height of 9 m. The leaves are pinnate, obliquely ovate and asymmetrical. The branches are covered with very sharp curved thorns. The bark is smooth and grey with white lentils on young branches. The plant is native to Ethiopia, Saudi Arabia and commonly found throughout western, eastern and southern Africa (Nonyane, 2013).

Medicinal use

A. mellifera has been used widely in traditional African medicines against various diseases. The stem-bark of *A. mellifera* is used in Africa as a treatment for pneumonia, malaria, primary infection of syphilis, sterility and stomach ache (Lalitha et al., 2010). A community in Kenya known as "Kipsigis" uses the water extract from the plant for the treatment of various skin diseases, coughs and gastrointestinal ailments (Mutai et al., 2009).

Figure 3.2.3: *Acacia mellifera* (www.plantzafrica.com)

Biological activity and phytochemistry

Previous studies have reported the antimicrobial activity of chloroform and methanol extract of *A. mellifera* against *S. aureus*, *Cryptococcus neoformans*, *Candida albicans* and *Microsporum gypseum.*

The fractionation of crude dichloromethane (DCM) and methanolic extract yielded three triterpenoids, namely, (20*S*)-oxolupane-30-al, (20*R*)-oxolupane-30-al, and betulinic acid. The three compounds showed activity against *S. aureus* and only (20*S*)-oxolupane-30-al showed activity against clinical isolate of *M. gypseum* (Mutai et al., 2009).

3.2.4 *Aloe arborescens* Mill

Description

Aloe arborescens Mill belongs to the family Aloaceae and is commonly known as krantz aloe. It is a multiheaded shrub which grows up to a height of 2-3 m. The leaves are grey green in colour with armed margins and they are arranged in rosettes. The large colourful flower spikes are borne in profusion. The plant is native to southern Africa (Hankey and Notten, 2004).

Medicinal use

A. arborescence has been widely used for medicines and cosmetics. The leaves of this plant have been found to possess purgative properties. The leaf sap was traditionally used to treat burns and other skin inflammation in South Africa (Hutchings et al., 1996).

Figure 3.2.4: *Aloe arborescence* (www.plantzafrica.com)

Biological activity and phytochemistry

In a study conducted by Jia et al., 2008, a medium containing *A. arborescens* extract displayed growth inhibition zone of 0.5 ± 0.03 cm against fungus spore, *C. neoforman*. The leaves of *A. arborescens* were reported to contain secondary phenolic metabolites such as barbaloin, aloeresin and aloenin (Gutterman and Volfson, 2000).

3.2.5 *Aloe barbadensis* Mill

Description

Aloe barbadensis Mill belongs to the family Aloaceae and is commonly known as Aloe vera. It is a stem less plant with one to several rosettes of thick, fleshy and non-thorny leaves. The flowers are erect, yellow or red colour and found in clusters. The fruit is a triangular capsule containing numerous seeds. The plant is native to northern parts of Africa and Mediterranean region of southern Europe (Kane, 2006).

Medicinal Use

The leaf sap of Aloe vera has medicinal properties associated with wound healing and cosmetic application. It has significant ability in promoting vascularisation, reducing edema and inflammation (Davis et al., 1989; Hutchings et al., 1996; Lee et al., 2000).

Figure 3.2.5: *Aloe barbadensis* (http://www.google.co.za/ imghp)

Biological activity and phytochemistry

The hydroalcoholic leaves extract of *A. barbadensis* was found to exhibit minimum fungicidal concentration between 80 and 100 µl/ml against the mycelial growth of *Botrytis gladiolorum*, *Fusarium oxysporum gladioli*, *Heterosporium pruneti* and *Penicillium gladioli* (Casian et al., 2007). Phytochemical investigations showed that *A. vera* contains mono and polysaccharides, tannins, sterols, organic acids, enzymes, saponins, vitamins and minerals. The main constituent of *A. vera* plant extract was found to be aloine, an anthraquinone heteroside (Bajwa et al., 2007).

3.2.6 *Aloe ferox* Mill.

Description

Aloe ferox Mill. belongs to the family Aloaceae and is commonly known as Red aloe. It is a robust, single-stemmed succulent plant with broad and spiny leaves. The flowers are borne in large flower head between five and eight branches and each carry a spike like head of flowers. The plant is native to southern parts of South Africa (Aubrey, 2001).

Medicinal use

The leaf sap of *A. ferox* possesses wound healing properties and is used in cosmetic products. The plant is a common remedy for skin irritations, cuts, abrasions, minor burns, sunburn, bruises and acne (Klopper and Smith, 2010).

Figure 3.2.6: A*loe ferox* (www.plantzafrica.com)

Biological activity and phytochemistry

The previous studies have reported antimicrobial activity of methanol extract of *A. ferox* against *Neisseria gonorrhoea* (minimum inhibitory concentration (MIC) 0.5 mg/ml) and fungus spore, *C. neoformans* (growth inhibition zone of 1.2 ± 0.05 cm). The isolated pure compound aloin inhibited the growth of both *N. gonorrhoea* and *C. albicans* (Kambiz and Afolayan, 2008).

3.2.7 *Aloe sessiliflora* Pole-Evans.

Description

Aloe sessiliflora Pole-Evans also known as *Aloe spicata* L.f. belongs to family Aloaceae and is commonly known as Lemombo aloe. It is a shrub which usually grows less than 1 m in height. The stem is stout, erect and sometimes branched. The matured leaves spread horizontally or are recurved. The inflorescence is unbranched raceme with numerous flowers set closely. The plant is native to Zululand, Swaziland and northern Transvaal (Jeppe, 1969).

Medicinal Use

The plant is used traditionally to treat the uterus disorders and is believed to promote menstruation (Hutchings et al., 1996; Van Wyk and Gerick, 2000).

Figure 3.2.7: *Aloe sessiliflora* (http://www.google.co.za/ imghp)

Biological activity and phytochemistry

Based on literature search, no antimicrobial activity of *A. sessiliflora* was found. However, ethanol leaf extracts of *A. sessiliflora* exhibited 13% tyrosinase inhibitory activity at concentration of 500 μg/ml (Mapunya et al., 2012).

3.2.8 *Anchusa capensis* Thunb.

Description

Anchusa capensis Thunb. belongs to the family Boraginaceae and is commonly called as Cape-forget me not. It is a vigorous herb with tall stems of blue flowers shooting up from clumps of bright green leaves. The stem is thick at bottom, gets thinner as extended. The leaves are soft and hairy. The plant originated in USA and is also grown in Europe, North Africa, South Africa and Western Asia (Van der Walt, 2000).

Medicinal Use

The plant has been found to be a traditional phytomedicine. Other uses include neurotoxins and mutagenic (Wink and Van Wyk, 2008).

Figure 3.2.8: *Anchusa capensis* (www.plantzafrica.com)

Biological activity and phytochemistry

No biological activity and phytochemistry was found based in the literature.

3.2.9 *Annona senegalensis* Pers.

Description

Annona senegalensis Pers. belongs to the family Annonaceae and is commonly known as wild custard apple. It is a small tree of 2-6 m height. The bark is smooth to roughish with roughly circular flake exposing paler patches of under bark. The leaves are alternate, simple, oblong, ovate or elliptic. Flowers are crimson and are borne solitary. The fruits are cylindrical, fleshy and orange-brown in colour. It is native to tropical east and northeast, west and west-central, and southern Africa, as well as southern subtropical Africa, and islands in the western Indian Ocean. Specific to the nation of South Africa, it is found in KwaZulu-Natal, Limpopo and Mpumalanga (Agroforestry Tree Database, 2012).

Medicinal Use

The bark is used for treating guinea worms and other worms, diarrhoea, gastroenteritis, snakebite, toothache and respiratory infections. Gum from the bark is used in sealing cuts and wounds. The leaves are used for treating pneumonia and as a tonic to promote general well-being. The roots are used for stomach-ache, venereal diseases, chest colds and dizziness. Various plant parts are combined for treating dermatological diseases and ophthalmic disorders (Agroforestry Tree Database, 2012).

Figure 3.2.9: *Annona senegalensis* (http://www.google.co.za/ imghp)

Biological activity and phytochemistry

During previous studies, the water and methanol extracts of *A. senegalensis* showed activity against

Pseudomonas aeruginosa and *S. aurens* with MIC value of 62.5 mg/ml (Lino and Deogracious, 2006). From the bark extract of *A. senegalensis*, kauran-16α-ol, kaur-16-en-19-oic acid, kauran-19-al-17-oic acid, and 19-norkauran-4α-ol-17-oic acid were isolated by previous researchers (Eshiet and Akisanya, 1971).

3.2.10 *Arbutus unedo* L.

Description

Arbutus unedo L. belongs to family Eriaceae and is commonly known as strawberry tree. It is an evergreen shrub which grows up to a height of 2.5 m. The leaves are dark green and glossy with serrated margins. The hermaphrodite flowers are white, bell-shaped, 4-6 mm in diameter and produce panicles. The fruits are small berries, with globular shape and vary considerable in size. The plant is native to the coastal regions of Mediterranean extending from southern Europe to western Asia and northern Africa (Benhouhou, 2005).

Medicinal Use

The leaves, bark and root of *A. unedo* are reported to possess astringent and diuretic properties. The plant was traditionally used for treatment of sore and irritated throats. The leaves and fruit of the plant are reported to possess antioxidant properties (Ziyyat and Boussairi, 1998; Kivack and Mert, 2001; Pabuccuoglu et al., 2003).

Figure 3.2.10: *Arbutus unedo* (http://www.google.co.za/ imghp)

Biological activity and phytochemistry

The ethyl acetate, methanol and acetone extract of aerial parts of *A. unedo* inhibited the growth of *S. aureus*, *Enterococcus faecalis*, *Staphylococcus epidermis* and *Staphylococcus saprophiticus* with zones of inhibition ranging between 7-29 mm (Sassi et al., 2007). Seven phenolic compounds, namely, arbutin, β-D-glucogalline, gallic acid 4-*O*-β-D-glucopyranoside, 3-*O*-galloylquinic acid, 5-*O*-galloylquinic acid, 3-*O*-galloylshikimic acid and 5-*O*-galloylshikimic acid were isolated from methanol extract of *A. unedo* (Pawlowska et al., 2006).

3.2.11 *Aspalathus linearis* R.Dahlgren

Description

Aspalathus linearis R.Dahlgren belongs to the family Leguminosae and is commonly known as rooibos. It is a shrub of half to two meters with bright green and needle shaped leaves. The small, yellow, typically pea-shaped flowers are produced in spring and early summer. The species is exceptionally variable. The plant is endemic to western parts of Cape and can be found naturally from the Cape Peninsula northwards to Nieuwoudtville (Van Wyk et al., 1997).

Medicinal Use

Rooibos tea is a popular health beverage. It is reported to be beneficial in eczema and possesses antispasmodic activity. Traditional medicinal uses of rooibos in South Africa include alleviation of infantile colic, allergies, asthma and dermatological problems (Van Wyk et al., 1997).

Figure 3.2.11: *Aspalathus linearis* (www.plantzafrica.com)

Biological activity and phytochemistry

The hot water and ethyl acetate extract of *A. linearis* was reported to show a growth inhibitory effect against *E. coli*, *S. aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Streptococcus mutans* and *Saccharomyces cerevisiae* (Scheepers, 2001). Based on previous studies, many compounds have been isolated from this plant. Flavonoids isolated from rooibos tea include aspalathin, nothofagin, orientin, iso-orientin, rutin, isoquercitrin, vitexin, isovitexin, chrysoeriol, quercetin and luteolin. It was reported that the phenolic acids in rooibos tea consist of protocatechuic acid, caffeic acid, p-hydroxybenzoic acid, vanillic acid, p-coumaric acid, ferulic acid and syringic acid which possess high levels of minerals and free radical capturing properties. Most of these compounds are widely distributed in nature and have shown to possess antioxidative properties. Topical application of rooibos extract are believed to alleviate dermatological problems like acne and eczema (Van Wyk et al., 1997).

3.2.12 *Barleria albostellata* C. B. Clarke

Description

Barleria albostellata C. B. Clarke belongs to the family Acanthaceae and is commonly known as grey barleria. The plant is a medium to large, multi-stemmed shrub which grows up to a height of 2 m. The leaves are whitish green, velvety and arranged in opposites. The plant produces beautiful white tubular flowers (Froneman and Roux, 2007). The plant is native to Africa and is found in woodland areas of South Africa, as well as in Zimbabwe.

Figure 3.2.12: *Barleria albostellata* (www.plantzafrica.com)

Medicinal Use

The plant possesses antimicrobial, anti-inflammatory and antioxidant properties. Due to relatively high flavonoid content, with a contributing effect from iridoid and tannin compounds, this plant has medicinal and pharmacological importance (Stephen, 2010).

Biological activity and phytochemistry

During previous studies, the petroleum ether (PE), ethanol and DCM extract of leaves showed activity against *C. albicans* with MIC values ranging from 1.17-4.68 mg/ml. The stem extract was also found to be active against the same pathogen with MIC values ranging from 0.78-3.12 mg/ml (Amoo et al., 2011). Another study showed antibacterial activity of the ethanolic, DCM and PE extract of leaves of *B. albostellata* against *Bacillus subtilis, S. aurens, E. coli* and *Klebsiella pneumonia* with MIC values ranging from 3.12-0.78 mg/ml and only ethanolic extract of stem was reported to be active against these micro organisms (Amoo et al., 2009). The details of compounds and phytochemistry of this plant is not found in the literature.

3.2.13 *Barleria repens* Nees

Description

Barleria repens Nees belong to the family Acanthaceae and is commonly known as small bush violet. It usually forms a rounded to spreading busy shrub and grows to a height of 0.7 m. The leaves are soft, shiny and dark green in colour. The flowers are large and are deep- purple or pink-red in colour. The fruit is an explosive club-shaped capsule. It is found throughout woodland and forest, from KwaZulu-Natal northwards to tropical Africa (Joffe, 2003).

Figure 3.2.13: *Barleria repens* (www.plantzafrica.com)

Medicinal Use

The plant possesses antimicrobial, anti-inflammatory and antioxidant properties. Due to relatively high flavonoid content, with a contributing effect from iridoid and tannin compounds, this plant has medicinal and pharmacological importance (Stephen, 2010).

Biological activity and phytochemistry

Based on literature search, no biological activity and phytochemistry was found.

3.2.14 *Broussonetia papyrifera* (L.) Vent.

Description

Broussonetia papyrifera (L.) Vent. belongs to family Moraceae and is commonly known as paper mulberry. It is a small tree which may grow up to 12 m in height. The leaves are simple with three to five lobes. The flowers are elongated with male spikes up to 8 cm long and female axillary globose heads up to 2.5 cm long, and are present on separate male and female trees. The fruit is a globose to club-shaped syncarp. The plant is native to Japan and is found globally (Whistler and Elevitch, 2006).

Medicinal Use

The plant is reported to possess the following medicinal properties: astringent, for skin ailments, stomachic, tonic, diaphoretic, diuretic and stimulant (Whistler and Elevitch, 2006; Dweck, 2012).

Figure 3.2.14: *Broussonetia papyrifera* (http://www.google.co.za/ imghp)

Biological activity and phytochemistry

During previous studies, the antifungal activity of root extract of *B. papyrifera* was reported by Yu et al. (2001). Papyriflavonol A and a prenylated flavonoid [5,7,3`,4`- tetrahydroxy-6,5`-di-(γ,γ-dimethylallyl)-flavonol isolated from the root bark of *B. papyrifera* showed a broad-spectrum antimicrobial activity against *C. albicans*, *S. cerevisiae*, *E. co*li and *S. aureus* with MIC values ranging between 10-25 mg/ml (Sohn et al., 2010).

3.2.15 *Buxus macowanii* Oliv.

Description

Buxus macowanii Oliv. belongs to family Buxaceace and is commonly known as Cape box. The Cape box is a small, very slow-growing tree up to about 9 m tall. It has a clean slender, greeny-brown stem with shiny green leaves which create the crown. The twigs are angled and hairy. Male and female flowers are borne separately and are held on short axils. The fruit is a small brown capsule (Dlamini and Turner, 2002). *B. macowanii* mainly originates from the Eastern Cape forests and extends to the eastern Northern Province.

Medicinal Uses

The plant is reported to be a useful remedy for the following ailments: gout, malaria, rheumatism and skin disorders (Wink and Van Wyk, 2008).

Figure 3.2.15: *Buxus macowanii* (http://www.google.co.za/ imghp)

Biological activity and phytochemistry

In a recent study, the dichloromethane extract of *B. macawonii* afforded five novel triterpenoidal alkaloids, 31-hydroxybuxatrienone, macowanioxazine , 16α-hydroxyma-

cowanitriene, macowanitriene and macowamine, along with two known Buxus bases, Nb-demethylpapillotrienine and moenjodaramine (Lam, 2012). However, antimicrobial activity of the crude extract has not been reported.

3.2.16 *Carpobrotus edulis* (L.) L. Bolus

Description

Carpobrotus edulis (L.) L. Bolus belongs to family Azioaceae and is commonly known as sour fig. It is a fleshy succulent perennial mat-like creeper with juicy erect leaves. The yellow flowers are large and fleshy which develop into fragrant fleshy fruit. The original occurrence of *C. edulis* was in sandy areas of Western Cape Province and along the Cape south coast to the Eastern Cape (Van Wyk et al., 1997).

Figure 3.2.16: *Carpobrotus edulis* (www.plantzafrica.com)

Medicinal Use

The leaf juice is traditionally used to treat infections of mouth and throat, eczema, wounds and burns. The leaves are taken orally for dysentery, digestive troubles, tuberculosis, as diuretic and as styptic. The plant is an effective remedy against toothache, earache and vaginal thrush (Van Wyk et al., 1997).

Biological activity and phytochemistry

The crude methanolic extract of plant showed antibacterial activity against *Moraxella catharralis* at concentration of 50 mg/cm^3. Five compounds namely, rutin, neohesperidin, hyperoside, cactichin and ferulic acid were isolated from ethanolic extract of *C. edulis*, which showed growth inhibitory activities against *B. subtilis*, *S. epidermis*, *S. aureus*, *Streptococcus pneumoniae*, *E. coli*, *P. aeruginosa* and *Streptococcus pyogens* at concentration of 10 mg/cm^3 (Van der Watt and Pretorius, 2001).

3.2.17 *Ceratonia siliqua* L.

Description

Ceratonia siliqua L. belongs to the family Leguminosae and is commonly known as carob tree. This tree grows up to 10 m tall. The crown is broad and semi-spherical, supported by a thick trunk with brown rough bark and sturdy branches. Leaves are long, alternate, pinnate, and may or may not have a terminal leaflet. It is native to the Mediterranean region including Southern Europe, Northern Africa, the larger Mediterranean islands (Batlle and Tous, 1997).

Medicinal Use

The plant is used as common sweetner, aphrodisiac; the pods are used as a medicine for coughs and sore throat. Its bark and leaves are used as antidiarrheal and diuretic in Turkish folk medicine. The fruits of this plant are traditionally used as antitussive and against warts (Kivack et al., 2002).

Biological activity and phytochemistry

The methanol, hexane, ethyl acetate and ethanol extract of *C. siliqua* showed inhibitory activity against *E. coli*, *Salmonella typhirium*, *Enterobacter cloacae*, *P. aeruginosa*, *S. aureus*, *S. epidermis* and *C. albicans* with zones of inhibition ranging between 7-22 mm (Kivack et al., 2002). The presence of flavonoids like myricetin, quercetin, kaempferol, luteolin, genisten, taxifolin was reported in the leaf extract of *C. siliqua* (Vaya and Mahmood, 2006).

Figure 3.2.17: *Ceratonia siliqua* (http://www.google.co.za/ imghp)

3.2.18 *Combretum apiculatum* Sond.

Description

Combretum apiculatum Sond**.** belongs to the family Combretaceae and is commonly known as red bushwillow. It is a single or multi stemmed tree that grows up to a height of 3-10 m. The leaves are simple, shiny yellow-green in colour, alternate, opposite or in whorls. The flowers are yellow to creamy green and strongly scented. The fruit is four-winged, almost round to ovoid in shape, and yellowish-green in colour with a single seed in the centre. It is widespread in Africa that grows from KwaZulu-Natal and Mpumalanga and Limpopo to Botswana (Masupa and Rampho, 2011).

Figure 3.2.18: *Combretum apiculatum* (www.plantzafrica.com)

Medicinal Use

A decoction of the leaves is used as a traditional medicine to relieve stomach disorders. For the treatment of conjunctivitis, an ash from the burnt stem is mixed with white clay and water and the resulting paste is spread over the face (Hutchings et al., 1996).

Biological activity and phytochemistry

In a study, antibacterial activity of *C. apiculatum* leaves extract and isolated flavanones- alpinetin, pinocembrin and one chalcone- flavokawain have been reported to show activity against *E. coli*, *P. aeruginosa*, *S. aurens* and *E. faecalis* with MIC values ranging between 100-268 µg/ml (Serage, 2003).

3.2.19 *Combretum molle* Engl. & Diels.

Description

Combretum molle Engl. & Diels. belongs to the family Combretaceae and commonly known as velvet bushwillows. It is a wide spread, fairly common shrub, up to 4 m tall, which grows on both deep sand and loamy sand. The leaves are simple, leathery and with opposite arrangement. The flowers are small, pale green or greenish- yellow in colour and borne individuals. The fruits are yellowish-green in colour and borne abundantly. The plant is native to South African woodlands and is widely distributed from southern Africa to extreme north east and to west (Masupa, 2011).

Figure 3.2.19: *Combretum molle* (www.plantzafrica.com)

Medicinal Use

The Zulus use fresh or dry leaves for wound healing. In Ghana the leaves are used as wound dressings, abortifacients and anthelmintics (Hutchings et al., 1996).

Biological activity and phytochemistry

The acetone extract of stem bark of *C. molle* showed antimicrobial activity against *E. coli* and *Shigella* spp with MIC value of 50 mg/ml. The extract also showed inhibitory effects on the fungus *C. albicans* with complete inhibition at a concentration of 400 µg/ml. The antimicrobial activity of the extract reported was attributed to the high amount of hydrolysable tannins-ellagitannin, punicalagin present in the bark of the plant (Asres et al., 2006).

3.2.20 *Cotyledon orbiculata* L.

Description

Cotyledon orbiculata L. belongs to the family of Crassulaceae and is commonly known as pig's ear. This is a common succulent shrub with woody branches and thick leaves which vary from green to grey. The orange or red tubular flowers are borne on a long and slender stalk. It is widely distributed over practically whole of southern Africa but is usually confined to rocky outcrops in grassland fynbos and karoo regions. It is widespread in the Western, Eastern and Northern Cape Provinces and Namibia, usually on rocky slopes in open vegetation (Harris, 2004).

Figure 3.2.20: *Cotyledon orbiculata* (www.plantzafrica.com)

Medicinal Use

The plant is widely used for medicinal purpose. The fleshy part of leaf is applied to soften and remove corns and warts. The warmed leaf juice is used as drops for earache, toothache and to treat epilepsy, boils and inflammation (Hutchings et al., 1996; Van Wyk et al., 2007).

Biological activity and phytochemistry

In a study conducted by Aremu et al. (2010), it was found that the ethanol, PE and DCM extract of the leaves/stem of *C. orbiculata* showed antibacterial activity against *Bacillus subtilis*, *Staphylococcus aurens*, *E. coli* and *K. pneumonia*; and antifungal activity against *C. albicans* with MIC values ranging from 1.5-6.2 mg/ml. Based on literature search, no reports regarding the isolation of compound were found.

3.2.21 *Cryptocarya woodii* Engl.

Description

Cryptocarya woodii Engl**.** belongs to the family Lauraceae and is commonly known as Cape laurel. It is a small to medium sized tree with small, shiny and bright leaves which are green above while slightly pale green below. The small inconspicuous flowers develop into round, shiny, purple-black fruits. The plant is widely distributed from the Eastern Cape, KwaZulu-Natal and Swaziland to eastern side of Gauteng and further north on eastern side of the continent (Mbambezeli, 2005).

Medicinal Use

C. woodii is said to be the traditional medicine for magical and medicinal purpose. An infusion of powdered bark is used as a remedy for diarrhoea (Mbambezeli, 2005).

Figure 3.2.21: *Cryptocarya woodii* (www.plantzafrica.com)

Biological activity and phytochemistry

Based on literature search, no reports regarding the antimicrobial activity of crude extract and phytochemistry was found.

3.2.22 *Dahlia imperialis* Roezl

Description

Dahlia imperialis Roezl belongs to family Asteraceae and is commonly called as tree dahlia. The plant is delicate herbaceous perennial tree which bears huge leaves and shaggy lavender flowers with yellow

centres. The stem is tall, straight and thin, much like bamboo stems. The plant is native to Mexico, Central America and Colombia (Royal New Zealand Institute of Horticulture, 2012).

Medicinal Use

The petals are used for skin treatments like rashes, grazes, infected scratches and for feet problems (Roberts, 2007).

Figure 3.2.22: *Dahlia imperialis* (http://www.google.co.za/ imghp)

Biological activity and phytochemistry

Based on literature review no antimicrobial activity and phytochemistry of this plant was reported.

3.2.23 *Datura stramonium* L.

Description

Datura stramonium L. belongs to the family Solanaceae and is commonly known as thornapple. It is an exotic weed which has large, irregularly toothed leaves of unpleasant smell. The axil leaf matures into tubular, large purplish flower, followed by follicular fruit capsule which is covered with numerous spines. The plant is indigenous to topical America but has become a weed and is now widely distributed to South Africa (Van Wyk et al., 2007; Wink and Van Wyk, 2008).

Medicinal Use

The plant is traditionally used to relieve asthma, bronchitis, reduce pain, as aphrodisiacs. The fresh warmed leaf is used as poultice to relieve the pain of rheumatism, gout, boils, abscesses and wounds. The fruit is applied for toothache, sore throat and tonsillitis (Hutchings et al., 2006; Van Wyk et al., 2007; Wink and Van Wyk, 2008).

Figure 3.2.23: *Datura stramonium* (www.plantzafrica.com)

Biological activity and phytochemistry

In a study, the methanolic extract of *D. stramonium* exhibited growth inhibitory activities against *B. subtilis, S. aurens* and *E. faecalis* with zones of inhibition ranging between 8-10 mm (Eftekhar et al., 2005). The studies of phytochemical investigation showed the presence of tropane alkaloids: atropine, hyoscine, hyoscyamine and scopolamine in the plant extract (Van Wyk et al., 2007).

3.2.24 *Dichrostachys cinerea* L. White & Arn.

Description

Dichrostachys cinerea L.White & Arn. belongs to family Leguminosae and is commonly known as sickle bush. It is a spiny, deciduous shrub or small tree which grows up to 7 m high and has a rounded crown. The bark is rough, yellow to grey-brown in colour and frequently fissured. The compound and petiolate leaves are very variable in size. The flowers are 25 to 50 mm long, pendulous spikes that are borne in the leaf axils, singly or in bundles. The plant is native to Africa but can be found in India and the Caribbean and parts of Southeast Asia (Cheek, 2009).

Medicinal Use

The roots are used as a local anaesthetic for ailments such as snake bites, scorpion stings and toothache. In Botswana, parts of the tree are used as a cure for tapeworm infections (Hutchings et al., 1996; Van Wyk et al., 2007; Cheek, 2009).

Figure 3.2.24: *Dichrostachys cinerea* (www.plantzafrica.com)

Biological activity and phytochemistry

In a study conducted by Eisa et al. (2000), chloroform, methanol and aqueous extracts of *D. cinerea* showed activity against several bacterial such as *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. The MIC values obtained in this study ranged from 0.27-90 mg/ml. In another study, the tannins isolated from *D. cinerea* exhibited antibacterial activity against *Shigella boydii, Shigella flexneri, E. coli, S. aurens* and *P. aeruginosa* using agar diffusion method. The MIC values ranged between 4.0-5.5 mg/ml (Banso and Adeyemo, 2007).

3.2.25 *Diospyros lycioides* Desf.

Description

Diospyros lycioides Desf. belongs to family Ebenaceae and commonly called as bluebush. This exciting shrub or small tree is a very tough plant with attractive features of its smooth bark, blue-green leaves, fragrant flowers and colourful fruits. The plant is native to the tropics, with only a few species extending into temperate regions. It occurs in Central Africa, southern Tanzania, throughout southern Africa, including South Africa, Lesotho and Swaziland. (Rambuwani, 2005).

Medicinal Use

The roots are chewed, as a toothbrush and used medicinally by local people of Namibia. The roasted powdered roots are also used to ease body pain (Hutchings et al., 1996; Rambuwani, 2005).

Figure 3.2.25: *Diospyros lycioides* (www.plantzafrica.com)

Biological activity and phytochemistry

In a study conducted by Lining et al. (2000), the crude methanolic extract of *D. lycioides* showed activity against *Streptococcus mutans* and *Prevotella intermedia* at MIC value of 1.25 mg/ml. The isolated compounds namely, naphthalene glycosides, diospyrosides, naphthoquinones, juglone and 7-methyljuglone inhibited the growth of oral cariogenic bacteria (*S. mutans* and *Streptococcus sanguis*) and periodontal pathogens (*Porphyromonas gingivalis* and *Prevotella intermedia*) with MIC values ranging from 0.01-1.25 mg/ml.

3.2.26 *Dodonaea viscosa* Jacq.

Description

Dodonaea viscosa Jacq. belongs to the family Sapindaceae and is commonly called as hopbush. *D. viscosa* is a shrub growing to 1–3 m tall and rarely grows to a small tree of 9 m tall. The leaves are simple elliptical, alternate in arrangement, and secrete a resinous substance. The flowers are yellow to orange-red and are produced in panicles. The fruit is a capsule, red ripening brown, with two to four wings. *D. viscosa* is native to Australia, but it occurs throughout the tropics and subtropics. In Africa, it

occurs along the coasts of West Africa, East Africa and in Madagascar (Harris, 2012).

Medicinal Use

D. viscosa is a traditional medicine which is utilized in folklores-medicine for the treatment of sore throats. The powdered leaf is applied to treat wounds of burns and scalds and is also useful for different skin diseases (Hutchings et al., 1996; Van Wyk et al., 2007).

Figure 3.2.26: *Dodonaea viscosa* (www.plantzafrica.com)

Biological activity and phytochemistry

The previous researchers have reported the antimicrobial activity of acetone extract of *D. viscosa* against 41 strains of *C. albicans*. The MIC values obtained in the study ranged between 6.25–25 mg/ml (Patel and Coogan, 2008). The kaempferol methyl esters isolated from *D. viscosa* exhibited antibacterial activity against *S. aureus*, *E. coli*, *E. faecalis* and *P. aeruginosa* with MIC values ranging from 16-250 µg/ml (Teffo et al., 2010).

3.2.27 *Erythrophleum lasianthum* Corbishley

Description

Erythrophleum lasianthum Corbishley belongs to family Leguminosae and is commonly known as Swazi ordeal tree. It has a rough bark and the foliage which forms a rounded crown. The leaves are divided into hairy leaflets. The small cream or greenish-yellow flowers are arranged in clusters. The species has a limited distribution in north-eastern parts of KwaZulu-Natal, Swaziland and southern Mozambique and is native to South Africa (Van Wyk et al., 1997).

Medicinal Use

The powdered bark is a traditional remedy for headaches, migraine, general body pains, intestinal spasm and fever. The snuff, known as "mbhemiso" is sold in traditional herbal shops (Hutchings et al., 1996).

Biological activity and phytochemistry

In a study conducted by Nielsen et al. (2012), the crude extract of *E. lasianthum* inhibited the growth of several microbes such as *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *C. albicans* and *Microsporum audouinii* with MIC values varying from 125-156 µg/ml. The presence of alkaloids namely, cassaine, erythrophleine, 3 beta-hydroxynorerythrosuamine and 3-O-beta-D-glucopyranoside from the leaves of *E. lasianthum* has been reported by previous researchers (Verotta et al., 1995; Van Wyk et al., 1997).

Figure 3.2.27: *Erythrophleum lasianthum* (http://www.google.co.za/ imghp)

3.2.28 *Euclea divinorum* Hiern

Description

Euclea divinorum Hiern belongs to the family Ebenaceae and is commonly known as magic gwarra. It is a shrub or small tree which grows up to 6 m tall. The branching often occurs from the base. The leaves are simple, dark grey or green in colour. Flowers are small, cup-shaped and creamy in colour. The fruit is round, thin and fleshy berry. The shrub is native to Botswana, Kenya, Namibia, South Africa and Swaziland (Agroforestry Tree Database, 2012).

Figure 3.2.28: *Euclea divinorum* (http://www.google.co.za/ imghp)

Medicinal Use

The branches are traditionally used as chewing sticks for oral care. In Kenya the root decoction is used as a purgative and the bark infusion as an appetizer. The decoctions of the root are used by the Zulu, a South African tribe, as a remedy for toothache (Agroforestry Tree Database, 2012).

Biological activity and phytochemistry

In a study conducted by More et al. (2008), the ethanol extract of *E. divinorum* exhibited antimicrobial activity against *Actinomyces naeslundii*, *Actinomyces israelii*, *S. mutans*, *C. albicans*, *P. gingivalis* and *P. intermedia* with MIC values varying from 0.8-25.0 mg/ml. The pentacyclic triterpenes and naphthoquinones, lupeol, lupene, betulin, 7-methyljuglone, isodiospyrin, shinalone, catechin and 3 beta-(5-hydroxyferuloyl) lup-20(30)-ene were previously isolated from the roots of *E. divinorum* (Mebe et al., 1998).

3.2.29 *Euclea natalensis* A. DC.

Description

Euclea natalensis A. DC. belongs to the family Ebenaceae and is commonly known as Natal guarri. It is a shrub or tree of height 10 m with darker, thin bark that gets fissured with age. The leaves are hard and leathery, dark green above and paler underneath. The flowers are small, bell shaped and creamy in colour. The fruits are round and fleshy berries. *E. natalensis* is widespread, from Clanwilliam in the Western Cape, southwards and eastwards along the coast through the Eastern Cape to KwaZulu-Natal,

Swaziland, Mozambique, Mpumalanga, Limpopo, Gauteng and further north to tropical east Africa into Ethiopia (Notten, 2010).

Figure 3.2.29: *Euclea natalensis* (www.plantzafrica.com)

Medicinal Use

The roots possess the medicinal properties and are used for curing bronchitis, pleurisy, chronic asthmas and UTI. The roots are used as a dye for skin infections caused by *Mycobacterium leprae* and also to relieve headache and toothache (Hutchings et al., 1996).

Biological activity and phytochemistry

During previous studies, the acetone bark extract of *E. natalensis* inhibited the growth of *Mycobacterium tuberculosis*, *B. cereus*, *Bacillus pumilus*, *B. subtilis*, *Micrococcus kristinae* and *S. aureus* at concentrations ranging between 1.0-6.0 mg/ml (Lall and Meyer, 1999; Lall and Meyer, 2000). Two compounds namely, β- sitosterol and octahydroeuclein isolated from the ethanolic extract of *E. natalensis* root bark inhibited the growth of *Cladosporium cladsporioides* significantally at 0.01 mg/ml (Lall et al., 2006).

3.2.30 *Galenia africana* L.

Description

Galenia africana L. is locally known as “kraalbos” or “geelbos” and belong to the family Aizoaceae. It is an aromatic, woody perennial sub-shrub which grows up to a height of 0.5-1 m. The leaves are green,

5 cm long and hairless with opposite arrangement. Tiny open inflorescences are borne at the ends of branches and are 3-10 cm long with many small, greenish yellow flowers. It is a dominant plant found throughout South Africa (Adamson, 1956).

Medicinal Use

The plant is used in the treatment of venereal diseases and a decoction as a lotion for skin diseases, including ringworms, and for the relief of inflammation of the eyes (Vries et al., 2005).

Figure 3.2.30: *Galenia africana* (http://www.google.co.za/ imghp)

Biological activity and phytochemistry

In a study conducted by Mativandlela et al. (2009), the ethanol extract of *G. africana* and its purified compounds namely, (2S)-5,7,2′-trihydroxyflavanone, (E)-3,2′,4′-trihydroxychalcone and (E)-2′,4′-dihydroxychalcone showed antituberculosis activity against *Mycobacterium smegmatis* and *M. tuberculosis* with MIC values varying between 0.78-1.2 mg/ml.

3.2.31 *Gomphocarpus fruticosus* R. Br.

Description

Gomphocarpus fruticosus R. Br. belongs to the family Asclepiadaceae and is commonly known as milkweed. It is an herbaceous, perennial, spindly shrub, often with watery or milky sap and can grow up to 1.5-2 m in height. The light brown stem branches higher up to form the crown. The attractive, creamy yellow flowers are carried in pendulous clusters. The fruit is an inflated, light brown, papery

follicle and is covered with bristle-like hairs containing dark seeds (Naidoo, 2005). The plant is native to South Africa.

Medicinal Use

The leaves are used as snuff and as a sedative in the treatment of headache and tuberculosis. Roots are used to relieve stomach pain and general aches in the body (Naidoo, 2005).

Figure 3.2.31: *Gomphocarpus fruticosus* (www.plantzafrica.com)

Biological activity and phytochemistry

A study showed that methanol and hexane fruit extract of *G. fruticosus* significantly inhibited the growth of *P. aeruginosa* with MIC value of 31 µg/ml (Madureria et al., 2011). Another study conducted by Heneidak et al. (2006) showed the presence of quercetin glycosides, kaempferol, rutin and isorhamenetin from *G. fruticosus* extract.

3.2.32 *Greyia flanaganii* Bolus

Description

Greyia flanaganii Bolus belongs to the family Greyiacece and is commonly known as Kei bottlebrush. It is a stunning shrub or small, much-branched, evergreen tree that grows up to 3 m in height with smooth and pale brown bark. The leaves are ovate to almost circular and are crowded at the ends of the branches. It has bright red, bell-shaped flowers while the fruits are cylindrical and brown in colour. It is an evergreen shrub endemic to Eastern Cape (Mbambezeli, 2002).

Figure 3.2.32: *Greyia flanaganii* (www.plantzafrica.com)

Medicinal Use

The plant is traditionally believed to have the ability to ward off sickness (Mbambezeli, 2002).

Biological activity and phytochemistry

Mapunya et al. (2011) reported antibacterial activity of ethanol extract of *G. flanaganii*. The crude extract and isolated compounds namely, 2,4,6-trihydroxydihydrochalcone; (3S)-4-hydroxyphenethyl3-hydroxy-5-phenylpentanoate;2,6,4-trihydroxy-4methoxydihydrochalcone inhibited the growth of *P. acnes* with MIC values ranging between 250-500 µg/ml.

3.2.33 *Greyia sutherlandii* Hook. & Harv.

Description

Greyia sutherlandii Hook. & Harv. belongs to the family Greyiacece and is commonly known as Natal bottlebrush. It is a small tree which may grow up to 3 to 7 m in height. The leaves are simple, alternate, rather leathery, slightly lobed and coarsely toothed with glandular surface. The flowers are red and open in closely packed racemes. The fruit is long, pale brown in color, cone-shaped and cylindrical capsule. The plant grows on the slopes and rocky ridges of the Drakensberg, in the Eastern Cape, the eastern Free State, KwaZulu-Natal, Swaziland and eastern Gauteng (Mbambezeli, 2006).

Medicinal Use

The bark of *G. sutherlandii* is used as a traditional medicine while root infusions are taken as emetics for biliousness (Hutchings et al, 1996).

Figure 3.2.33: *Greyia sutherlandii* (www.plantzafrica.com)

Biological activity and phytochemistry

Based on literature search, no antimicrobial activity and phytochemistry of *G. sutherlandii* were found.

3.2.34 *Harpephyllum caffrum* Bernh. ex Krauss

Description

Harpephyllum caffrum Bernh. ex Krauss belongs to the family Anacardiaceae and is commonly known as wild plum. This is a large tree with rough and dark brown bark. The dark green shiny leaves are divided into leaflets. The flowers are small, whitish inconspicuous with separate male and female flowers. The fruits are edible and sour. The natural distribution is restricted to southern Africa from the Eastern Cape northwards through KwaZulu-Natal, Swaziland, southern Mozambique, and Limpopo and into Zimbabwe (Van Wyk et al., 1997).

Medicinal Use

The decoctions of the bark are traditionally used as blood purifiers, for facial saunas, skin washes, to

treat acne and eczema. To treat sprains and fractures, powdered burnt bark is applied to scarification (Van Wyk et al., 1997).

Figure 3.2.34: *Harpephyllum caffrum* (www.plantzafrica.com)

Biological activity and phytochemistry

The ethanolic extract of *H. caffrum* was reported to be active against four bacterial species namely, *B. subtilis*, *E. coli*, *K. pneumoniae*, and *S. aureus* while an aqueous extract showed activity against *C. albicans* (Buwa and Van Staden, 2007). The chemical constituents of *H. caffrum* are poorly known but based on literature review, the presence of polyphenolics, flavonoids and organic acids like protocatechuic acid was reported (Van Wyk et al., 1997).

3.2.35 *Helichrysum argyrophyllum* DC.

Description

Helichrysum argyrophyllum DC. belongs to family Asteraceae and is commonly known as golden guinea flower. It is a shrub that grows to a height of 12-18 inches. The leaves are small and grey in colour. The flowers are daisy like with yellow rays surrounding a darker yellow centre. The plant originated in South Africa and is found in abundance in Eastern Cape (Smith, 2001).

Medicinal Use

The plant possesses tissue regenerating properties and is used traditionally to heal scars. Various parts of the plant are used to treat intestinal troubles (Van Wyk et al., 1997; Herbal Africa, 2012).

Figure 3.2.35: *Helichrysum argyrophyllum* (www.plantzafrica.com)

Biological activity and phytochemistry

During the previous studies, gyrosanol (belonging to diterpenoid group) was isolated from the aerial parts and the roots of *H. argyrophyllum*. However, no antimicrobial activity of the crude extract and isolated compound was reported (Cheng et al., 2010).

3.2.36 *Helichrysum glomeratum* Klatt

Description

Helichrysum glomeratum Klatt belongs to family Asteraceae and is commonly known as everlastings. These are aromatic perennial herbs with densely hairy or woolly leaves. The small flowers are white in colour and arranged in form of central persistent heads surrounded by ray flowers. They are distributed all over South Africa (Van Wyk et al., 1997; Herbal Africa, 2012).

Medicinal Use

Many ailments are treated including coughs, colds, fever, infections, wound dressing, headache and menstrual pain (Van Wyk et al., 1997; Herbal Africa, 2012).

Figure 3.2.36: *Helichrysum glomeratum* (http://www.google.co.za/ imghp)

Biological activity and phytochemistry

In a study conducted by Mathekga and Meyer (1998), the acetone extract of *H. glomeratum* was found to be active against *Cladosporium cladosporioides* and *Cladosporium cucumericum* exhibiting MIC value of 1.0 mg/ml. The detail of phytoconstituents was not reported.

3.2.37 *Heteropyxis natalensis* Harv.

Description

Heteropyxis natalensis Harv. belongs to family Myrtaceae, commonly known as lavender tree. It is a medium to large sized tree which may grow up to a height of 10 m. The dense leafy branches make drooping foliage. The leaves are narrowing elliptical and aromatic. The flowers are yellowish, inconspicuous followed by small dry capsules. It is a common tree of north-eastern parts of South Africa (Dlamini and Hankey, 2002).

Medicinal Use

Leaf infusions are traditionally used to treat colds. The roots are used to treat nose bleeding, bleeding gums and against menorrhagia. The dried powdered leaf are used as vermifuge and traditional tea is prepared from the fresh ones (Hutchings et al., 1996; Van Wyk et al., 1997).

Figure 3.2.37: *Heteropyxis natalensis* (www.plantzafrica.com)

Biological activity and phytochemistry

In a study conducted by Gundidza (1993), the essential oil of *H. natalensis* showed growth inhibitory activity against a variety of microorganisms such as, *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *Streptococcus faecalis* at concentrations of 50, 20 and 10 ml/l. The presence of monoterpenoids namely, β-ocimene, 1,8-cineole, limonene, linalool and myrcene was also reported (Van Wyk et al., 1997).

3.2.38 *Hyaenanche globosa* Lamb.

Description

Hyaenanche globosa Lamb. belongs to the family Euphorbiaceae. This plant is the single species of *Hyaenanche* and is commonly known as hyaena-poison. It is a small, rounded tree, with dark green, leathery leaves, characteristically arranged in four along the stems. Male and female flowers are both small and occur on separate trees. The fruits are large rounded capsules with several segments. The plant is confined to northern Bokkeveld Escarpment Mountain Plateau (Jaarsveld, 2011).

Medicinal Use

The plant contains several sesquiterpene lactones which were traditionally used against vermin and are known to cause convulsions (Wink and Van Wyk, 2008).

Biological activity and phytochemistry

In a study conducted by Momtaz et al. (2010), the ethanolic extract of the leaves and fruits of *H. globosa* showed antibacterial activity against *M. smegmatis* exhibiting MIC value of 3.1 mg/ml. Two compounds known as: ′Tutin′ and ′hyenanchin′ were isolated but no antimicrobial activity was reported.

Figure 3.2.38: *Hyaenanche globosa* (www.plantzafrica.com)

3.2.39 *Knowltonia vesicatoria* Sims.

Description

Knowltonia vesicatoria Sims belongs to family Ranunculaceae and is commonly known as Blisterleaf. It is perennial herb with short rhizome and fleshy roots which usually grows on shady slopes and forests. The leaves are dark green and possess leaflets. The white or yellowish flowers are produced in winter and spring. The plant can widely be found in western and southern parts of South Africa (Van Wyk et al., 1997).

Medicinal Use

The plant is used as a remedy for lumbago and rheumatism. The fresh roots and leaves of *K. vesicatoria* are sniffed for headache, are used to alleviate toothache and are also applied to skin to treat blisters (Van Wyk et al., 1997).

Biological activity and phytochemistry

During previous studies the ethanol extract of *K. vesicatoria* and isolated compound stigmasta-5,23-

dien-3-ol showed antimycobacterial activity with MIC value of 50.00 µg/ml against *M. tuberculosis* (Labuschagne et al., 2009).

Figure 3.2.39: *Knowltonia vesicatoria* (www.plantzafrica.com)

3.2.40 *Leucosidea sericea* Eckl. & Zeyh.

Description

Leucosidea sericea Eckl. & Zeyh. belongs to the family Rosacece and is commonly known as oldwood. This is a straggly shrub which grows up to 7m tall to 5m wide. The bark is rough and reddish brown in colour. The leaves are alternately and covered with silky, silver hairs. The flowers are greenish-yellow in colour, star-shaped and grow in spike. The fruits are nut-like. The plant is native to South Africa and grows in Eastern Cape, Lesotho, western KwaZulu-Natal, the eastern Free State, North West, Gauteng, Mpumalanga, Limpopo provinces, Swaziland and Zimbabwe (Stern, 2002).

Figure 3.2.40: *Leucosidea sericea* (www.plantzafrica.com)

Medicinal Use

L. sericea is used as a vermifuge and in the treatment of ophthalmia by Zulu traditional healers in southern African countries. It is also used as an astringent in combination with other plants (Hutchings et al., 1996).

Biological activity and phytochemistry

The leaf extract of *L. sericea* exhibited antibacterial activity against *B. subtilis* and *S. aureus* at concentrations ranging from 0.02-6.25 mg/ml (Aremu et al., 2010). Previous researchers have isolated two phloroglucinols, namely, aspidinol and desaspidinol from the leaves and flowers, while the presence of *β*-sitosterol and *β*-sitostenone were reported from the stems (Bosman et al., 2004; Nair et al., 2012).

3.2.41 *Magnolia grandiflora* L.

Description

Magnolia grandiflora L. belongs to the family Magnoliaceae and is commonly known as magnolia, Bull bay. *M. grandiflora* is a large evergreen tree which grows up to a height of 30 m. The leaves are alternate and simple. The flowers are creamy white and the fruits are borne as cylindrical cone. *M. grandiflora* is native to North America and occurs from North Carolina to Florida to Texas (Ruth, 2012).

Figure 3.2.41: *Magnolia grandiflora* (http://www.google.co.za/ imghp)

Medicinal Use

This plant has been reported to have beneficial effects on several ailments, such as high blood pressure, heart disturbances, dyspnoea, abdominal discomfort, muscle spasm, infertility and epilepsy (Martinez, 1959; Mellado et al., 1980).

Biological activity and phytochemistry

Previous studies have reported antifungal activity of DCM extract of leaves of *M. grandiflora* and isolated compound, 'costunolide', against *Nigrospora* spp., *Rhizocotania solani* and *Helminthosporiun* spp. at varying concentrations from 2.9-46.6 µg/ml (Ahmed and Abdelgaleil, 2005).

3.2.42 *Myrsine africana* L.

Description

Myrsine africana L**.** belongs to the family Myrsinaceae and is commonly known as african boxwood. It is an evergreen shrub which grows up to 1-2 m in height. From the few, woody, upright stems, many short and thinner side branches shoot that point upwards. The leaves are small, oval shaped and glossy. The flowers are cream coloured and form group at the base. The purple coloured fruits are fleshy and borne in abundance. The plant is found throughout South Africa (Van der Walt, 2005).

Figure 3.2.42: *Myrsine africana* (www.plantzafrica.com)

Medicinal Use

Traditionally, the decoction of the leaf is used as a *'blood purifier'* (Van der Walt, 2005).The concoctions made from the bark, roots and fruits of this tree have been widely used as anthelmintics in humans and livestock (Beentje, 1994).

Biological activity and phytochemistry

In a study conducted by Kang et al. (2007), the the ethanolic extract of *M. africana* and isolated compounds myrsininone A, B inhibited growth of *S. aureus* and *S. mutant* with MIC values ranging between 1.9-15.6 µg/ml.

3.2.43 *Parinari curatellifolia* Planch. ex Benth.

Description

Parinari curatellifolia Planch. ex Benth. belongs to family Chrysobalanaceae and is commonly known as mobola-plum. It is an evergreen tree which may grow up to a height of 10-13 m. The bark is rough and corky with the presence of silica crystals. The leaves are distinctly bicoloured. The sweetly scented bell shaped inflorescences appear in shades of white, yellow or pink, the fruits are edible. The plant is native to Africa and is very widespread, ranging from the south in Mpumalanga and Swaziland, towards Zimbabwe and the Limpopo Province in the north, and into central Africa (Maharaj and Glen, 2008).

Medicinal Use

The leaf extracts and bark are used as a remedy for the symptoms of pneumonia or to treat ailments of the eye or ear (Maharaj and Glen, 2008).

Biological activity and phytochemistry

The previous studies reported the antimicrobial activity of ethanol extract of *P. curatellifolia* against oral pathogens namely, *Actinobacillus actinomycetemcomitans, A. naeslundii, A. israelii, P. gingivalis* and *S. mutans* with MIC values varying from 1.6-12.5 mg/ml (More et al., 2008). Two diterpenoids, 13-methoxy-15-oxozoapatlin and 13-hydroxy-15-oxozoapatlin, were isolated from the root bark of *P. curatellifolia*, together with 15-oxozoapatlin (Lee et al., 1996).

Figure 3.2.43: *Parinari curatellifolia* (http://www.google.co.za/ imghp)

3.2.44 *Ranunculus repens* L.

Description

Ranunculus repens L. belongs to the family Ranunculaceae and is commonly known as creeping buttercup. It is a perennial weed of 10-30 cm tall. The stalks are ascending, hollow, hairy and grow from shortened rhizome. The long above-ground, creeping shoots and roots emerge at the nodes. The glabrous leaves are located on long petioles. Flowers are golden-yellow in colour and the fruits are rounded-ovoid with flat lateral surface. The plant is native to Europe, Asia and north-western Africa (Millspaugh, 1974).

Figure 3.2.44: *Ranunculus repens* (http://www.google.co.za/ imghp)

Medicinal Use

A poultice of the chewed leaves has been used traditionally in the treatment of sores, muscular aches and rheumatic pains. The entire plant possesses analgesic and rubefacient properties (Moreman, 1998).

Biological activity and phytochemistry

The previous researchers have reported antimicrobial activity of water and chloroform extracts of *R. repens.* The extracts exhibited zones of growth inhibition ranging from 1-15 mm against various microbes such as, *B. subtilis*, *Proteus vulgaris*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi*, *Aspergillus niger* and *C. albicans* (Hussain et al., 2011). In another study by Khan et al. (2006), methyl 3,4,5-trihydroxybenzoate; 4-methoxydalbergione and dalbergiophenol were isolated from roots of *Ranunculus repens*.

3.2.45 *Rhus lancea* L.f.

Description

Rhus lancea is also known as *Searsia lancea* (L.f.) F. A. Barkley. It belongs to family Anacardiaceae and is commonly known as Karee. It is a small to medium sized evergreen tree that usually grows to a height of 7 m with course textured bark. The leaves are trifoliate and lanceolate. The small, inconspicuous flowers are greenish-yellow. The fruit are small, round, slightly flattened and covered with a thin fleshy layer which is glossy and yellowish to brown when ripe (Stern, 2008). The plant is native to South Africa.

Figure 3.2.45: *Rhus lancea* (http://www.google.co.za/ imghp)

Medicinal Use

The oil of *R. lancea* is reported to possess antimicrobial and antioxidant properties (Gundidza et al., 2008).

Biological activity and phytochemistry

During previous studies, the essential oil of *R. lancea* indicated antibacterial and antifungal activities against a variety of microbes such as, *B. subtilis*, *E. coli*, *S. typhii*, *K. pneumoniae*, *C. albicans*, *A. niger* and *Aspergillus flavus* at varying concentrations of 2.5-200 µg/ml. The major phytoconstituents of its essential oil, β-pinene, benzene and β-3-carene showed antibacterial and antifungal activities against *E. coli*, *Clostridium perfringens* and *A. flavus* (Gundidza et al., 2008).

3.2.46 *Sclerocarya birrea* Hochst.

Description

Sclerocarya birrea Hochst. belongs to the family Anacardiaceae and is commonly known as Marula tree. It is a single stemmed, medium-sized tree which may grow to a height of 15 meters. The bark is smooth, fissured, grey in colour and the leaves are divided into leaflets. The fruit found on female trees are small and plum-like, with a pale-yellow leather-like rind, and contain juicy mucilaginous flesh. The plant is commonly found in sub-Saharan Africa preferably in semi-arid, deciduous and savannah regions. They can be found in the lowlands of KwaZulu-Natal in South Africa (Van Wyk et al., 1997).

Figure 3.2.46: *Sclerocarya birrea* (www.plantzafrica.com)

Medicinal Use

The bark of marula has been found to be used in traditional medicine to treat skin ailments such as eczema, acne and boils (Hutchings et al., 1996; Van Wyk et al., 1997).

Biological activity and phytochemistry

The acetone extract of bark and leaves of *S. birrea* demonstrated antibacterial activity against *S. aureus*, *P. aeruginosa*, *E. coli* and *E. faecalis* with MIC values from 0.15-3 mg/ml (Eloff, 2001). Phytochemical studies undertaken by a group of investigators on *S. birrea* stem-bark resulted in the identification and isolation of (-)-epicatechin-3-galloyl ester, catechin derivatives and procyanidin compounds (polyphenols) which may be responsible for its activity (Galvez et al., 1993; Van Wyk et al., 1997).

3.2.47 *Sideroxylon inerme* L.

Description

Sideroxylon inerme L. belongs to family Sapotaceae and is commonly called as white milkwood. It is a small to medium evergreen tree which grows to a height of 10-15 m. The bark is normally grey-brown to black in colour. The leaves are leathery and spirally arranged dark green above and dull beneath. The tree has small greenish white flowers with a strong, unpleasant smell. Fruits are purplish black, small, round and fleshy. It is a South African costal tree scattered through the coastal woodlands and littoral forests of South Africa as far as Zimbabwe, along the coast and bays of Cape Town, especially at Noordhoek, Macassar and Gordon's Bay (Bosman, 2006).

Figure 3.2.47: *Sideroxylon inerme* (www.plantzafrica.com)

Medicinal Use

Bark and roots of this plant possess medicinal value and are used to cure broken bones, to treat fevers, to dispel bad dreams, and to treat gall sickness in stock (Bosman, 2006).

Biological activity and phytochemistry

In a study conducted by Momtaz et al. (2008), the methanol and acetone extracts of the stem bark of *S. inerme* showed significant inhibition of monophenolase activity with IC_{50} values of 63 µg/ml and 82 µg/ml, respectively. The methanolic extract also exhibited 37% reduction of melanin content at 6.2 µg/ml in melanocytes.

3.2.48 *Symphytum officinale* L.

Description

Symphytum officinale L. belongs to the family Boraginaceae and is commonly known as knitboneor comfrey. It is an erect perennial vigorous plant with broad lanceolate leaves. The flowers are bell-shaped mauve or white in colour which curve downwards. The fruits are borne in four greyish-brown nutlets. The rhizome of the plant is quite short and thick with black, finger-thick branched roots. The plant is native to Europe (Buchman, 1988).

Figure 3.2.48: *Symphytum officinale* (http://www.google.co.za/ imghp)

Medicinal Use

The roots of *S. officinale* are traditionally used for the topical treatment of contusions, strains and sprains The plant is used for healing wounds, skin conditions, insect bites, bedsores, inflamed bunions, nosebleeds, sunburn, rheumatism, arthritis and bruises (Buchman, 1988).

Biological activity and phytochemistry

In a study conducted by Izzo et al. (1995), the ethanol root extract of *S. officinale* inhibited the growth of *B. subtilis*, *Proteus mirabilis* and *S. typhi* at concentrations of 12 and 9 µg/ml. The previous studies reported isolation of three pyrrolizidine alkaloids namely, symlandine, symphytine and echimidine from the roots of *S. officinale* (Kim et al., 2001).

3.2.49 *Syzygium jambos* (L.) Alston

Description

Syzygium jambos (L.) Alston belongs to the family Myrtaceae and is commonly known as rose apple. It is a large shrub or small tree with spreading branches and simple lanceolate leaves. The flowers are greenish white which are borne in terminal clusters. The fruits are succulent pericarp. The plant is native to East Indies, Malaysia and is found in India, Sri Lanka, Africa and Pacific Islands (Longman, 1996).

Medicinal Use

The bark of *S. jambos* is reported to possess astringent, antidiarrheal and anthelmintic properties. The plant is useful in the treatment of gout, syphilis, leprosy and dermatopathy (Longman, 1996).

Figure 3.2.49: *Syzygium jambos* (http://www.google.co.za/ imghp)

Biological activity and phytochemistry

Acetone and aqueous bark extracts of *S. jambos* have been reported to be active against *S. aureus*, *Yersinia enterocolitica*, *Staphylococcus hominis*, *Staphylococcus cohnii* and *Staphylococcus warneri* with MIC values ranging between 62-750 µg/ml. The activity of the extract was explained due to the presence of high tannin content (Djipa et al., 2000). In another study done by Kuiate et al. (2007), it was found that the ethanol bark extract of *S. jambos* and its isolated triterpenoids such as, friedelin, β-amyrin acetate, betulinic acid and lupeol exhibited antidermatophytic activity against *M. audouinii*, *Trichophyton mentagrophytes* and *Trichophyton soudanense.*

3.2.50 *Warburgia salutaris* (G.Bertol.) Chiov.

Description

Warburgia salutaris (G.Bertol.) Chiov. belongs to the family Cancellaceae and is commonly known as pepper-bark tree. It is a medium sized tree of about 10 m in height with rough and molten bark which is reddish on inner side. The leaves are oblong, long and glossy. The flowers are small-greenish and yellow in colour and the fruits are green containing several flat seeds. The tree is known only from a few localities in north-eastern parts of South Africa (Van Wyk et al., 1997).

Medicinal Use

The plant is a popular and widely used remedy for cough, colds and chest complaints. The other ailments include influenza, rheumatism, malaria, venereal diseases, headache, toothache, dermatological disorder and gastric ulcers (Hutchings et al., 1996; Van Wyk et al., 1997).

Figure 3.2.50: *Warburgia salutaris* (www.plantzafrica.com)

Biological activity and phytochemistry

In a study done by Motsei et al. (2003), the leaves extract of *W. salutaris* inhibited growth of *C. albicans* at MIC values ranging from 12.5-25 mg/ml and the bark extracts and isolated sesquiterpenoid muzigadial showed growth of inhibition against *S. aurens, S. epidermis, B. subtilis* and *E. coli* (Rabe and Van Staden, 1997).

3.2.51 *Zanthoxylum capense* Harv.

Description

Zanthoxylum capense Harv. belongs to the family Rutaceae and is commonly known as small knob wood. It is a small branched tree with sharp thorns on bark and stem. The leaves are divided into leaflets and are citrus scented when crushed. The flowers are greenish-white and inconspicuous while the minute fruits are present in clusters. *Z. capense* is native to South Africa and is widely distributed in the eastern and northern parts of South Africa, Zimbabwe and Mozambique (Kondlo, 2012).

Medicinal Use

The plant is widely used traditionally for the treatment of flatulent colic, stomach ache, toothache, fever and as a mouthwash. It is an old remedy for epilepsy (Kondlo, 2012).

Figure 3.2.51: *Zanthoxylum capense* (www.plantzafrica.com)

Biological activity and phytochemistry

The water and ethanol extract of leaves of *Z. capense* exhibited antibacterial activity against *B. subtilis*, *E. coli*, *K. pneumonia* and *S. aurens* with MIC values ranging from 3-12.5 mg/ml (Buwa and Van Staden, 2006). In another study the isolated compounds from the methanol extract of the roots of *Zanthoxylum capense* namely, 2-arylbenzofuran neolignans decarine, norchelerythrine, dihydrochelerythrine, 6-acetonyldihydrochelerythrine, tridecanonchelerythrine and 6-acetonyldihydronitidine inhibited the bacterial growth of *S. aureus* and *E. coli* with MIC values ranging between 3-25 µg/ml (Luo et al., 2012).

Table 3.1: Medicinal use, biological activity and phytochemistry of plants selected for the study

Plant Name	Medicinal use	Biological activity and phytochemistry
Acacia caffra Willd.	blood disorders, infantile abdominal disorders	Proteracacinidin *ent*-oritin-(4β → 5) epioritin-4β-ol, 8-*O*-methylepioritin-4α-ol, 3-*O*-methyl-7,8,4′-trihydroxy-flavone reported.
Acacia galpinii Burtt Davy.	demulcent, mucilaginous	Inhibited growth of *Staphylococcus aureus* and *Escherichia coli*. Proanthocyanins, teracacidin and proteracacinidin epioritin reported.
Acacia mellifera Benth.	coughs, gastrointestinal ailments, malaria, pneumonia, stomach-ache, sterility, skin diseases	*Inhibited S. aureus, Cryptococcus neoformans, Candida albicans* and *Microsporum gypseum.* (20*S*)-oxolupane-30-al, (20*R*)-oxolupane-30-al and betulinic acid reported.
Aloe arborescens Mill.	cosmetics, treat burns, purgative	Inhibited *C. neoformans.* Barbaloin, aloeresin and aloenin reported.
Aloe barbadensis Mill.	cosmetic application, wound healing, anti-inflammatory	Inhibited *Botrytis gladiolorum, Fusarium oxysporum gladioli, Heterosporium pruneti* and *Penicillium gladioli*. Aloine reported.
Aloe ferox Mill.	skin irritation, wound healing, acne	Inhibited *Neisseria gonorrhoea* and *C. neoformans*. Aloin reported.
Aloe sessiliflora Pole-Evans.	believed to promote menstruation	Tyrosinase inhibitory activity.
Anchusa capensis Thunb.	mutagenic, traditional phytomedicine,	Not found in literature.

	neurotoxin	
Annona senegalensis Pers.	dermatological diseases and ophthalmic disorder	Inhibited *Pseudomonas aeruginosa* and *S. aureus*. Kauran-16α-ol, kaur-16-en-19-oic acid, kauran-19-al-17-oic acid, and 19-norkauran-4α-ol-17-oic acid reported.
Arbutus unedo L.	anti-diarrhoeal, astringent, antioxidant, against diabetes, as depurative, for hypertension, diuretic	Inhibited *S. aureus, Enterococcus faecalis, Staphylococcus epidermis* and *Staphylococcus saprophiticus.* Arbutin, β-D-glucogalline, gallic acid 4-*O*-β-D-glucopyranoside, 3-*O*-galloylquinic acid, 5-*O*-galloylquinic acid, 3-*O*-galloylshikimic acid and 5-*O*-galloylshikimic acid reported.
Aspalathus linearis (Burm.f.) R.Dahlgren	alleviation of infantile colic, allergies, asthma, dermatological problems	Inhibited *E. coli, S. aureus, Bacillus cereus, Listeria monocytogenes, Streptococcus mutans* and *Saccharomyces cerevisiae*. Aspalathin, nothofagin, orientin, iso-orientin, rutin, isoquercitrin, vitexin, isovitexin, chrysoeriol, quercetin and luteolin reported.
Barleria albostellata C. B. Clarke	antimicrobial, anti-inflammatory, antioxidant	Inhibited *C. albicans, Bacillus subtilis, S. aurens, E. coli* and *Klebsiella pneumonia.*
Barleria repens Nees	antimicrobial, anti-inflammatory, antioxidant	Not found in literature.
Broussonetia papyrifera (L.) Vent.	astringent, skin ailments, diuretic	Inhibited *C. albicans, S. cerevisiae, E. co*li and *S. aureus.* Papyriflavonol A and a prenylated flavonoid [5,7,3`,4`-tetrahydroxy-6,5`-di-(γ,γ-dimethylallyl)-flavonol reported.
Buxus macowanii Oliv.	gout, malaria, rheumatism, skin disorders	31-hydroxybuxatrienone, macowanioxazine , 16α-hydroxyma-cowanitriene, macowanitriene, macowamine, Nb-demethylpapillotrienine and moenjodaramine reported.

Carpobrotus edulis (L.) L.Bolus	infections of mouth and throat, eczema, wounds and burns	Inhibited *Moraxella catharralis, B. subtilis, S. epidermis, S. aureus, Streptococcus pneumoniae, E. coli, P. aeruginosa* and *Streptococcus pyogens*. Rutin, neohesperidin, hyperoside, cactichin and ferulic acid reported.
Ceratonia siliqua L.	anti-diarrhoeal, antitussive, against warts, diuretic	Inhibited *E. coli, Salmonella typhirium, Enterobacter cloacae, P. aeruginosa, S. aureus, S. epidermis* and *C. albicans.* Myricetin, quercetin, kaempferol, luteolin, genisten and taxifolin reported.
Combretum apiculatum Sond.	conjunctivitis, stomach disorders	Inhibited *E. coli, P. aeruginosa, S. aurens* and *E. faecalis.* Alpinetin, pinocembrin and flavokawain reported.
Combretum molle Engl. & Diels	anthelmintic, coughs, fever, stomach. ailments, wounds	Inhibited *E. coli, Shigella* spp and *C. albicans.* Ellagitannin and punicalagin reported.
Cotyledon orbiculata L.	earache, toothache and to treat epilepsy, boils and inflammation	Inhibited *Bacillus subtilis, Staphylococcus aurens, E. coli* and *K. pneumonia.*
Cryptocarya woodii Engl.	diarrhoea, magical and medicinal purpose	Not found in literature.
Dahlia imperialis Roezl	skin treatments like rashes, grazes, infected scratches	Not found in literature.
Datura stramonium L.	abscesses and wounds, relieves asthma and reduces pain, remedy for boils	Inhibited *B. subtilis, S. aurens* and *E. faecalis*. Atropine, hyoscine, hyoscyamine and scopolamine reported.
Dichrostachys cinerea (L.) Wight & Arn.	anaesthetic, toothache	Inhibited *B. subtilis, S. aureus, E. coli, P. aeruginosa, Shigella boydii* and *Shigella flexneri.*
Diospyros lycioides Desf.	chewed and used as a toothbrush, ease the body	Inhibited *Streptococcus mutans, Prevotella intermedia,*

	pains	*Streptococcus sanguis* and *Porphyromonas gingivalis.* Naphthalene glycosides, diospyrosides, naphthoquinones, juglone and 7-methyljuglone reported.
Dodonaea viscosa Jacq.	skin disease, sore throat, wound healing	Inhibited *C. albicans, S. aureus, E. coli, E. faecalis* and *P. aeruginosa.* Kaempferol methyl esters reported.
Erythrophleum lasianthum Corbishley.	fever, general body pains, headaches, intestinal spasm, migraine	Inhibited *S. aureus, E. coli, K. pneumoniae, P. aeruginosa, C. albicans* and *Microsporum audouinii*. Cassaine, erythrophleine, 3 beta-hydroxynorerythrosuamine and 3-O-beta-D-glucopyranoside reported.
Euclea divinorum Hiern.	chewing sticks for toothache, headaches, a purgative and the bark infusion as an appetizer	Inhibited *Actinomyces naeslundii, Actinomyces israelii, S. mutans, C. albicans, P. gingivalis* and *P. intermedia*. Lupeol, lupene, betulin, 7-methyljuglone, isodiospyrin, shinalone, catechin and 3 beta-(5-hydroxyferuloyl) lup-20(30)-ene reported.
Euclea natalensis A.DC.	bronchitis, chronic asthmas, pleurisy, toothache, UTI	Inhibited *Mycobacterium tuberculosis, B. cereus, Bacillus pumilus, B. subtilis, Micrococcus kristinae, Cladosporium cladsporioides* and *S. aureus* β- sitosterol and octahydroeuclein reported.
Galenia africana L.	skin diseases, to relieve eye inflammation, venereal sores	Inhibited *Mycobacterium smegmatis* and *M. tuberculosis.* (2S)-5,7,2′-trihydroxyflavanone, (E)-3,2′,4′-trihydroxychalcone and (E)-2′,4′-dihydroxychalcone reported.
Gomphocarpus fruticosus R.Br.	headache and tuberculosis, to relieve stomach pain, general aches in the body	Inhibited *P. aeruginosa*. Quercetin glycosides, kaempferol, rutin and isorhamenetin

		reported.
Greyia flanaganii Bolus.	ward off sickness	Inhibited *P. acnes.* 2,4,6-trihydroxydihydrochalcone; (3S)-4-hydroxyphenethyl3-hydroxy-5-phenylpentanoate and2,6,4-trihydroxy-4methoxydihydrochalcone reported.
Greyia sutherlandii Hook. & Harv.	traditional medicine, emetics for biliousness[7]	Not found in literature.
Harpephyllum caffrum Bernh. ex Krauss	blood purifiers, for facial saunas, skin washes, to treat acne and eczema	Inhibited *B. subtilis*, *E. coli*, *K. pneumoniae*, *C. albicans* and *S. aureus*. Polyphenolics, flavonoids and organic acids like protocatechuic acid reported.
Helichrysum argyrophyllum DC.	heal scars, intestinal troubles	Gyrosanol reported.
Helichrysum glomeratum Klatt	cough, cold, fever, infections, wound dressing	Inhibited *Cladosporium cladosporioides* and *Cladosporium cucumericum.*
Heteropyxis natalensis Harv.	colds, bleeding gums, nose bleeding, vermifuge	Inhibited *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and *Streptococcus faecalis*. β-ocimene, 1,8-cineole, limonene, linalool and myrcene reported.
Hyaenanche globosa Lamb.	against vermins, arrow posion	Inhibited *M. smegmatis*. Tutin and hyenanchin reported.
Knowltonia vesicatoria Sims.	headache, to alleviate toothache, to treat blisters on skin	Inhibited *M. tuberculosis.* Stigmasta-5,23-dien-3-ol reported.
Leucosidea sericea Eckl. &	astringent, treatment of opthalmia	Inhibited *B. subtilis* and *S. aureus.*

Zeyh.		Aspidinol, desaspidinol, β-sitosterol and β-sitostenone reported.
Magnolia grandiflora L.	abdominal discomfort, blood pressure, dyspnoea, epilepsy, heart disturbances, infertility, muscle spasm	Inhibited *Nigrospora* spp., *Rhizocotania solani* and *Helminthosporiun* spp. Costunolide reported.
Myrsine africana L.	anthelmintics, blood purifier	Inhibited *S. aureus* and *S. mutans.* Myrsininone A, B reported.
Parinari curatellifolia Planch. ex Benth.	ailments of the eye or ear, pneumonia	Inhibited *Actinobacillus actinomycetemcomitans, A. naeslundii, A. israelii, P. gingivalis* and *S. mutans*. 13-methoxy-15-oxozoapatlin and 13-hydroxy-15-oxozoapatlin reported.
Ranunculus repens L.	muscular aches, rheumatic pains, sores	Inhibited *B. subtilis, Proteus vulgaris, S. aureus, E. coli, P. aeruginosa, S. typhi, Aspergillus niger* and *C. albicans.* Methyl 3,4,5-trihydroxybenzoate; 4-methoxydalbergione and dalbergiophenol reported.
Rhus lancea L.f.	antimicrobial and antioxidant	Inhibited *B. subtilis, E. coli, S. typhii, K. pneumoniae, C. albicans, A. niger, Aspergillus flavus, Clostridium perfringens* and *A. flavus.* β-pinene, benzene and β-3-carene reported.
Sclerocarya birrea Hochst.	skin ailments such as eczema, acne, boils	Inhibited *S. aureus, P. aeruginosa, E. coli* and *E. faecalis.* (-)-epicatechin-3-galloyl ester, catechin derivatives and procyanidin reported.
Sideroxylon inerme L.	to treat fevers, to treat gall sickness in stock	Monophenolase activity.
Symphytum officinale L.	arthritis, bruises, insect bites, inflamed bunions,	Inhibited *B. subtilis, Proteus mirabilis* and *S. typhi.*

	healing wounds, skin conditions, nosebleeds, sunburn, rheumatism	Symlandine, symphytine and echimidine reported.
Syzygium jambos (L.) Alston	astringent, antidiarrheal, anthelmintic, leprosy, gout	Inhibited *S. aureus*, *Yersinia enterocolitica*, *Staphylococcus hominis*, *Staphylococcus cohnii*, *Staphylococcus warneri*, *M. audouinii*, *Trichophyton mentagrophytes* and *Trichophyton soudanense.* Friedelin, β-amyrin acetate, betulinic acid and lupeol reported.
Warburgia salutaris (G. Bertol.) Chiov.	influenza, rheumatism, malaria, venereal diseases, headache, toothache, dermatological disorder, gastric ulcers	Inhibited *S. aurens*, *S. epidermis*, *C. albicans*, *B. subtilis* and *E. coli.* Muzigadial reported.
Zanthoxylum capense Harv.	colic, stomach ache, toothache, fever, epilepsy	Inhibited *B. subtilis*, *E. coli*, *K. pneumonia* and *S. aurens*. 2-arylbenzofuran neolignans decarine, norchelerythrine, dihydrochelerythrine, 6-acetonyldihydrochelerythrine, tridecanonchelerythrine and 6-acetonyldihydronitidine reported.

3.3 References

Adamson, R., 1956. The genus *Galenia*. Journal of South African Botany, 22(3), 88-123.

Agroforestry Tree Database, 2012. http://www.worldagroforestrycentre.org/Sea/Products/AFDbases/AF/asp/BotanicSearch.asp. Accessed : November 2011.

Ahmed, S.M. and Abdelgaleil, S.A.M., 2005. Antifungal activity of extracts and sesquiterpene lactones from *Magnolia grandiflora* L. (Magnoliaceae). International Journal of Agriculture and Biology. 7(4), 638-642.

Amoo, S.O., Ndhlala, A.R., Finnie, J.F., Van Staden, J., 2011. Antifungal, acetylcholinesterase inhibition, antioxidant and phytochemical properties of three *Barleria* species. South African Journal of Botany, 77(2), 435-445.

Amoo, S.O., Finnie, J.F., Van Staden., 2009. *In vitro* pharmacological evaluation of three *Barleria* species. Journal of Ethnopharmacology, 121(2), 274-277.

Aremu, A.O., Fawole, O.A., Chukwujekwu, J.C., Light, M.E., Finnie, J.F., Van Staden, J., 2010. In vitro antimicrobial, anthelmintic and cyclooxygenaseinhibitory activities and phytochemical analysis of *Leucosidea sericea*. Journal of Ethnopharmacology, 131, 22-27.

Arnold, T.H. and De Wet, B.C., 1993. Plants of Southern Africa: Names and Distribution. Memoirs of the Botanical Survey of South Africa, vol. 62. National Botanical Institute, Pretoria.

Aubrey, A. 2001. *Acacia caffra*. http://www.plantzafrica.com/plantab/acaciacaffra.htm Accessed: November 2011.

Aubrey, A. 2001. *Aloe ferox*. http://www.plantzafrica.com/plantab/aloeferox.htm. Accessed: November 2011.

Asres, K., Mazumder, A., Bucar, F., 2006. Antibacterial and antifungal activities of *Combretum molle*. Ethopian Medicinal Journal, 44(3), 269-77.

Ayyanar, M. and Ignacimuthu, S., 2011. Ethnobotanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats, India. Journal of Ethnopharmacology, 134, 851-864.

Bajwa, R., Shafique, S., Shafique, S., 2007. Appraisal of antifungal activity of *Aloe vera*. Mycopath, 5(1), 5-9.

Banso, A. and Adeyemo, S.O., 2007. Evaluation of antibacterial properties of tannins isolated from *Dichrostachys cinerea*. African Journal of Biotechnology, 6(15), 1785-1787.

Batlle, I. And Tous, J., 1997. In: Carob tree: *Ceratonia siliqua* L. Promoting the conservation and use of underutilised and neglected crops. 17. Institure of Plant Genetics and Crop plant research, Gatersleben/ international Plant Genetic Resources Institute, Rome, Italy.

Bennie, L., Coetzee, J., Malan, E., Ferreria, D., 2002. (4-6)-Coupled proteracacinidins and promelacacinidins from *Acacia galpinii* and *Acacia caffra*. Phytochemistry, 60(5), 521-532.

Beentje, H.J., 1994. In: Kenya Trees, Shrubs and Lianas. National Museums of Kenya, Nairobi.

Benhouhou, S., 2005. In: A Guide to Medicinal plants of North America. IUCN Centre of Mediterranean Cooperation, Spain. Pg: 37.

Bosman, A.A., Combrinck, S., Roux-van der Merwe, R., Botha, B.M., McCrindle, R.I., 2004. Isolation of an anthelmintic compound from *Leucosidea sericea*. South African Journal of Botany, 70, 509-511.

Bosman, F., 2006. *Sideroxylon inerme.* http://www.plantzafrica.com/plantqrs/sideroxinerm.htm. Accessed: November 2011.

Buchman, D.D., 1988. In: Herbal Medicine. Gramercy: Random House Value Publishing, 101-171.

Buwa, L.V. and Van Staden ,J., 2006. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. Journal of Ethnopharmacology, 103(1), 139-142.

Buwa, L.V. and Van Staden ,J., 2007. Effects of collection time on the antimicrobial activities of *Harpephyllum caffrum* bark. South African Journal of Botany, 73(2), 242-247.

Casian, O.R., Parvu, M., Vlase, L., Tamas, M., 2007. Antifungal activity of *Aloe vera* leaves. Fitoterapia, 78(3), 219-222.

Cheek, M., 2009. *Dichrostachys cinerea.* http://www.plantzafrica.com/plantcd/dichroscinerea.htm. Accessed: November 2011.

Cheng, S.Y., Chuang, C.T., Wang, S.K., Wen, Z.H., Chiou, S.F., Hsu, C.H., Dai, C.F., Duh, C.Y., 2010. Antiviral and anti-inflammatory diterpenoids from the soft coral *Sinularia gyrosa.* Journal of Natural Products, 73, 1184-1187.

Dalmini, M.D. and Turner, S., 2002. *Buxus macowanii.* http://www.plantzafrica.com/plantab/buxusmacowen.htm. Accessed: November 2011.

Dalmini, M.D. and Hankey, A., 2002. Heterophyxis natalensis. http://www.plantzafrica.com/planthij/heteropnatal.htm. Accessed: November 2011.

Davis, R.H., Rosental, K.Y., Cesario, L.R ., Rouw, G.A., 1989. Processed *Aloe vera* administered topically inhibits inflammation. Journal of the American Podiatric Medical Association 79, 395–397.

Djipa, C.D., Delmee, M., Quetin-Leclercq, J., 2000. Antimicrobial activity of bark extracts of *Syzygium jambos* (L.) Alston (Myrtaceae). Journal of Ethnopharmacology, 17, 307-313.

Dweck, A.C., 2012. A review of the paper mulberry (*Broussonetia papyrifera*) (L.) Hert. ex Vent. http://www.rarefruit.org/PDF_files/Broussonetia_papyrifer.pdf

Eftekhar, F., Yousefzadi, M., Tafakori, V., 2005. Antimicrobial activity of *Datura innoxia* and *Datura stramonium.* Fitoterapia, 76(1), 118-120.

Eisa, M.M., Almagboul, A.Z., Omer, M.E.A., Elegami, A.A., 2000. Antibacterial activity of *Dichrostachys cinerea*. Fitoterapia, 71(3), 324-327.

Eloff, J.N. and Katerere, D.R., 2004. Variation in chemical composition, antibacterial and antioxidant activity of fresh and dried *Acacia* leaf extracts. South African Journal of Botany, 70(2), 303-305.

Eshiet, I.T.U. and Akisanya, A., 1971. Diterpenes from *Annona senegalensis*. Phytochemistry, 10(12), 3294-3295.

Froneman, W. and Roux, L.N., 2007. *Barleria albostellata*. http://www.plantzafrica.com/plantab/barleralbo.htm. Accessed: November 2011.

Galvez, J., Crespo, M.E., Zarzuelo, A., 1993. Pharmacological activity of a procyanidin isolated from *Sclerocarya birrea* bark: antidiarrhoeal activity and effects on isolated guinea-pig ileum. Phytotherapy Research, 7, 25–28.

Gundidza. M., 1993. The essential oil from *Heteropyxis natalensis* Harv: Its antimicrobial activities and phytoconstituents. Journal of the Science of Food and Agriculture, 63, 361-364.

Gundidza, M., Gweru, N., Mmbengwa, V., Ramalivhana, N.J., Magwa, Z., Samie, A., 2008. Phytoconstituents and biological activities of essential oil from *Rhus lancea* L.F. African Journal of Biotechnology, 7(16), 2787-2789.

Gutterman, Y. and Volfson, E.C., 2000. The distribution of the phenolic metabolites barbaloin, aloeresin and aloenin as a peripheral defense strategy in the succulent leaf parts of Aloe arborescens. Biochemical Systematics and Ecology, 28, 825-838.

Harris, S., 2004. *Cotyledon orbiculata*. http://www.plantzafrica.com/plantcd/cotyledorbic.htm Accessed: November 2011.

Harris, S., 2012. *Dodonea viscose*. http://www.plantzafrica.com/plantcd/dodonaeavisangust.htm. Accessed: November 2011.

Hankey, A. and Notten, A. 2004. *Aloe arborescens.* http://www.plantzafrica.com/plantab/aloearbor.htm Accessed: November 2011.

Heneidak, S., Grayer, R.J., Kite, G.C., Simmonds, M.S.J., 2006. Flavonoid glycosides from Egyptian species of the tribe Asclepiadeae (Apocynaceae, subfamily Asclepiadoideae). Biochemical Systematics and Ecology, 34(7), 575-584.

Herbal Africa, 2012. *Helichrysum*. http://herbalafrica.co.za/HERBSHELICHRYSUM.HTM. Accessed: December, 2011.

Hussain, I., Khattak, M.U.R., Ullah, R., Muhammad, Z., Khan, N., Khan, F.A., Ullah, Z., Haider, S., 2011. Phytochemical screening and antimicrobial activities of selected medicinal plants of Khyberpakhtunkhwa Pakistan. African Journal of Pharmacy and Pharmacology, 5(6), 746-750.

Hutchings, A. et al. 1996. Zulu Medicinal Plants. University of Natal Press, Pietermaritzburg.

Iwu, M.M., 1993. Handbook of African Medicinal Plants. CRC Press, Boca Raton, Florida, USA.

Izzo, A.A., Carlo, G,D., Biscardi,D., Fusco, R. D., Mascolo, N., Borrelli, F., Capasso, F., 1995. Biological screening of Italian Medicinal plants for antibacterial activity. Phytotherapy Research, 9, 281-286.

Jeppe, B., 1969. Swaziland's flora database, *Aloe spicata*. http://www.sntc.org.sz/flora/speciesinfo.asp?spid=412. Accessed: November 2011.

Jia, Y., Zhao, G., Jia, Jicheng., 2008. Preliminary evaluation: The effect of *Aloe ferox* Miller and A*loe arborescens* Miller on wound healing. Journal of Ethnopharmacology, 120(2), 181-189.

Joffe, P., 2003. *Barleria repens.* http://www.plantzafrica.com/plantab/barlerrepens.htm Accessed: November 2011.

Kambiz, L. and Afolayan., 2008. Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhoea*. African Journal of Biotechnology, 7(1), 12-15.

Kane, C.W., 2006. In: Herbal Medicine of the American southwest. Lincoln Town press. Page 13.

Kang, L., Zhou, J.X., Shen, Z.W., 2007. Two novel antibacterial flavonoids from *Myrsine africana* L. Chinese Journal of Chemistry, 25, 1323-1325.

Khan, W.N., Lodhi, M.A., Ali, I., Haq, A.Q., Malik, A., Bilal, S., Gul, R., Choudhary, M.I., 2006. New natural urease inhibitors from *Ranunculus repens*. Journal of Enzymen inhibition and Medicinal Chemistry, 21(1), 17-19.

Kim, N.C., Oberlies, N.H., Brine, D.R., Handy, R.W., Wani, M.C., Wall, M.E., 2001. Isolation of symladine from the roots of common Comfrey (*Syphytum officinale*) using counter countercurrent chromatography. Journal of Natural Products, 64(2), 251-253.

Kivcak, B. and Mert, T., 2001. Quantitative determination of tocopherol in *Arbutus unedo* by TLC-densitometry and colorimetry. Fitoterapia, 72, 656-661.

Kivack, B., Mert, T., Ozturk, H.T., 2002. Antimicrobial and cytotoxic activities of *Ceratonia siliqua* L. extracts. Turkish Journal of Biology, 26, 197-200.

Klopper, R.R. and Smith, G.F., 2010. *Aloe* L. http://www.plantzafrica.com/plantab/aloe.htm. Accessed: November 2011.

Kuiate, J.R., Mouokeu, S., Wabo, H.K., Tane, P., 2007. Antidermatophytic triterpenoids from *Syzygium jambos* (L.) Alston (Myrtaceae). Phytotherapy Research, 21,149-152.

Labuschangne, A., Hussein, A.A., Rodriguez, B., Lall, N., 2012. Synergistic antimycobacterial actions of *Knowltonia vesicatoria* (L.f) Sims. Evidence-based complementary and Alternative medicine, Volume 2012 (2012), Article ID 808979, 9 pages.

Lalitha, S., Rajeshwaran, K., Kumar, P.S., Deepa, K., and Gowthami, K. 2010. *In vivo* Screening of Antibacterial Activity of *Acacia mellifera* (BENTH) (Leguminosae) on Humna Pathogenic Bacteria. Global Journal of Pharmacology, 4(3), 148-150.

Lall, N. and Meyer, J.J.M., 1999. In vitro inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South african plants. Journal of Ethnopharmacology, 66, 347-354.

Lall, N. and Meyer, J.J.M., 2000. Antibacterial activity of water and acetone extracts of the roots of *Euclea natalensis*. Journal of Ethnopharmacology, 72, 313-316.

Lall, N., Weiganand, A.A., Hussein, A.A., Meyer, J.J.M., 2006. Antifungal activity of napthoquinones and triterpenes isolated from the root bark of *Euclea natalensis*. South African Journal of Botany, 72(4), 579-583.

Lam, C.W., 2012. Phytochemical studies on *Buxus macowanii* and microbial transformation of glycyrrhetinic acid. MSc Thesis, University of Manitoba, Canada.

Lee, K.Y., Weintraub, S.T., Yu, B.P., 2000. Isolation and identification of a phenolic antioxidant from *Aloe barbadensis*. Free Radical Biology and Medicine 28, 261-265.

Lee, K.S., Shamon, L.A., Chai, H.B., Chagwedera, T.E., Besterman, J.M., Fransworth, N.R., Cordell, G.A., Pezzuto, J.M., Kinghorn, A.D., 1996. Cell-cycle specific cytotoxicity mediated by

rearranged ent-kaurene diterpenoids isolated from *Parinari curatellifolia*. Chemico-Biological Interactions, 99(1-3), 193-204.

Lining, C., Guo-Xian, W., Van der Bijl, P., D Wu, C., 2000. Namibian chewing stick, *Diospyros lycioides*, contains antibacterial compounds against oral pathogens. Journal of Agricultural Food Chemistry, 48(3), 909-914.

Lino, A. and Deogracious, O., 2006. The in-vitro antibacterial activity of Annona senegalensis, Securidacca longipendiculata and Steganotaenia araliacea- Ugandan medicinal plants. Africal Health Sciences, 6(1), 31-35.

Longman, O., 1996. Indian Medicinal Plants: a compendium of 500 species. Pg: 229.

Luo, X., Pedro, L., Milic, V., Mulhovo, S., Duarte, A., Duarte, N., Ferreira, M.J.U., 2012. Antibacterial benzofuran Neolignans and benzophenanthridine alkaloids from the roots of *Zanthoxylum capense*. Planta Medica, 78(2), 148-153.

Madureira, A.M., Ramalhete, C., Mulhovo, S., Duarte, A., Ferreira, M.U., 2011. Antibacterial activity of some African medicinal plants used traditionally against infectious disease. Pharmaceutical Biology, 1-9.

Maharaj, V. and Glen, H.F., 2008. *Parinari curatellifolia*. http://www.plantzafrica.com/plantnop/parinaricurat.htm. Accessed: November 2011.

Malan, E., 1995. A (4β-5)-linked proteracacinidin dimer from the heartwood of *Acacia caffra*. Phytochemistry 40(5), 1519-1521.

Mapunya, M.B., Hussein, A.A., Rodriguez, B., Lall, N., 2011. Tyrosinase activity of *Greyia flanaganii* Bolus constituents. Phytomedicine, 18, 1006-1012.

Mathekga, A.D.M. and Meyer, J.J.M., 1998. Antibacterial activity of South African *Helichrysum* species. South African Journal of Botany. 64(5), 293-295.

Mbambezeli, G., 2002. *Greyia flanaganii*. http://www.plantzafrica.com/plantefg/greyiaflan.htm. Accessed: November 2011.

Mbambezeli, G., 2005. *Cryptocarya woodii*. http://www.plantzafrica.com/plantcd/crytocarwood.htm. Accessed: November 2011.

Mbambezeli, G., 2006. *Greyia sutherlandii*. http://www.plantzafrica.com/plantefg/greyiasuther.htm. Accessed: November 2011.

Mander, M., 1998. Marketing of indigenous medicinal plants in South Africa. A case study in KwaZulu-Natal. FAO, Rome.

Mapunya, M.B., Nikolova, R. V., Lall, N., 2012. Melanogenesis and antityrosinase activity of selected South African plants. Evidence-based complementary and Alternative medicine, 2012, 1-6.

Martinez. M., 1959. Las plantas medicinales de Mexico. Eds Botas, 4th edition., 343–347.

Mativandlela, S.P.N., Muthivhi, T., Kikuchi, H., Oshima, Y., Hamilton, Y., Hussein, A.A., Van der Walt, M.L.,Houghton, P.J., Lall, N., 2009. Antimycobacterial Flavonoids from the Leaf Extract of *Galenia africana*. Journal of Natural Products, 72, 2169-2171.

Masupa, T., 2011. *Combretum molle*. http://www.plantzafrica.com/plantcd/combretummolle.htm. Accessed: November 2011.

Masupa, T. and Rampho, E., 2011. *Combretum apiculatum.* http://www.plantzafrica.com/plantcd/combretumapicapic.htm. Accessed: November 2011.

Mebe, P.P., Cordell, G.A., Pezzuto, J.M., 1998. Pentacyclic triterpenes and naphthoquinones from *Euclea divinorum*. Phytochemistry. 47(2), 311-313.

Mellado, V., Chavez, S.M.A., Lozoya, X., 1980. Pharmacological screening of the aqueous extracts of *Magnolia grandiflora* L. Archives of Medical Research, 11(3), 335–346.

Millspaugh, C.F., 1974. In: American medicinal plants. Dover Publications, Canada.

Momtaz, S., Mapunya, B.M., Houghton, P.J., Edgerly, C., Hussein, A., Naidoo, S., Lall, N., 2008. Tyrosinase inhibition by extracts and constituents of *Sideroxylon inerme* L. bark, used in South Africa for skin lightening. Journal of Ethnopharmacology, 119, 507-512.

Momtaz, S., Lall, N., Ahmed, H., Sayed, O., Abdollahi, M., 2010. Investigation of the possible biological activities of a poisonous South African plant; *Hyaenanche globosa* (Euphorbiaceae). Pharmacognsy Magzine, 6(21), 34-41.

More, G., Tshikalange, T.E., Lall, N., Botha, F., Meyer, J.J.M., 2008. Antimicrobial activity of medicinal plants against oral microorganisms. Journal of Ethnopharmacology, 119(3), 473-477.

Moreman, D., 1998. In: Native American Ethnobotany. Portland: Timber Press.

Motsei, M.L., Lindsey, K.L., Van Staden, J., Jager, A.K., 2003. Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. Journal of Ethnopharmacology, 86(2–3), 235-241.

Mutai, C., Bii, C., Rukunga, G., Ondicho, J., Mwitari, P., Abatis, D., Vagias, C., Roussis, V., and Kirui, J. 2009. Antimicrobial activity of pentacyclic triterpenes isolated from *Acacia mellifera*. African Journal of Traditional, Complemantary and Alternative Medicine, 6(1), 42-48.

Mutshinyalo, T., 2003. *Acacia galpinii*. http://www.plantzafrica.com/plantab/acaciagalpin.htm Accessed: November 2011.

Nair, J.J., Aremu, A.O., Van Staden J., 2012. Anti-inflammatory effects of *Leucosidea sericea* (Rosaceae) and identification of the active constituents. South African Journal of Botany, 80, 75-76.

Nielsen, T.R.H., Kuete, V., Jager, A.K., Meyer, J.J.M., Lall, N., 2012. Antimicrobial activity of selected South African medicinal plants. BMC Complementary and Alternative Medicine, 12, 1-6.

Notten, A., 2010. *Euclea natalensis*. http://www.plantzafrica.com/plantefg/eucleanatal.htm. Accessed: November 2011.

Pabuccuoglu, A., Kivcak, B., Bas, M., Mert, T., 2003. Antioxidant activity of *Arbutus unedo* leaves, Fitoterapia 74, 597-599.

Pawlowska, A.M., Leo, M.D., Braca, A., 2006. Phenolics of *Arbutus unedo* L. (Ericaceae) fruits: identification of anthocyanins and gallic acid derivatives. Journal of Agricultural Food chemistry, 54(26), 34-38.

Prakash, P., Gupta, N., 2005. Therapeutic uses of Ocimum sanctum linn (Tulsi) with a note on eugenol and its pharmacological actions. Indian Journal of Physiological Pharmacology 49, 125-131.

Rabe, T. and Van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology, 56(1), 81-87.

Rambuwani, L.D., 2005. *Diospyros lycioides.* http://www.plantzafrica.com/plantcd/diospyroslyc.htm. Accessed: November 2011.

Roberts, M., 2007. Dahlia, In: Edible and Medicinal Flowers. Claremont, Spearhead, P-25.

Royal New Zealand Institute of Horticulture, 2012. http://www.rnzih.org.nz/pages/Dahlia-imperialis.htm. Accessed: November 2011.

Ruth, A.P., 2012. Genus: *Magnolia.* http://www.discoverlife.org/nh/tx/Plantae/Dicotyledoneae/Magnoliaceae/Magnolia/grandiflora/#Odenwald and Turner, 1996. Accessed: November 2011.

Sassi, A.B, Harzallah-Skhiri, F., Aouni, M., 2007. Investigation of some medicinal plants from Tunisia for antimicrobial activities. Pharmaceutical Biology, 45(5), 421-428.

Scheepers, S., 2001. Anti-microbial activity of rooibos tea (*Aspalathus linearis*) on food spoilage organisms and potential pathogens. M.Sc. (Food Science) Thesis. Stellenbosch University, Stellenbosch, South Africa.

Serage, A., 2003. Isolation and characterization of antibacterial compounds present in *Combretum apiculatum* subsp. *apiculatum*. MSc Thesis, Programme for Phytomedicine, University Of Pretoria.

Smith, R., 2001. *Helichrysum argyrophyllum.* http://www.plantzafrica.com/planthij/helichryargry.htm. Accessed: November 2011.

Sohn. H.Y., Kwon, C.S., Kun, H.S., 2010. Fungicidal effect of prenylated flavonol, Papriflavonol A, isolated from *Broussonetia paprifera* (L.) Vent. against *Candida albicans*. Journal of Microbiology and Biotechnology, 20(10), 1397-1402.

Stephen, A.O., 2010. Micro propagation and medicinal properties of *Barleria greenii* and *Huernia hystrix*. PhD Thesis. University of KwaZulu-Natal, Pietermaritzburg.

Stern, M., 2002. *Leucosidea sericea.* http://www.plantzafrica.com/plantklm/leucosidseri.htm. Accessed: November 2011.

Stern, M., 2008. *Searsia lancea.* http://www.plantzafrica.com/plantqrs/searsialancea.htm. Accessed: November 2011.

Teffo, L.S., Aderogba, M.A., Eloff, J.N., 2010. Antibacterial and antioxidant activities of four kaempferol methyl ethers isolated from *Dodonaea viscosa* Jacq. var. *angustifolia* leaf extracts. South African Journal of Botany, 76(1), 25-29.

Van der Walt, L., 2000. *Anchusa capensis.* http://www.plantzafrica.com/plantab/anchusacapensis.htm. Accessed: November 2011.

Van der Walt, L., 2005. *Myrsine africana*. http://www.plantzafrica.com/plantklm/myrsinafr.htm. Accessed: November 2011.

Van der Watt, E. and Pretorius, J.C., 2001. Purification and identification of active antibacterial components in *Carpobrotus edulis* L. Journal of Ethnopharmacology, 76(1), 87-91.

Van Wyk, B.E, Oudtshoorn, B.V., Gericke, N., 1997. In: Medicinal plants of South Africa. Briza Publications.

Van Wyk, B.E. and Gerick, N., 2000. People's Plants. A Guide To Useful Plants of Southern Africa 1st ed. Briza Publications, South Africa.

Vaya, J. And Mahmood, S., 2006. Flavonoid content in leaf extracts of the fig (*Ficus carica* L.), carob (*Ceratonia siliqua* L.) and pistachio (*Pistacia lentiscus* L.). Biofactors, 28, 169-175.

Verotta, L., Aburjai, T., Rogers, C.B., Dorigo, P., Maragno, I., Fraccarollo, D., Santostasi, G., Gaion, R.M., Floreani, M., Carpenedo, F., 1995. Chemical and pharmacological characterization of *Erythrophleum lasianthum* alkaloids. Planta Medica, 61(3), 271-274.

Vries, F.A., Bitar, H.E.L., Green, I.R., Klassen, J.A., Mabusela, W.T., Bodo, B., Johnson, Q., 2005. An antifungal extract from the aerial parts of *Galenia africana*. 11th Napreca Symposium Book of Proceedings Antananarivo. Madagascar, 123-131.

Wink, M. and Van Wyk, B.E., 2008. Mind-altering and Poisonous Plants of the World. First Edition. South Africa, Briza Publications.

Whistler, W.A. and Elevitch, C.R., 2006. *Broussonetia papyrifera* (paper mulberry). Species profiles for Pacific Island Agroforestry. http://www.agroforestry.net/tti/Broussonetia-papermulb.pdf. Accessed on December 2010.

Yu, P., Shao, H., Feng, J., Zhu, M., Zhang, X., 2001. Preliminary study on antifungal activity of 62 plant extracts. Journal of Northwest Sci-Tech University of Agriculture and Forestry, 1-6.

Ziyyat, A. and Boussairi, E.H., 1998. Cardiovascular effects of *Arbutus unedo* L. in spontaneously hypertensive rats, Phytotherapy Research, 12, 110-113.

Chapter 4

Antibacterial, antioxidant activities and cytotoxicity of plants against *Propionibacterium acnes*

CHAPTER 4

Antibacterial, antioxidant activities and cytotoxicity of plants against *Propionibacterium acnes*

A section of this chapter has been accepted for publication in journal, South African Journal of Science

Richa Sharma[a], Namrita Lall[a*]

[a]Department of Plant Science, Faculty of Agricultural and Biological Science, University of Pretoria, Pretoria 0002, South Africa.

[*]Corresponding Author. Department of Plant Science, Plant Sciences Complex, University of Pretoria, Pretoria-0002, South Africa. Tel.: +27 12 420 2524; fax: +27 12 420 6688.
Email address: Namrita.Lall@up.ac.za

Abstract

The usage of plants to treat skin ailments has strong support in the current trend of drug discovery. *Propionibacterium acnes*, an anaerobic pathogen, plays an important role in the occurrence of acne. The present study was conducted to evaluate the antimicrobial, antioxidant activity and cytotoxic effects of medicinal plants grown in South Africa against *P. acnes.* The broth dilution and DPPH radical scavenging methods were used for antibacterial and antioxidant activity, respectively. The cytotoxicity was determined on mouse melanoma (B16-F10) cells. The 'GraphPad Prism 4' statistical program was used to analyse the concentration at which fifty percent cells were viable (EC_{50}). The ethanolic bark extract of *Acacia galpinii* Burtt Davy. (Leguminosae) exhibited the lowest minimum inhibitory concentration (MIC) value of 62.25 µg/ml. Excellent antioxidant activity was shown by *Aspalathus linearis* (Burm.f.) R.Dahlgren (Leguminosae), *Combretum apiculatum* Sond. (Combretaceae), *Harpephyllum caffrum* Bernh. ex Krauss (Anacardiaceae), and *Sclerocarya birrea* Hochst. (Anacardiaceae) with 50% radical scavenging activity (EC_{50}) at concentrations ranging from 1.6 to 3.5 µg/ml. *Greyia sutherlandii* Hook. & Harv. (Greyiaceae) also exhibited good antioxidant activity with an EC_{50} value of 7.9 µg/ml. *A. linearis, G. sutherlandii,* and *S. birrea* showed low toxicity with 50% viability of cells (EC_{50}) at concentrations of 125, 107.8 and 92 µg/ml, respectively. The extracts of *A. linearis, G. sutherlandii* and *S. birrea* showed good antibacterial, antioxidant activity and

low toxicity. Therefore, these plants can be considered as possible anti-acne agents and warrant further investigations.

Key words: antibacterial; antioxidant; cytotoxicity; mouse melanoma; *Propionibacterium acnes*

4.1 Introduction

Acne, one of the most common disorders of the skin, is a polymorphic disease with non-inflammatory (blackhead or whitehead) and inflammatory (papules, pustules, or nodules) aspects and a wide spectrum of severity. It can have a significant impact on psychosocial and physical aspects of life. It affects up to 85% of adolescents to some extent but is less common among infants. Its prevalence has been estimated to be 95—100% in males and 83—85% in females (Bloch, 1931; Munro-Ashman, 1963; Burton et al., 1971; Rademaker et al., 1989).

Propionibacterium acnes, a Gram-positive anaerobic bacterium is a normal component of the micro biota of human skin. *P. acnes* causes an increase in the secretion of sebum from sebaceous glands and this is accompanied by the thickening of the epidermis at the outlet to the pilosebaceous follicles. As a result, there is an obstruction to the flow of sebum outwards, and a comedone develops. Colonization of the follicles with *P. acnes* and the host's inflammatory response plays a pivotal role in the development of typical inflammatory papulopustular lesions (Shaw and Kennedy, 2007). In an anaerobic environment, the bacteria secretes nucleases, nuraminidases, hyaluronidases, acid phosphatises, lecithinases and other lipases. Due to action of these enzymes, the sebum content changes and reactive oxygen species (ROS) may be released from the damaged follicular walls. This may also be the reason for the progression of inflammation in the pathogenesis of disease (Arican et al., 2005).

Conventional drugs such as tetracycline, erythromycin, minocycline and metronidazole, commonly used in acne treatment, act as antioxidants and antibacterials. Benzoyl peroxide, a topical agent for the treatment of acne, shows the ability to induce an inflammatory reaction mediated by ROS in addition to its antibacterial activity (Arican et al., 2005).

These drugs also have various known side effects. The topical antibiotics can lead to dryness, redness and irritation of the skin, as well as hypopigmentation while oral antibiotics have age restrictions, can cause gastrointestinal disorders and increase the risk of venous thromboembolism

(Shaw and Kennedy, 2007).

Herbal medicines are an important part of African tradition and have very deep roots in dermatological ailments as well. Also, ethnobotanical studies have documented the use of plants by the local people for the treatment of various skin ailments (Hutchings et al., 1996). Different plant parts commonly used as cosmetics or face masks, known as *umemezis*, are widely used in southern Africa for skin problems like inflammation, wounds, burns, eczema and puberty acne (Van Wyk and Gerick, 2006).

Since many skin disorders like atopic dermatitis and acne are associated with inflammation and the release of free radicals, which lead to oxidative/cellular damage and bacterial infection such as *P. acnes*, the presence of antioxidant and antimicrobial agents can explain the effectiveness of plants in the treatment of skin infections. In order to develop the therapeutic potential and as well as for drug development, it is important to know whether there are any cytotoxic effects. Therefore, the ethanol extracts of the selected plants were evaluated for antibacterial, antioxidant activities and for cytotoxicity using mouse melanoma.

Limitations in the usage of some drugs and the prevailing side effects of the various chemically derived compounds have led to the search for alternate herbal agents to treat acne. The aim of this study was to test the effect of selected plant extracts on the pathogenic bacteria *P. acnes*, and to identify which plant extract could be considered as possible anti-acne agents.

4.2 Materials and methods

4.2.1 Chemicals, microbial strain and culture media

Tetracycline, vitamin C, *p*-iodonitrotetrazolium salt and DPPH (2,2-diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich (Kempton Park, South Africa). Nutrient agar, nutrient broth, Anaerocult A and Gram stain were obtained from Merck SA (Pty) Ltd. *P. acnes* (ATCC 11827) was purchased from Anatech Company South Africa. The cell culture reagents and the equipment were purchased from Highveld Biological (Sandringham, South Africa), Labotech (Midrand, South Africa) and The Scientific Group (Midrand, South Africa). The B16-F10 mouse melanoma cell line was obtained from Highveld Biological (Sandringham, South Africa).

4.2.2 Plant material

Different plants parts (leaves, roots, bark, twigs) were collected from the Botanical Garden of the University of Pretoria. The plants were identified by a taxonomist (Prof A.E. [Braam] van Wyk) at the H.G.W.J. Schweickerdt Herbarium (PRU) of the University of Pretoria. The shade-dried plant material (80 g) was ground with a mechanical grinder, then soaked in 300 ml of ethanol and was left on a shaker for three days. The plant material was then filtered and the solvent was evaporated under vacuum (Buchi Rotavapor, Labotech, Switzerland) to yield dry extracts. Eight plant extracts were prepared while the remaining were obtained (stored at -4°C protected from light) from the phytochemists of the Medicinal Plant Science program.

4.2.3 Antibacterial bioassay

4.2.3.1 Bacterial strain and culture

The antibacterial activity of 51 ethanol plants extracts was investigated against *P. acnes* (ATCC 11827). Prior doing the antibacterial assay, the bacteria was cultured from a Kwik-Stick on nutrient agar and incubated at 37°C for 72 h under anaerobic conditions in an anaerobic jar with Anaerocult A.

4.2.3.2 Gram stain for the determination of the identity of bacteria

The Gram stain test was performed for the identification of bacteria in pure cultures. A smear of *P. acnes* culture suspended in nutrient broth was heat fixed on a glass slide. Crystal violet was used as primary stain. Iodine resublime solution was subsequently added as mordant. A mixture of acetone and ethyl alcohol were then added as a decolourant. Finally, a counterstain Safranine O was applied to the smear (Gerhardt et al., 1981).

The cell wall structure of microorganism determines the ability to be stained differentially by Gram's method. The cell wall of Gram positive bacteria possess a higher peptidoglycan and lower lipid content, in contrast, high lipids and a thinner peptidoglycan layer is found in Gram negative bacteria. Therefore, the purple colour of crystal violet is retained by Gram positive bacteria whereas the same is washed by alcohol in Gram negative bacteria which appears red due to the counter stain (Gerhardt et al., 1981).

4.2.3.3 Cell count

To determine the colony forming units (CFU) for *P. acnes*, the serially diluted cell suspension of bacteria from the reference inoculums of 0.5 McFarland standard (550 nm) was plated by spread plate technique (Gerhardt et al., 1981). Ten-fold serial dilutions were made in a sterile quarter-strength Ringer solution. One hundred micro litres (100 µl) of 10^4 to 10^7 dilutions were plated on agar and spread using a "L" shaped loop. The plates were incubated at 37°C for 10 days under anaerobic conditions. The plates were divided into sections and approximate colonies were counted (Figure 4.1). The approximate counted colonies are listed in Table 4.1.

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

Figure 4.1: Agar plates with the colonies of *Propionibacterium acnes* at (a) 10^6 dilution, (b) 10^5 dilution and (c) 10^4 dilution

Table 4.1: The approximate counted colonies of *Propionibacterium acnes* on agar plates for the determination of colony forming units (CFU)/ml

Dilutions	No. Of Colonies (approx.)
10^4	1310
10^5	137
10^6	5-6
10^7	-

The CFU/ml for *P. acnes* was calculated using the following formula:

$$\frac{\text{No. of colonies x dilution of plate}}{\text{Volume of culture on plate}} = \frac{\text{CFU}}{1\text{ml}}$$

The plate with between 50-150 colonies was selected. Substituting the values in the above formula,

$$\frac{137 \times 10^5}{0.1} = \frac{\text{CFU}}{1\text{ml}}$$

Thus, the CFU/ml was evaluated to be 10^5 - 10^6.

4.2.3.4 Determination of antibacterial activity

The ethanol extract of the plant samples were tested against *P. acnes* by determining the minimum inhibitory concentration (MIC) values obtained by a microdilution method. This assay was done using the methods as described by Mapunya et al. (2011), with slight modifications. For this purpose, *P. acnes* were cultured as explained in section 4.2.4.1. The ethanolic extracts were dissolved in 10% DMSO to obtain a stock solution of 2 mg/ml. The positive control (tetracycline) was dissolved in sterile distilled water to obtain a stock solution of 0.2 mg/ml. The 96 well plates were prepared by dispensing 100 µl of the nutrient broth into each well. Hundred micro litres (100 µl) of the plant stock samples and positive control were added to the first row of wells in triplicates. Twofold serial dilutions were made in broth over a range to give concentrations of 500 to 3.9 µg/ml and 50 to 0.3 µg/ml for the plant extracts and positive control, respectively. The 72 h culture of bacteria was dissolved in nutrient broth and the suspensions were adjusted to 0.5 McFarland standard turbidity at 550 nm. About 100 µl of this bacterial inoculum with 10^5—10^6 CFU/ml was then added to all the wells. The wells with 2.5% DMSO and bacterial suspension without samples served as the solvent and negative controls, respectively. The plates were then incubated at 37°C for 72 h under anaerobic conditions. The MIC value was determined by observing the colour change in the wells after the addition of *p*-iodonitrotetrazolium salt (INT) (defined as the lowest concentration that showed no bacterial growth).

4.2.4 Antioxidant assay

The antioxidant activity of selected plant extracts was investigated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging method as previously described by Du Toit et al. (2001), with slight

modifications. DPPH is a free radical, which is stable at room temperature, and produces a violet solution in ethanol. When reduced in the presence of an antioxidant molecule, it gives rise to a colourless solution.

DPPH was dissolved in ethanol to obtain a solution of 0.04% w/v. The selected plant samples and the positive control (Vitamin C) stock solutions (2 mg/ml) were serially diluted to final concentrations ranging from 100 to 0.78 µg/ml. Ethanol and DPPH without any plant material were used as blanks while plant samples diluted in distilled water were used as controls. DPPH solution (90 µg/ml) was then added to all the wells except for the controls and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 515 nm using Biotek Power-wave XS multi well reader (A.D.P., Weltevreden Park, South Africa). The values were converted into the percentage antioxidant activity (AA) using the formula given below. The 50% inhibitory concentration (EC_{50}) values were then calculated by linear regression of the plots using 'GraphPad Prism 4' statistical program.

$$AA\% = \{Abs_{blank}(Abs_{sample} - Abs_{control}) / Abs_{blank}\}*100$$

4.2.5 Cytotoxicity assay

The cytotoxicity of selected plant extracts was determined following a previously described method (Mapunya et al., 2011). Briefly, mouse melanoma (B16-F10) cells were plated in complete Roswell Park Memorial Institute (RPMI) medium (10% fetal bovine serum and 1% gentamycin) directly in the wells of a 96-well plate (10^5 cells per well). After an overnight incubation at 37 ºC in 5% CO_2 and a humidified atmosphere, extract samples and the positive control (actinomycin D) were added to the cells to give the final concentrations of plant extract and positive control ranging from 400-3.13 and 0.05-0.03 x 10^{-2} µg/ml, respectively. Plates were incubated at 37°C in 5% CO_2 in a humidified atmosphere for three days. The toxicity effects of the extracts on the B16-F10 cells were assayed using the sodium 3'-[1-(phenyl amino-carbonyl)-3,4-tetrazolium]-bis-[4-methoxy-6-nitrobenzene sulfonic acid hydrate (XTT) cytotoxicity assay. Thereafter, 50 µl of XTT reagent (1 mg/ml XTT with 0.383 mg/ml PMS) was added to the wells and incubated for 1 h. The optical densities of the wells were measured at 450 nm with background subtraction at 690 nm. By referring to the controls (medium with DMSO), the cell survival was assessed. The EC_{50} value, which represents the concentration of plant extract that causes 50% death in the cells was analysed using 'GraphPad Prism 4', statistical program.

4.2.6 Statistical analysis

All the assays were performed in triplicates. The EC_{50} values for antioxidant and cytotoxicity tests were derived from a nonlinear regression model (curve fit) based on sigmoidal dose response curve (variable) and computed using GraphPad Prism 4 (Graphpad, San Diego, CA, USA).

4.3 Results and discussions

4.3.1 Identification of *Propionibacterium acnes* using Gram's staining

Figure 4.2 shows the pictures of *P. acnes* with Gram's stain. The bacteria appeared blue in colour as they retained crystal violet. *P. acnes* are pleomorphic, coryneform, anaerobic and occasionally branching Gram positive bacilli (Feingold and Meislich, 2012).

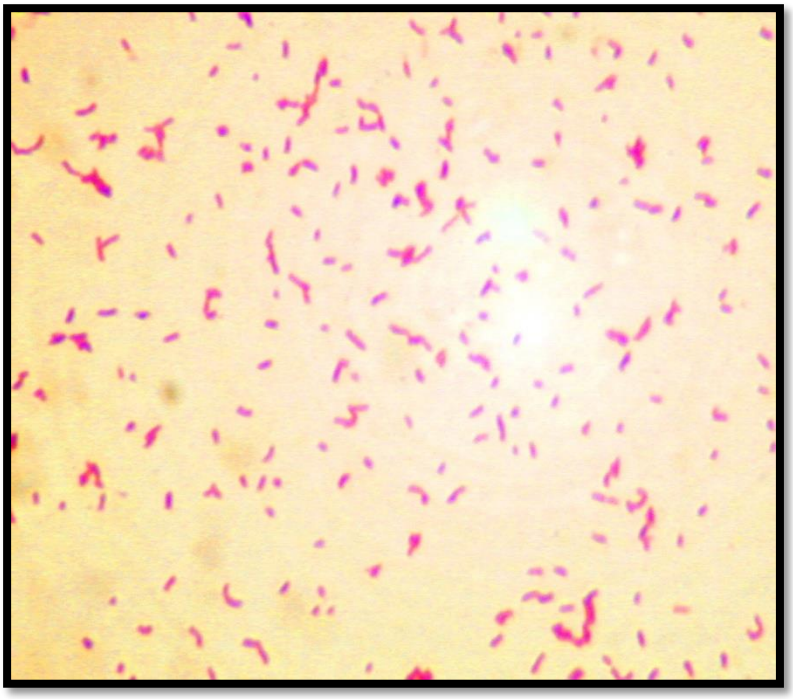


Figure 4.2: Gram staining of *Propionibacterium acnes* showing purple coloured bacilli

4.3.2 Antibacterial activity of ethanolic extracts

The antibacterial activity of the selected plants against *P. acnes* are summarised in Table 4.2. After the addition of INT, the MIC value of positive drug control (tetracycline) was determined to be 3.12 µg/ml.

Most of the plants exhibited antibacterial activity with MIC values ranging from 15.62-500 µg/ml. Similar to the findings by other researchers, Kumar et al., (2007) found ethanolic extract of *Coscinium fenestratum* (Gaertn.) Colebr. with the MIC value of 0.046 mg/ml against *P. acnes*. Tsung-Hsien Tsai et al., (2010) found a methanolic extract of *Rosa damascena* Mill, *Eucommia ulmoides* Oliv. and *Ilex paraguariensis* A. St.-Hil. inhibited the growth of *P. acnes* at MIC values of 2, 0.5, and 1 mg/ml respectively.

In the present study, the best activity was shown by the *Leucosidea sericea* Eckl. & Zeyh. and *Syzygium jambos* (L.) Alston leaves extract with MIC values of 15.62 and 31.25 µg/ml respectively. The bark extract of *Acacia galpinii* Burtt Davy. inhibited the bacterial growth at an MIC of 62.25 µg/ml. It is worth noting that threshold MIC values of 100 µg/ml have been recommended for plant extracts to rate them as having significant antimicrobial activity (Kuete, 2010). Therefore, the MIC values of all these three plants can be considered significant. Some of the plant extracts such as *Aspalathus linearis* (Burm.f.) R.Dahlgren, *Combretum apiculatum* Sond., *Combretum molle* Engl. & Diels, *Galenia africana* L., *Greyia sutherlandii* Hook. & Harv., *Harpephyllum caffrum* Bernh. ex Krauss, *Ranunculus repens* L., *Sclerocarya birrea* Hochst. and *Warburgia salutaris* (G. Bertol.) Chiov. exhibited an MIC of 125 µg/ml. Another 30 extracts showed MIC values ranging from 500-250 µg/ml whereas the remaining 10 extracts did not show any antibacterial activity even at the highest concentration (500 µg/ml) tested.

This is the first scientific report for these plants' inhibitory activity against the acne causing bacteria, *P. acnes*. However, for some of the plants used in the present study, the antimicrobial activities on other pathogens have been reported by researchers. In a previous study *A. linearis* showed a zone of inhibition against *Bacillus cereus, Micrococcus luteus,* and *Candida albicans* (7, 6.4 and 8.5 mm respectively) (Almajano et al., 2008). The antibacterial activity of *C. apiculatum* against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* were reported by Serage (2003). The acetone extract of the stem bark of *C. molle* showed antimicrobial activity against *Escherichia coli* and *Shigella* spp with an MIC value of 50 mg/ml. The extract also showed inhibitory effects on the fungus *Candida albicans* with complete inhibition at a concentration of 400 µg/ml. In a study done by Lining Cai et al. (2000), the crude methanolic extract of *Diospyros lycoides* Desf. showed activity against *Streptococcus mutans* and *Prevotella intermedia* at 1.25 mg/ml. In contrast, our results showed no activity of the ethanolic extract of *D. lycoides* against *P. acnes*.

In a previous study by Mativandlela et al., (2008) the ethanolic extract of *Galenia Africana* L. showed antimycobacterial activity against *Mycobacterium tuberculosis* and *M. smegmatis* with MIC values of 0.78 and 1.2 mg/ml, respectively. However, in the present study the ethanolic extract of *G. africana* showed an MIC of 125 µg/ml against *P. acnes*. The ethanolic extract of *H. caffrum* was reported to be very active against four bacterial species namely, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* while an aqueous extract showed activity against *Candida albicans* (Buwa and Van Staden, 2007). In the present study, the ethanolic extract of *H. caffrum* showed antibacterial activity with MIC of 125 µg/ml. The acetone extract of the bark and leaves of *S. birrea* was reported to be active against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus faecalis* with MIC values from 0.15 to 3 mg/ml (Eloff, 2001).

Table 4.2: Minimum inhibitory concentrations (MICs) of plant extracts against *Propionibacterium acnes* as determined by micro dilution assay

Plant Name	**Voucher no.**	**Part used**	**MIC µg/ml**
Acacia caffra Willd.	PRU 90700	Leaves	250
Acacia galpinii Burtt Davy.	PRU 16209	Bark	62.25
Acacia mellifera Benth.	PRU 078373	Leaves	250
Aloe arborescens Mill.	MN 5	Leaves	500
Aloe barbadensis Mill.	PRU 118947	Leaves	Not active
Aloe ferox Mill.	PRU 110308	Leaves	Not active
Aloe sessiliflora Pole-Evans.	PRU 118948	Leaves	Not active
Anchusa capensis Thunb.	Not available	Leaves	Not active
Annona senegalensis Pers.	PRU 074974	Bark	250
Arbutus unedo L.	PRU 6211000	Leaves	500
Aspalathus linearis (Burm.f.) R.Dahlgren	PRU 110523	Leaves	125
Barleria albostellata C. B. Clarke	PRU 096399	Leaves	500
Barleria repens Nees	PRU 081712	Leaves	250
Broussonetia papyrifera (L.) Vent.	PRU 51221	Leaves	500

Buxus macowanii Oliv.	PRU 110526	Leaves	Not active
Carpobrotus edulis (L.) L.Bolus	PRU 096398	Leaves	Not active
Ceratonia siliqua L.	SM 95502	Leaves	Not active
Combretum apiculatum Sond.	PRU 110531	Leaves	125
Combretum molle Engl. & Diels	EP 81	Leaves	125
Cotyledon orbiculata L.	PRU 096402	Leaves	Not active
Cryptocarya woodii Engl.	PRU 064439	Leaves	250
Dahlia imperialis Roezl	PRU 3311010	Leaves	500
Datura stramonium L.	MN 8	Leaves	500
Dichrostachys cinerea (L.) Wight & Arn.	PRU 096403	Leaves	500
Diospyros lycioides Desf.	PRU 118949	Twigs	Not active
Dodonaea viscosa Jacq.	PRU 096404	Leaves	500
Erythrophleum lasianthum Corbishley.	PRU 110525	Leaves	250
Euclea divinorum Hiern.	AJ 64	Leaves	250
Euclea natalensis A.DC.	PRU 95059	Leaves	250
Euclea natalensis A.DC.	NL 22	Roots	250
Galenia africana L.	SM 93723	Leaves	125
Gomphocarpus fruticosus R.Br.	MN 1	Leaves	250
Greyia flanaganii Bolus.	P.Van Wyk 2274	Leaves	250
Greyia sutherlandii Hook. & Harv.	PRU 118946	Leaves	125
Harpephyllum caffrum Bernh. ex Krauss	PRU 118950	Leaves	125
Helichrysum argyrophyllum DC.	Not available	Leaves	250
Helichrysum glomeratum Klatt	M5055	Leaves	250
Heteropyxis natalensis Harv.	PRU 096405	Leaves	250
Hyaenanche globosa Lamb.	SM 95499	Leaves	250
Knowltonia vesicatoria Sims.	PRU 096499	Roots	250

Leucosidea sericea Eckl. & Zeyh.	PRU 119052	Leaves	15.62
Magnolia grandiflora L.	PRU 2651000	Leaves	250
Myrsine africana L.	SM 95503	Stalks	500
Parinari curatellifolia Planch. ex Benth.	PRU 096215	Bark	250
Ranunculus repens L.	PRU 096416	Leaves	125
Rhus lancea L.f.	PRU 110530	Leaves	250
Sclerocarya birrea Hochst.	NH 1910	Bark	125
Sideroxylon inerme L.	PRU 96216	Bark	250
Symphytum officinale L.	PRU 096414	Leaves	250
Syzygium jambos (L.) Alston	PRU 119053	Leaves	31.25
Warburgia salutaris (G. Bertol.) Chiov.	PRU 110529	Leaves	125
Zanthoxylum capense Harv.	PRU 096406	Leaves	Not active
Tetracycline (positive control)			3.12

*Not active: at the highest concentration of 500 µg/ml tested

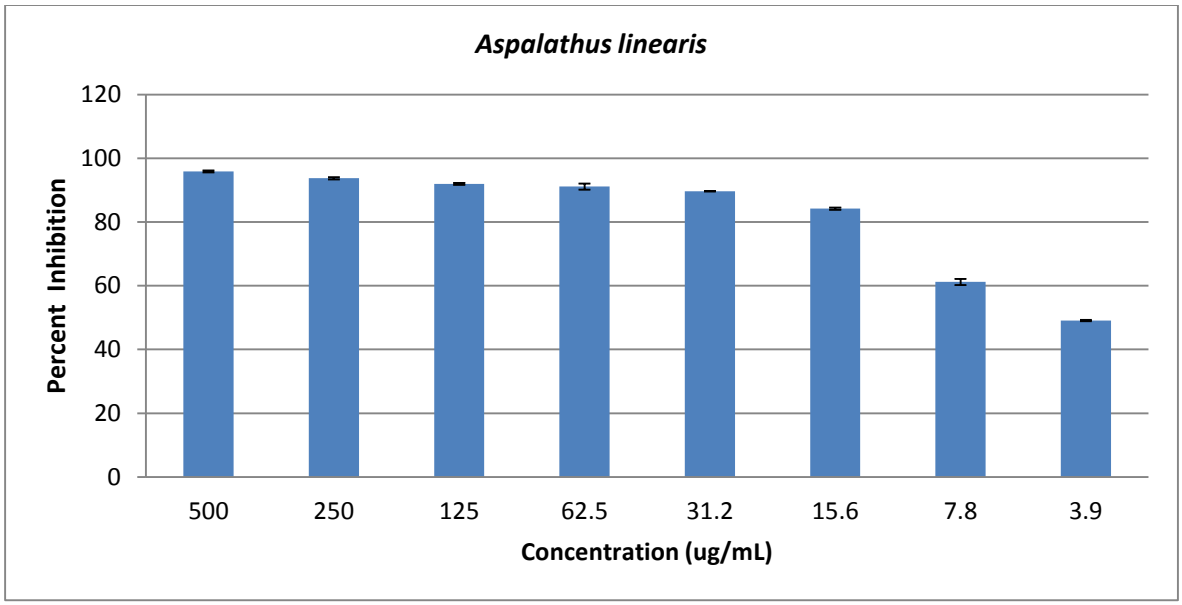
In a study, leaf extracts of *W. salutaris* inhibited growth of *Candida albicans* with an MIC value ranging from 12.5-25 mg/ml (Motsei et al., 2003) and the bark extracts showed growth of inhibition against *Staphylococcus aurens, Staphylococcus epidermis, Bacillus subtilis,* and *Escherichia coli* (Rabe and Van Staden, 1997). Based on the literature search, no reports regarding the antimicrobial activity for *G. sutherlandii* and *R. repens* were found. However, in the present study, both these plants showed growth inhibitory activity against *P. acnes* at MIC value of 125 µg/ml. In a study conducted by Eloff and Katerere (2004), the acetone and chloroform leaves extract of *A. galpinii* inhibited the growth of *S. aureus* and *E. coli*. Similar to our findings, the ethanol bark extract of *A. galpinii* exhibited good inhibitory effect on *P. acnes* (MIC 62.25 µg/ml). However, based on literature search, no antimicrobial activity of bark extract of *A. galpinii* was found.

4.3.3 The antioxidant activity of selected extracts

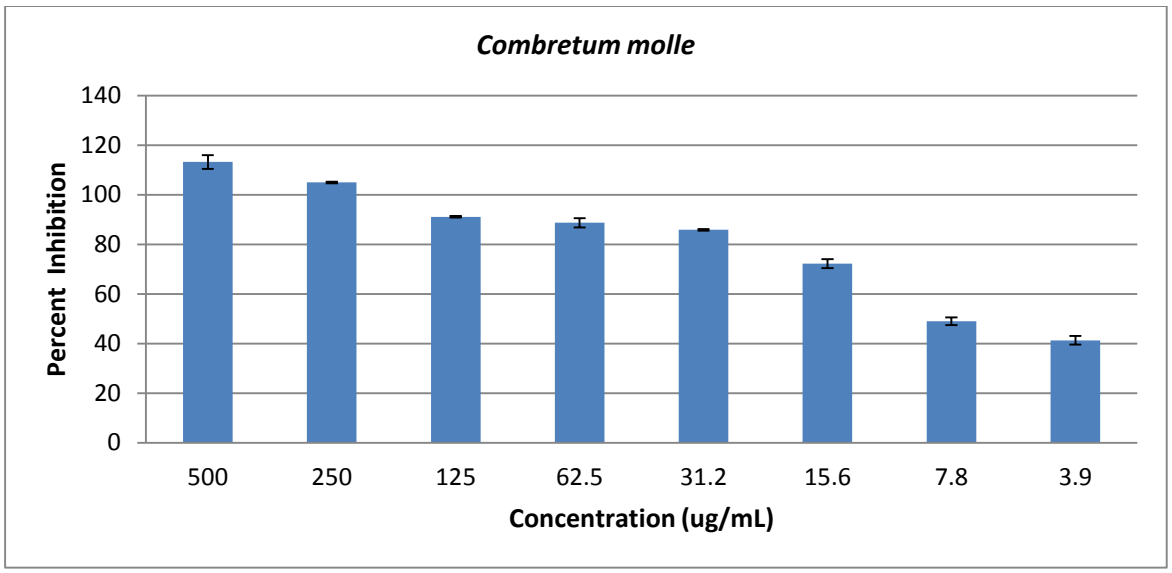
Vitamin C, a widely used antioxidant compound, was used as the positive control (EC_{50} 2 µg/ml)

(Figure 5.7e). The plant extracts which demonstrated excellent radical scavenging activity as comparable to vitamin C were *A. linearis* (EC_{50} 3.5 µg/ml), *C. apiculatum* (EC_{50} 1.6 µg/ml), *H. caffrum* (EC_{50} 2.6 µg/ml), and *S. birrea* (EC_{50} 2 µg/ml) (Figure 4.3). The plant extracts of *C. molle* and *G. sutherlandii* also showed good antioxidant activity with EC_{50} values of 9.8 and 7.9 µg/ml, respectively (Figure 4.3). *A. galpinii* and *R. repens* exhibited comparatively higher antioxidant activity with EC_{50} values of 16 and 24.7 µg/ml, respectively. The extracts of *G. africana* and *W. salutaris* exhibited lowest radical scavenging activity with the highest EC_{50} values of 90.9 and 111 µg/ml, respectively.

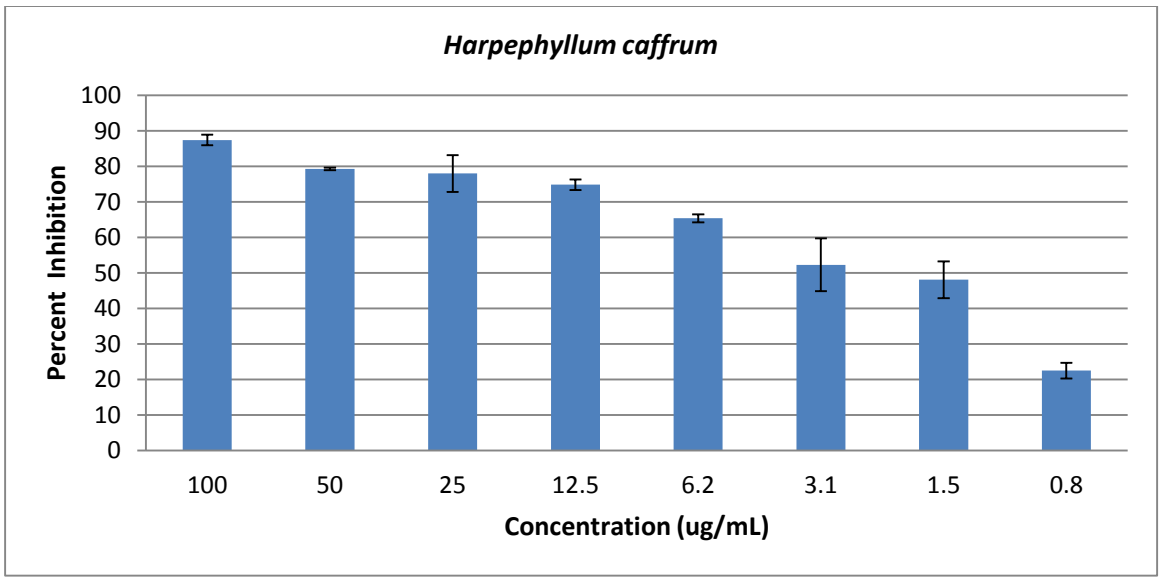
Acne is associated with the production of free radicals along with the infection of *P. acnes*. Reactive oxygen species (ROS) are produced as a result of action of hydrolytic enzymes released from the bacteria on the follicular walls of pilosebaceous unit. Therefore, the plant extracts were evaluated for antioxidant activity along with the antibacterial activity. In our study, the ethanol extracts of *A. linearis*, *C. apiculatum*, *H. caffrum*, *S. birrea*, *C. molle*, and *G. sutherlandii* exhibited significant antioxidant activity with EC_{50} values of ≤10 µg/ml. Our results were in agreement with other researchers. During a previous study by Joubert et al. (2004), the DPPH radical scavenging activity of *A. linearis* and its constituents were confirmed. The polar fractions of *C. apiculatum* showed antioxidant activity with EC_{50} value of 3.9 µg/ml (Aderogba et al., 2012).



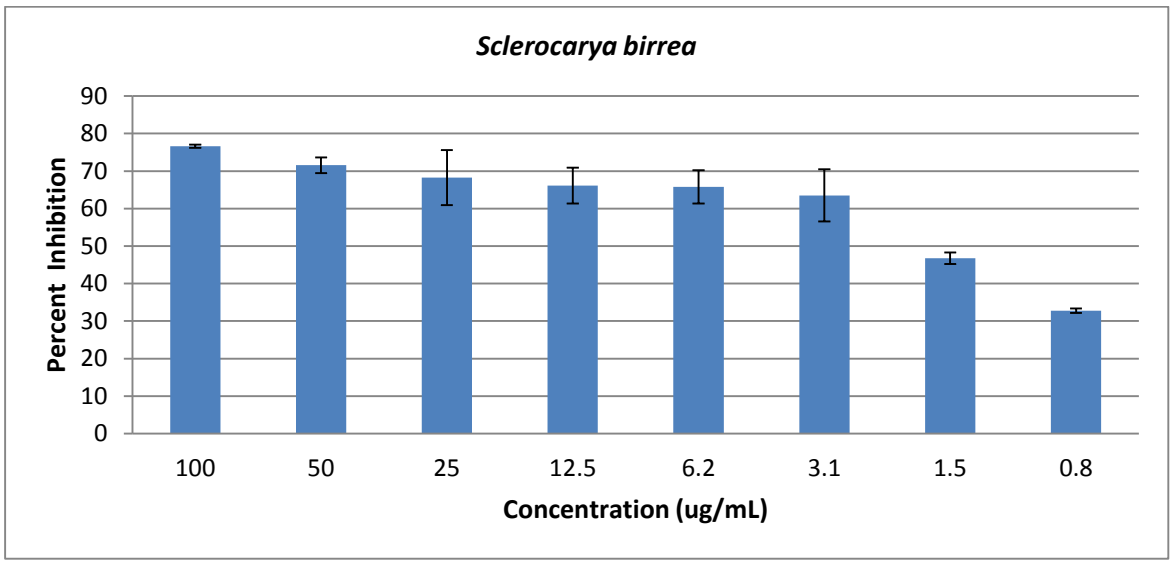
(a)



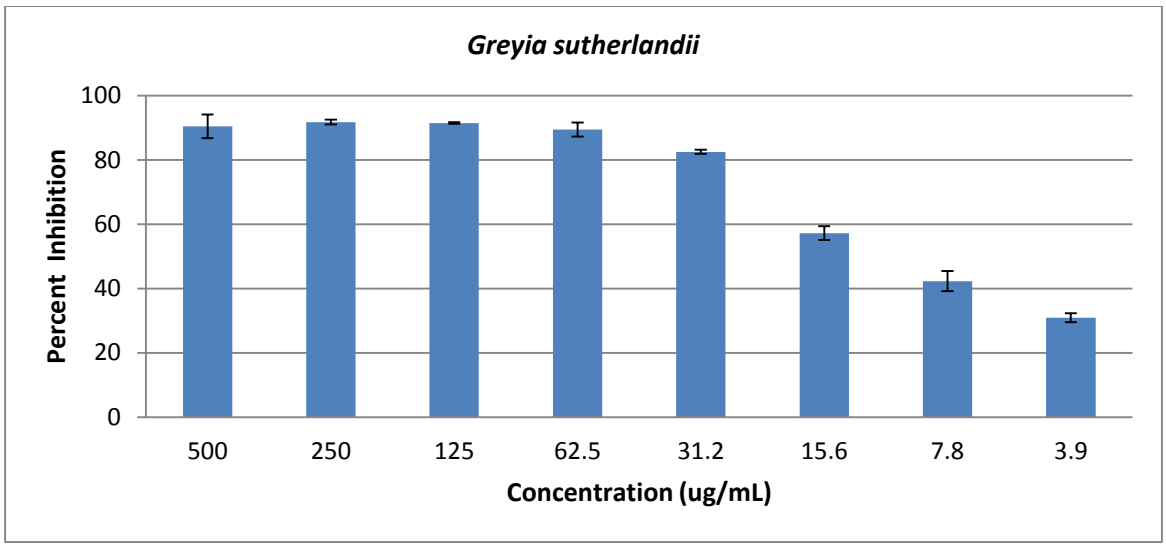
(b)



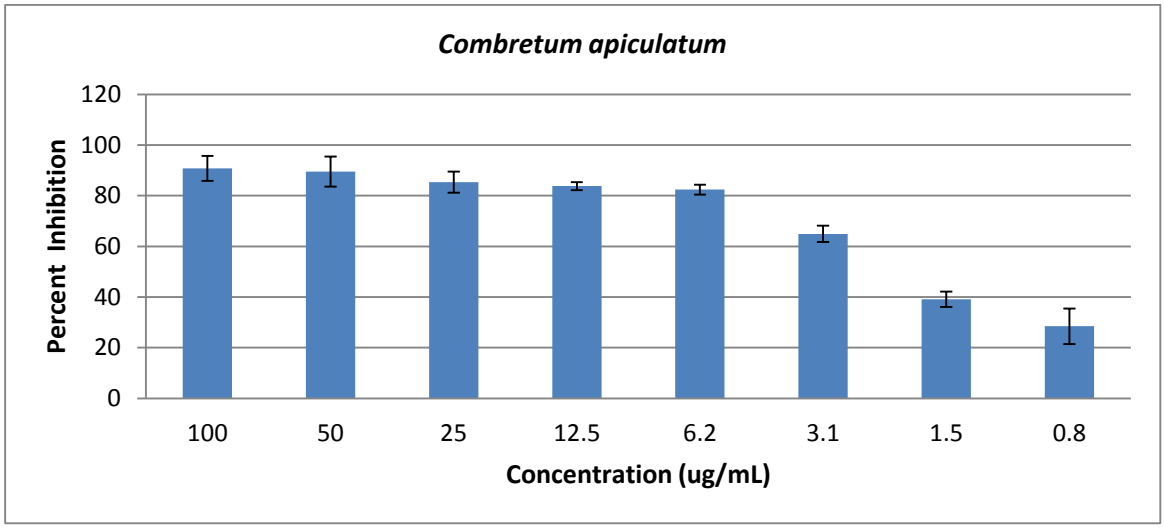
(c)



(d)



(e)



(f)

Figure 4.3: The DPPH radical scavenging activity of the potential extracts and the positive controls: (a) *Aspalathus linearis* (EC_{50} 3.5 µg/ml); (b) *Combretum molle* (EC_{50} 9.8 µg/ml); (c) *Harpephyllum caffrum* (EC_{50} 2.6 µg/ml); (d) *Sclerocarya birrea* (EC_{50} 2 µg/ml); (e) *Greyia sutherlandii* (EC_{50} 7.9 µg/ml) and (f) *Combretum apiculatum* (EC_{50} 1.6 µg/ml)

The DPPH radical scavenging activity of *H. caffrum* and *S. birrea* was confirmed by Moyo et al. (2010) with EC_{50} values of 6.8 and 5 µg/ml, respectively. In another study, acetone and DCM extract of *C. molle* displayed antioxidant activity after spraying with DPPH (P-Masoko, 2007). Studies have reported that DPPH free radical traps the phenolic hydrogen of the electron-donating molecule and this could be the general mechanism for the scavenging action of flavonoids (Ratty et al., 1998). Based on the mechanism of reduction of the DPPH molecule that is correlated with the presence of hydroxyl groups on the antioxidant molecule, the antioxidant activity of the polar plant extracts in the present study can be explained due to the presence of their phyto constituents (phenolics or flavonoids) as

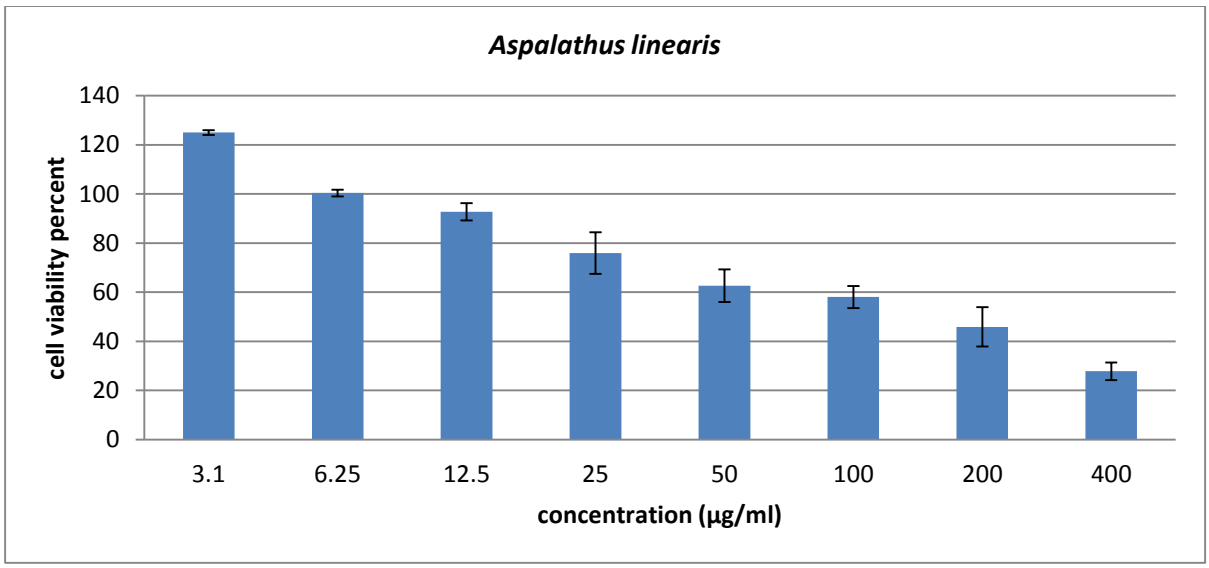
radical scavengers with an available hydroxyl group which are known to occur abundantly in plant species.

4.3.4 The cytotoxicity of selected extracts on mouse melanoma (B16-F10)

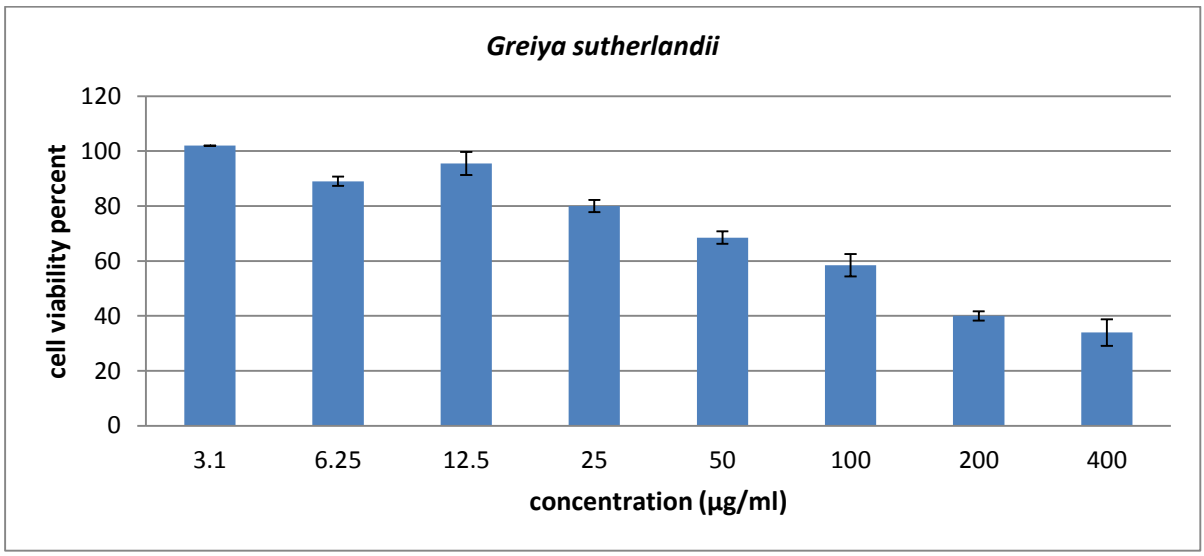
The cytotoxicity was performed on the plant extracts which demonstrated EC_{50} values of ≤10 µg/ml for radical scavenging activity. The plant extracts of *A. linearis*, *G. sutherlandii*, and *S. birrea* showed low toxicity with 50% viability of the cells (EC_{50}) at concentrations of 125, 107.8 and 92 µg/ml, respectively (Figure 4.4). During a previous study from our research group, the leaves extract of *H. caffrum* showed toxicity to B16-F10 cells at a concentration of 100 µg/ml (Manyatja-Brenda et al., 2012). The plant extract of *C. molle* showed moderate toxicity with an EC_{50} value of 48.8 µg/ml whereas, *C. apiculatum* was found to be most toxic with an EC_{50} value of 12.1 µg/ml and was lethal to almost all cells at the highest concentration of 400 µg/ml. Actinomycin D, the positive control showed an EC_{50} value of 4.5 ng/ml (Figure 4.4).

In order to know the therapeutic potential of plants, cytotoxicity of the selected samples were done on B16-F10 cells. To the best of our knowledge, the cytotoxicity of the extracts described in the present study is reported for the first time. However, previous researchers have documented similar cytotoxic effects on different cell lines. In a study by McGaw et al. (2007), *A. linearis* showed low toxicity on vero cells and brine shrimp larva with LD_{50} (dose/concentration required to kill 50% of cells) value of >1000 µg/ml. *S. birrea* showed low cytotoxicity on vero cells with IC_{50} value of 361.24 µg/ml (Gathirwa et al., 2008). According to previous studies on cytotoxicity of *C. molle* by Fyhrquist et al. (2006), the extract showed IC_{50} values of 27.7, 72.6, and 42.6 µg/ml on T 24 (bladder carcinoma), HeLa (cervical carcinoma), and MCF 7 (breast carcinoma) cells, respectively while for *C. apiculatum* the IC_{50} values were reported to be 65.0 and 40.1 µg/ml for T 24 and MCF 7 cells, respectively. Based on the literature search, no records of cell cytotoxicity for *G. sutherlandii* were found.

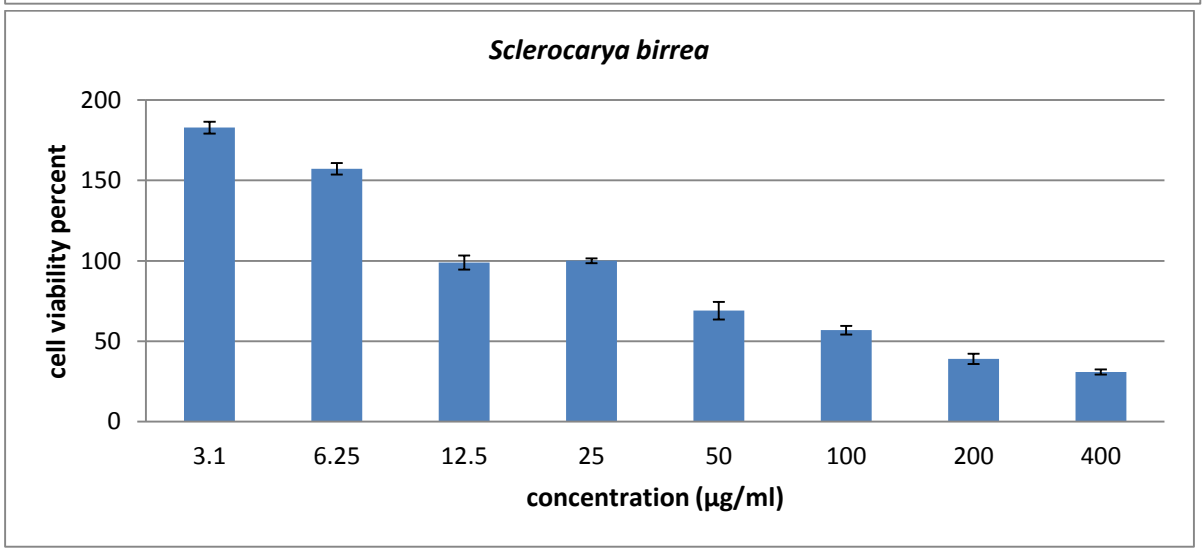
The results shown in this study proves the capability of medicinal plants to be used as anti-acne agents, though the mode of action and *in vivo* studies are required to support further the aforesaid statement. The antioxidant activity and cytotoxicity of two lead plant extracts (*S. jambos* and *L. sericea*) are discussed in chapter 5 and 6, respectively.



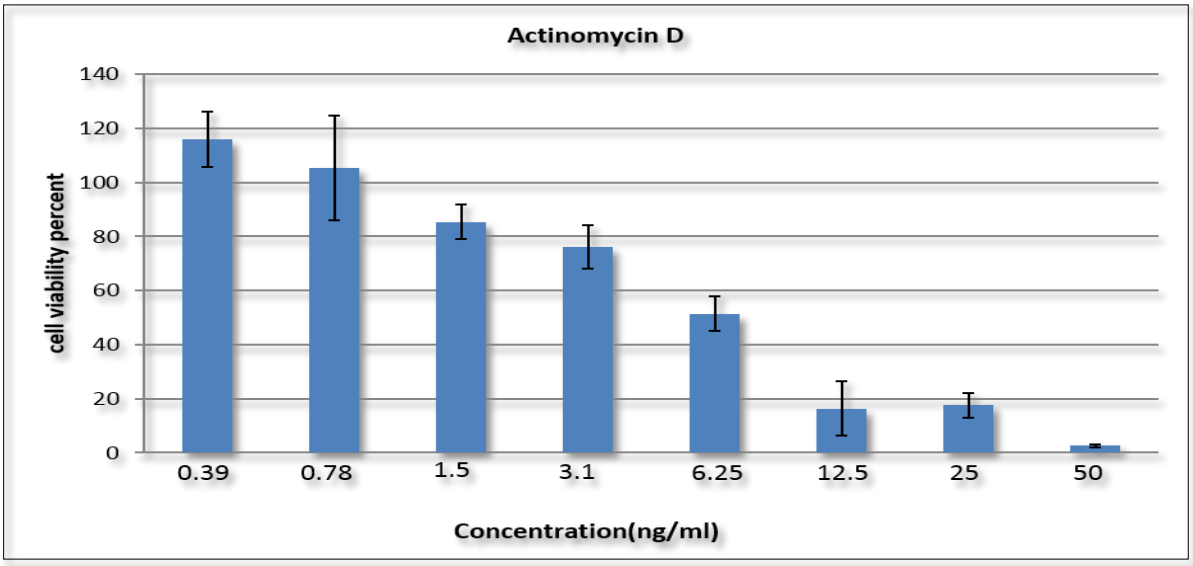
(a)



(b)



(c)



(d)

Figure 4.4: The cytotoxic effects of the plant extracts and the positive controls on mouse melanoma B16-F10 cells: (a) *Aspalathus linearis* (EC_{50} 125 µg/ml); (b) *Greyia sutherlandii* (EC_{50} 107.8 µg/ml); (c) *Sclerocarya birrea* (EC_{50} 92 µg/ml) and (d) Actinomycin D-drug control (EC_{50} 4.5 ng/ml)

4.4 Conclusion

The antibacterial study performed in this chapter gives an indication of the potency of the selected plants as actives against *P. acnes* infection. Based on the results obtained, it can be concluded that ethanol plant extracts of *L. sericea*, *S. jambos* and *A. galpinii* inhibited *P. acnes* growth and exhibited significant MIC values below 100 µg/ml. The plant extracts of *H. caffrum, C. apiculatum,* and *C. molle* showed good antibacterial and excellent antioxidant activity, these samples also showed moderate toxicity to mouse melanoma cells. The plant extracts of *A. linearis*, *S. birrea,* and *G. sutherlandii* also exhibited good antibacterial and antioxidant activity but had low toxicity to the mouse melanoma cells therefore, proving their potential as anti-acne agents either alone or in combination with each other.

4.5 References

Aderogba, M.A., Kgatle, D,T., McGaw, L.J., Eloff, J.N., 2012. Isolation of antioxidant constituents from *Combretum apiculatum* subsp. *apiculatum*. South African Journal of Botany, 79, 125-131.

Almajano, M.P., Carbo, R., Jimrnez, J.A.L., Gordon, M.H., 2008. Antioxidant and antimicrobial activities of tea infusions. Food Chemistry, 108(1), 55-63.

Arican, O., Kurutas, E.B., Sasmaz, S., 2005. Oxidative stress in patients with acne vulgaris. Mediators of Inflammation, 6, 380-384.

Bloch, B., 1931. Metabolism, endocrine glands and skin disease, with special reference to acne vulgaris and xanthoma. British Journal of Dermatology, 43, 61-87.

Burton, J.L., Cunliffe, W.J., Stafford, I., Shuster, S., 1971. The prevalence of acne vulgaris in adolescence. British Journal of Dermatology, 85, 119-26.

Buwa, L.V. and Van Staden, J., 2007. Effects of collection time on the antimicrobial activities of *Harpephyllum caffrum* bark. South African Journal of Botany, 73(2), 242-247.

Dimayuga, R.E. and Garcia, S.K., 1991. Antimicrobail screening of medicinal plants from Baja California Sur, Mexico. Journal of Ethnopharmacology 31,181-192.

Du Toit, R., Volsteedt, Y., Apostolides, Z., 2001. Comparison of the antioxidant content of the fruits, vegetables and teas measured as Vitamin C equivalents. Toxicology166, 63-69.

Eloff, J.N., 2001. Antibacterial activity of Marula (*Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro, (Anacardiaceae) bark and leaves. Journal of Ethnopharmacology, 76(3), 305-308.

Eloff, J.N. and Katerere, D.R., 2004. Variation in chemical composition, antibacterial and antioxidant activity of fresh and dried *Acacia* leaf extracts. South African Journal of Botany 70(2), 303-305.

Feingold, A.R. and Meislich, D., 2012. Anaerobic Gram-Positive, Nonsporulating Bacilli (inducing Actinomycosis) in Principlea ans Practice of pediatric infectious disease. P 990.

Fyhrquist, P., Mwasumbi, L., Vuorela, P., Vuorela, H., Hiltunen, R., Murphy, C., Adlercreutz, H.,(2006. Preliminary antiproliferative effects of some species of *Terminalia*, *Combretum*, and *Pteleopsis* collected in Tanzania on some human cancer cell lines. Fitoterapia, 77(5), 358-366.

Gathirwa, J.W,, Rukunga, G.M., Njagim E.N.M., Omar, S.A., Mwitari, P.G., Guantai, A.N., Tolo, F.M., Kimani, C.W., Muthaura, C.N., Kirira, P.G., Ndunda, T.N., Amalemba, G., Mungai, G.M., Ndiege, I.O., 2008. The *in vitro* anti-plasmodial and *in vivo* anti-malarial efficacy of combinations of some medicinal plants used traditionally for treatment of malaria by the Meru community in Kenya. Journal of Ethnopharmacology, 115(2), 223-231.

Gehardt, P., Murray, R.G.E., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R., Briggs Phillips, G., 1981. Manual of methods for General Biotechnology. American society for Microbiology, P 26-27 and 186-187.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. Zulu Medicinal Plants. An Inventory. Pietermaritzburg: University of Natal Press.

Joubert, E., Winterton, P., Britz, T.J., Ferreira, D., 2004. Superoxide anion and α,α-diphenyl-β-picrylhydrazyl radical scavenging capacity of rooibos (*Aspalathus linearis*) aqueous extracts, crude phenolic fractions, tannin and flavonoids. Food Research International, 37(2), 133-138.

Kuete, V., 2010. Potential of cameroonian plants and derived-products against microbial infections. A review. Planta Medica, 76, 1479-1491.

Kumar, G.S., Jayaveera, K.N., Kumar, C.K., Sanjay, U.P., Swamy, B.M., Kumar, D.V., 2007. Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. Tropical Journal of Pharmaceutical Research, 6(2), 717-723.

Lining, C., Guo-Xian, W., Pieter van der Bijl, Christine, D.Wu., 2000. Namibian chewing stick, *Diospyros lycioides*, contains antibacterial compounds against oral pathogens. Journal of Agriculture and Food Chemistry, 48(3), 909-914.

Manyatja-Brenda, M., Vassileva, R.N., Lall, N., 2012. Melanogenesis and antityrosinase activity of selected South African Plants. Evidence Based Complementary and Alternative Medicine., 2012, Article ID 374017, 6 pages, doi:10.1155/2012/374017.

McGaw, L.J., Steenkamp, V., Eloff, J.N., 2007. Evaluation of Athrixia bush tea for cytotoxicity, antioxidant activity, caffeine content and presence of pyrrolizidine alkaloids. Journal of Ethnopharmacology, 110(1), 16-22.

Mapunya, M.B., Hussein, A.A., Rodriguez, B., Lall, N., 2011., Tyrosinase activity of *Greyia flanaganii* Bolus constituents. Phytomedicine, 18, 1006-1012.

Mativandlela, S.P.N., Meyer, J.J.M., Hussein, A.A., Houghton, P.J., Hamilton, C.J., Lall, N., 2008., Activity against *Mycobacterium smegmatis* and *M. tuberculosis* by extracts of South African medicinal plants. Phytotherapy Research, 22(6), 841-845.

Motsei, M.L., Lindsey, K.L., Van Staden, J., Jager, A.K.. 2003. Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. Journal of Ethnopharmacology, 86(2–3), 235-241.

Moyo, M., Ndhlala, A.R., Finnie, J.F., Van Staden, J., 2010. Phenolic composition, antioxidant and acetylcholinesterase inhibitory activities of *Sclerocarya birrea* and *Harpephyllum caffrum* (Anacardiaceae) extracts. Food Chemistry, 123(1), 69-76.

Munro-Ashman, D., 1963. Acne vulgaris in public schools. Trans St John's Hospital Dermatology Society, 49, 144-8.

P-Masoko, J.E., 2007. Screening of twenty-four South African *Combretum* and six *Terminalia* species (Combretaceae) for antioxidant activities. African Journal of Traditional Complementary and Alternative Medicine, 4(2), 231-239.

Rabe, T. and Van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology, 56(1), 81-87.

Rademaker, M., Garioch, J.J., Simpson, N.B., 1989. Acne in school children: no longer a concern for dermatologists. British Medical Journal, 298,1217-1219.

Ratty, A.K. and Sunamoto, J., Das, N.P., 1998. Interaction of flavonols with 1,1 – diphenyl- 2-picrylhydrazyl free radical liposomal membranes and soyabean lipoxygenase- 1. Biochemical Pharmacology, 37, 989-995.

Samaranayake, L.P, 2002. Essential microbiology for dentistry; with a contribution by B.M. Jones; foreword by Crispian Scully. Edinburg, New York, Churchill Livingstone.

Serage, A., 2003., Isolation and characterization of antibacterial compounds present in *Combretum apiculatum* subsp. *apiculatum*. MSc Thesis, Programme for Phytomedicine, University Of Pretoria.

Shaw, L. and Kennedy, C., 2007. The treatment of acne. Paediatrics and Child Health, 17, 385-389.

Srivastava, J., Lambert, J., N. Vietmeyer, 1996. Medicinal plants: An expanding role in development. World Bank Technical Paper. No. 320. 20.

Tsai, T.H., Tsai, T.H., Wu, W.H., Tseng, J.P., Tsai, P.J., 2010. *In vitro* antimicrobial and anti-inflammatory effects of herbs against *Propinonibacterium acnes*. Food Chemistry, 119, 964-968.

Uniyal, S.K., K.N. Singh, P. Jamwal and B. Lal, 2006. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. Journal of Ethnobiology and Ethnomedicine, 2, 1-14.

Van Wyk, B.E. and Gerick, N., 2006. People's Plants. A Guide to Useful Plants of Southern Africa. First edition. South Africa: Briza Publications.

Chapter 5

Antibacterial and anti-inflammatory effects of *Syzygium jambos* (L.) Alston and isolated compounds on acne vulgaris

CHAPTER 5

Antibacterial and anti-inflammatory effects of *Syzygium jambos* (L.) Alston and isolated compounds on acne vulgaris

A section of this chapter is published in BMC Complementary and Alternative Medicine.

Richa Sharma[a], Navneet Kishore[a], Ahmed Hussein[a,b], Namrita Lall[a*]

[a]Department of Plant Science, Faculty of Agricultural and Biological Science, University of Pretoria, Pretoria 0002, South Africa.

[b]Chemistry Department, University of Western Cape, Private Bag X17, Bellville, 7535 Cape Town, South Africa.

[*]Corresponding Author. Department of Plant Science, Plant Sciences Complex, University of Pretoria, Pretoria-0002, South Africa. Tel.: +27 12 420 2524; fax: +27 12 420 6688.
Email address: Namrita.Lall@up.ac.za

Abstract

Background: Acne vulgaris is a chronic skin disorder leading to inflammation as a result of the production of reactive oxygen species due to the active involvement of *Propionibacterium acnes* (*P. acnes*) in the infection site of the skin. The current study was designed to assess the potential of the leaf extract of *Syzygium jambos* (L.) Alston and its compounds for antibacterial and anti-inflammatory activity against the pathogenic *P. acnes*.

Methods: The broth dilution method was used to assess the antibacterial activity. The cytotoxicity investigation on mouse melanoma (B16-F10) and human leukemic monocyte lymphoma (U937) cells was done using sodium 3'-[1-(phenyl amino-carbonyl)-3,4-tetrazolium]-bis-[4-methoxy-6-nitrobenzene sulfonic acid hydrate (XTT) reagent. The non-toxic concentrations of the samples was investigated for the suppression of cytokines interleukin-8 (IL-8) and tumour necrosis factor (TNF- α) by testing the supernatants in the co-culture of the human U937 cells and heat killed *P. acnes* using enzyme immunoassay kits (ELISA). The statistical analysis was done using the Graph Pad Prism 4 program.

Results: Bioassay guided isolation of ethanol extract of the leaves of *S. jambos* led to the isolation of three known compounds namely; squalene, an anacardic acid analogue and ursolic acid which are reported for the first time from this plant. The ethanol extract of *S. jambos* and one of the isolated compound namely, anacardic acid analogue were able to inhibit the growth of *P. acnes* with a noteworthy minimum inhibitory concentration (MIC) value of 31.25 and 7.81 µg/ml, respectively. The ethanol extract and three commercially acquired compounds namely; myricetin, myricitrin, gallic acid exhibited significant antioxidant activity with fifty percent inhibitory concentration (IC_{50}) ranging between 0.8-1.9 µg/ml which was comparable to that of vitamin C, the reference antioxidant agent. The plant extract, compounds ursolic acid and myricitrin (commercially acquired) significantly inhibited the release of inflammatory cytokines IL-8 and TNF-α by suppressing them by 74 - 99%. TEM micrographs showed the lethal effects of selected samples against *P. acnes*.

Conclusions: The interesting antibacterial, antioxidant and anti-inflammatory effects of *S. jambos* shown in the present study warrant its further investigation in clinical studies for a possible alternative anti-acne agent.

Keywords: *Syzygium jambos*, *Propionibacterium acnes*, Antibacterial, Interleukin 8, Tumour necrosis factor, Cytotoxicity, Transmission electron microscopy

5.1 Introduction

Syzygium jambos (L.) Alston belongs to the family Myrtaceae and is commonly known as rose apple which is widespread in sub-Saharan Africa (Adjanohoun, 1989), Central America and Asia (Maskey and Shah, 1982). The plant is reported to be useful for a variety of ailments and is known for its antipyretic and anti-inflammatory properties. All parts of the plant are reported to have medicinal value. In Indo-China all parts of the plant are used for digestive and tooth ailments. A decoction of the leaves is used as a diuretic, a remedy for sore eyes and for rheumatism. The seeds are used to treat diarrhoea, dysentery, diabetes and catarrh. A decoction of bark is administered to relieve asthma and bronchitis (Lim, 2012).

Previous researchers who have investigated the plant have documented its potential pharmacological value. Acetone and aqueous bark extracts of this plant have been reported to be active against

Staphylococcus aureus, *Yersinia enterocolitica*, *Staphylococcus hominis*, *Staphylococcus cohnii* and *Staphylococcus warneri* (Djipa et al., 2000). Researchers have investigated the antimicrobial activity of both acetone and aqueous extracts of the leaves, bark and seeds against eight microorganisms namely, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Vibrio cholera.* The acetone bark extract showed growth inhibitory activity against all the microorganisms tested whereas the leaf extract inhibited only *S. aureus*, and the seed extract did not show any inhibitory activity. The aqueous bark extract exhibited a growth inhibitory effect against *S. aureus, E. coli* and *S. typhi*, whereas the seed extract inhibited the growth of *P. aeruginosa* and *V. cholerae*, and leaf extract exhibited an inhibitory effect only against *S. typhi* (Murugan et al., 2011). In another study the ethanol bark extract of *S. jambos* and its isolated triterpenoids such as friedelin, β-amyrin acetate, betulinic acid and lupeol exhibited antidermatophytic activity against *Microsporum audouinii*, *Trichophyton mentagrophytes* and *Trichophyton soudanense* (Kuiate et al., 2007). Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit with multifactorial etiology. It affects almost everybody during the course of their life. There are four key processes in the pathogenesis of acne: 1. Increased sebum production, 2. Follicular hyperkeratinization which leads to follicular obstruction, 3. Colonization by the causative agent, *Propionibacterium acnes* and 4. Host inflammatory responses triggered as a results of bacterial infection (Shaw and Kennedy, 2007; Arican et al., 2005).

As a result of the increased sebum production due to high androgen levels, *P. acnes*, a gram positive anaerobic commensal, produces various hydrolytic enzymes that act on the sebum to release free fatty acids. These free fatty acids acts as chemokines and increase the release of pro-inflammatory cytokines like interleukins-8 (IL-8) and tumour necrosis factor- α (TNF-α) which attract macrophages and lead to severe inflammation. Follicular wall ruptures due to the action of hydrolytic enzymes cause oxidative damage with the release of free radicals (Shaw and Kennedy, 2007; Arican et al., 2005). Therefore, an agent that can inhibit the growth of *P. acnes*, scavenge free radicals and suppress the inflammatory response is promising.

The conventional drugs available to treat acne act as antibacterial and anti-inflammatory agents. But these drugs have various side effects such as dryness, itching and hypopigmentation and also have age restrictions. Moreover, bacterial resistance is an ongoing problem. The use of medicinal plants dates back thousands of years. In light of previous reports regarding *S. jambos* as an antimicrobial agent on a

variety of micro-organisms, also, as reported in the previous chapter, the ethanol leaves extract of *S. jambos* exhibited noteworthy activity against Gram-positive *P. acnes* with significant MIC value of 31.3 µg/ml, the present study was conducted to explore its efficiency as an anti-acne agent. Therefore, the plant extract was further subjected to isolation of compounds and investigated for its anti-inflammatory and antioxidant activity. The transmission electron microscopy (TEM) was performed to visualise the lethal effects of plant extract/bioactive compound on *P. acnes.* To investigate the therapeutic potential of extract/compound, cytotoxicity was performed on human/mouse cell lines.

5.2 Materials and methods

5.2.1 Chemicals, microbial strain and culture media

Silica gel 60 (70-230 mesh); sephadex LH-20 and all the analytical grade chemicals were purchased from Sigma-Aldrich and Merck SA Pty Ltd. Three chemical compounds namely, myricetin, myricitrin and gallic acid were acquired from Sigma-Aldrich.The ELISA kits were bought from BD Biosciences, Johannesburg, South Africa. Cell proliferation Kit II (XTT) was supplied by Roche diagnostics Pty Ltd., Johannesburg, South Africa. The cell lines and medium were purchased from Highveld Biological Pty Ltd., Johannesburg, South Africa. Nitrate/nitrite assay colorimetric kit was bought from Sigma-Aldrich.

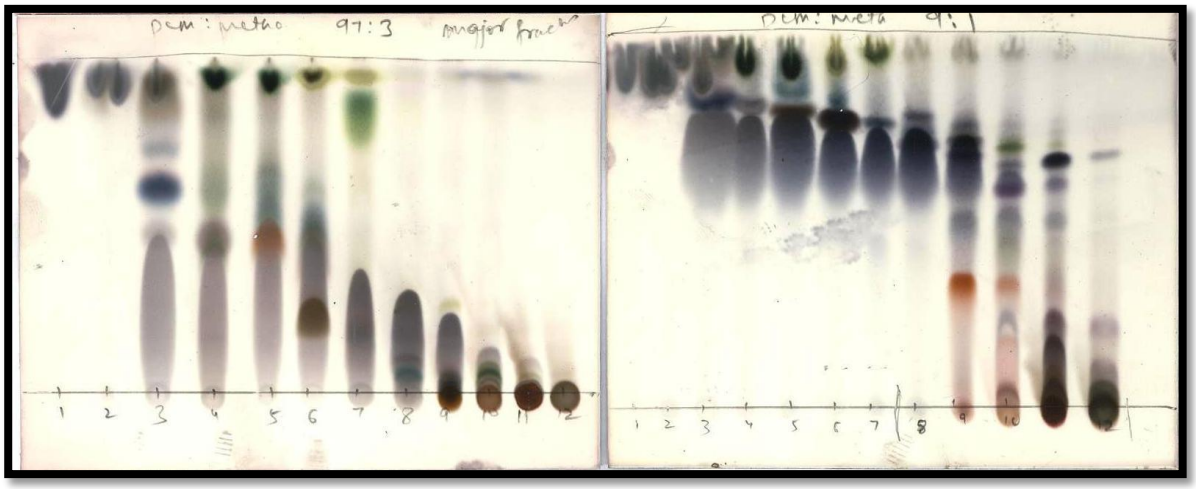
5.2.2 Plant material

The leaves of *S. jambos* were collected in August 2010 from the botanical garden of the University of Pretoria. The plant was identified at the H.G.W.J Schweicherdt Herbarium (University of Pretoria, Pretoria) where a voucher, specimen number (PRU 119053), has been deposited for future reference.

5.2.3 Extraction and purification

The air-dried and powdered leaves (1.9 kg) of *S. jambos* were soaked in 7.5 L ethanol for three days at room temperature. The crude ethanol extract (70.5 g) was obtained by concentrating the filtrate under reduced pressure. About 60 g of this ethanol extract was applied to a silica gel column (70 cm× 120 cm) using hexane fractions (Hex): ethyl acetate (EtOAc) of increasing polarity (100:0 to 0:100)

followed by 100% methanol (MeOH) as eluents. In total forty three (43) fractions (500 ml) were collected. Similar fractions were combined, according to the thin-layer (TLC) profiles, which resulted in twelve (12) major fractions (MF) (Figure 5.1). All the twelve (12) major fractions were tested for antibacterial activity against the pathogenic *P. acnes* using the broth dilution method. The results are listed in Table 5.1. The active MF (2, 4, and 6) were subjected to chromatographic separations to isolate the individual components. MF 2 (200 mg) was applied to a silica gel column using Hex: EtOAc (100:0 to 0:100) gradient as eluents. Eighty five (85) fractions of 50 ml each were collected. The sub-fractions 9-13 were combined on the basis of the TLC analysis which led to the isolation of the compound **1** (10 mg, 0.02%). MF 4 (600 mg) was subjected to a sephadex LH 20 column using 0.5% methanol in dichloromethane (DCM) as the eluent. One hundred and seventeen (117) fractions of 20 ml each were collected. Sub-fractions 80-88 were combined which yielded the compound **2** (8 mg, 0.01%). MF 6 (1.4 g) was subjected column chromatography similar to MF 4. One hundred (100) fractions of 20 ml each were collected. Sub-fractions 39-56 were combined on the basis of the TLC profiles which yielded the compound **3** (56.5 mg, 0.1%).



(a) **(b)**

Figure 5.1: Thin layer chromatography of the 12 pooled fractions from the ethanol extract of *Syzygium jambos* developed in (a) dichloromethane:methanol (97:3) and (b) dichloromathane:methanol (9:1)
Detection: Vanillin in H_2SO_4

5.2.4 Antibacterial and antioxidant activity

The antibacterial and antioxidant activity of the isolated compounds were done as described in Chapter 4. For antioxidant assay, the results were also expressed as the mg Vit C equivalents/g dry weight and calculated as follows: VitEAC (mg AA/100 g) = (EC_{50}(vit c)/EC_{50}(sample)) x 1000.

5.2.5 Transmission electron microscopy (TEM)

The TEM procedures followed the protocol of a previous publication (Pan et al., 2009). Briefly, bacteria was concentrated by centrifugation at 10 000 rpm for 1 min. The pellet was resuspended in nutrient broth to a final OD550 nm of 1. The concentrations of plant extract were 1.3 and 4 times the MIC; and 5 times the MIC for pure compound in order to visualise the lethal effects of tested samples against bacteria. The bacterial suspension (5 ml) was mixed with plant extract and pure compound to a final concentration of 300 and 100 µg/ml for plant extract and 50 µg/ml for pure compound. Tetracycline (50 µg/ml) and DMSO (2.5 %) were used as positive and solvent control. The pathogen was treated for 72 h; the control group consisted of only bacterial suspension in nutrient broth. Treated and untreated *P. acnes* cultures were centrifuged and fixed in 2.5% glutraldehyde in phosphate buffer at room temperature for 1 h. Samples were washed with phosphate buffer and postfixed in both 1% osmium tetraoxide and uranyl acetate. The cells were dehydrated in ethanol and embedded in quetol resin. Thin sections were prepared with a microtome and micrographs were taken using a JEOL JEM-2100F field emission electron microscope.

5.2.6 *In vitro* cytotoxicity assay

The mouse melanoma (B16-F10) cells were cultured as described in Chapter 4. The human U937 cells were cultured in Roswell Park Memorial Institute (RPMI) containing 10% fetal bovine serum (FBS) and 1% gentamycin. B16-F10 (10^5 cells per well) and U937 (10^6 cells per well) were seeded into a 96-well plate. After an overnight incubation at 37 ºC in 5% CO_2 and a humidified atmosphere, the extract, compounds and the positive control (actinomycin D) were added to the cells. The final concentrations of plant extract and pure compounds ranged from 400-3.13 µg/ml and 100-1.5 µg/ml, respectively. The highest concentration of positive control (0.05 µg/ml) was serially diluted to eight consecutive wells. The plate was then incubated at 37°C in 5% CO_2, and a humidified atmosphere after which the toxic effects of the extracts were assayed using the XTT (sodium 3'-[1-(phenyl amino-carbonyl)-3,4-tetrazolium]-bis-[4-methoxy-6-nitrobenzene sulfonic acid hydrate) cytotoxicity assay. Fifty micro litres of XTT reagent (1 mg/ml XTT with 0.383 mg/ml PMS) was added to the wells and incubated for 1 h. The optical densities of the wells were measured at 450 nm (690 nm reference wavelength) using BIOTEK Power-wave XS multi well reader (A.D.P., Weltevreden Park, South Africa). By referring to

the control (medium with DMSO), the cell survival rate was assessed. A statistical program (Graph Pad Prism 4) was used to analyze the 50% inhibitory concentration (EC_{50}) values.

5.2.7 Anti-inflammatory activity

5.2.7.1 Anti-inflammatory activity by suppression of IL-8 and TNF-α

5.2.7.1.1 Preparation of heat-killed P. acnes and measurement of cytokine production

The effect of selected samples on cytokine production (IL-8 and TNF-α) was evaluated using the respective enzyme immunoassay kits (ELISA) by a previously described method (Tsai et al., 2010). Briefly, the log phase culture of *P. acnes* was harvested, washed three times with phosphate buffer saline (PBS) and incubated at 80°C for 30 minutes to kill the bacteria. The heat-killed bacteria were stored at 4°C until use. The U937 cells were seeded at 10^6 cells per well in a 24-well plate and was stimulated with heat killed *P. acnes* (wet weight 100 µg/ml) alone and in combination with the different test samples. Pentoxifylline was used as a control. After 18-h incubation, the cell-free supernatants were collected and the concentrations of IL-8 and TNF-α were analysed. Cytokine standards were serially diluted to facilitate the construction of calibration curves necessary for determining protein concentration after treatment with test samples.

5.2.7.2 Anti-inflammatory activity by suppression of Nitric oxide

5.2.7.2.1 Isolation of macrophages from mouse

Macrophages were derived from three Balb mice by Marleze Rheeder (Faculty of Veterinary Science, Onderstepoort Campus, Pretoria) as previously described (Zang et al., 2008). Mice were sacrificed (CO_2 euthanasia) for another study for which ethics was approved. The abdomen was sterilized by 70% ethanol and a small incision along the midline was made with sterile blade. The abdominal skin was manually retracted to expose the intact peritoneal wall. With the help of a 10 ml syringe, the peritoneal cavity was filled with cold DMEM medium carefully without puncturing any of the organs (Figure 5.2). Using the same syringe and needle, the fluid was aspirated from peritoneum. The cells in the medium from peritoneal fluid (Peritoneal exudate cells- PEC) were dispensed in a polypropylene centrifuge tube on ice. The cells were kept cold throughout the procedure and were transported to Department of Plant Science, Hatfield Campus, Pretoria for culturing.

The PEC were centrifuged in ice cold tubes and 4 x 10^5 cells/well were added to 24 well plate. The

cells were allowed to adhere to the substrate by culturing them in DMEM medium for 2 h at 37°C. Nonadherent cells were removed by gentle washing three times with warm PBS. At this stage, 90% of the cell population comprised of macrophages.

5.2.7.2.2 Determination of Nitric oxide production

The mouse derived macrophages were stimulated with LPS (100 µg/ml) to elicit the Nitric oxide (NO) production. Nitro arginine was used as NO inhibitor and standard solution (provided in the kit) were prepared following manufacturer's protocol. The supernatants were collected after incubating the cells with plant extract (100, 50 and 10 µg/ml), nitroarginine (200, 100 and 50 µM) and LPS for16 h. The presence of NO was determined by adding Griess reagent in equal volume to that of supernatant. The absorbance at 540 nm was measured by a microplate reader.

Figure 5.2: Mouse peritoneal cavity filled with cold DMEM medium for isolation of macrophages

5.2.8 Glutathione reductase (GR) enzyme assay

Glutathione (GSH) is considered to play key role in protecting cells against oxygen toxicity and is mainly found in most eukaryotes. GSH inactivates potentially damaging free radicals and is oxidised to symmetrical glutathione disulphide (GSSG). NADPH dependent GR maintains the reaction by reducing GSSG back to GSH (Figure 5.3).

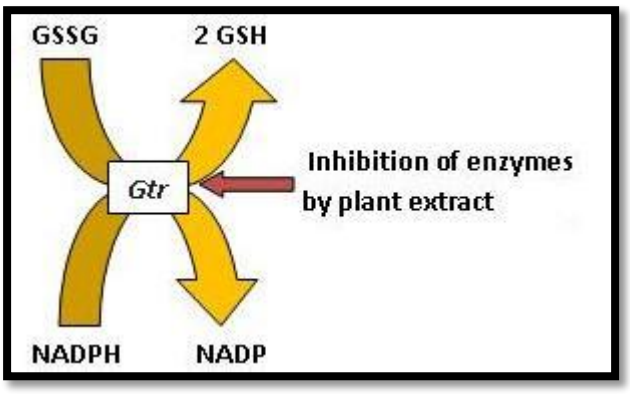


Figure 5.3: Enzyme reaction of glutathione disulphide (GSSG) reduced to glutathione (GSH) in NADPH dependent manner by glutathione reductase (GR) enzyme

The inhibition of ethanol extract of *S. jambos* on the activity of glutathione reductase (GR) was carried out at room temperature following a previous method with slight modifications (Mahapatra et al., 2007). The reaction mixture consisted of 50 mM Hepes (pH 7.6), 0.1 nM EDTA containing Gtr (0.2268 units), NADPH (140 µM) with varying concentrations of plant extract. The percentage of DMSO was kept constant (2% (v/v)) in all assay mixtures. GR was pre-incubated with NADPH for 5 min before initiating the reaction with oxidised glutathione (1mM). The activity of enzyme was monitored by decrease in absorbance at 340 nm due to consumption of NADPH. The IC_{50} (concentration of plant extract when fifty percent of enzyme activity is inhibited) value was determined by analysis of dose-dependent sigmoidal curve.

5.3 Results and discussions

5.3.1 Identification of isolated compounds

The structure elucidation of the isolated compounds was established on the basis of physical and spectroscopic techniques, especially NMR spectra and direct comparison with spectroscopic measurements to published literature values. The compounds isolated from the ethanol extract of leaves of *S. jambos* were identified as squalene (**1**; yellow oil; m.p. -75°C) (Tchinda et al., 2006) and ursolic acid (**3**; Greenish powder; m.p. 284-286 °C) (Guvenalp et al., 2006). Compound **2** was obtained as pale yellow liquid. The UV spectrum of compound **2** exhibited maximum absorption (λ_{max}) at 243 and 302 nm and in the IR spectrum showed absorption bands at 3448 and 1617 cm^{-1} indicating the characteristics of hydroxybenzoic acid (Yalpini and Tyman, 1983). The ^{1}H NMR spectrum of

compound **2** showed a triplet at δ 7.40 (J= 8.2), and two doublets at 6.90 (J= 8.2), 6.80 (J= 7.2), and the characteristic signals for a 1, 2, 6-trisubstituted benzene ring. Additionally, the ^{13}C NMR spectra showed the signals at δ 176.3 (COOH), 163.5 (C-6), 147.7 (C-2), 135.3 (C-4), 122.7 (C-3) 115.8 (C-1) and 110.3 (C-5), also supported the presence six carbons and a carboxylic group constituting the backbone, although a few signals indicating the presence of long aliphatic chain could not be established. There are many anacardic acids that have been reported by previous researchers which differ in the length of side chain (Liua and Abreu, 2006; Kubo et al., 1993). Therefore, in the current study compound **2** is being reported as anacardic acid analogue with a side chain R. The ethanol leaves extract of *S. jambos* exhibited significant antibacterial activity against *P. acnes*; therefore, it was decided to acquire three commercially known compounds namely, myricetin **(**yellow needles; m.p. 356-358 °C), myricitrin (white *powder*; m.p. 206-208 °C) and gallic acid (colourless crystals; m.p. 212-214 ºC) which have been previously isolated from 90% ethanol extract of *S. jambos* (Jayprakasham, 2010), to investigate if any of these compounds contribute towards the total extract activity. The chemical structures of all the compounds are illustrated in Figure 5.4.

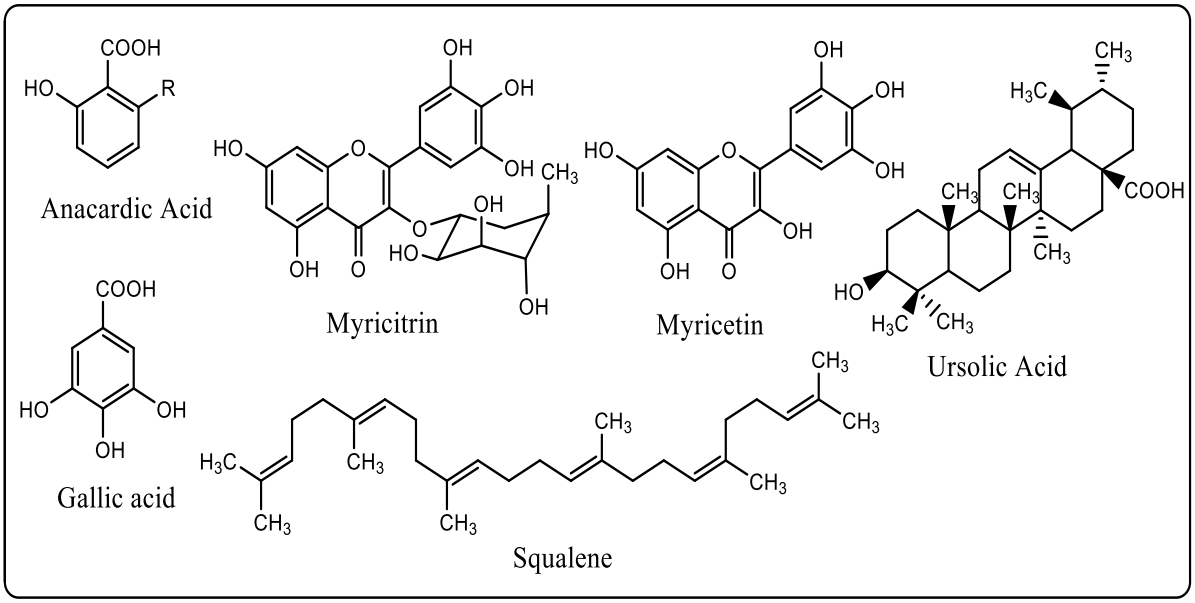


Figure 5.4: The chemical structures of the compounds from *Syzygium jambos*: Anacardic acid analogue, myricitrin, myricetin, ursolic acid, gallic acid and squalene

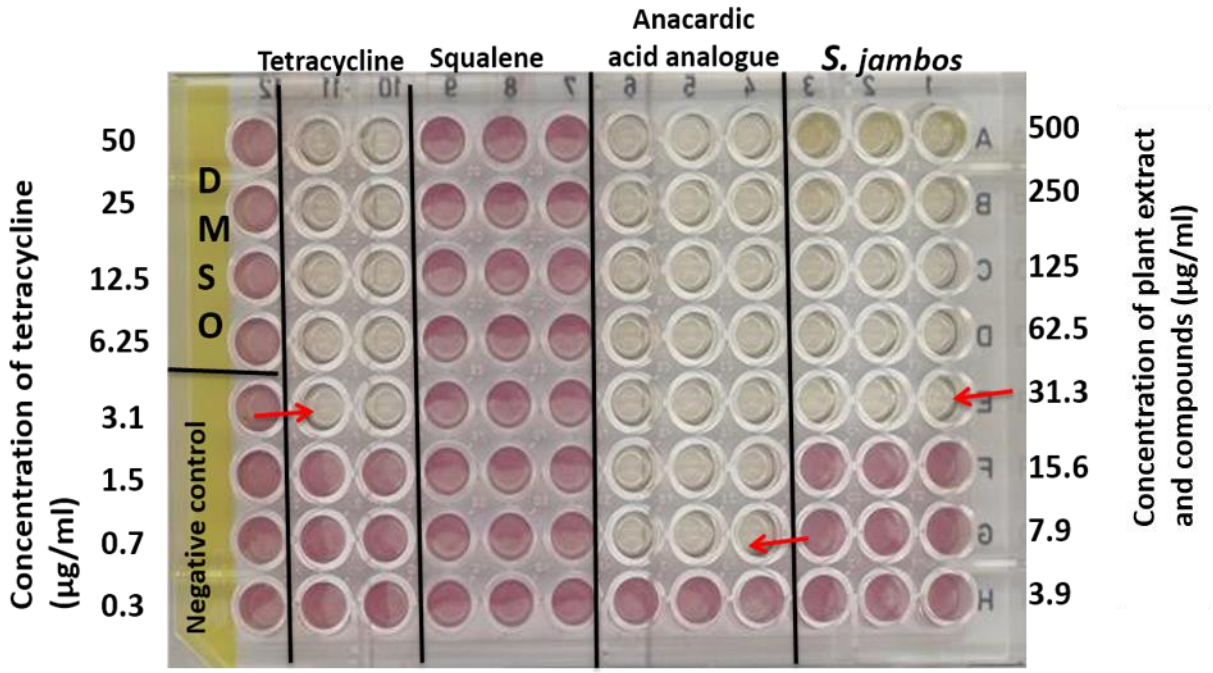
The present study reports the isolation of squalene, ursolic acid and analogue of anacardic acid for the first time from *S. jambos*. The presence of the compounds discussed in this study is known since antiquity. Ursolic acid was first time reported in 1920 from epicuticular waxes of apple (Belding et al., 1998). Squalene was first reported in 1917 from shark liver oil by Mitsumaru Tsujimoto (Tsujimoto, 1917). Myricetin and myricitrin was first isolated from the bark of *Myrica nagi* and subsequently was

reported in leaves of *Rhus coriaria*, *Myrica gale*, *Pistachia lentiscus* and *Haematoxylon campeachianum* (Perkin, 1902; Wurdack, 1924). Gallic acid was initially found in the nutgalls of *Rhus toxicodendron* and later in the leaves of the same plant by M. Aschoff and M. Bracconnot in early 19th century (Breuster, 1837). Anacardic acid was first reported from shells of cashew nuts by Stadler in 1847 (Harvey and Caplan, 1940).

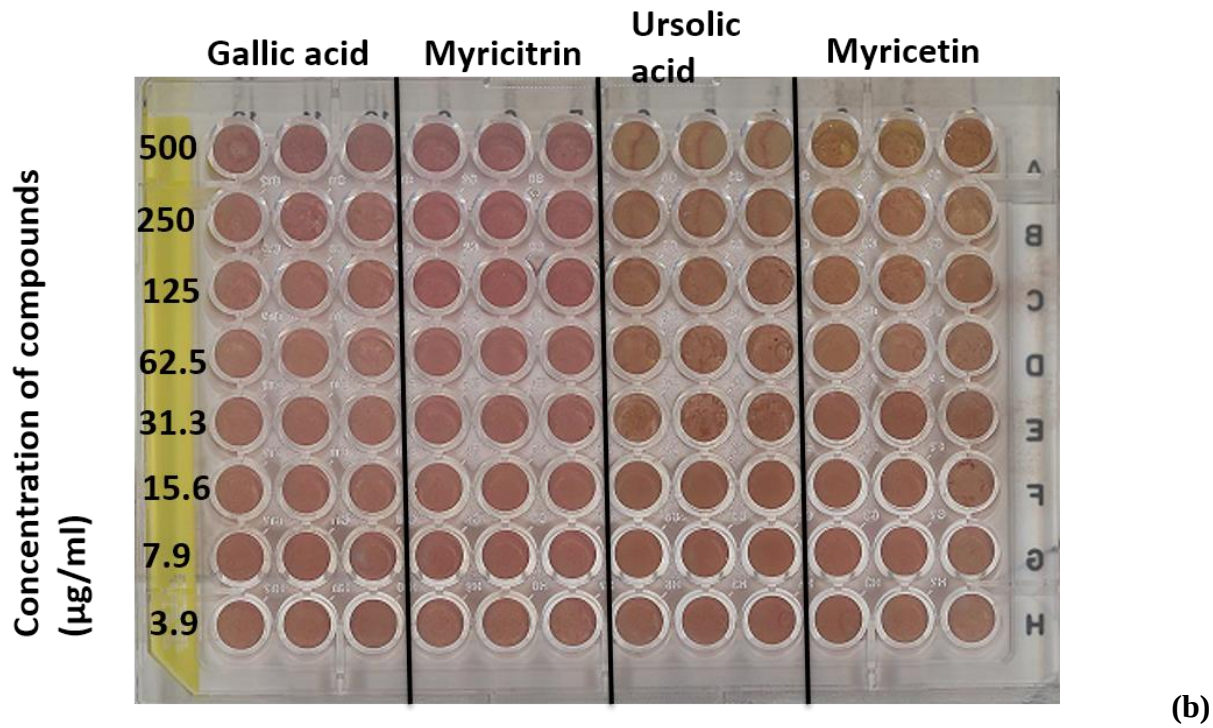
5.3.2 Antibacterial bioassay

The MIC value of the ethanol extract *S. jambos*, twelve MF and isolated compounds are listed in Table 5.1.The ethanol extract of *S. jambos* inhibited bacterial growth and exhibited a noteworthy MIC value of 31.25 µg/ml. Anacardic acid analogue was found to be the most active compound against *P. acnes* at MIC value of 7.81 µg/ml as compared to tetracycline (positive control) which exhibited the MIC value of 3.12 µg/ml. Compounds squalene, ursolic acid, myricetin, myricitrin and gallic acid did not show any inhibitory activity at the highest concentration tested (500 µg/ml) (Figure 5.5 a, b).

To the best of our knowledge, the antibacterial activity of *S. jambos* and the compounds (squalene, ursolic acid, myricetin, myricitrin and gallic acid) against *P. acnes* is reported for the first time. In the present study anacardic acid analogue significantly inhibited the growth of *P. acnes*. Our results corroborate well with previous investigations where reports regarding antibacterial activity of a series of synthetic anacardic acids possessing different side-chain lengths against *P. acnes* were found. Four anacardic acids namely, pentadecatrienyl salicylic acid, pentadecadienyl salicylic acid, pentadecenyl salicylic acid and pentadecyl salicylic acid were reported to be active against *P. acnes* with MIC value of 0.78 µg/ml, very similar to our results (Kubo et al., 1993). In another study, ursolic acid showed MIC value of 4 µg/ml against vancomycin-resistant enterococci (Horiuchi et al., 2007), myricetin inhibited the growth of methicillin-resistant *S. aureus*, *Burkholderia cepacia* and *K. pneumoniae* (Xu and Lee, 2001), different strains of *Staphylococcus epidermidis* exhibited sensitivity towards myricitrin (Pistelli et al., 2009) and gallic acid showed growth inhibitory behaviour on *S. aurens*, *Bacillus cereus*, *E. coli* and *Candida albicans* (Panizzi et al., 2002). No scientific report on the antimicrobial activity of squalene was found in the literature.



(a)



(b)

Figure 5.5: The 96 well plates showing antibacterial activities against *Propionibacterium acnes* and MIC values (red arrow) of (a) ethanol extract of *Syzygium jambos* (31.25 µg/ml), anacardic acid analogue (7.81 µg/ml), squalene (not active at 500 µg/ml) and drug control tetracycline (3.12 µg/ml); (b) myricetin, ursolic acid, myricitrin, gallic acid (not active at 500 µg/ml)

Table 5.1: Antibacterial, antioxidant and cytotoxic effects of ethanol extract of *Syzygium jambos*, fractions and isolated compounds

Test samples	Antibacterial	Antioxidant		Cytotoxicity	
	[a]MIC µg/ml	[b]EC_{50} µg/ml/(**µM**)	Mg Vit C equivalents/g dry weight	B16-F10 mouse melanoma	U937 human macrophage
				EC_{50} µg/ml/(**µM**)	
Syzygium jambos	31.25	0.9	450	60	440
[c]MF 1,3,5,7-12	[d]Not active	[e]-	-	-	-
MF 2	500	-	-	-	-
MF 4	62.5	-	-	-	-
MF 6	250	-	-	-	-
Squalene (**1**)	Not active	>100	-	>100	>100
Anacardic acid analogue (**2**)	7.81	>100	-	[f]Not tested	57.8
Ursolic acid (**3**)	Not active	>100	-	Not tested	38/(**83**)
Myricetin	Not active	0.9/(**3**)	2105	11/(**35**)	19/(**60**)
Myricitrin	Not active	1.8/(**4**)	1081	259/(**557**)	318/(**684**)
Gallic acid	Not active	0.8/(**4.8**)	2444	2.2/(**13**)	28/(**169**)
[g]PC	3.12/(**7**)	2/(**11**)	-	4.5 x 10^{-3}/(**3.5 x 10^{6}**)	4.5 x 10^{-3}/(**3.5 x 10^{6}**)

[a]MIC: minimum concentration of sample that inhibits bacterial growth; [b]EC_{50}: concentration at which 50% DPPH radicals are scavenged (for antioxidant)/ 50% cells are viable (for cytotoxicity); [c]MF: major fractions; [d]not active at the highest concentration tested (500 µg/ml); [e]not applicable: for MF as not tested for antioxidant activity and cytotoxicity, mg equivalent could not be detected for non-antioxidant compounds; [f]not tested due to low yield of the compound; [g]positive drug controls where tetracycline for antibacterial, vitamin C for antioxidant, actinomycin D for cytotoxicity

5.3.3 Transmission electron microscopy (TEM)

For the microscopy studies, the plant extract and isolated compound anacardic acid analogue, which showed inhibitory activity against the bacteria, were selected. The TEM micrograph showed clear differences between untreated and treated *P. acnes*. The untreated *P. acnes* had a distinct cell wall which was long, spindle shaped, smooth and lined with cell membrane. A centrally located nucleoid surrounded by ribosomes was observed (Figure 5.6 a). The TEM micrograph showed cell injury caused to *P. acnes* after treatment with ethanol extract of S. *jambos* for 72 h. *P. acnes* showed breaks in the cell wall when treated with an ethanol extract of *S. jambos* at 100 µg/ml (Figure 5.6 b) while a complete loss of cell wall was also observed at a higher concentration of 300 µg/ml (Figure 5.6 c).The TEM micrographs of *P. acnes* treated with anacardic acid analogue at a concentration of 50 µg/ml showed abnormal changes in cell content material such as shrinkage of intracellular inclusions and the hollow appearance of the bacteria. The outer membrane was found to be irregular and distortions in the cell structure were observed (Figure 5.6 d). Tetracycline at a concentration of 50 µg/ml caused significant damages to the cells of *P. acnes*, leading to damages in the cell membrane, distortion in the cell structure and shrinkage of cell content material (Figure 5.6 e). DMSO at 2.5% exhibited no lethal effects to bacteria (Figure 5.6 f). The TEM micrograph confirms the antibacterial activity of *S. jambos* and bioactive compound (anacardic acid analogue) against *P. acnes.*

5.3.4 Antioxidant assay

The DPPH assay indicated the free radical scavenging properties of the samples. Antioxidants are able to stabilize the free DPPH radicals due to their proton donating ability. The scavenging effect of the *S. jambos* ethanol extract and the compounds (myricetin, myricitrin and gallic acid) on DPPH increased with increasing concentrations. These samples showed significant antioxidant activity with EC_{50} values ranging between 0.7-1.9 µg/ml, very similar to that of Vitamin C, a widely used antioxidant compound exhibiting an EC_{50} value of 2 µg/ml (Figure 5.7). The isolated compounds (squalene, anacardic acid analogue and ursolic acid) did not exhibit any radical scavenging activity at highest concentration tested (100 µg/ml). The results are summarised in Table 5.1. Our results were in agreement with previous reports. The DPPH radical scavenging activity of *S. jambos* has been reported previously with IC_{50} value of 14.10 µg/ml (Islam et al., 2012) and can be explained due to the presence of flavonoids and polyphenol compounds namely, myricetin, myricitrin and gallic acid. The antioxidant activity of

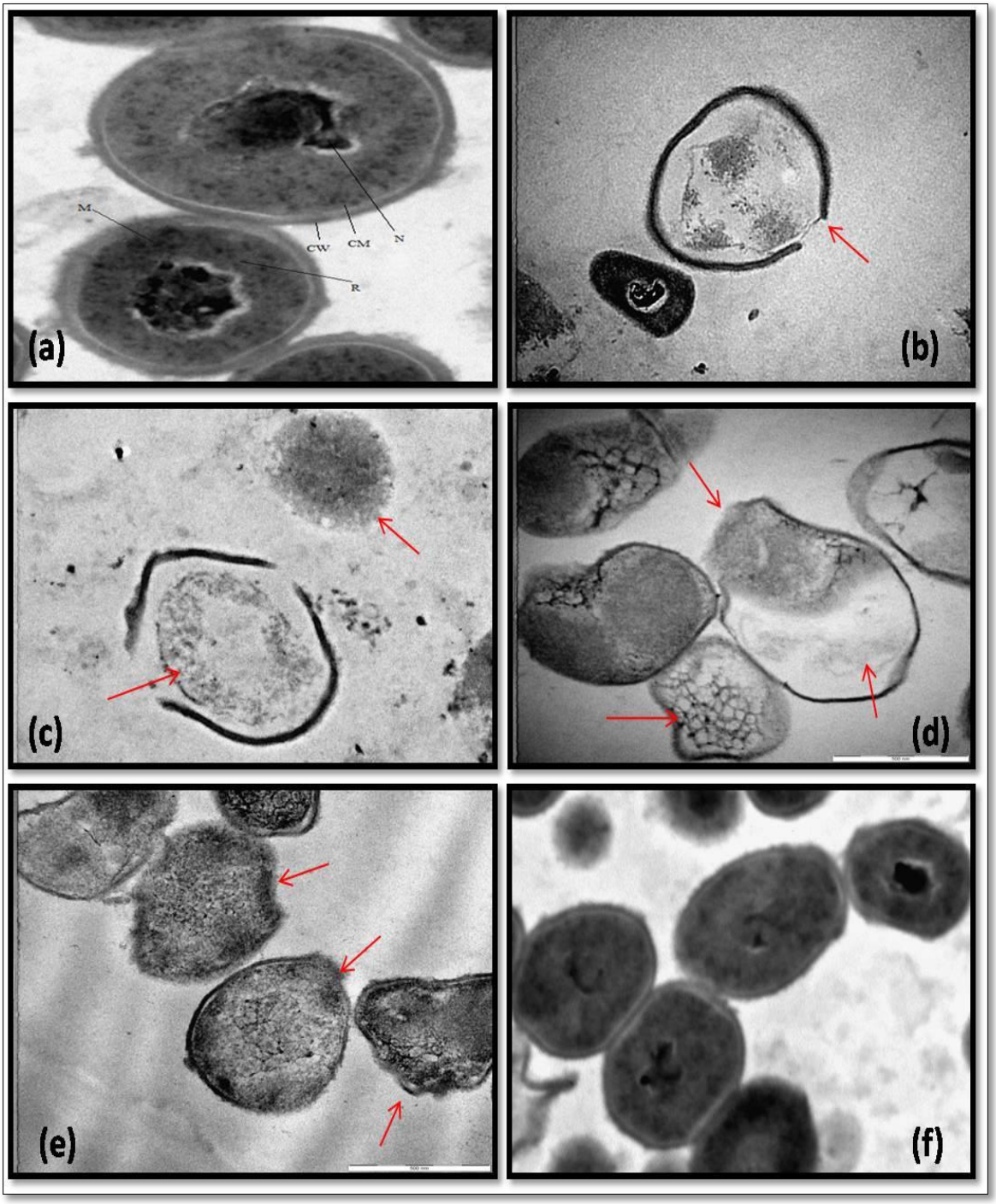
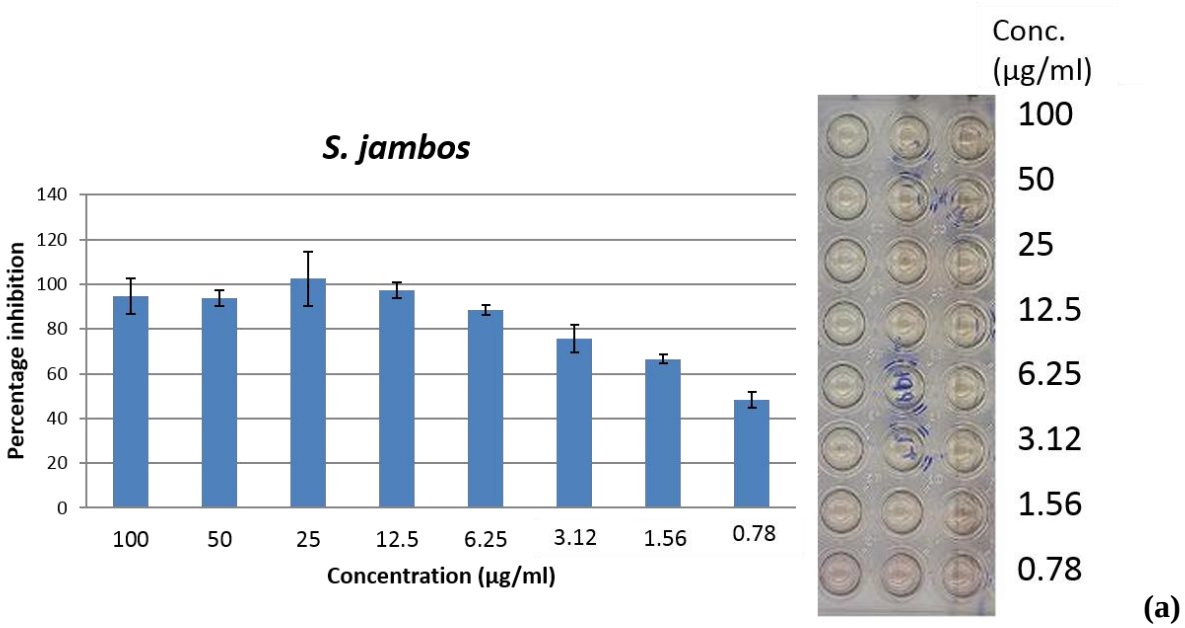
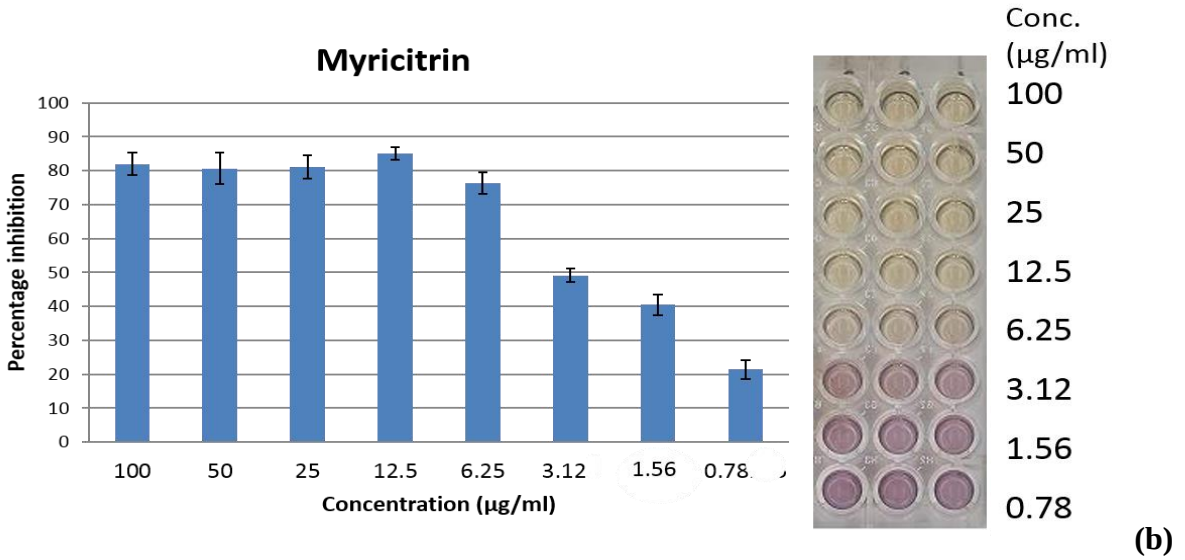


Figure 5.6: Transmission electron micrograph of a thin section of *Propionibacterium acnes* at 60 K magnification: (a) untreated bacteria, labelled structures: cell wall (CW); cytoplasmic membrane (CM); nucleoid (N); ribosomes (R); mesosomes (M); (b) *P. acnes* treated with *Syzygium jambos* at 100 µg/ml; (c) *P. acnes* treated with *S. jambos* at 300 µg/ml; (d) *P. acnes* treated with anacardic acid analogue at 50 µg/ml; (e) *P. acnes* treated with positive control (tetracycline) at 50 µg/ml; (f) *P. acnes* treated with solvent (DMSO at 2.5%). The arrows indicate cell injuries to the *P. acnes*

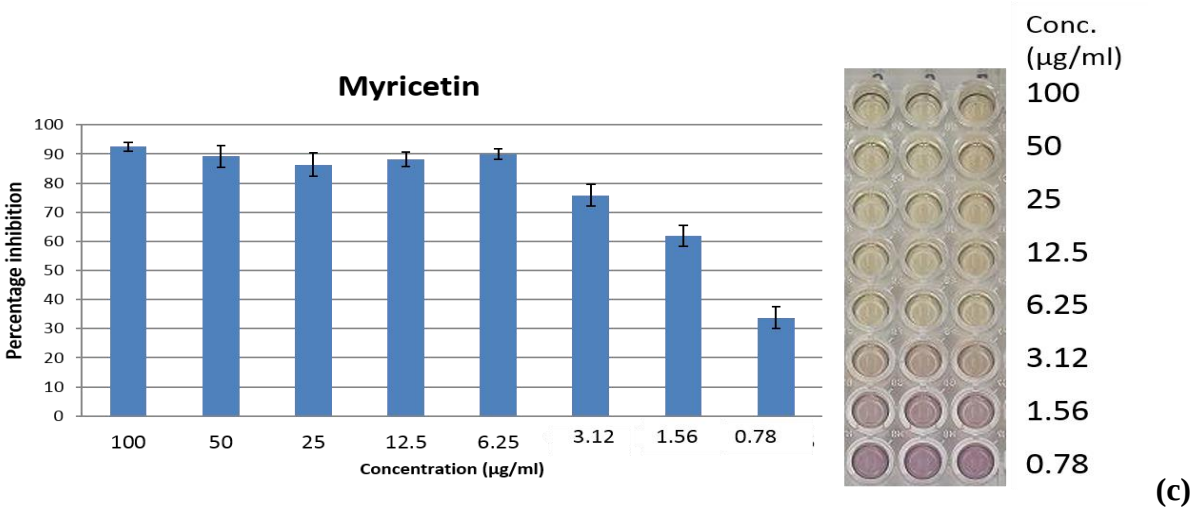
flavonoids and polyphenols is ascribed to the presence of free hydroxyl (-OH) substitutes. Myricetin possess six free hydroxyl radicals at 3, 5, 7, 3′, 4′, 5′ carbon positions and gallic acid possess three free hydroxyl radicals at 3, 4, 5 carbon positions. In the current study, ursolic acid did not demonstrate any antioxidant activity even at its highest concentration of 100 µg/ml. Similar to our findings, another researcher found ursolic acid to be inactive in inhibiting the generation of free radicals at concentrations of 0.25 and 0.5 mg/ml (Jung et al., 1999). In our study squalene did not show any DPPH free radical scavenging activity and this can be explained as it is a single oxygen scavenger and lacks free hydroxyl groups, therefore cannot scavenge a DPPH radical (Ko et al., 2002). In the current study, no antioxidant activity for anacardic acid analogue was found. Similar to our findings,



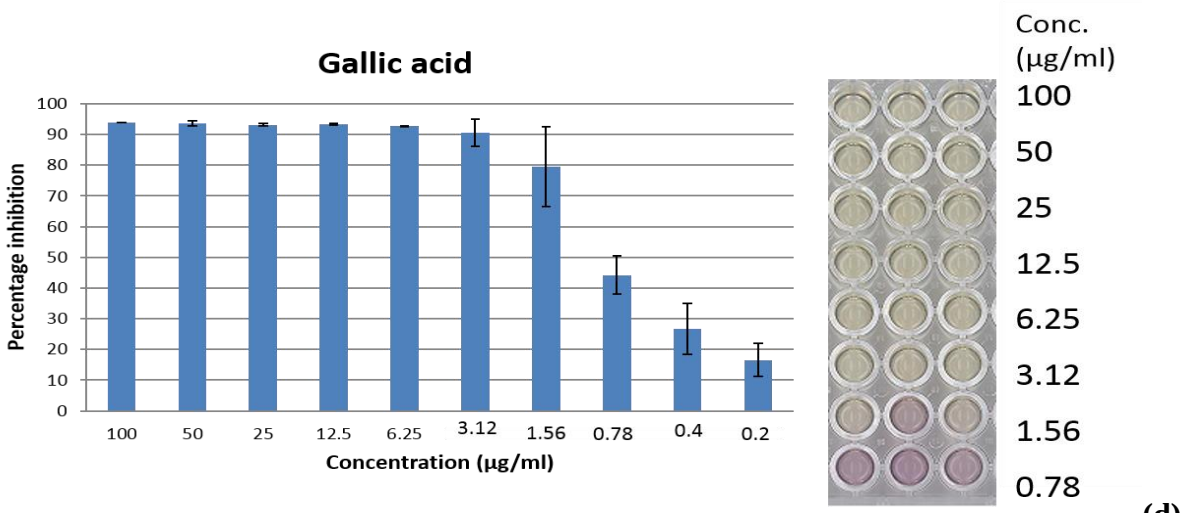
(a)



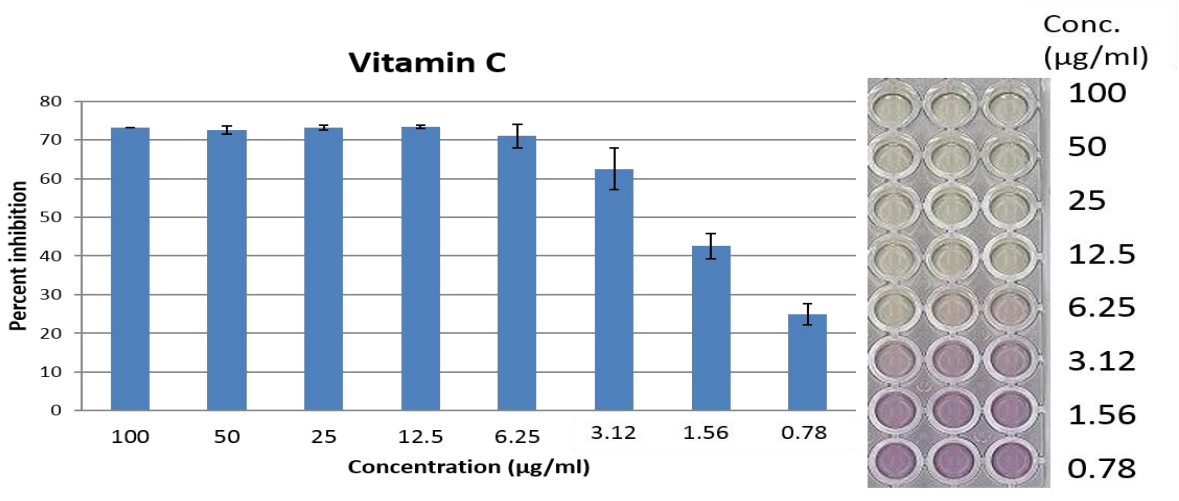
(b)



(c)



(d)



(e)

Figure 5.7: DPPH radical scavenging activity of (a) *Syzygium jambos* (EC_{50} 0.9 µg/ml); (b) Myricitrin (EC_{50} 0.9 µg/ml); (c) Myricetin (EC_{50} 1.8 µg/ml); (d) Gallic acid (EC_{50} 0.8 µg/ml) and (e) Vitamin C positive control (EC_{50} 2 µg/ml)

6-pentadecenylsalicylic acid isolated from *Anacardium occidentale* did not exhibit notable DPPH radical scavenging activity (Kubo et al., 2006).

5.3.5 *In vitro* cytotoxicity assay

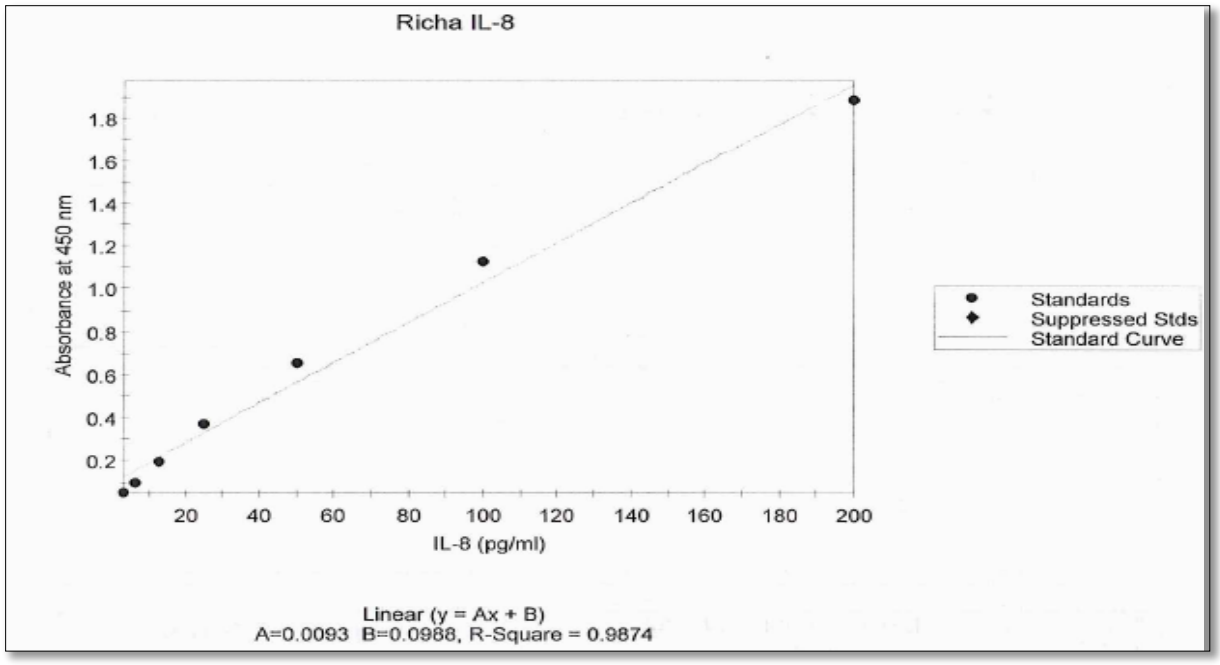
The cytotoxicity assay of the extracts and the compounds was done on B16-F10 mouse melanoma and U937 human macrophage cells. Due to the low yield, no further test could be done on the isolated compound anacardic acid analogue. All the results are listed in Table 1. To the best of our knowledge, the cytotoxicity results of ethanol extract of *S. jambos* and the compounds (squalene, ursolic acid, myricetin, myricitrin and gallic acid) on the cell viability of B16-F10 and U937 cells obtained in the present study are reported for the first time. *S. jambos* exhibited moderate toxicity to B16-F10 cells and no toxicity to U937 cells. In contrary, previous studies have reported strong cytotoxic effects of 70% acetone extract of *S. jambos* on human leukemia cells (HL-60) with an IC_{50} value of 10.2 µg/ml (Yang et al., 2000). In the current study, squalene was not found to be toxic on both the cell lines with 100% viability of cells even at the highest concentration of 100 µg/ml tested. Similar to our findings, squalene was reported to be non toxic to human mammary epithelial cells (MCF10A) (Warleta et al., 2010). In the present study, ursolic acid was found to be moderately toxic to U937 cells. Contrary to our findings, in a study, significant cytotoxic effects of ursolic acid against lymphocytic leukemia cells (P 388 and L 1210), human colon cells (HCT 8) and mammary (MCF 7) tumour cells were reported (Lee et al., 1988). Myricetin and gallic acid showed significant toxicity to B16-F10 cells and moderate toxicity on U937 cells. Similar to our results, myricetin showed strong toxicity to human A549 lung cells (Lu et al., 2006) and gallic acid was not reported to be toxic on human lymphocytes derived from fresh blood (Yen et al., 2002). In our study myricitrin did not show any toxicity to either of the cell lines and similar results are reported in a study where myricitrin was not found to be toxic on murine fibro sarcoma (L929) cells (Hsu et al., 2006).

5.3.6 Anti-inflammatory activity

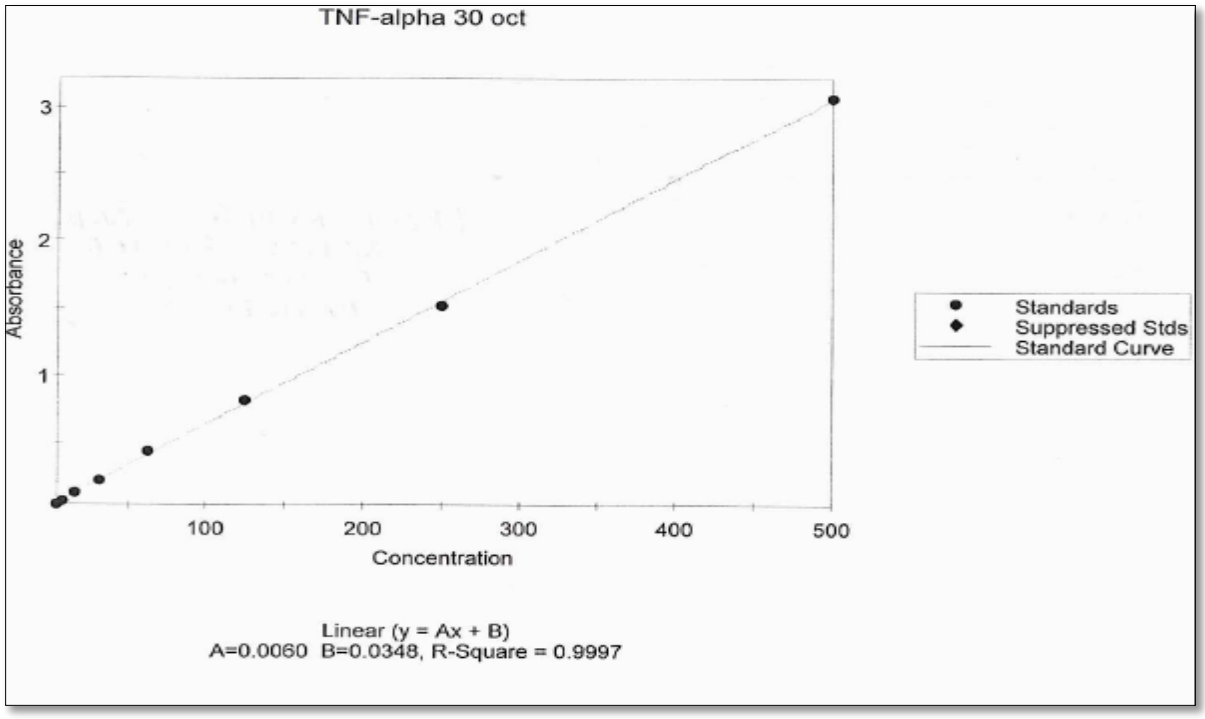
5.3.6.1 Effect of ethanol extract of Syzygium jambos and its compounds on the pro-inflammatory cytokines

P. acnes contribute to the inflammatory nature of acne by inducing macrophages to secrete pro-inflammatory cytokines like IL-8 and TNF-α. In the current study, U937 cells treated with heat-killed

P. acnes resulted in an increase in the secretion of IL-8 and TNF-α (Figure 5.9). These results confirmed that *P. acnes* are capable in eliciting the inflammatory response which plays an important role in acne pathogenesis. The standard graph for IL-8 and TNF-α are shown in Figure 5.8 with R-square value as 0.9.



(a)



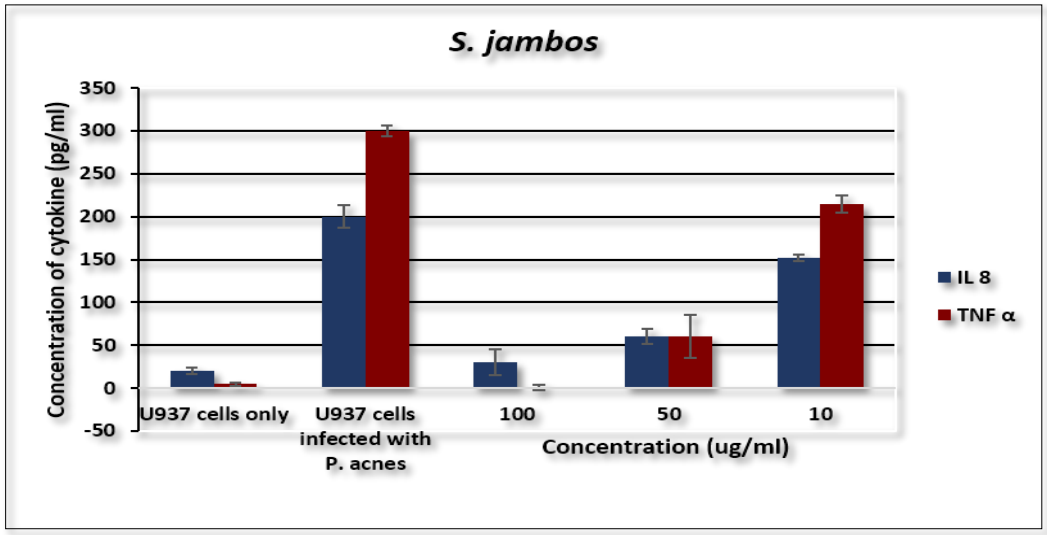
(b)

Figure 5.8: Standard curve of (a) IL-8 and (b) TNF-α developed by using ELISA method

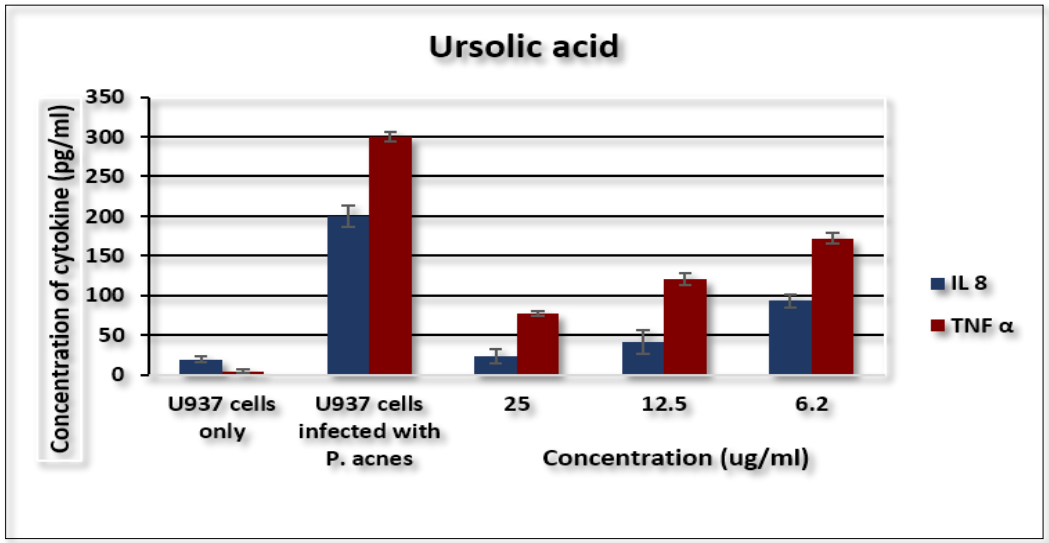
To test the anti-inflammatory effects of *S. jambos* and its compounds, *in vitro* screening at three non-cytotoxic concentrations of the samples to the cells were applied. As shown in Figure 5.9 a-e, the ethanol extract of *S. jambos* and the compounds (ursolic acid, myricetin, myricitrin and gallic acid) decreased the production of IL-8 and TNF-α in a dose-dependent manner. A significant inhibition of IL-8 and TNF-α was observed for the *S. jambos* extract, ursolic acid and myricitrin at their highest concentrations. Gallic acid, although suppressed TNF-α drastically, had no significant suppression of IL-8 at its highest concentration. Myricetin moderately decreased the release of TNF-α and showed low inhibition of IL-8 at the highest concentration tested. Pentoxifylline, which was used as a control, behaved differently on the cytokines. Based on previous reports, it down regulated the secretion of TNF-α and caused no change in the IL-8 release (D' Hellencourt et al., 1996). As shown in Figure 6.6 (b), our results corroborate well with previous investigations. Very high inhibition of TNF-α was observed at 100 and 50 µg/ml of pentoxifylline whereas no significant change in IL-8 concentration was observed. Furthermore, the test samples did not increase the secretion of the cytokines in the culture of U937 cells in the absence of the heat killed *P. acnes*. Other researchers have previously reported the anti-inflammatory potential of the samples isolated in this study. Myricetin was reported to inhibit the release of IL-8 and TNF-α from human umbilical cord blood-derived cultured mast cells (Kempuraj et al., 2005), myricitrin and myricetin suppressed TNF-α production in LPS/IFN-γ stimulated J774.A1cell line (Ferreria et al., 2013).

Gallic acid inhibited the production of IL-8 and TNF-α from *Fusobacterium nucleatum* activated human mouth epithelial cell line and human mast cells, respectively (Kang et al., 2009; Kim et al., 2006). Ursolic acid inhibited IL-8 secretion from HT29 cells (Thuong et al., 2005). To the best of our knowledge, no reports about *S. jambos* in context with suppression of cytokines were found. Although, similar to our results, other plants such as *Eucommia ulmoides* and *Ilex paraguariensis* extracts were reported to reduce the secretion of IL-8 and TNF-α in human monocytic THP-1 cells pre-treated with *P. acnes* at concentration of 0.1 mg/ml (Tsai et al., 2010).

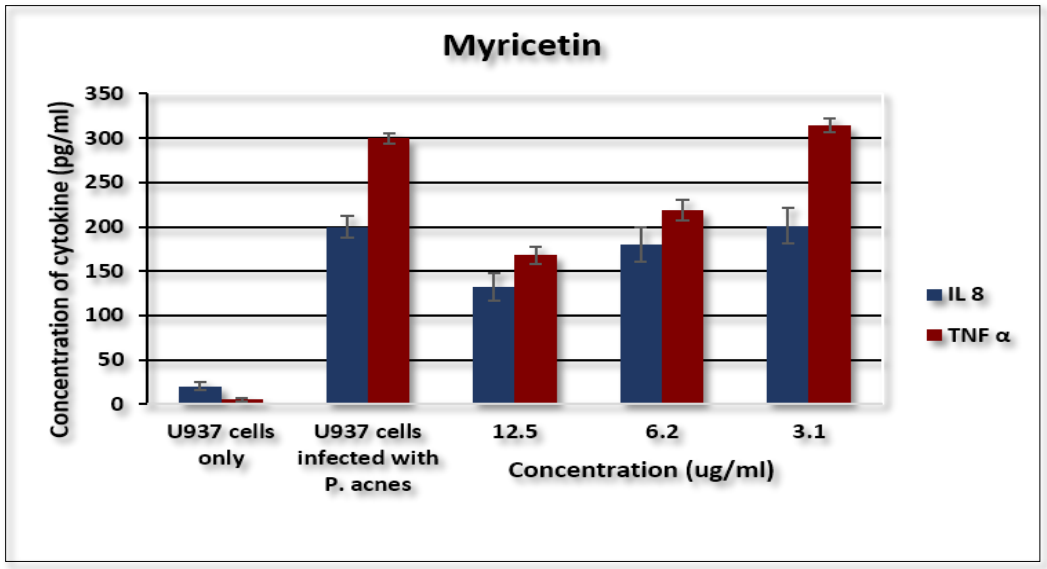
The anti-inflammatory activity and release of cytokines like IL-8 and TNF-α is linked with an inflammatory mediator nuclear factor-kappa B (NF-κB). NF-κB is a transcription factor that resides in the cytoplasm of every cell and its constitutive activation is linked with *P. acnes* infection. The suppression of the cytokines discussed in this study can possibly be due to blocking activation of common transcription factor such as NF-κB involved in their induction.



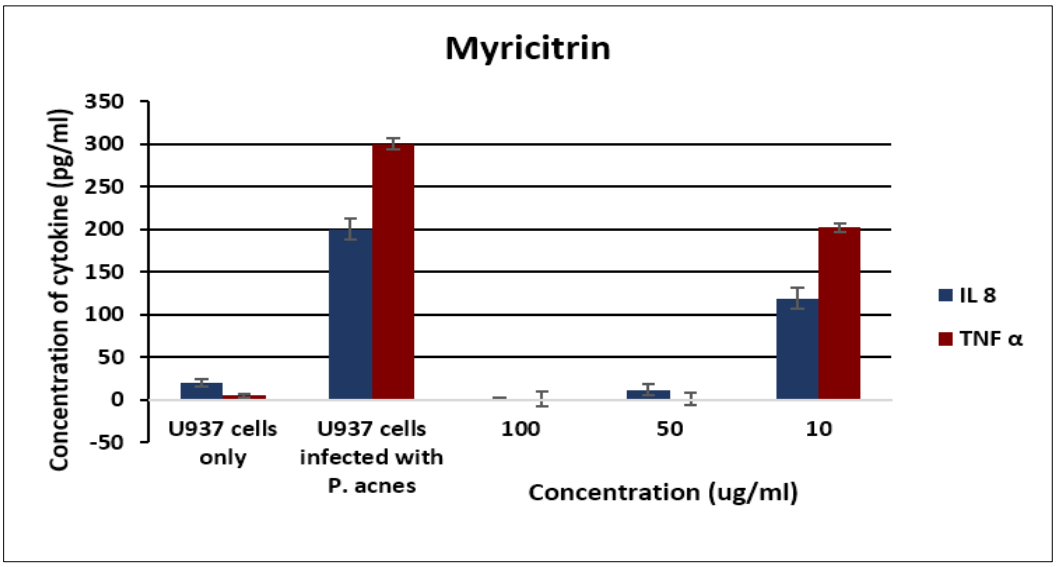
(a)



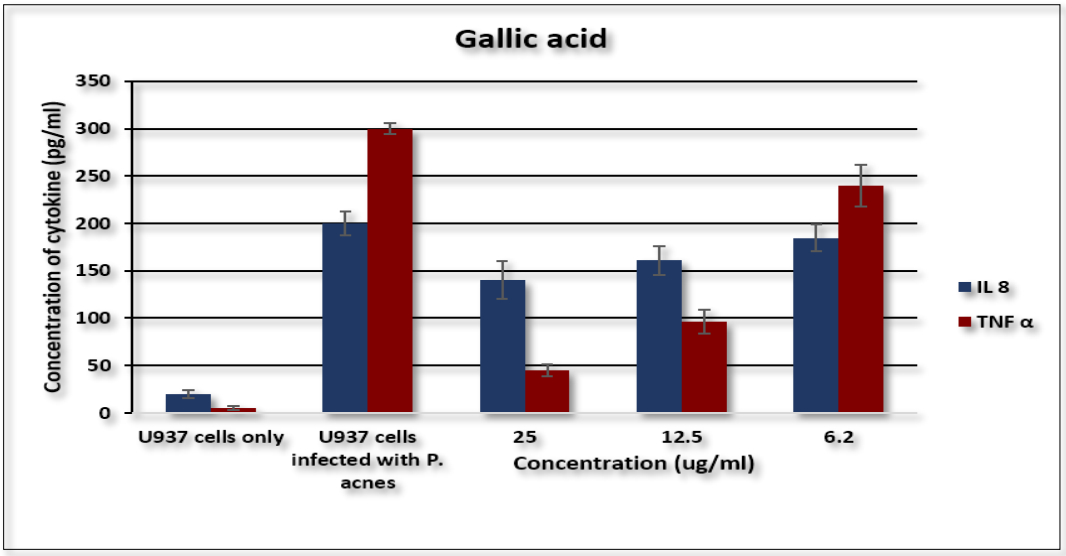
(b)



(c)



(d)



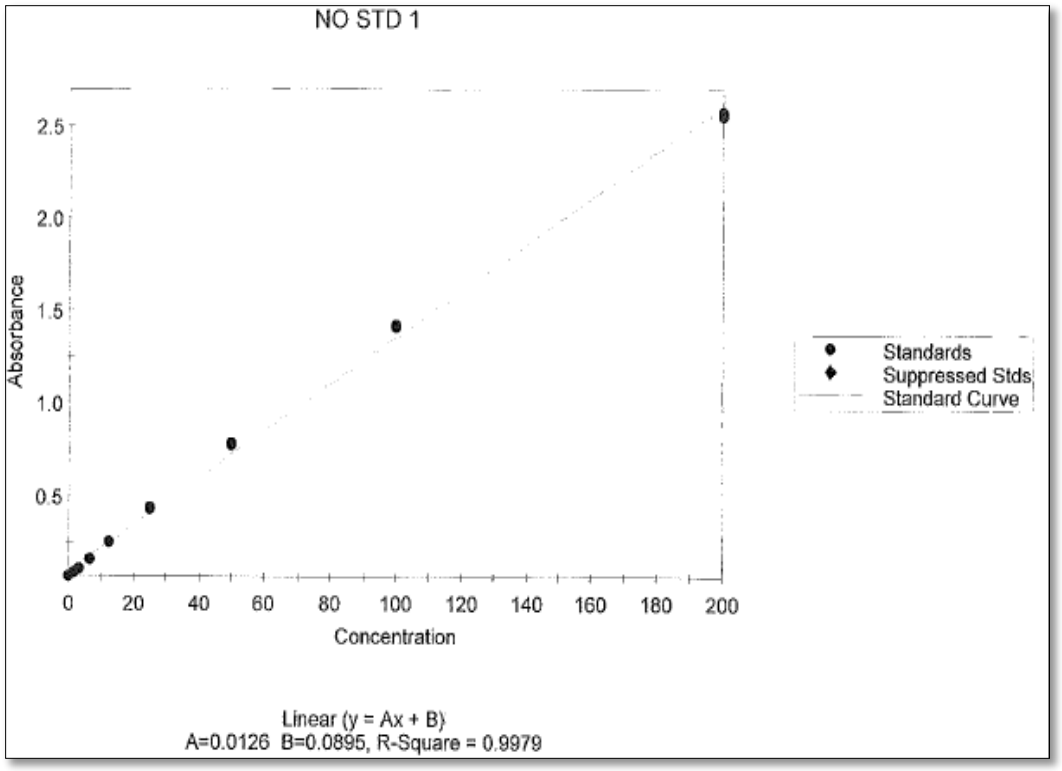
(e)

Figure 5.9: Dose-dependent inhibition of IL-8 and TNF-α by (a) ethanol extract of *Syzygium jambos*; (b) Ursolic acid; (c) Myricetin; (d) Myricitrin and (e) Gallic acid

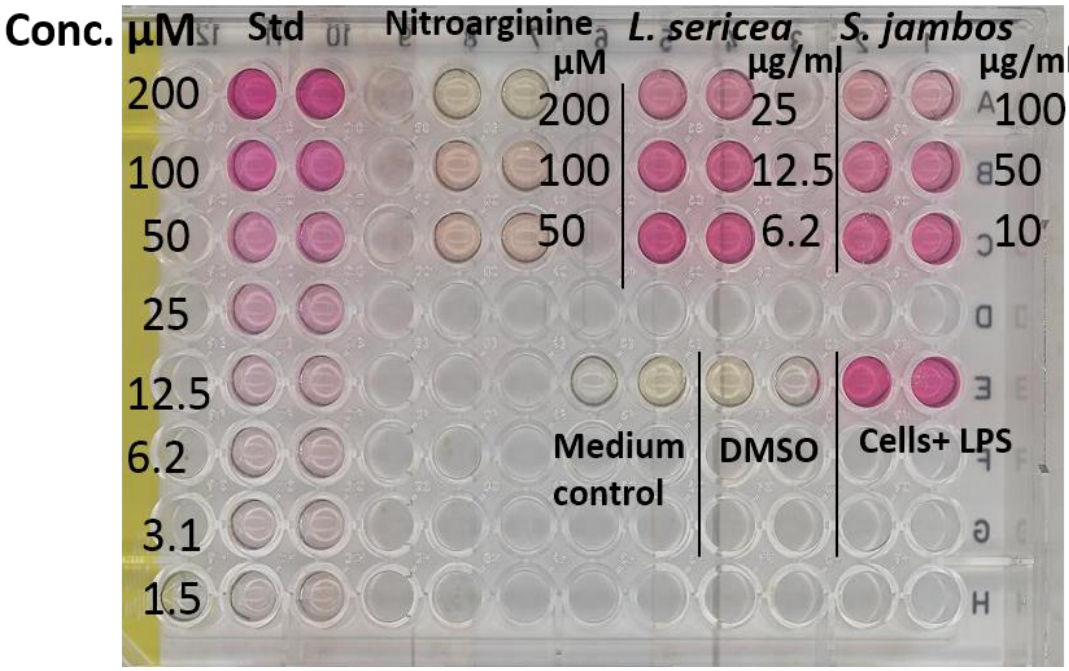
5.3.6.2 Suppression of Nitric oxide

The standard graph was plotted by preparing a gradient of the standard solution provided in the kit from 200 to 1.5 µM (Figure 5.10 a, b). The mouse derived macrophages when stimulated with LPS triggered the release of Nitric oxide. The ethanol extract of *S. jambos* dose dependently suppressed the production of Nitric oxide (Figure 5.10 c). However, the solvent and cells alone did not showed any production of Nitric oxide. The positive control significantly inhibited Nitric oxide at all the concentration tested.

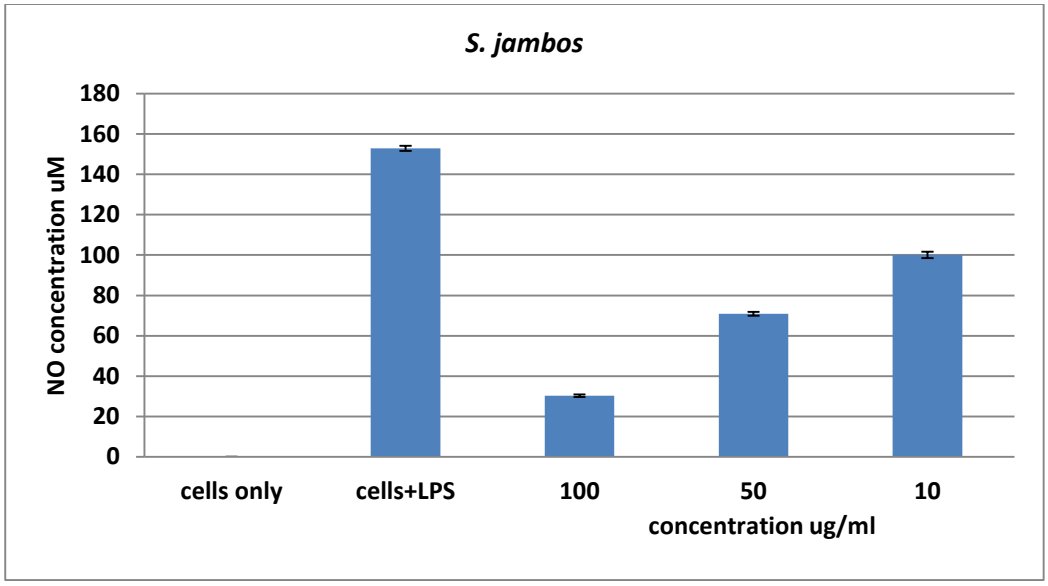
Nitric oxide is a messenger molecule that plays an important role in orchestrating normal regulatory processes of the skin. Nitric oxide is synthesized by an intracellular enzyme, NO synthase (NOS). The activity of NOS is enhanced in response to skin wounding or infection which appears to be important in directing the infiltrated defence cells and initiate the inflammation.



(a)



(b)



(c)

Figure 5.10: (a) Standard graph of nitric oxide (NO) as developed using nitrite/nitrate kit; (b) A microtitre plate with supernatents treated with griess reagents for the detection of Nitric oxide along with standard dilutions and (c) dose dependent suppression on Nitric oxide by ethanol extract of *Syzygium jambos*

5.3.7 Enzyme assay

The ethanol extract of *S. jambos* dose dependently decreased the activity of GR enzyme with IC_{50} value of 10.4 µg/ml. The sigmoidal graph shown in Figure 5.11 depicts an increase in percentage activity of GR at lower concentrations of plant extract and at higher concentrations; the percentage activity of GR was reduced. The inhibitory activity of extract on GR enzyme might be due to blocking of active sites of GR enzyme by compounds present in the extract. This is the first report of plant *S. jambos* inhibiting glutathione reductase enzyme. No reports in the literature regarding the GR enzyme and plant extracts were found.

The role of glutathione is indirectly linked with acne vulgaris. Glutathione constitutes first line cellular defence against oxidative injury. Also, intracellular glutathione in macrophages is important for the release of various cytokines which may play role in host defence during infection of *P. acnes* (Murata et al., 2002). Some studies have reported relation between severity of acne vulgaris and glutathione enzyme levels. A significant decreased level of glutathione was reported in the patients with acne vulgaris (Aybey et al., 2005).

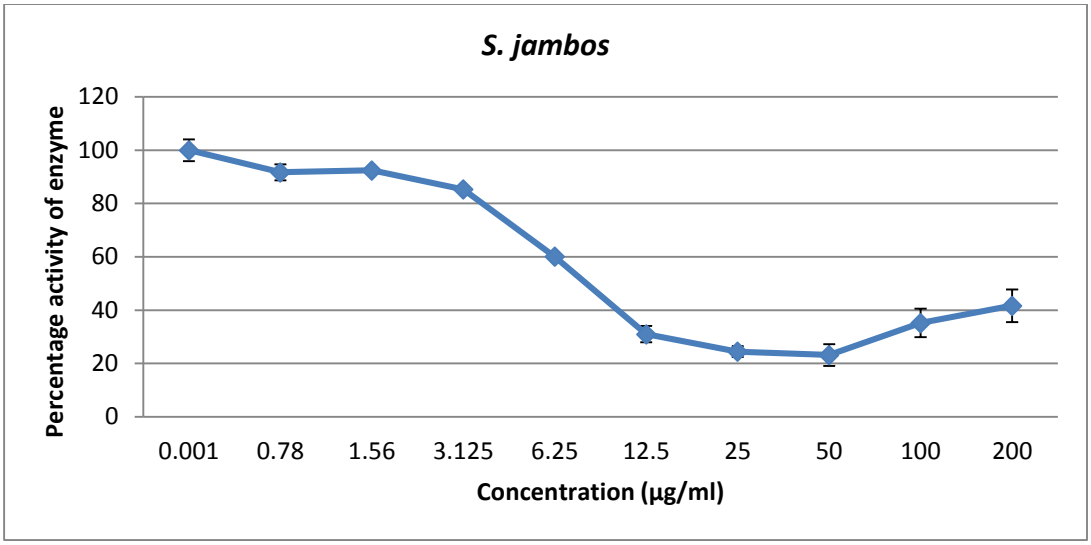


Figure 5.11: A dose dependent graph showing decrease in the activity of glutathione reductase (GR) with increasing concentration of ethanol extract of *Syzygium jambos* (IC_{50} 10.4 µg/ml)

5.4 Conclusion

The present study provided an important insight on the plants and compounds to be promising source of alternative medicine. Effective anti-acne agents possess three essential capabilities of antibacterial, antioxidant and anti-inflammatory activities. The experimental data gathered in this study provided an important insight on the plant *S. jambos* which might be due to the synergistic action of compounds present in it. Additionally, the plant extract was not found to be toxic to human cells. Therefore, *S. jambos* could be an ideal concomitant for an alternative anti-acne agent. This study will be helpful to understand this important herbal medicine and further clinical trials are under way.

5.5 References

Adjanohoun, E.J., 1989. Contribution auxetudes ethnobotaniques et floristiques en Republique Populaire du Benin. Medecine traditionnelle et pharmacopee. Agence de cooperation culturelle et technique, France.

Arican, O., Kurutas, E.B., Sasmaz, S., 2005. Oxidative stress in patients with acne vulgaris. Mediators of Inflammation, 6, 380-384.

Aybey, B., Ergenekon, G., Hekim, N., Yarat, S., Kural, Y., Onsun, N., 2005. Glutathione peroxidase enzyme levels of patients with acne vulgaris. European Academy of Dermatology and Verenology, 19, 763-784.

Belding, R.D., Blankenship, S.M., Young, E., 1998. Composition and variability of epicuticular waxes in apple cultivars. Journal of American Society for Horticultural Science, 123, 348–356.

Brewster, D., 1837. In: The London and Edinburgh Philosophical Magazine and Journal of Science, 11, 163.

D'Hellencourt, C.L., Diaw, L., Cornillet, P., Guenounou, M., 1996. Differential regulation of TNFα, IL-1β, IL-6, IL-8, TNFβ and IL-10 by pentoxifylline. International Journal of Immunopharmacology, 18, 739-748.

Djipa, C.D., Delmee, M., Quetin-Leclercq, J., 2000. Antimicrobial activity of bark extracts of *Syzygium jambos* (L.) Alston (Myrtaceae). Journal of Ethnopharmacology, 17,307-313.

Du Toit, R., Volsteedt, Y., Apostolides, Z., 2001. Comparison of the antioxidant content of the fruits, vegetables and teas measured as Vitamin C equivalents. Toxicology,166, 63-69.

Ferreria, L.C., Guimaraes, A.G., Paula, C.A., Michel, M.C.P., Guimaraes, R.G., Rezende, S.A., Filho, J.D.S., Guimaraes, D.A.S., 2013. Anti-inflammatory and antinociceptive activities of *Compomanesia adamantium*. Journal of Ethnopharmacology, 145, 100-108.

Guvenalp, Z., Kilic, N., Kazaz, C., Kaya, Y., Demirezer, L.O., 2006. Chemical Constituents of *Galium tortumense*. Turkish Journal of Chemistry, 30, 515-523.

Harvey, M.T. and Caplan. S., 1940. Cashew Nutshell liquid**.** Journal of Industrial and Engineering Chemistry, 32(10), 1309.

Horiuchi, K., Shiota, S., Hatano, T., Yoshida, T., Kuroda, T., Tsuchiya, T., 2007. Antimicrobial activity of oleanolic acid from *Salvia officinalis* and related compounds on vancomycin-resistant enterococci (VRE). Biological and Pharmaceutical Bulletin, 30, 1147-1149.

Hsu, L.W., Chang, S.C., Shen, C.H., Liao, Y.X., Chuang, K.S., 2006. Flavone derivatives as TNF alpha inhibitors or antagonists. US Patent Application number 10/992, 178.

Islam, M.R., Parvin, M.S., Islam, M.E., 2012. Antioxidant and hepatoprotective activity of an ethanol extract of *Syzygium jambos* (L.) leaves. Drug discovery and Therapeutics 6, 205-11.

Jayprakasham, R., 2010. Chapter 5- Phytochemical examination of *Syzygium jambos*. 192-213.

Jung, H.A., Park, J.C., Chung, H.Y., Kim, J., Choi, J.S., 1999. Antioxidant flavonoids and chlorogenic acid from the leaves of *Eriobotrya japonica.* Archives of Pharmcal Research, 22,213-218.

Kang, M.S., Jang, H.S., Oh, J.S., Yang, K.H., Choi, N.K., Lim, H.S., Kim, S.M., 2009. Effects of methyl gallate and gallic acid on the production of inflammatory mediators interleulin-6 and interleulin-8 by oral epithelial cells stimulated with *Fusobacterium nucleatum*. Journal of Microbiology, 47(6), 760-767.

Kempuraj, D., Madhappan, B., Christodoulou, S., Boucher, W., Cao, J., Papadopoulou, N., Cetrulo, C.L., Theoharides, T.C., 2005. Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. British Journal of Pharmacology, 145, 934-944.

Kim, S.H., Jun, C.D., Suk, K., Choi, B.J., Lim, H., Park, S., Lee, S.H., Shin, H.Y., Kim, D.K., Shin, T.Y., 2006. Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cells. Toxicological Sciences, 91(1), 123-131.

Ko, T.F., Weng, Y.M., Chiou, R.Y., 2002. Squalene content and antioxidant activity of *Terminalia catappa* leaves and seeds. Journal of Agricultural and Food Chemistry, 50, 5343-5348.

Kubo, I., Muroi, H., Himejima, M., 1993. Structure-Antibacterial Activity Relationships of Anacardic Acids. Journal of Agricultural and Food Chemistry, 41, 1016-1019.

Kubo, I., Masuoka, N., Ha, T.J., Tsujimoto, K., 2006. Antioxidant activity of anacardic acids. Food Chemistry, 99, 555-562.

Kuiate, J.R., Mouokeu, S., Wabo, H.K., Tane, P., 2007. Antidermatophytic triterpenoids from *Syzygium jambos* (L.) Alston (Myrtaceae). Phytotherapy Research 21, 149-152.

Lee, K.H., Lin, Y.M., Wu, T.S., Zhang, D.C., Yamagishi, T., Hayashi, T., Hall, I.H., Chang, J.J., Wu, R.Y., Yang, T.H., 1988. The cytotoxic principles of *Prunella vulgaris*, *Psychotria serpens* and *Hyptis capitata*: Ursolic acid and relative derivatives. Planta Medica, 54(4), 308-311.

Lu , J., Papp, L.V., Fang, J., Nieto, S.R., Zhivotovsky, B., Holmgren, A., 2006. Inhibition of mammalian thioredoxin reductase by some flavonoids: Implications for myricetin and quercetin anticancer activity. Cancer Resarch, 66, 4410-4418.

Lim, T.K., 2012. Edible Medicinal and Non-medicinal Plants: Fruits. Volume 3, Dordrecht Heidelberg, London, New York, P 765.

Liua, Y. and Abreu, P.J.M., 2006. Long Chain Alkyl and Alkenyl Phenols from the Roots of *Ozoroa insignis*. Journal of Brazilian Chemical Society, 17,527-532

Mahapatra, A., Mativandlela, S.P., Binneman, B., Fourie, P. B., hamilton, C.J., Meyer, J.J, Van der Kooy, F., Houghton, P., Lall, N., 2007. Activity of 7-methyljuglone derivatives against *Mycobacterium tuberculosis* and as subversive substrates for mycothiol disulfide reductase. Bioorganic med Chemistry, 15(24), 7638-46.

Maskey, K., Shah, B.B., 1982. Sugars in some Nepalese edible wild fruits. Journal of Nepal Chemical Society, 2, 23-30.

Murata, Y., Ohteki, T., Koyasu, S., Hamuro, J., 2002. IFN-gamma and pro-inflammatory cytokine production by antigen-presenting cells is dictated by intracellular thiol redox status regulated by oxygen tension. European Journal of Immunology, 32, 2866-2873.

Murugan, S., Uma Devi, P., Parameshwari, K.N., Mani, K.R., 2011. Antimicrobial activity of *Syzygium jambos* against selected human pathogens. International Journal of Pharmacy and Pharmaceutical Sciences, 44-47.

Pan, C.Y., Chen, J.Y., Lin, T.L., Lin, C.H., 2009. *In vitro* activities of three synthetic peptides derived from epinecidin-1 and an anti-lipopolysaccharide factor against *Propionibacterium acnes*, *Candida albicans*, and *Trichomonas vaginalis*. Peptides, 30, 1058-1068.

Panizzi. L., Caponi, C., Catalano, S., Cioni, P.L., Morelli, I., 2002. In vitro antimicrobial activity of extracts and isolated constituents of *Rubus ulmifolius*. Journal of Ethnopharmacology,79, 165-168.

Perkin, A.G., 1902. Myricetin Part II. Journal of Chemical Society, Transactions, 203.

Pistelli, L., Bertoli, A., Noccioli, C., Mendez, J., Musmanno, R.A., Maggio, T.D., Coratza, G., 2009. Antimicrobial activity of *Inga fendleriana* extracts and isolated flavonoids. Natural Product Communication, 4, 1679-1683.

Shaw, L., Kennedy, C., 2007. The treatment of acne. Paediatrics and Child Health, 17, 385-389.

Tchinda, A.T., Teshome, A., Dagne, E., Arnold, N., Wessjohann, L.A., 2006. Squalene and amentoflavone from *Antidesma laciniatum*. Bulletin of the Chemical Society of Ethiopia, 20, 325-328.

Thuong, P.T., Jin, W.Y., Lee, J.P., Seong, R.S., Lee, Y.M., Seong, Y.H., Song, K.S., Bae, K.H., 2005. Inhibitory effect on TNF- α induced IL-8 production in the HT29 cell of constituents from the leaf and stem of *Weigela subsessilis*. Archives of Pharmacal Research, 28(10), 1135-1141.

Tsai, T.H., Tsai, T.H., Wu, W.H., Tseng, J.P., Tsai, P.J., 2010. *In vitro* antimicrobial and anti-inflammatory effects of herbs against *Propionibacterium acnes*. Food Chemistry, 119, 964-968.

Tsujimoto, M., 1917. Saturated hydrocarbons in basing-shark liver oil. Journal of Industrial and Engineering Chemistry, 9(12), 1098.

Warleta, F., Campos, M., Allouche, Y., Sanchez-Quesada, C., Ruiz-Mora, J., Baltran, G., Gaforio, J.J., 2010. Squalene protects against oxidative DNA damage in MCF10A human mammary epithelial cells but not in MCF7 and MDA-MB-231 human breast cells. Food and Chemical Toxicology, 48, 1092-1100.

Wurdack, J.H., 1924. The natural plant colouring matters**.** Journal of American Pharmacist Association, 13(4), 307-315.

Yalpani, M., Tyman, J.P., 1983. The phenolic acids of *Pistachia vera*. Phytochemistry, 22, 2263-2266.

Yang, L.L., Lee, C.Y., Yen, K.Y., 2000. Induction of apoptosis by hydrolyzable tannins from *Eugenia jambos* L. on human leukaemia cells. Cancer Letters, 157, 65-75.

Yen, G.C., Duh, P.D., Tsai, H.L., 2002. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food Chemistry, 79, 307-313.

Xu, H.X., Lee, S.F., 2001. Activity of plant flavonoids against antibiotic resistant bacteria. Phytotherapy Research, 15, 39-43.

Zang, X., Gincalves, R., Mosser, D.M., 2008. The isolation and characterization of Murine macrophages. Currrent Protocols in Immunology, chapter: Unit 14.1.

Chapter 6

The potential of *Leucosidea sericea* against *Propionibacterium acnes*

CHAPTER 6

The potential of *Leucosidea sericea* against *Propionibacterium acnes*

A section of this chapter is published in Phytochemistry Letters.

Richa Sharma[a], Navneet Kishore[a], Ahmed Hussein[a,b], Namrita Lall[a*]

[a]Department of Plant Science, Faculty of Agricultural and Biological Science, University of Pretoria, Pretoria 0002, South Africa.

[b]Chemistry Department, University of Western Cape, Private Bag X17, Bellville, 7535 Cape Town, South Africa.

[*]Corresponding Author. Department of Plant Science, Plant Sciences Complex, University of Pretoria, Pretoria-0002, South Africa. Tel.: +27 12 420 2524; fax: +27 12 420 6688.
Email address: Namrita.Lall@up.ac.za

Abstract

The present study reports on the potential of *Leucosidea sericea* addressing acne vulgaris. Four known compounds namely, phytol acetate, triacontanol, phytol and alpha kosin and one new compound namely, (*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,1 7-diol have been isolated for the first time from this plant. The ethanol extract of leaves and one of the isolated compounds, alpha kosin exhibited significant minimum inhibitory concentration (with MIC values 15.62 and 1.95 µg/ml, respectively) against acne inducing bacteria, *Propionibacterium acnes*. Moreover, the transmission electron micrographs showed the efflux of intracellular content of the cells of *P. acnes* caused by plant extract and alpha kosin. The ethanol extract of *L. sericea* exhibited significant anti-inflammatory activity by suppressing interleukin-8 (IL-8) and tumour necrosis factor (TNF-α) in coculture of human U937 cells and heat killed *P. acnes* at concentrations of 25.0, 12.5 and 6.2 µg/ml.

Keywords: *Propionibacterium acnes*, *Leucosidea sericea*, antibacterial, Interleukin-8, Tumour necrosis alpha, Transmission electron microscopy

6.1 Introduction

Acne is an inflammatory disease caused by gram-positive bacterium *Propionibacterium acnes* (*P. acnes*). It is the most common skin disease that affects areas covering the oil glands and hair follicles usually found on the face, chest, upper arm, back and trunk (Leydon, 1997). *P. acnes* is an obligate anaerobic organism that has capability to metabolize sebaceous triglycerides into fatty acids inside sebaceous gland. Also, due to the increased production of sebum, thickening of epidermis at the outlet of pilosebaceous unit occurs resulting in obstruction to the flow of sebum outwards and a comedo develops (Chomnawang et al., 2012; Coenye et al., 2012). Due to increased fatty acids content, the production of various reactive oxygen species (ROS) from the damaged follicular walls lead to the release of various cytokines like interleukin-8 (IL-8) and tumour necrosis factor (TNF-α) as host immune response. All these events lead to inflammation and pathogenesis of the disease. The usual drugs used in the treatment of acne have various side effects. The topical antibiotics can lead to dryness, redness, irritation of skin and hypopigmentation whereas oral antibiotics can cause gastrointestinal disorders and increase the risk of venous thromboembolism (Arican et al., 2005; Shaw and Kennedy, 2007).

The plant *Leucosidea sericea* Eckl. & Zeyh. (Rosaceae) is a single species of the genus *Leucosidea*, found in Eastern Cape, Free State and KwaZulu-Natal provinces of South Africa (Van Wyk et al., 2008). It is used against various ailments including severe inflammation of the eyes and in the treatment of ophthalmia (Aremu et al., 2010). The Zulus, a South African tribe, use the plant as an astringent in combination with other plants (Fouche et al., 2008). Earlier researchers have reported *in vitro* antimicrobial, antioxidant, acetyl-cholinesterase inhibitory (Aremu et al., 2011) and moderate anticancer activity (Fouche et al., 2008) of *L. sericea*. There are very few reports on the phytochemical investigation of the plant. Previous researchers have isolated two phloroglucinols, namely, aspidinol and desaspidinol from the leaves and flowers, while the presence of *β*-sitosterol and *β*-sitostenone were reported from the stems (Bosman et al., 2004; Nair et al., 2012). In our continuing search for bioactive molecules from plant resources, present chapter describes the isolation and characterization of four long chain fatty alcohols with one phloroglucinol derivative and their anti-bacterial, antioxidant and anti-inflammatory activity. Also, as reported in Chapter 4, the ethanol leaves extract of *L. sericea* exhibited noteworthy activity against Gram-positive *Propionibacterium acnes* with significant MIC

value of 15.6 µg/ml. Therefore, the plant extract was further subjected to isolation of compounds and investigated for its anti-inflammatory and antioxidant activity.

6.2 Materials and methods

All the methods for antibacterial, antioxidant, Transmission electron microscopy, cytotoxicity, anti-inflammatory and enzyme assays were followed as described in Chapter 5.

6.2.1 Plant material

The twigs and leaves of *L. sericea* were collected from the botanical garden of University of Pretoria, Pretoria, in March 2011. A voucher specimen (PRU 119052) was deposited at H.G.W.J Schwelckerdt Herbarium, Department of Plant Science, University of Pretoria, Pretoria.

6.2.2 Extraction and purification

The air-dried and powdered leaves (2.3 kg) were soaked in 9 L of ethanol for 3 days at room temperature. The filtrates were collected and concentrated under reduced pressure by a rotavapor at 40 °C to produce 73 g of crude ethanol extract. About 60 g of the ethanolic extract of *L. sericea* was subjected to silica gel column chromatography (70 cm× 120 cm) with hexane fraction (Hex): ethyl acetate (EtOAc) mixtures of increasing polarity (100:0 to 0:100) followed by 100% methanol (MeOH) as eluent. In total 51 fractions (500 ml) were collected and similar fractions were combined, according to thin-layer (TLC) profile, which resulted into 20 major fractions (MF) (Figure 6.1). All the 20 major fractions were tested for antibacterial activity using broth dilution method against pathogenic *P. acnes.* The results are shown in Table 6.1. Fractions 6, 10, 15 and 16 showed inhibitory activity against *P. acnes*; hence were subjected further to chromatographic columns to isolate the bioactive compounds. The isolation of compound from only one of the major fractions (MF 6); was done by our post-doctoral phytochemist, Dr. Navneet Kishore. MF 6 (600 mg) was separated on a silica gel column eluted with Hex: dichloromethane (DCM) mixtures of increasing polarity (100:0 to 0:100) which yielded twenty three sub fractions (Sf). Sf 3-5 led to the isolation of compound **1** (6 mg, 0.01%), Sf 7-8 eluted compound **2** (10 mg, 0.02%) and Sf 9-12 led to the separation of compound **3** (9 mg, 0.02%) and compound **4** (8 mg, 0.01%) was obtained from Sf 13-14. The separation of MF 10 (1.4 g) was done

using silica gel column chromatography eluting with mixture of Hex: (DCM: MeOH:: 99:1) in equal ratio, which yielded forty six Sf. Sf 4 and 5 were combined according to TLC analysis, consequently compound **5** (34 mg, 0.06%) was obtained. Further, MF 15 and 16 (2.3 g) were combined based on TLC profile and were separated similar to MF 10, which yielded one hundred and forty Sf. From the Sf. 46-51, compound **5** (15 mg,
0.03%) was obtained for the second time.

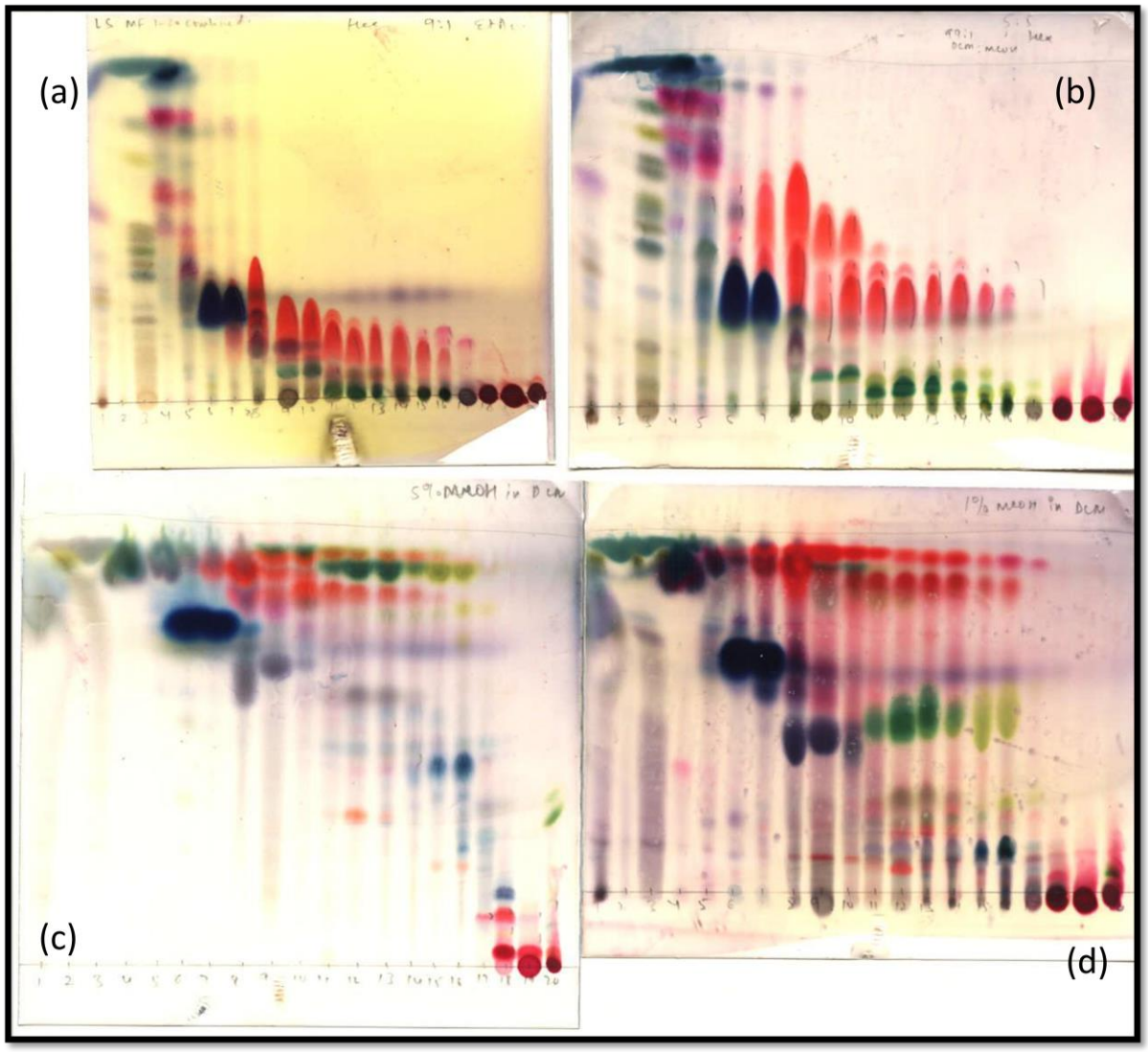


Figure 6.1: Thin layer chromatography of the 20 pooled fractions from the ethanol extract of *Leucosidea sericea* developed in (a) Hexane:Ethyl acetate (9:1); (b) (Dichloromethane: Methanol:: 99:1): Hexane (1:1); (c) 5% methanol in dichloromethane and (d) 1% methanol in dichloromethane
Detection: Vanillin in H_2SO_4

6.3 Results and discussions

6.3.1 Identification of isolated compounds

The ethanol extract of dried and powdered leaves of *L. sericea* subjected to chromatographic purification resulted into isolation of compounds **1-5** (Figure1). Structural assessment of these compounds was characterized by Mass, ^{1}H and ^{13}C NMR spectroscopic data. Assignment of signals was facilitated by COSY, HSQC and HMBC experiments. The known compounds obtained in this study, phytol acetate (**1**) (Itoh et al., 2003), triacontanol (**2**), (Tsai et al., 2007), phytol (**3**) (Itoh et al., 2003), and alpha kosin (**5**) (Woldemariam et al., 1992) were identified by comparison of their physical and spectroscopic data with literature reports. However, the compounds discussed in the present study have been previously reported from other plant species. Alpha kosin was first time isolated from the female flowers of *Hagenia abyssinica* (Lounasmaa et al., 1972), phytol was initially isolated from ether soluble substances of cabbage leaf cytoplasm (Chibnall and Channon, 1929) and triacontanol was isolated from the leaf wax of *Medicago sativa* (Chinball et al., 1933).

Compound **4** was obtained as an oily liquid from a variety of chromatographic separations. The IR spectrum (KBr) exhibited absorption bands at 3417 and 3300 (corresponding to two hydroxyl groups) along with other absorption bands at 2954 and 2849 cm^{-1}. Elemental analysis (Found: C, 77.21%; H, 12.93%/requires: C, 77.24%; H, 12.96%) in combination with 21 carbons resonance and a molecular ion peak $[M]^{+}$ observed at m/z 327.3021 in positive mode of EI-MS, established the molecular formula to be $C_{21}H_{42}O_2$. A peak at 295.2727 appeared due to loss of [M—CH_2OH] moiety. ^{1}H NMR spectrum (200 MHz in $CDCl_3$) of compound **4** exhibited the signal of H-2 at δ 5.39 (J = 8) vicinally coupled to the two protons doublet on C-1 at δ 4.15 (J = 8) and allylically to the protons on C-4 and the vinyl methyl (C-21). The methylene protons doublet at δ 4.15 (J = 8) showed correlation with an olefinic proton δ 5.39 in ^{1}H-^{1}H COSY experiment, suggested the presence of a double bond between C-2 and C-3. In ^{13}C NMR spectrum (50 MHz, $CDCl_3$) of compound **4**, the two carbon resonance signals observed at δ 141.2 and 123.9 were evidenced for olefinic carbon positioned at C-3 and C-2, respectively. The chemical shift values corresponding to protons and olefin methyl group attached to respective hetero-nuclear carbons were evidenced on the basis of HSQC spectrum. In HMBC spectrum the proton H-2 was found to be correlated to C-3, Similarly, H-3 showed connectivity to C-4 and C-17. Also, the correlations were observed between olefin methyl protons at δ 1.65 to C-2 and C-4.

On the basis of identical chemical shift values as well as in DEPT and HMBC signals, compound **4** was

found to be almost related to compound **3**. The cross-signals in the proton spectrum showed a two proton multiplet at δ 3.67 (J = 6) and a carbon spectrum signal which appeared at δ 63.9 evidenced a hydroxyl group bearing carbon. The protons appeared at δ 3.67 were found strongly attached to the carbon signal at δ 63.9 as evidenced in the HSQC spectrum. The protons H-17 at δ 3.67 was found to be correlated with C-15 in hetero-nuclear multiple bond coherence. Hence, a new phytol analogue (*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol, was identified from the combined spectral analysis of compound **4**. To the best of our knowledge this is the new compound which has been isolated first time from the plant *L. sericea*. The structure of the isolated compounds is shown in Figure 6.2.

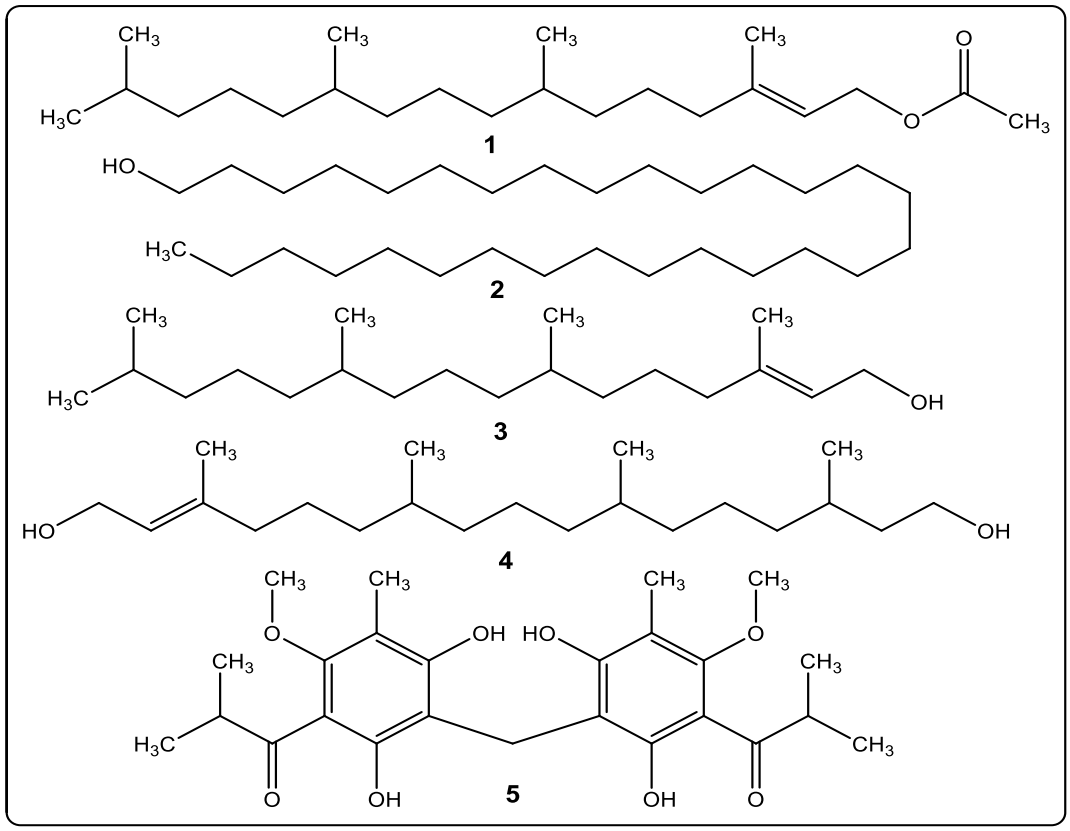


Figure 6.2: Structures of isolated compounds from ethanol extract *Leucosidea sericea*: phytol acetate (1), triacontanol (2), phytol (3), (*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol (4) and alpha kosin (5)

6.3.1.1 Characteristic data of compound 4

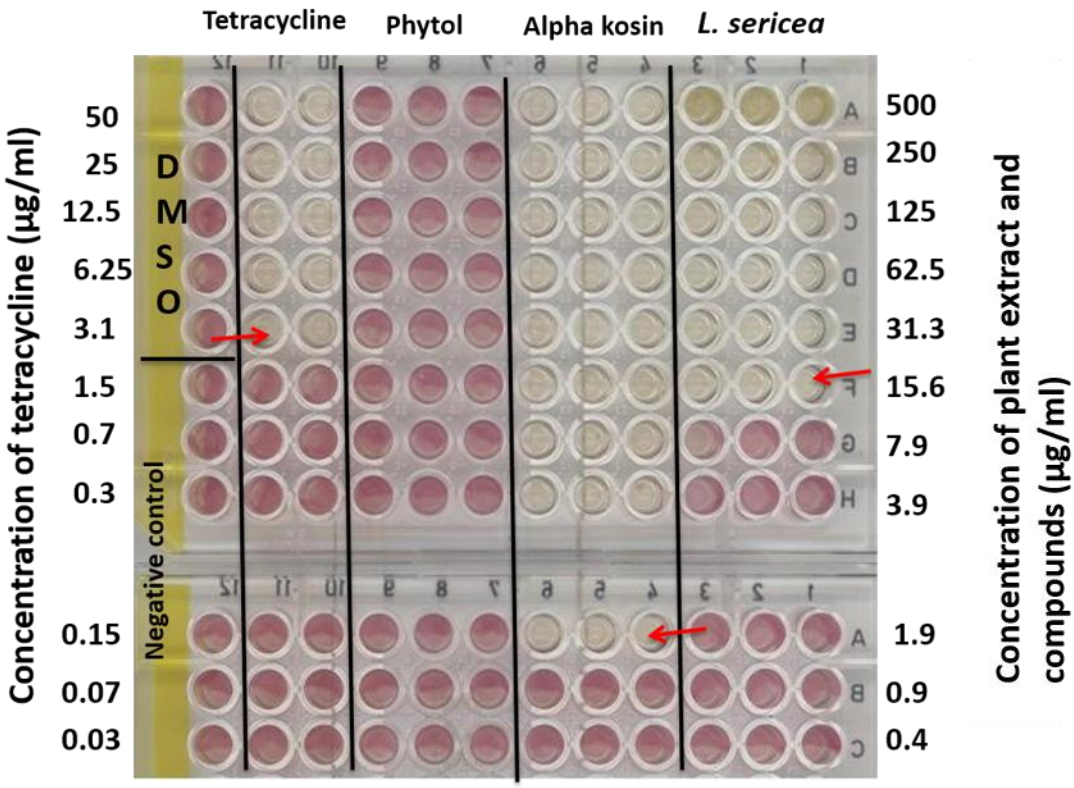
(E)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol (**4**): Colourless oil; UV ($CDCl_3$) λ_{max}(log ε) 217 nm; IR (KBr) υ_{max}: 3417, 3300, 2954, 2923, 2849, 1462, 1384 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, δ in ppm, J in Hz): δ_H 5.43 (1H, *t*, J = 8, H-2), 4.12 (2H, *d*, J = 8, H-1), 3.62 (2H, *t*, J = 6, H-17) 2.01 (2H, 2x -OH), 1.97 (2H, *t*, J = 8, H-4), 1.65 (3H, s, H-21), 1.42-1.10 (21H, m, CH_2 , -CH-, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15 and H-16), 0.86-0.83 (9H, 3x -CH_3, H-18, H-19 and H-20).

^{13}C NMR (50 MHz, $CDCl_3$): δ_C 141.2 (C-3), 123.9 (C-2), 63.9 (C-17), 60.2 (C-1), 40.7, 40.2, 38.2, 37.5, 33.6, 33.5, 32.7, 30.5, 30.4, 28.8, 25.9, 25.6, 25.3 (C-4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16), 23.5 (C-18), 23.4 (C-19), 20.6 (C-20), 17.0 (C-21). HREIMS $[M+H]^+$ *m/z* 327.3021 (calcd. for $C_{21}H_{42}O_2$+H, 327.3017 required 326 for $C_{21}H_{42}O_2$).

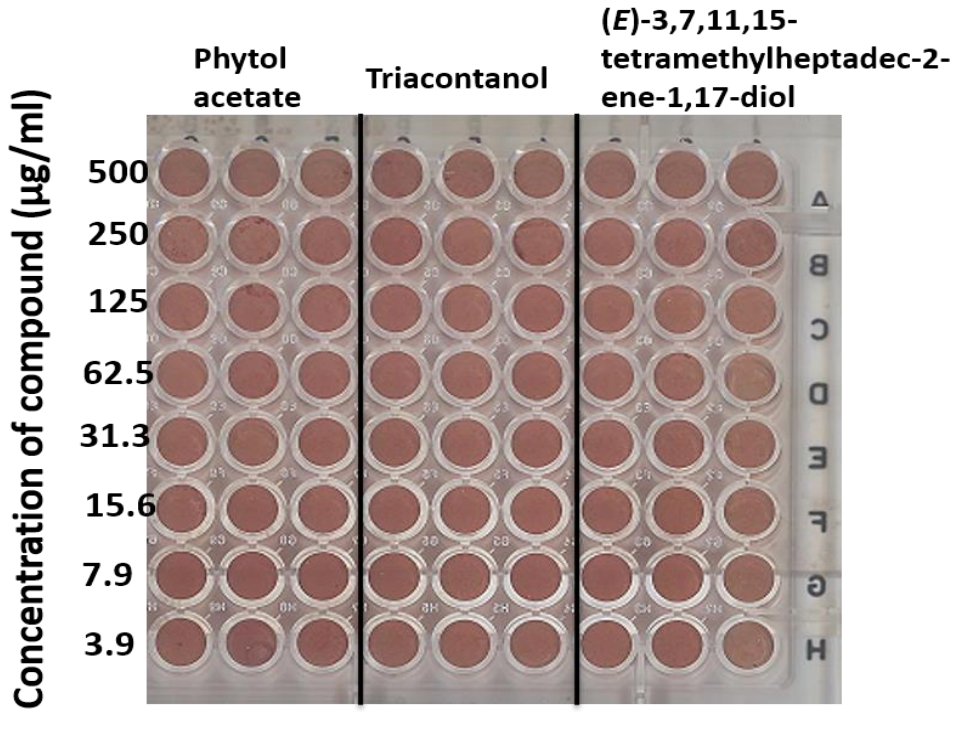
6.3.2 Antibacterial bioassay

The antibacterial activity of ethanol extracts of *L. sericea* and isolated compounds have been summarised in Table 6.1. The ethanol extract of *L. sericea* inhibited the bacterial growth and exhibited noteworthy MIC value of 15.62 µg/ml. Alpha kosin was found to be the most active compound against *P. acnes* with an MIC value of 1.95 µg/ml as compared to tetracycline (positive control) with MIC value of 3.12 µg/ml (Figure 6.3). It is worth noting that threshold MIC values of 100 and 10 µg/ml have been recommended for plant extracts and pure compounds, respectively, to rate them as having significant antimicrobial activity (Kuete, 2010). Thus the MIC values measured for the activity of crude extract and bioactive compound (alpha kosin) can be considered significant. Compounds phytol acetate, triacontanol, phytol and (*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol did not show any growth inhibitory activities at highest concentration tested (500 µg/ml).

To the best of our knowledge, the antibacterial activity of *L. sericea* and isolated compounds (phytol acetate, triacontanol, phytol, (*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol and alpha kosin) against *P. acnes* is being reported for the first time. However, petroleum ether and dichloromethane leaves extract of *L. sericea* was found to be active against *Bacillus subtilis* and *Staphylococcus aureus*, respectively, with MIC value of 0.025 mg/ml (Aremu et al., 2010). In the present study no activity for phytol and triacontanol was found. Contrary to this in a study, phytol exhibited activity against *Mycobacterium tuberculosis* and *M. avium* with MIC values of 2 and 16 µg/ml, respectively (Rugutt and Rugutt, 2012); and triacontanol showed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Lactobacillus acidophilus* with MIC values ranging between 0.01- 0.1 µg/ml (Upadhyay et al., 2010). Based on literature search, no antimicrobial activity of phytol acetate and alpha kosin was found.



(a)



(b)

Figure 6.3: The 96 well plates showing antibacterial activities against *Propionibacterium acnes* and MIC values (red arrow) of (a) ethanol extract of *Leucosidea sericea* (15.62 µg/ml), alpha kosin (1.95 µg/ml), phytol (not active at 500 µg/ml) and drug control tetracycline (3.12 µg/ml); (b) (*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol triacontanol, phytol acetate (not active at 500 µg/ml)

6.3.3 Transmission electron microscopy (TEM)

For microscopy studies, the plant extract and alpha kosin which showed activity against the bacteria were selected. The TEM micrograph represents clear differences between untreated and treated *P. acnes*. The untreated *P. acnes* had a distinct cell wall which was long, spindle shaped, smooth and lined with cell membrane. A centrally located nucleoid surrounded by ribosomes was observed (Figure 6.4 a). The TEM micrograph showed cell injuries caused to *P. acnes* after treatment to the ethanol extract of *L. sericea* for 72 h. *P. acnes* treated with the ethanol extract of *L. sericea* at a concentration of 100 µg/ml exhibited abnormal changes in cell content material whereas at higher concentration of 300 µg/ml, the cell wall of bacteria was found lysed and cell debris was observed (Figure 6.4 b, c). Alpha kosin caused significant damage to the cells of *P. acnes* at a concentration of 50 µg/ml. The intracellular content was found to be effluxed due to breaks in the cell wall. The intact cells showed changes in the appearance of cell organelles. Due to extensive lysis of the bacteria, the debris was observed all over (Figure 6.4 d). Tetracycline at a concentration of 50 µg/ml caused significant damages to the cells of *P. acnes*, leading to damages in the cell membrane, distortion in the cell structure and shrinkage of cell content material (Figure 6.4 e). DMSO at 2.5% exhibited no lethal effects to bacteria (Figure 6.4 f). The TEM micrograph confirms the antibacterial activity of *L. sericea* and alpha kosin against *P. acnes.*

6.3.4 Antioxidant assay

DPPH assay provides antiradical properties of the samples. The antioxidants are able to stable the free DPPH radical due to their proton donating ability. The scavenging effect of *L. sericea* ethanol extract and compound alpha kosin on DPPH increased with their increasing concentrations (Figure 6.5 a, b). These samples showed very significant antioxidant activity with EC_{50} values very similar to Vitamin C, a widely used antioxidant compound (Figure 5.7 e). The results are shown in Table 6.1. The mg vitamin C equivalents/g dry weight for alpha kosin was calculated to be 392. Compounds phytol acetate, triacontanol, phytol and (*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol did not show any antioxidant activity. Concerning the structure-activity relationship, it was clear that the presence of four free hydroxyl groups in alpha kosin could be responsible for its antioxidant activity in comparison with phytol acetate, triacontanol, phytol and (*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol which possess less or lacks any free hydroxyl groups. Similar to our results, it has been reported that the

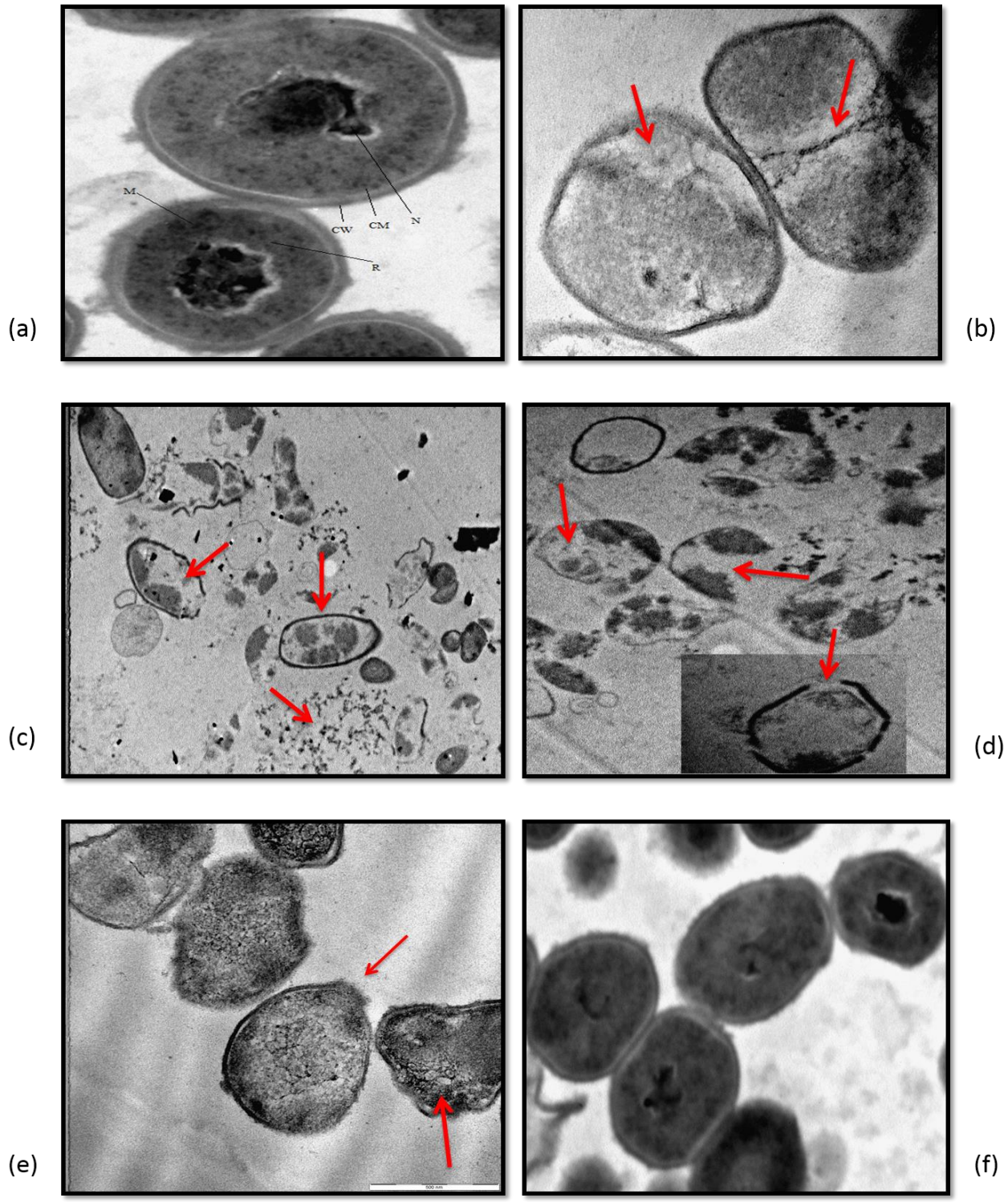


Figure 6.4:Transmission electron micrograph of a thin section of *Propionibacterium acnes*: (a) untreated bacteria, labelled structures: cell wall (CW); cytoplasmic membrane (CM); nucleoid (N); ribosomes (R); mesosomes (M) x 60 K ; (b) *P. acnes* treated with *Leucosidea sericea* at 100 µg/ml x 60 K; (c) *P. acnes* treated with *L. sericea* at 300 µg/ml x 40 k; (d) *P. acnes* treated with alpha kosin at 50 µg/ml x 50 K; (e) *P. acnes* treated with positive control (tetracycline) at 50 µg/ml x 60 K ; (f) *P. acnes* treated with solvent (DMSO at 2.5%) x 60 K. The arrows indicate cell injuries to the *P. acnes*

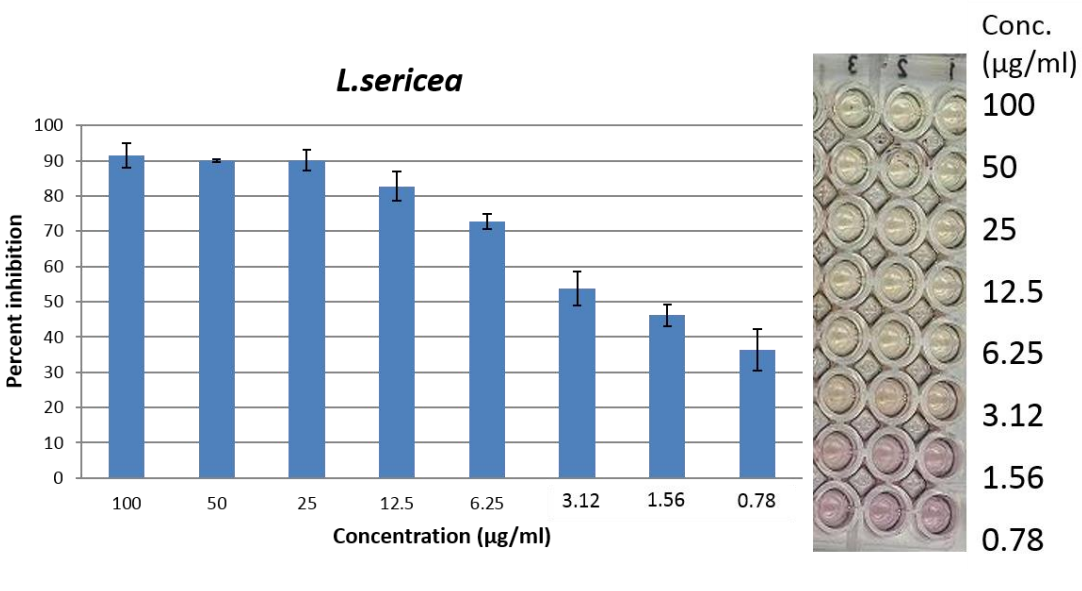
Table 6.1: Antibacterial, antioxidant and cytotoxic effects of ethanol extract of *Leucosidea sericea* and isolated compounds

Test samples	**Antibacterial** [a]MIC µg/ml/(**µM**)	**Antioxidant** [b]EC_{50} µg/ml/(**µM**)	**Cytotoxicity** EC_{50} µg/ml/(**µM**)	
			B16-F10 mouse melanoma	U937 human macrophage
Leucosidea sericea	15.62	2	55	26
[c]MF 1-5,7-9, 11-14, 17-20	[d]Not active	[e]-	-	-
MF 6	500	-	-	-
MF 10	1.9	-	-	-
MF 15, 16	31.25	-	-	-
Phytol acetate (**1**)	Not active	>100	[f]Not tested	Not tested
Triacontanol (**2**)	Not active	>100	>100	>100
Phytol (**3**)	Not active	>100	>100	>100
(*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol (**4**)	Not active	>100	Not tested	Not tested
Alpha kosin (**5**)	1.9/**(2.1)**	5.1/**(10)**	< 3.12	<3.12
[g]**PC**	3.12/(**7**)	2/(**11**)	4.5×10^{-3}/**3.5×10^{6}**	4.5×10^{-3}/**3.5×10^{6}**

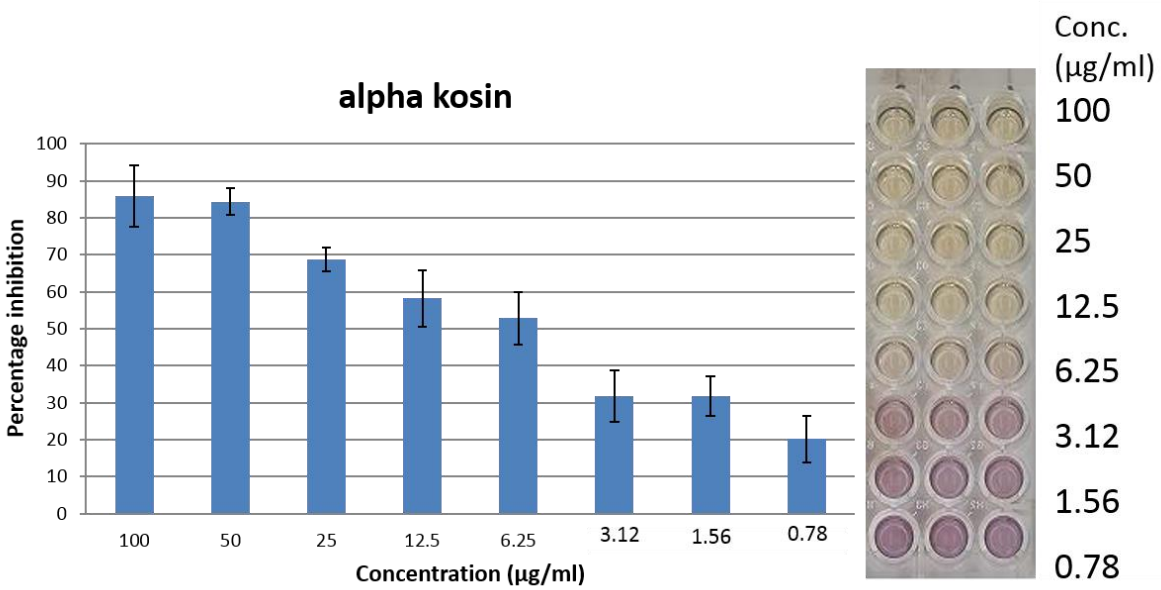
[a]MIC: minimum concentration of sample that inhibits bacterial growth; [b]EC_{50}: concentration at which 50% DPPH radicals are scavenged (for antioxidant)/ 50% cells are viable (for cytotoxicity); [c]MF: major fractions; [d]not active at the highest concentration tested (500 µg/ml); [e]not applicable: for MF as not tested for antioxidant activity and cytotoxicity, mg equivalent could not be detected for non-antioxidant compounds; [f]not tested due to low yield of the compound; [g]positive drug controls where tetracycline for antibacterial, vitamin C for antioxidant, actinomycin D for cytotoxicity

methanol extract of the leaves of *L. sericea* exhibited antioxidant activity with EC_{50} value of 3.0 µg/ml. However, petroleum ether and DCM extract exhibited a higher EC_{50} value of 26.2 and 27.7 µg/ml,

respectively (Aremu et al., 2010). To the best of our knowledge, this is the first report of DPPH scavenging activity of all the compounds isolated in this study.



(a)



(b)

Figure 6.5: DPPH radical scavenging activity of (a) *Leucosidea sericea* (EC_{50} 2 µg/ml) and (b) Alpha kosin (EC_{50} 5.1 µg/ml)

6.3.5 In vitro cytotoxicity assay

The cytotoxicity of the extracts and compound triacontanol, phytol and alpha kosin was done on B16-F10 mouse melanoma and U937 human macrophage cells. The cytotoxicity analysis of compounds phytol acetate and (*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol was not conducted due to

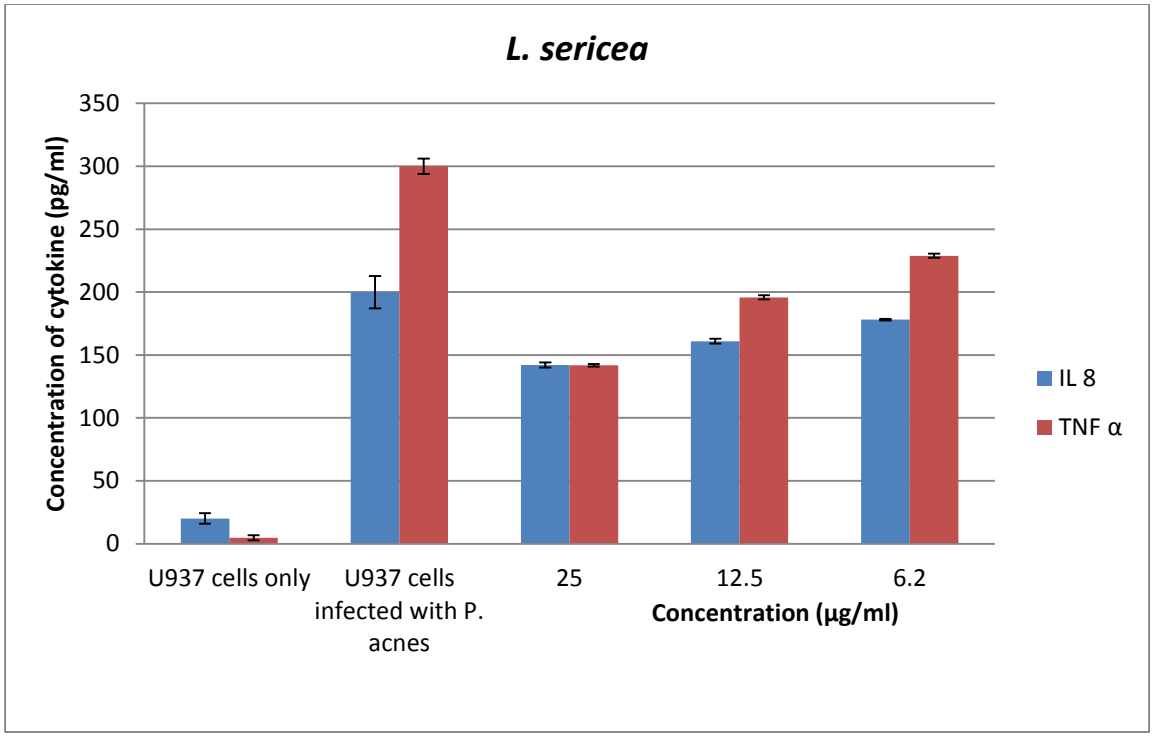
unavailability of sufficient amount of the samples. All the results are listed in Table 6.1. To the best of our knowledge, the cyotoxicity in the present study of the ethanol extract of *L. sericea* and three isolated compounds against B16-F10 mouse melanoma and U937 human macrophage cells is reported for the first time. *L. sericea* exhibited moderate toxicity on B16-F10 cells and comparatively higher toxicity on U937 cells. Alpha kosin showed significant toxicity on both the cell lines with EC_{50} value of <3.12 µg/ml. However, tricontanol and phytol did not exhibit any toxicity on both the cell lines with 100% viability of cells at their highest concentration of 100 µg/ml. Similar to our findings, strong cytotoxic effects of alpha kosin against murine adenocarcinoma (MAC) tumour cells with EC_{50} value of 1.5 µg/ml are reported by Woldemariam et al. (1992). Triacontanol was reported as non-toxic constituent of *Viburnum jucundum* (Rios et al., 2001). Phytol showed toxicity against skin cancer cells (SK-MEL-2), CNS cancer cells (XF498) and colorectal cancer cells (HCT15) with EC_{50} values ranging from 6.2-11.2 µg/ml (Sung et al., 1999). No reports about cell toxicity have been found for *L. sericea* in the literature.

6.3.6 Anti-inflammatory activity

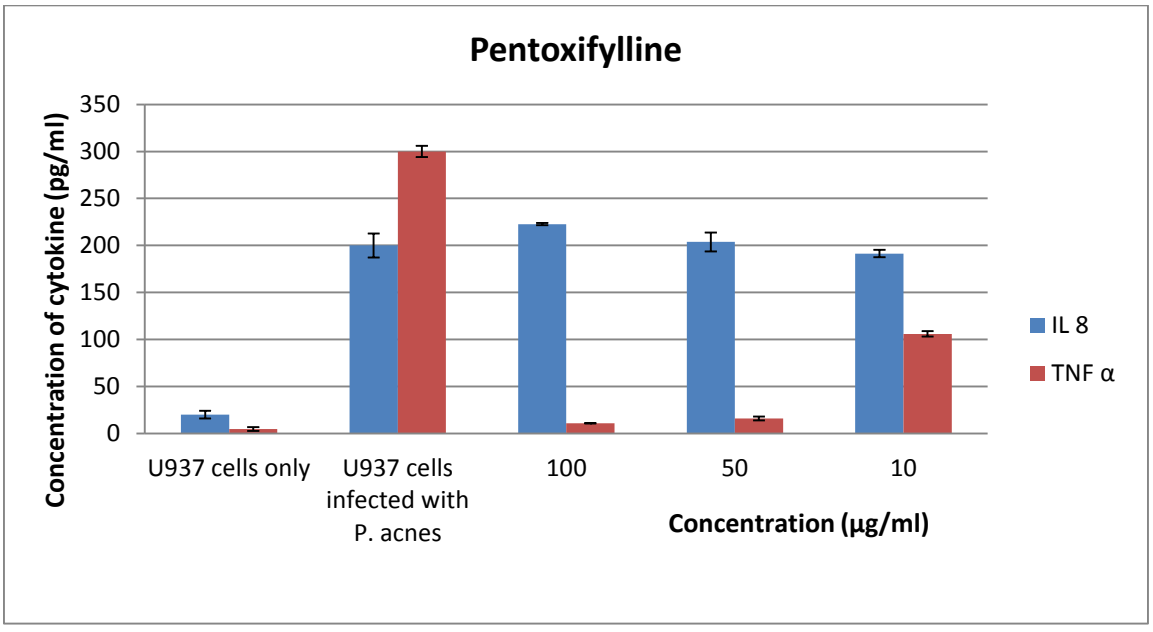
6.3.6.1 Effect of ethanol extract of Leucosidea sericea on the pro-inflammatory cytokines

P. acnes stimulate macrophages for increased production of pro-inflammatory cytokines such as IL-8 and TNF-α which contributes to the induction of mediators of inflammatory response. In the present study, IL-8 and TNF-α were used as major criteria for evaluation of anti-inflammatory activity. The U937 cells co-cultured with *P. acnes* caused an increase in the production of IL-8 and TNF-α (Figure 6.6a). To test the anti-inflammatory effects of *L. sericea* an *in vitro* screening at three nontoxic concentrations were applied. As shown in Figure 6.6a, the ethanol extract of *L. sericea* decreased the production of IL-8 and TNF-α in dose-dependent manner. Furthermore, the plant extract did not increase the secretion of either of the cytokines in culture of U937 cells in the absence of heat killed *P. acnes* (data not shown). Pentoxifylline which was used as a control behaves differently on the cytokines. Based on previous reports, it down regulated the secretion of TNF-α and caused no change in IL-8 release (D'Hellencourt et al., 1996). As shown in Figure 6.6b, our results were in agreement with other researchers. Significant inhibition of TNF-α was observed at 100 and 50 µg/ml of pentoxifylline whereas, no change in IL-8 concentration was observed. To the best of our knowledge, no reports about *L. sericea* in context with suppression of cytokines were found. Although, similar to our results, other plants *i.e. Eucommia ulmoides* and *Ilex paraguariensis* extracts were reported to

reduce the secretion of IL-8 and TNF-α in human monocytic THP-1 cells pre-treated with *P. acnes* at concentration of 0.1 mg/ml (Tsai et al., 2010).



(a)



(b)

Figure 6.6: (a) Dose-dependent inhibition of IL-8 and TNF-α by ethanol extract of *Leucosidea sericea*; (b) Differential response of pentoxifylline on the release of IL-8 and TNF-α by U937 cells infected with *Propionibacterium acnes*

6.3.6.2 Suppression of Nitric oxide

The standard graph was plotted by preparing a gradient of standard solution provided in the kit from 200 to 1.5 µM (Figure 5.10 a, b). The mouse derived macrophages when stimulated with LPS triggered the release of Nitric oxide. The ethanol extract of *L. sericea* dose dependently suppressed the production of Nitric oxide (Figure 6.7). However, the solvent and cells alone did not show any production of Nitric oxide. The positive control significantly inhibited Nitric oxide at all the concentration tested.

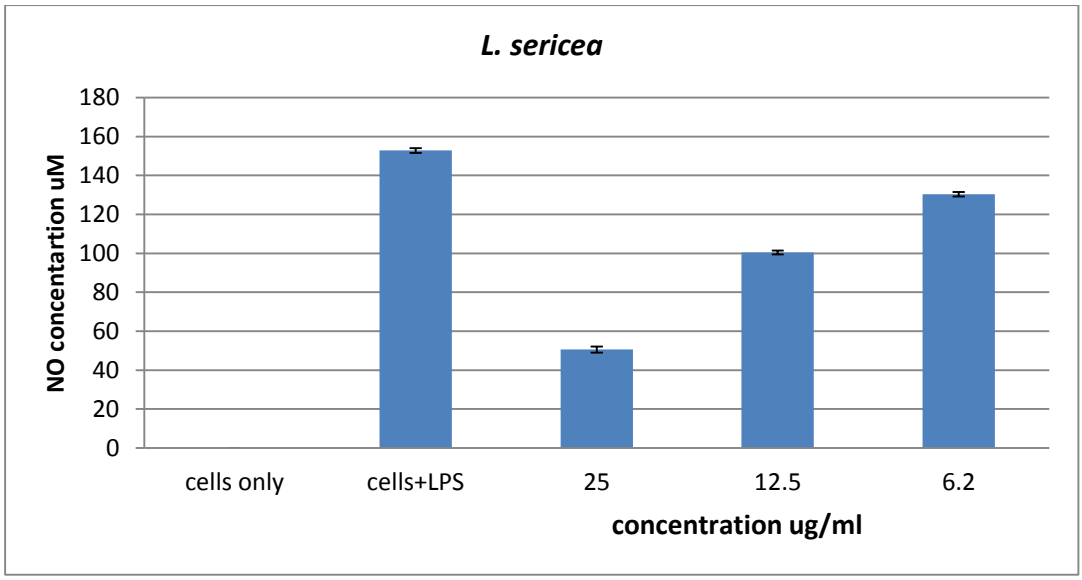


Figure 6.7: Dose-dependent suppression on nitric oxide (NO) by ethanol extract of *Leucosidea sericea*

6.3.7 Enzyme assay

The ethanol extract of *L. sericea* dose dependently decreased the activity of GR enzyme with IC_{50} value of 3.1 µg/ml. The sigmoidal graph shown in Figure 6.8 depicts an increase in percentage activity of GR at lower concentrations of plant extract and at higher concentrations; the percentage activity of GR was reduced. This is the first report of plant *L. sericea* inhibiting glutathione reductase enzyme. No reports in the literature regarding the GR enzyme and plant extracts were found.

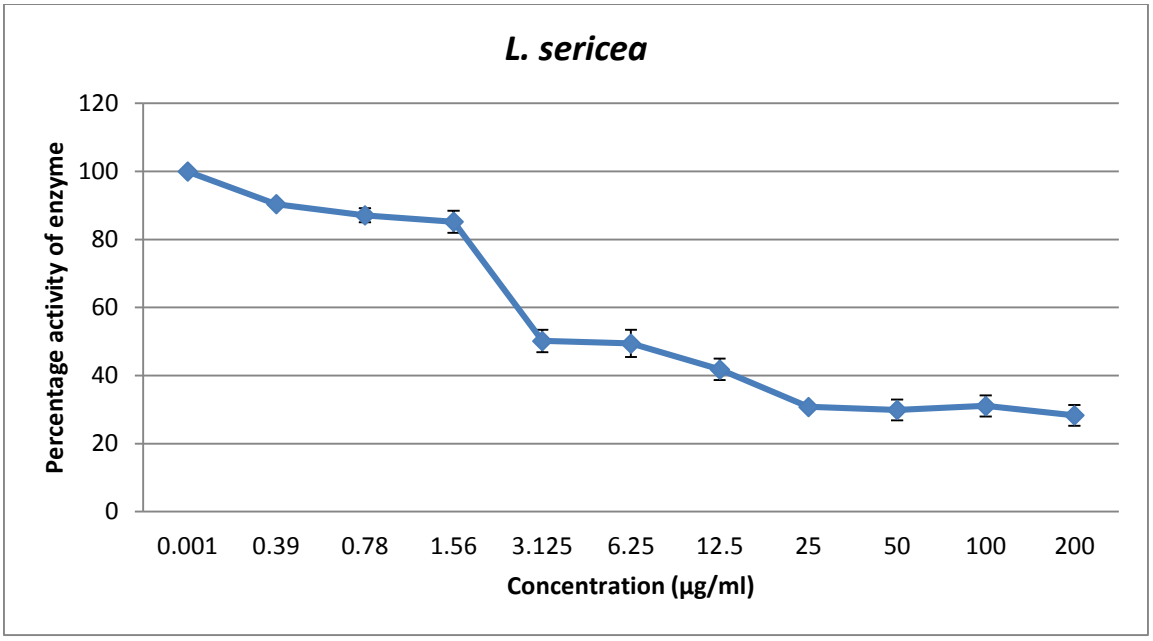


Figure 6.8: A dose-dependent graph showing decrease in the activity of glutathione reductase (GR) with increasing concentration of ethanol extract of *Leucosidea sericea* (IC_{50} 3.1 µg/ml)

6.4 Conclusion

The ethanol extract of *L. sericea* and isolated compound alpha kosin demonstrated significant activity against *P. acnes* which was also confirmed by TEM micrographs. The plant extract exhibited noteworthy antioxidant and anti-inflammatory activity which are crucial for an anti-acne agent.

6.5 References

Aremu, A.O., Fawole, O.A., Chukwujekwu, J.C., Light, M.E., Finnie, J.F., Van Staden, J., 2010. In vitro antimicrobial, anthelmintic and cyclooxygenaseinhibitory activities and phytochemical analysis of *Leucosidea sericea*. Journal of Ethnopharmacology, 131, 22–27.

Aremu, A.O., Amoo, S.O., Ndhlala, A.R., Finnie, J.F., Van Staden, J., 2011. Antioxidant activity, acetylcholinesterase inhibition, iridoid content and mutagenic evaluation of *Leucosidea sericea*. Food and Chemical Toxicology, 49, 1122–1128.

Bosman, A.A., Combrinck, S., Roux-van der Merwe, R., Botha, B.M., McCrindle, R.I., 2004. Isolation of an anthelmintic compound from *Leucosidea sericea*. South African Journal of Botany, 70, 509–511.

Chibnall, A.C., Channon, H.J., 1929. The ether-soluble substances of cabbage leaf cytoplasm. Biochemical Journal, 23(2), 176-184.

Chinball, A.C., Williams, E.F., Latner, A.L., Piper, S.H., 1933. The isolation of n- triacontanol from Lucerne wax. Biochemical Journal, 27(6): 1885-1888.

D'Hellencourt, C.L., Diaw, L., Cornillet, P., Guenounou, M., 1996. Differential regulation of TNFα, IL-1β, IL-6, IL-8, TNFβ and IL-10 by pentoxifylline. International Journal of Immunopharmacology, 18, 739-748.

Fouche, G., Cragg, G.M., Pillay, P., Kolesnikova, N., Maharaj, V.J., Senabe, J., 2008. *In vitro* anticancer screening of South African plants. Journal of Ethnopharmacology, 119, 455- 461.

Itoh, D., Kawano, K., Nabeta, K., 2003. Biosynthesis of chloroplastidic and extrachloroplastidic terpenoids in liverwort cultured cells: ^{13}C serine as a probe of terpene biosynthesis via mevalonate and non-mevalonate pathways. Journal of Natural Products, 66, 332-336.

Lounasmaa, M., Widen, C.J., Huhtikangas, A., 1973. Phloroglucinol derivatives of *Hagenia abyssinica*. Phytochemistry, 12, 2017-2025.

Kuete, V., 2010. Potential of Cameroonian plants and derived-products against microbial infections. A review. Planta Medica, 76, 1479-1491.

Nair, J.J., Aremu, A.O., Van Staden J., 2012. Anti-inflammatory effects of *Leucosidea sericea* (Rosaceae) and identification of the active constituents. South African Journal of Botany, 80, 75-76.

Rios, M.Y., Gonzalez-Morales, A., Villarreal, L., 2001. Sterols, triterpenes and biflavonoids of *Viburnum jucundum* and cytotoxic activity of ursolic acid. Planta Medica, 67, 683-684.

Rugutt, J. K., Rugutt, K.J., 2012. Antimycobacterial activity of steroids, long-chain alcohols and lytic peptides. Natural Product Research, 26(11), 1004-1011.

Sung, J.H., Lee, J.O., Son, J.K., Park, N.S., Kim, M.R., Kim, J.G., Moon, D.C., 1999. Cytotoxic constituents from *Solidago virga-aurea* var. *gigantea* MIQ. Archives of Pharmacal Research, 22(6), 633-637.

Tsai, I.L., Cheng, M.J., Hung, H.W., Cheng, H.I., Chen, I.S., 2007. Chemical constituents from the leaves of *Litsea acutivena*. Jouranl of Chinese Chemical Society, 54, 503-506.

Tsai, T.H., Tsai, T.H., Wu, W.H., Tseng, J.P., Tsai, P.J., 2010. *In vitro* antimicrobial and anti-inflammatory effects of herbs against *Propionibacterium acnes*. Food Chemistry, 119, 964-968.

Upadhyay, R.K., Ahmad, S., Tripathi, R., Rohtagi, L., Jain, S.C., 2010. Screening of antimicrobial potential of extracts and pure compounds isolated from *Capparis decidua.* Journal of Medicinal Plant Research, 4(6), 439-445.

Van Wyk, B., Van Wyk, P., Van Wyk, B.E., 2008. Photo Guide to Trees of Southern Africa. Briza Publications, Pretoria, South Africa.

Woldemariam, T.Z., Fell, A.F., Linley, P.A., Bibby, M.C., Phillips, R.M., 1992. Evaluation of the anti-tumour action and acute toxicity of kosins from *Hagenia abyssinica*. Journal of Pharmaceutical and Biomedical Anaysis, 10, 555-560.

Chapter 7

Synergistic activity of *Syzygium jambos* and *Leucosidea sericea*

CHAPTER 7

Synergistic activity of *Syzygium jambos* and *Leucosidea sericea*

7.1 Introduction

The synergistic interactions have known to possess therapeutic value since antiquity. The use of polyherbal and their application has been carried down through the centuries. The basic principle of using plants in combination is adopted from the concept that the plants in combination may enhance efficacy and reduce the dosage in overall formulation (Vuuren and Viljoen, 2011).

There has been an increasing interest and various upcoming researchers are exploring the use of medicinal plants in combination to potentiate antimicrobial activity. There is a thought that plants when used in combinations provide multiple compounds and that act at many target sites and thus aid in enhancing overall activity and efficacy (Al-Bayati, 2008; Biavatti, 2009). For topical application of cosmetics, the organic plant extract in liquid form is accepted by the companies. Therefore, in the current study the antibacterial activity of aqueous extract of the two lead plants i.e. *Leucosidea sericea* and *Syzygium jambos* was investigated.

7.2 Materials and methods

7.2.1 Preparation of aqueous extract

The aqueous extract of *Leucosidea sericea* and *Syzygium jambos* was prepared by weighing 12.5 g of dried leaves powder and soaking in 150 ml of autoclaved deionised water. The soaked leaves were kept on a shaker for 3 days and then were filtered four times to obtain the aqueous extract. Euxyl 9010 (1%) was added as preservative.

7.2.2 Antibacterial activity of aqueous extract of *Leucosidea sericea* and *Syzygium jambos*

The antibacterial activity of aqueous extract of *L. sericea* and *S. jambos* was carried out separately as explained in Chapter 4, section 4.2.4.4 with few modifications. Briefly, the bacteria was cultured from

a Kwik-Stick on nutrient agar and incubated at 37°C for 72 h under anaerobic conditions before the assay. The 72 h culture of the bacteria was dissolved in nutrient broth and the suspension was adjusted to 0.5 McFarland standard turbidity. This resulted in 10^5-10^6 colony forming units (CFU)/ml. In a sterile 96-well plate, 100 µl of sterile liq aq. extract (sterilised by micro filter with 0.2 micron pore size) was diluted with 100 µl of broth. The serial dilutions were made in the broth to give percentage concentrations ranging from 0.3%-50%. Tetracycline was used as positive control and was serially diluted to give concentration ranging from 0.3-50 and µg/ml. The bacterial suspension (100 µl) was added to the wells. The bacterial suspension without samples served as the negative control. The plates were incubated at 37°C for 72 h in an anaerobic environment. The MIC value was determined by observing colour change in the wells after the addition of INT (defined as the lowest concentration that showed no bacterial growth).

7.2.3 Antibacterial activity of plants in combination and determination of synergism

The antibacterial activity in combination was done following the same procedure as explained in section 2.9.2. The aqueous extract of *Leucosidea sericea* (LS) and *Syzygium jambos* (SJ) were combined in different ratios to make nine combinations (LS:SJ :: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1). The activity of plants in combination was evaluated by calculating the Fraction Inhibitory Concentration (FIC) value (Suliman et al., 2010). The MIC value of aqueous extract of *L. sericea* and *S. jambos* alone and in nine different combinations were used to calculate FIC by the following equation:

FIC 1= MIC (a) in combination with (b) / MIC (a) independently

FIC 2= MIC (a) in combination with (b) / MIC (b) independently

Where (a) represents *L. sericea* and (b) represents *S. jambos*. The FIC of all the combinations was calculated and sum of FIC known as FIC index was calculated by adding all the FIC.

The FIC index was used to determine the correlation of the two plants in combination and may be classified as synergistic (≤ 0.5), additive (> 0.5-1.0), non-interactive (> 1.0- ≤ 4.0) or antagonistic (> 4.0).

In order to investigate the potential of various combinations of LS:SJ, a comparative *in vitro* antibacterial test was conducted with Cytobiol Iris A^2. Cytobiol Iris A^2 is a commercially available

active which fights against the functional and physical signs of acne. It is a synergistic blend of propanediol, water, alcohol, *Iris florentina* root extract, zinc sulphate and retinyl palmitate.

7.2.4 Clinical studies

The clinical studies were performed by experts at Future Cosmetics, Pretoria. The aqueous extract of *L. sericea* and a combination of *L. sericea* and *S. jambos* in ratio 1:1 was selected for the following clinical studies.

7.2.4.1 Patch testing

A 24 h occlusive irritancy patch testing was performed on 20 subjects.

7.2.4.2 In Vivo Soothing and Calming efficacy

An in vivo study to determine the soothing and calming efficacy of the samples for 24 to 72 hours post-application on tape stripped skin was done.

7.2.4.3 In Vivo acne reduction testing

The objective of the study was to determine the acne reduction efficacy of the samples on the face of human subjects.

7.3 Results and discussions

7.3.1 Antibacterial activity in combination

The results of MIC value of aqueous extract of *L. sericea* and *S. jambos* individually and in nine combinations along with the calculated FICs are depicted in Table 7.1. The aqueous extract of *L. sericea* inhibited the growth of *P. acnes* with MIC value of 3.1% (2603 µg/ml) whereas *S. jambos* exhibited a comparable higher MIC value of 6.2% (5206 µg/ml). The commercially acquired Cytobiol Iris A^2 blend inhibited the growth of *P. acnes* at 0.7%. (Figure 7.1).

All the combinations where *L. sericea* was dominant (i.e. LS:SJ :: 9:1, 8:2, 7:3, 6:4) inhibited the bacterial growth with MIC value of 1.5% (1305 µg/ml) and showed additive interactions with FIC

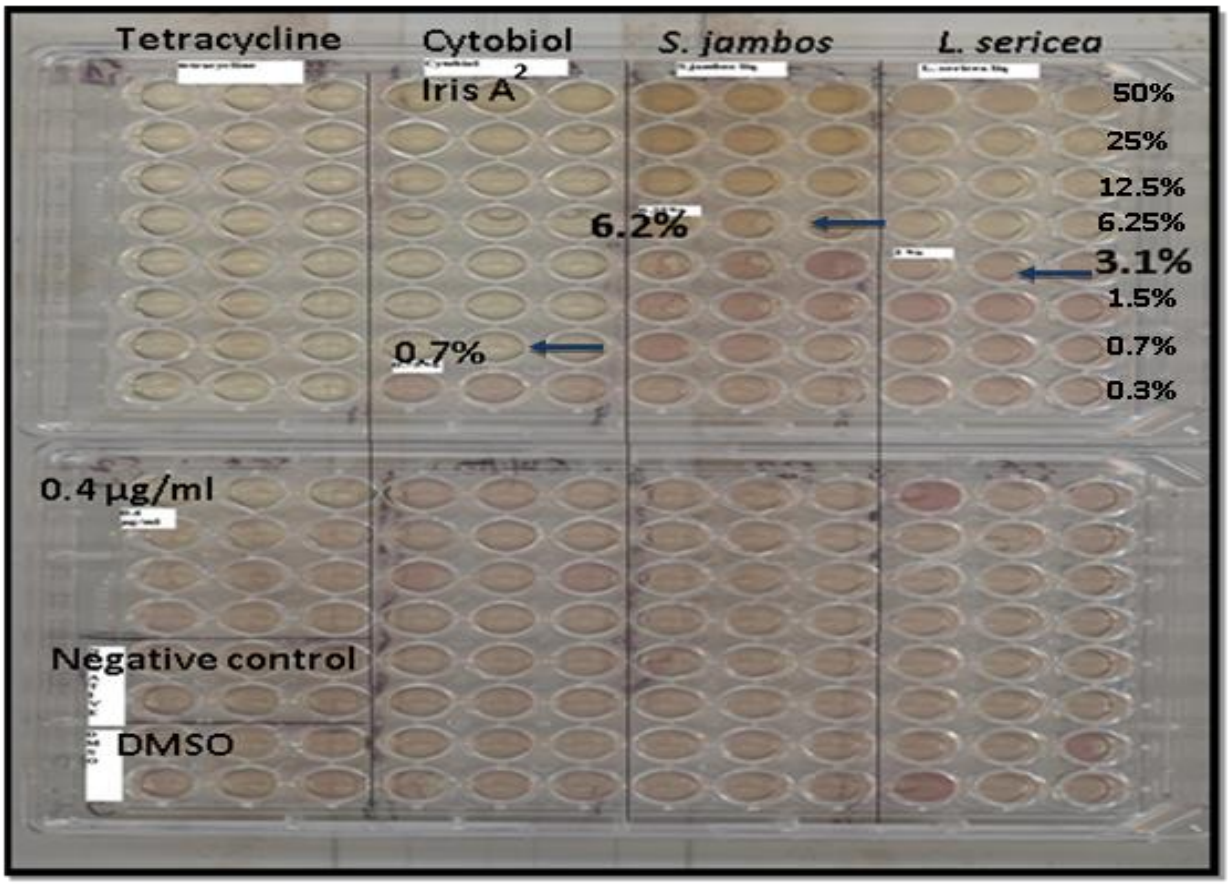


Figure 7.1: Antibacterial activity of the aqueous extract of *Syzygium jambos* (MIC 6.2% (5206 µg/ml)) and *Leucosidea sericea* (MIC 3.1% (2603 µg/ml)) in comparison with Cytobiol Iris A^2 (MIC 0.7%) against *Propionibacterium acnes*, tetracycline drug control (0.4 µg/ml)

Table 7.1: The minimum inhibitory concentration (MIC) and Fraction inhibitory concentration (FIC) index values of aqueous extract *Syzygium jambos* and *Leucosidea sericea*

Plant samples	**MIC % / µg/ml**	**∑ FIC**
L. sericea	3.1 / 2603	
S. jambos	6.2 / 5206	
Combinations of LS:SJ ratios		
9:1, 8:2, 7:3, 6:4	1.5 / 1305	0.72
5:5, 4:6, 3:7, 2:8, 1:9	0.7 / 650	0.33
Cytobiol Iris A^2 blend	0.7	

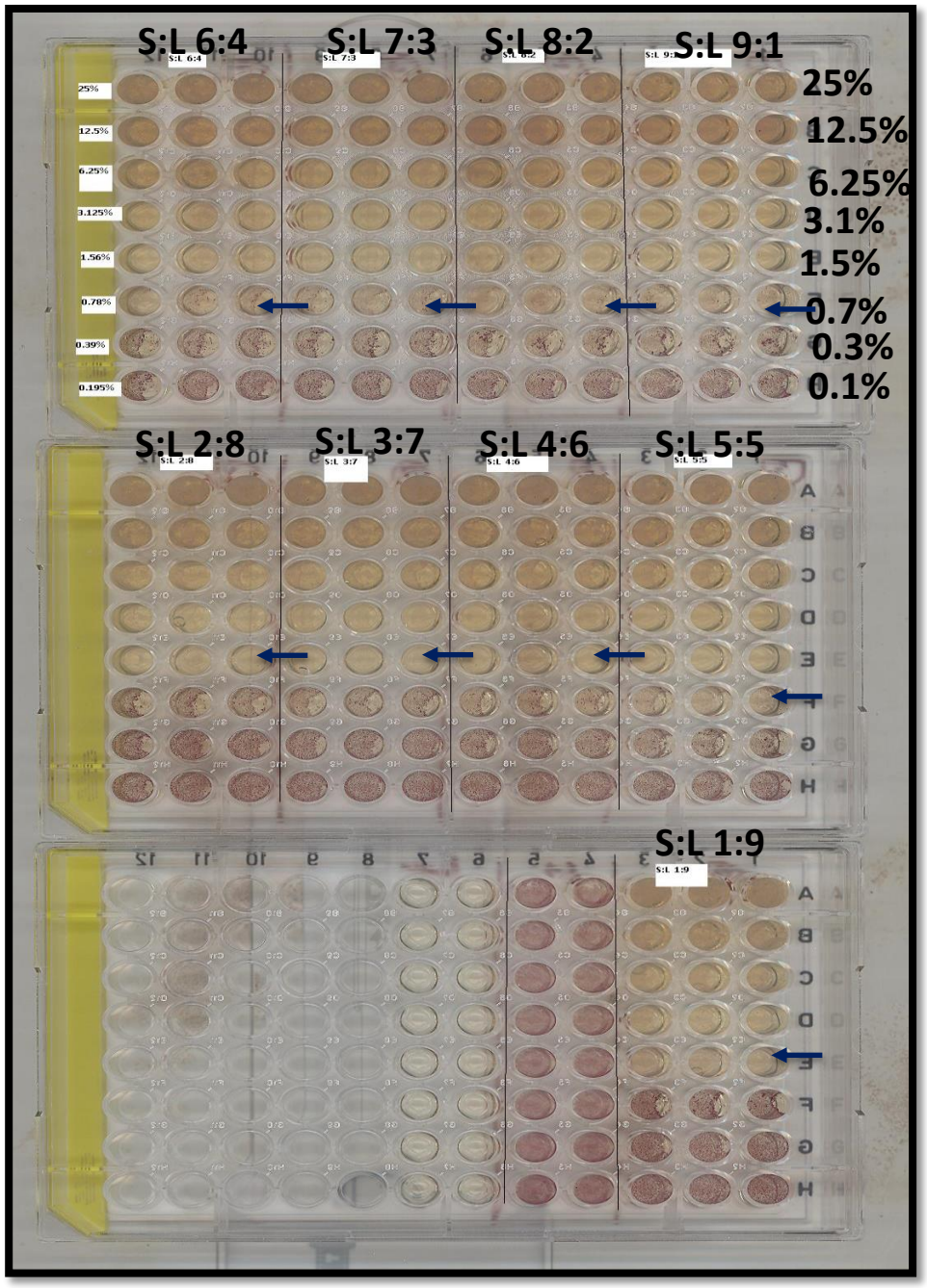


Figure 7.2: Antibacterial activity of aqueous extract of *Syzygium jambos* (SJ) and *Leucosidea sericea* (LS) in combinations - LS:SJ :: 9:1, 8:2, 7:3, 6:4 (MIC 1.5% / 1305 µg/ml); LS:SJ :: 5:5, 4:6, 3:7, 2:8, 1:9 (MIC 0.7% / 650 µg/ml). (MIC values of different combinations are indicated by arrows)

index value of 0.72. The other combinations of LS:SJ where *S. jambos* was dominant and in equal ratio to *L. sericea* (i.e. LS:SJ :: 5:5, 4:6, 3:7, 2:8, 1:9) inhibited the bacterial growth with MIC value of 0.7% (650 µg/ml) (Figure 7.2) and depicted synergism with FIC index value of 0.33. The results of MIC and

FIC index values of *L. sericea* and *S. jambos* showed that the aqueous extract of both these plants have exhibited improved antibacterial activity when tested in combination.

The MIC and FIC index values of *L. sericea* and *S. jambos* therefore, proves that the aqueous extract of both these plants have shown improved antibacterial activity when tested in combination.

This study hence provide an evidence to the concept that plants when used in combinations provide multiple compounds and that act at many target sites and thus aid in enhancing overall activity and efficacy (Al-Bayati, 2008; Biavatti, 2009; Vuuren and Viljoen, 2011).

To the best of our knowledge, the study performed here on the aqueous extract of *L. sericea* and *S. jambos* in combination and as alone has been reported for the first time. Although previous researchers have reported similar studies on different plants and pathogens where combinations have demonstrated different interactions of synergism, additive, antagonism and non-interactive.

The commercially acquired synergistic blend Cytobiol Iris A^2, demonstrated MIC value of 0.7% (Figure 7.1). Similar results were found for LS:SJ combinations (5:5, 4:6, 3:7, 2:8, 1:9) (Figure 7.2). Therefore, it can be said that the MIC values obtained in this study are significant and comparable to commercial anti-acne actives. The combination of LS:SJ (1:1) was thus selected for clinical trials.

7.3.2 Clinical studies

The following samples were selected for the clinical studies:

1. Aqueous extract of *Leucosidea sericea* (8% diluted in distilled water)
2. Aqueous extract of *Leucosidea sericea* and *Syzygium jambos* in ratio 1:1.

Refer results to Appendix B.

The aqueous extract of *L. sericea* (8% diluted in distilled water) and combination of *L. sericea* and *S. jambos* in ratio 1:1 (8% diluted in distilled water) showed positive results for 24 h hydration. Hydration is an important factor in the treatment of acne.

Acne affects barrier function directly through the impact of the inflammatory process on epidermal growth and maturation. Medications used to treat a number of conditions indirectly disrupt barrier

function. Conventional acne therapeutics such as benzoyl peroxide and retinoic acid decreases stratum corneum hydration leading to a dry itchy skin. Hydration therefore, strongly benefits the acne prone skin. A skin hydrating agent acts as stabilized epidermal barrier which means less potential for skin irritation. Restoring and maintaining barrier function is critical in patients with dermatologic diseases. The addition of moisturizers/hydrating calming agents to acne treatment products helps maintain barrier function and will provide better outcomes. For comedonal acne, combination products containing moisturizers/ hydrating agents may be effective first-line agents (Tanghetti, 2005). Therefore the liq aq. extract of *L. sericea* and combination of *L. sericea* and *S. jambos* in ratio 1:1 could be an ideal concomitant alternative topical treatment for acne.

The INCI (International Nomenclature of Cosmetic Ingredients) is a system of names for various ingredients in a cosmetic formulation. The ingredients can be the names of chemicals, pigments, scientific names, oils or waxes. The possible INCI for LS:SJ (1:1) can be: Water, *Leucosidea sericea*, *Syzygium jambos*; whereas possible INCI for *L. sericea* aqueous extract can be: Water and *Leucosidea sericea*. Figure 7.3 shows a possible product prototype for hydration which include Water, *Leucosidea sericea*, *Syzygium jambos* or Water, *Leucosidea sericea* as active ingredients.

Figure 7.3: Possible product prototype which include Water, *Leucosidea sericea*, *Syzygium jambos* or Water, *Leucosidea sericea* as active ingredients

The *in vivo* study to determine the acne reduction efficacy of the selected samples on the face of human subjects was found to be negative (Appendix B, Figure 9.7). The possible reason might be the formulation (aqueous based cream) that was used in the clinical study. The new range of cosmetics

available for acne treatment are gel based formulation which are restricted in their application to the problematic area (acne, comedones, blackheads or papules) unlike cream which can be applied over the full facial surface. Therefore, further pilot study was performed on *L. sericea* extract (300 mg in 40 ml of 70% ethanol at 10%) using the gel based formulation. The results showed that the gel based formulation was effective in reducing papules and blackheads after 14 and 28 days (Appendix B, Figure 9.8). Similar clinical trials are being considered for *L. sericea* + *S. jambos* combination in future.

7.4 Conclusion

From the combination study showed here it can be concluded that the aqueous extract of *L. sericea* and *S. jambos* exhibited synergistic and additive interactions. It was interesting to note that none of the combinations showed antagonism or non-interactions. Also, the synergistic combination of LS:SJ in ratio 1:1 and LS alone was clinically proven for 24 h hydration at Future Cosmetics, Pretoria and therefore, could be an ideal concomitant alternative topical treatment for acne.

7.5 References

Al-Bayati, F.A., 2008. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. Journal of Ethnopharmacology, 116, 403–406.

Biavatti, M.W., 2009. Synergy: an old wisdom, a new paradigm for pharmacotherapy. Revista Brasileira de Ciencias Farmaceuticas/Brazilian. Journal of Pharmaceutical Sciences 45(3), 371–378.

Suliman, S., Van Vuuren S.F., Viljoen, 2010. Validating the *in vitro* antimicrobial activity of *Artemisia afra* in polyherbal combinations to treat respiratory infections. South African Journal of Botany 76, 655-661.

Tanghetti, A., 2005. Acne vulgaris and Rosacea: Optimizing Therapy. Skin Disease Education Foundation's 29th Annual Hawaii Dermatology Seminar, on March 18-24, in Maui, Hawaii.

Vuuren, S.V. and Viljoen, A., 2011. Plant-based antimicrobial studies-Methods and approaches to study the Interaction between Natural Products. Planta Medica 77, 1168-1182.

Chapter 8

Conclusions

CHAPTER 8

Conclusions

- The antimicrobial activity of 51 medicinal plants grown in South Africa was determined against *Propionibacterium acnes* using broth dilution method. No evidence could be found in the literature of the selected plant extracts against the tested bacteria.
- The plant extracts demonstrating significant antibacterial activity (~100 µg/ml) were further tested for their antioxidant potential and cytotoxicity. The plant extracts of *H. caffrum, C. apiculatum,* and *C. molle* (MIC 125 µg/ml) showed noteworthy antibacterial and antioxidant activity, these samples also showed moderate toxicity to mouse melanoma B16-F10 cells. The plant extracts of *A. linearis, S. birrea,* and *G. sutherlandii* (MIC 125 µg/ml) also exhibited good antibacterial and antioxidant activity and had low toxicity to the mouse melanoma cells therefore, proving their potential as anti-acne agents either alone or in combination with each other.
- *S. jambos* and *L. sericea* were found to be most active against *P. acnes* with significant MIC values of 31.25 and 15.62 µg/ml, respectively. This is the first scientific report of both these plants against the tested bacteria.
- Three perviously isolated compounds were identified for the first time form the ethanol extract of leaves of *S. jambos*. The compounds were identified as squalene (**1**), anacardic acid analogue (**2**) and ursolic acid (**3**) (Figure 5.4). Anacardic acid analogue was found to be active against *P. acnes* with MIC value of 7.81 µg/ml. Squalene and ursolic acid were however not found to be active at highest concentration of 500 µg/ml tested. This is the first report of compound squalene and ursolic acid for their activity against *P. acnes.*
- Four perviously isolated compounds and one new compound were identified for the first time form the ethanol extract of leaves of *L. sericea*. The compounds were identified as phytol acetate (**1**), triacontanol (**2**), phytol (**3**), alpha kosin (**5**) and new compound (*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol (**4**) (Figure 6.2). Alpha kosin was found to exhibit very significant activity against *P. acnes* with MIC value of 1.95 µg/ml. Compounds phytol acetate, triacontanol, phytol and (*E*)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol were however not found to be active at highest concentration of 500 µg/ml tested. This is the first report of all these compounds for their activity against *P. acnes*.

- The transmission electron microscopy indicated the lethal effects of active samples on the cells of *P. acnes*. The extracts of *S. jambos*, *L. sericea*, isolated bioactive compounds anacardic acid analogue and alpha kosin caused notable damages to cell wall, cell structure and intracellular content of *P. acnes*. This interesting study showed visual effects of active samples on bacteria was performed for the first time.
- The extracts of *S. jambos*, *L. sericea* and isolated compound alpha kosin exhibited noteworthy DPPH radical scavenging activity with EC_{50} values of 0.9, 2 and 5.1 µg/ml, respectively, very similar to vitamin C (EC_{50} 2 µg/ml). All the other isolated compounds from both the plants did not show any antioxidant activity. The DPPH scavenging results of *L. sericea*, squalene and all compounds isolated from *L. sericea* are reported for the first time.
- The ethanol extract of *S. jambos* exhibited very low levels of toxicity to human macrophage U937 cells while moderate toxicity to mouse melanoma B16-F10 cells. Squlane was not found to be toxic to both cell lines whereas, anacardic acid analogue and ursolic acid was found to be moderately toxic to U937 cells. These study of cytotoxicity of selected samples on both cell lines is reported for the first time.
- The ethanol extract of *L. sericea* demonstrated moderate toxicity to both cell lines. Isolated compounds triacontanol and phytol were not found to be toxic whereas, alpha kosin showed high toxicity to both cell lines. The cytotoxicity of *L. sericea* and compounds on selected cell lines is reported for the first time.
- The potential of plant extracts of *S. jambos*, *L. sericea*, compounds myricetin, myricitrin, gallic acid (commercially acquired) and ursolic acid for suppression of inflammatory cytokines IL-8 and TNF-α in coculture of U937 cells and heat killed *P. acnes* was reported for the first time.
- Inhibitory activity of *S. jambos* and *L. sericea* extracts on glutathione reductase (GR) enzyme was performed for the first time. Both plant extracts were found to inhibit GR with IC_{50} values of 10.4 and 3.1 µg/ml, respectively.
- The combination study of aqueous extract of *S. jambos* and *L. sericea* indicated interesting results of synergism and additive interactions between these two plants. The combination of two plant extracts (1:1) and aqueous extract of *L. sericea* showed hydration for 24 h in an *in vivo* study, therefore, proves the potential of both these plants for acne problem.

Chapter 9

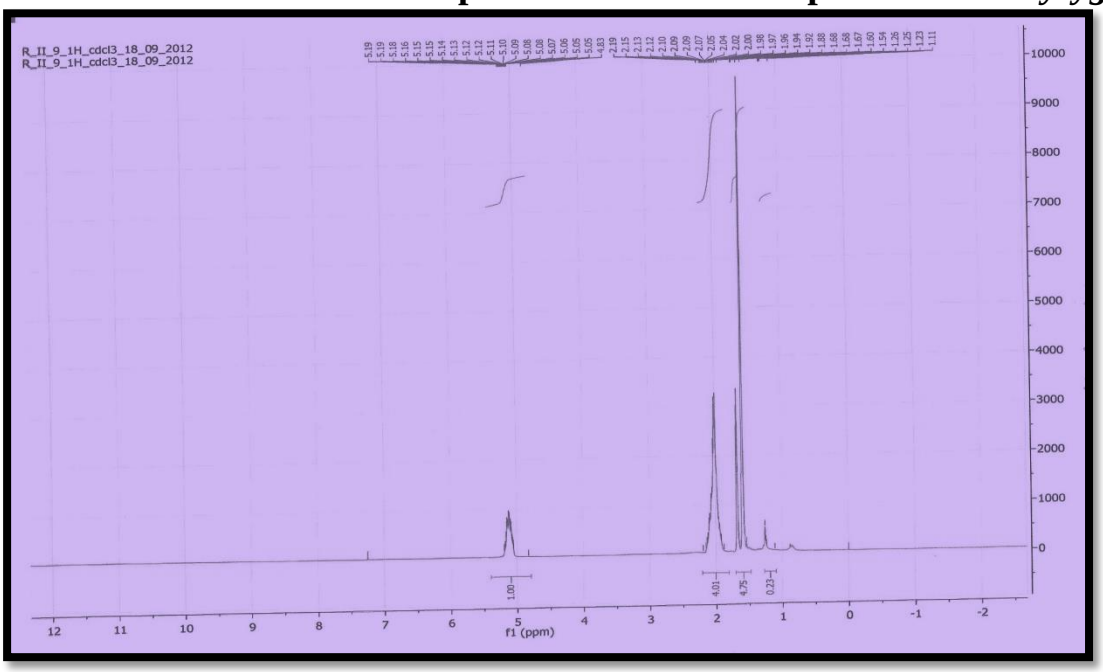
Appendix

CHAPTER 9

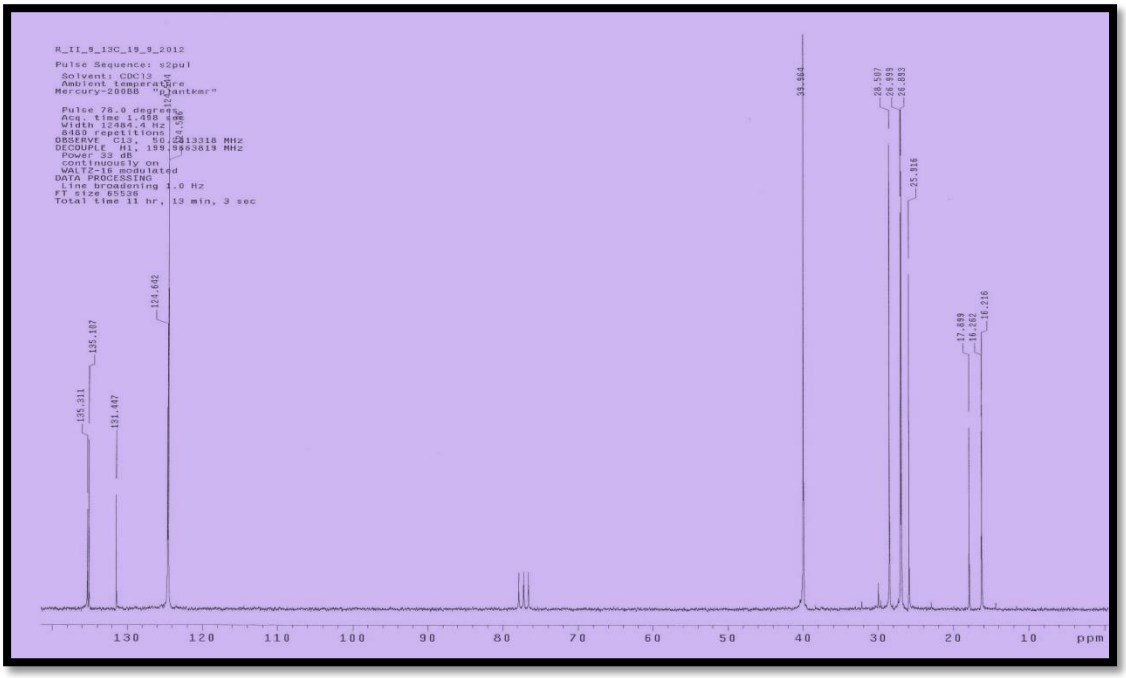
Appendix

Appendix A

9.1 ^{1}H-NMR and ^{13}C-NMR spectra of isolated compounds from *Syzygium jambos*

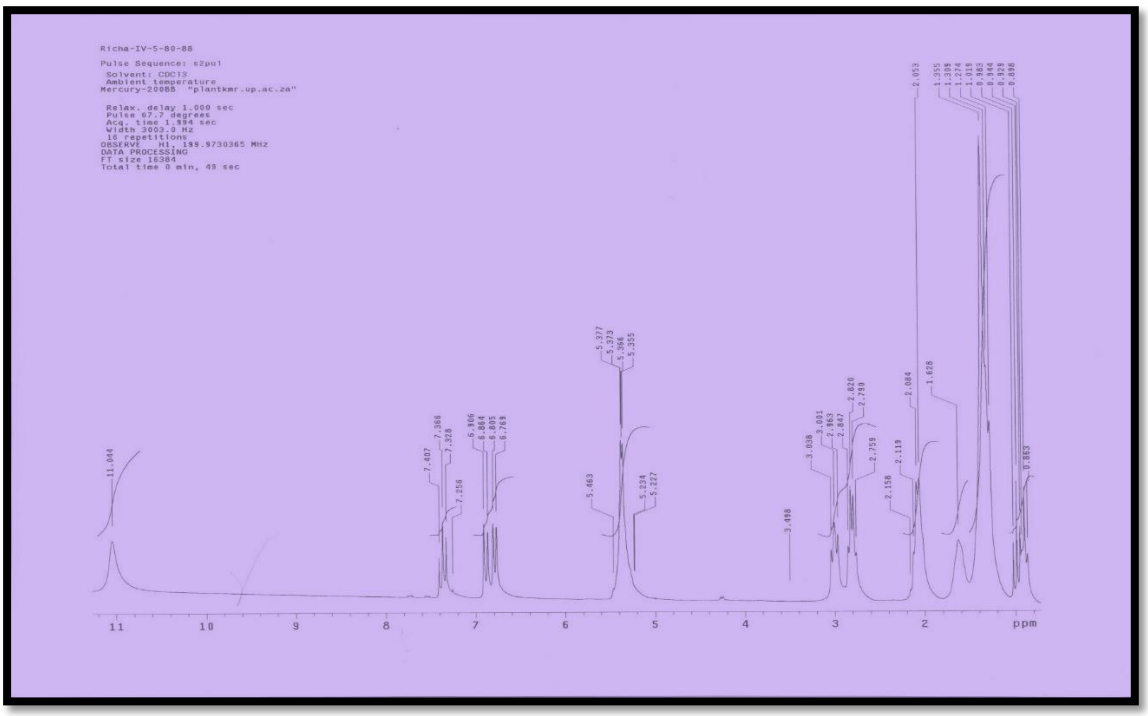


(a)

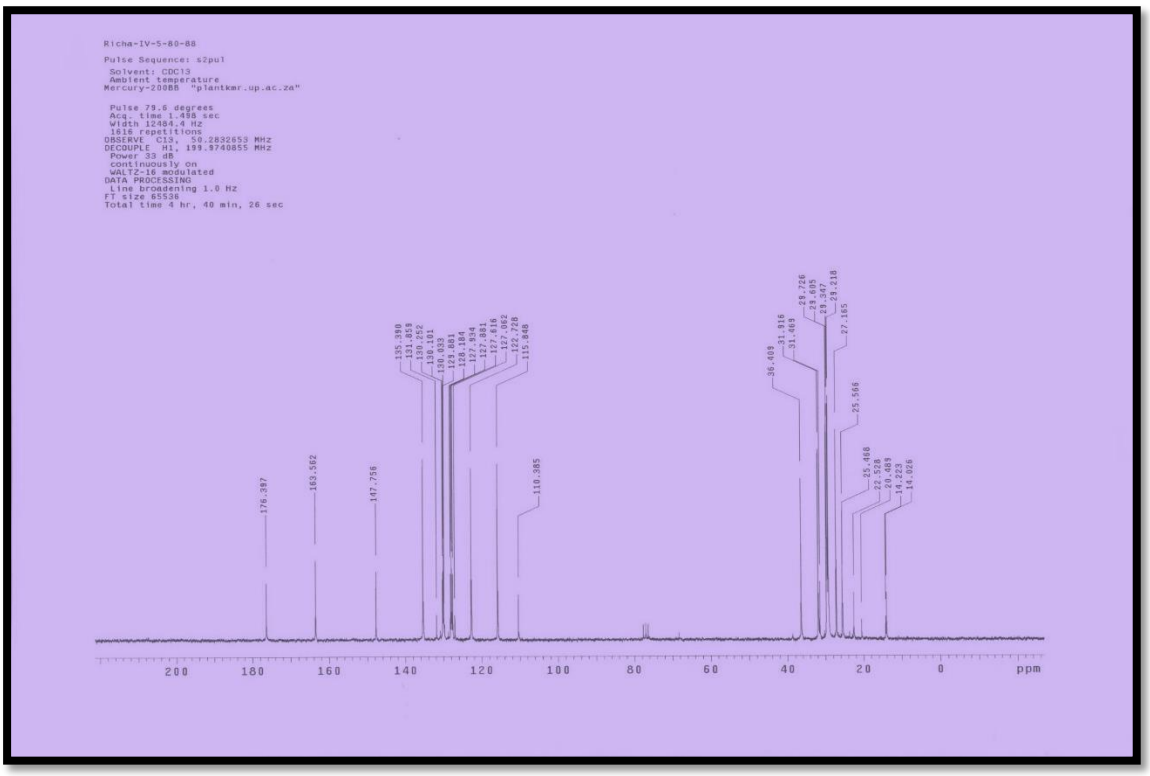


(b)

Figure 9.1: (a) ^{1}H-NMR and (b) ^{13}C-NMR of squalene

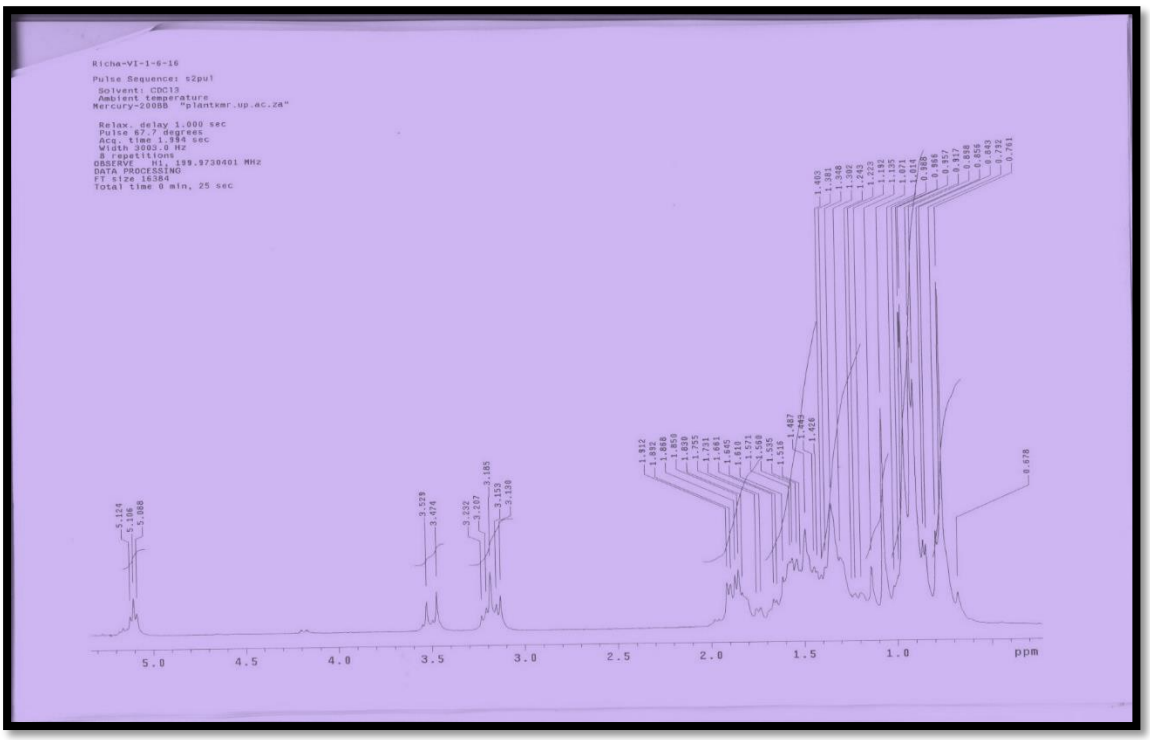


(a)

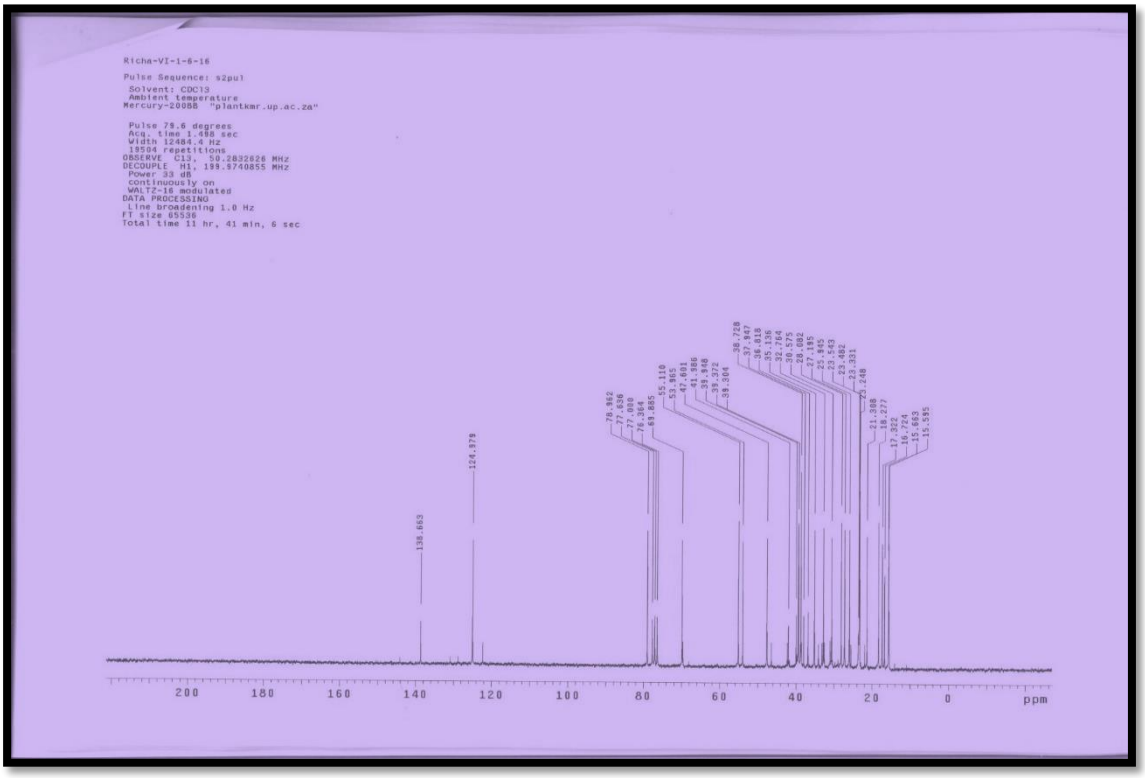


(b)

Figure 9.2: (a) ^{1}H-NMR and (b) ^{13}C-NMR of anacardic acid analogue



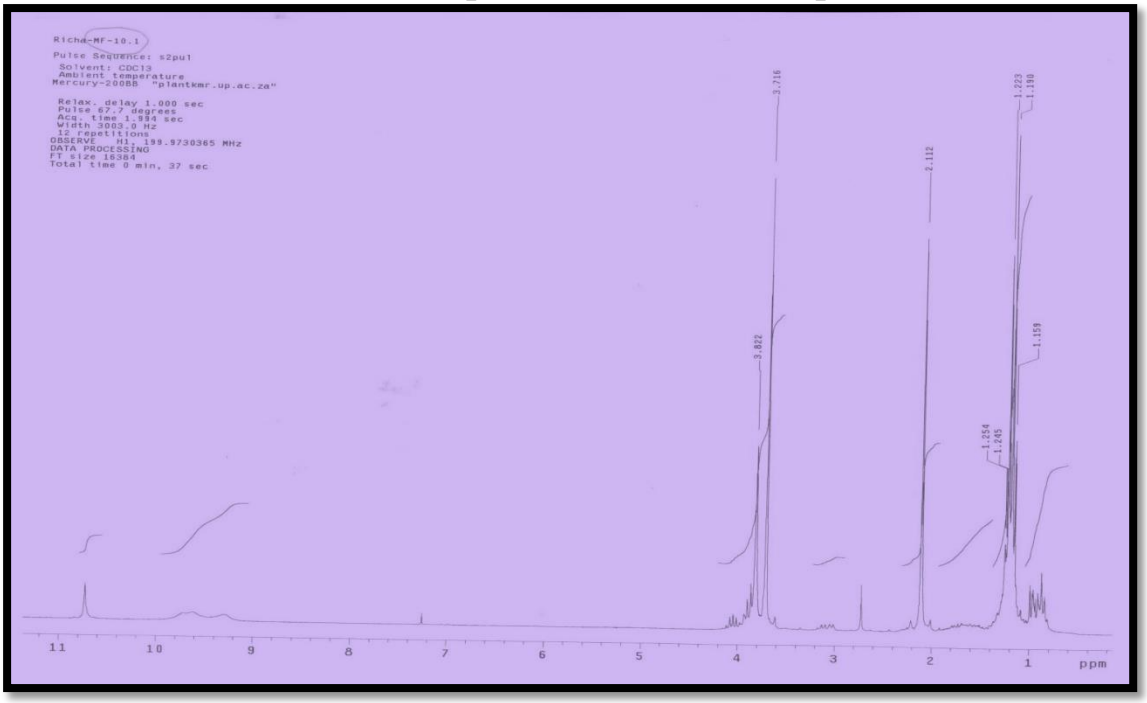
(a)



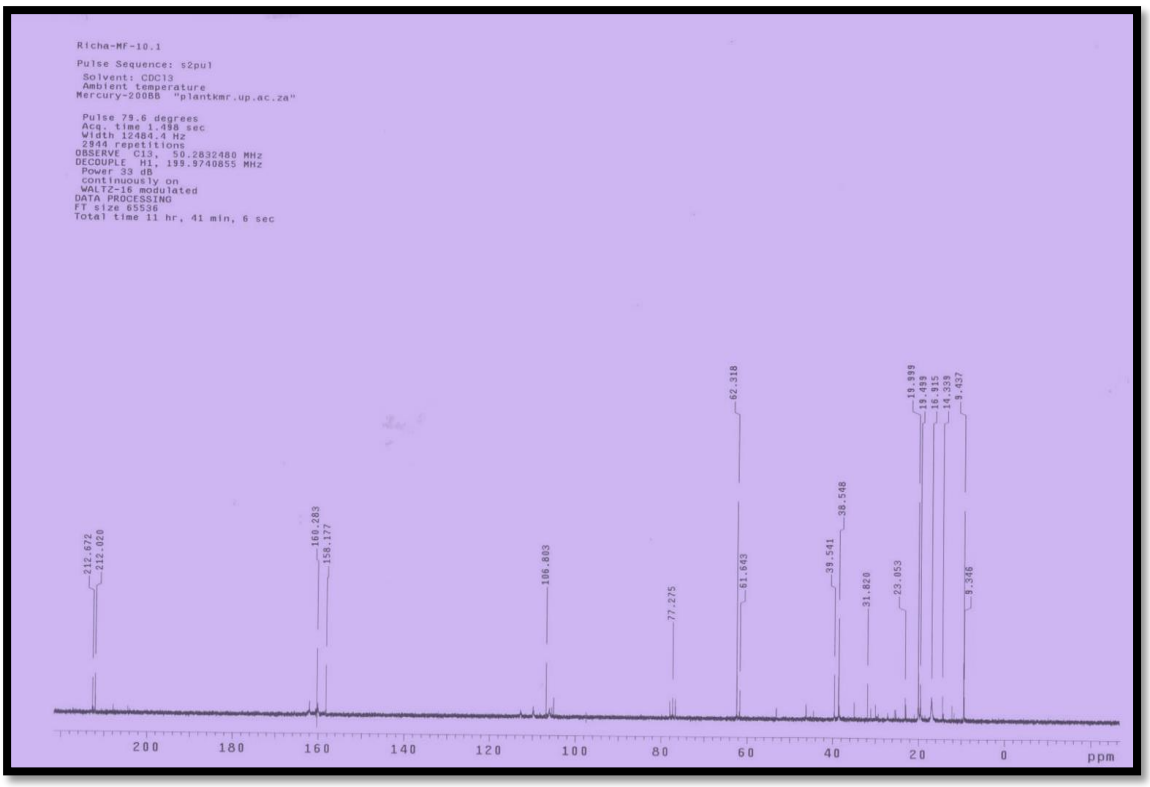
(b)

Figure 9.3: (a) ^{1}H-NMR and (b) ^{13}C-NMR of ursolic acid

9.2 ^{1}H-NMR and ^{13}C-NMR spectra of isolated compounds from *Leucosidea sericea*



(a)



(b)

Figure 9.4: (a) ^{1}H-NMR and (b) ^{13}C-NMR of alpha kosin

Appendix B
9.3 Efficacy of *Syzygium jambos* and *Leucosidea sericea* in clinical studies

FUTURE COSMETICS CC
From Concept to Product

Results and Conclusions:

A Summary of the results is given in Attachment E.
TEST RESULTS VALUES AFTER NINETY-SIX (96) HOURS

NO:	TEST PRODUCT NAME:			Average Value	Average Score	Number of subjects with reactions after 48 hrs	Irritancy Potential % (TP-NC)/(PC-NC)	Irritancy
1	FCSS219	/NCR	Negative contol	0.15	0.41	4	0.00	Non Irritant
12	FCSS219	/4049	Ls (dH20) 16-08-2012	0.21	0.61	4	12.89	Mild Irritant
13	FCSS219	/4050	Ls & Sj (dH20) 16-08-2012	0.27	0.70	5	18.31	Mild Irritant
20	FCSS219	/PCR	POSITIVE CONTROL RIGHT	1.18	1.99	19	100.00	Irritant

Kind regards

HEIBRIE LE ROUX
(MANAGING MEMBER)

Member:Heibrie Le Roux Registration No:2001/055088/23
Address:287 Sinovich Street, Grootfontein C.E, Garsfontein Road, Pretoria East, RSA
Tel:+27-128110621 Fax:+27-128110510 Mobile:+27-829613509 E-mail:heibrie@futuercosmetics.co.za

Figure 9.5: Irritancy patch testing results (mild irritant) of aqueous extract of *Leucosidea sericea* and *Leucosidea sericea+Syzygium jambos* from Future Cosmetics

FUTURE COSMETICS CC

From Concept to Product

Management Summary

Test Product	Test	Result
Ls & Sj (dH20) 16-08-2012 (FCLEN077/4050) compared to Distilled Water (FCLEN077/NC)	Reduction of Erythema Efficacy (Mexameter)	FAIL No significant reduction in erythema at all time points at a 5% level of confidence
	Increase in Hydration level (Corneometer)	PASSED Significant increase in hydration 24 hours (T24) at a 5% level of confidence FAIL No significant increase in hydration 48 hours (T48) and 72 hours (T72) at a 5% level of confidence
LS (DH20) 06-09-2012 (FCLEN077/4120) compared to Distilled Water (FCLEN077/NC)	Reduction of Erythema Efficacy (Mexameter)	FAIL No significant reduction in erythema at all time points at a 5% level of confidence
	Increase in Hydration level (Corneometer)	PASSED Significant increase in hydration 24 hours (T24) at a 5% level of confidence FAIL No significant increase in hydration 48 hours (T48) and 72 hours (T72) at a 5% level of confidence

Attached please find the report and calculations.

Please do not hesitate to contact me.

Kind Regards

Heibrie Le Roux
(Managing Member)

Member: Heibrie le Roux
Registration Number: 2001/055088/23
Physical Address: 287 Sinovich Street, Grootfontein Country Estates,
Garsfontein Road, Pretoria East, South Africa,
Postal Address: PO Box 39675, Garsfontein East, South Africa, 0060
Tel: +27-12-811 0621 Fax: +27-12-811 0510 Mobile: +27-82-9613509
E-mail: heibrie@futurecosmetics.co.za

2

Figure 9.6: *In vivo* soothing and calming results (passed 24 h hydration test) of aqueous extract of *Leucosidea sericea* and *Leucosidea sericea*+*Syzygium jambos* from Future Cosmetics

FUTURE COSMETICS CC

From Concept to Product

Management Summary

Test Product	Test	Result
LS @10% in Aqueous Cream (FCAH095/4049) compared to Aqueous Cream (FCAH095/NC)	Reduction of Comedones (Physical Count)	FAIL
	Reduction of Pustules (Physical Count)	FAIL
	Reduction of Papules (Physical Count)	FAIL
	Reduction of Blackheads (Physical Count)	FAIL
	Reduction of Whiteheads (Physical Count)	FAIL
	Reduction of Total Acne (Physical Count)	FAIL
	Reduction of Total Acne (Objective Sensory)	FAIL
	Reduction in Skin Roughness (Visioscan)	FAIL
	Increase in Skin Roughness (Visioscan)	FAIL

Attached please find the report and calculations and invoice. Please contact us if all is not included within 14 days of receipt of results.

Please do not hesitate to contact me.

Kind Regards

Helbrie Le Roux
Managing Member

Member: Helbrie le Roux
Registration Number: 2001/065088/23
Physical Address: 287 Sinovich Street, Grootfontein Country Estates, Garsfontein Road, Pretoria East, South Africa,
Postal Address: PO Box 39675, Garsfontein East, South Africa, 0060
Tel: +27-12-811 0621 Fax: +27-12-811 0510 Mobile: +27-82-9613509
E-mail: helbrie@futurecosmetics.co.za

2

Figure 9.7: *In vivo* acne reduction results (failed to reduce acne) of aqueous extract of *Leucosidea sericea* and *Leucosidea sericea+Syzygium jambos* from Future Cosmetics

FUTURE COSMETICS CC
From Concept to Product

Table 2: Comparison of objective sensory (physical count, PAPULES) values for FCAG008/4354 compared to FCAG008/AqC test sites:
Mean
(± Standard Deviation)

Time Interval	FCAG008/4354	Table Ref.	FCAG008/AqC	Table Ref.	(Diff) p-Value	Table Ref.
BASELINE	3.50 (± 2.83)	F5	2.65 (± 0.25)	F7	Not applicable	Not applicable
D14 - BASELINE	-2.00 (± 4.95)	F5	-2.15 (± 0.25)	F7	Not applicable	Not applicable
D28 - BASELINE	-3.39 (± 1.96)	F6	-1.16 (± 1.65)	F8	Not applicable	Not applicable

Conclusions:

Objective sensory (physical count, PAPULES)

It can therefore indicate that the Test Product (FCAG008/4354) could be effective in reducing the papule count after twenty-eight (28) days; should this study be conducted in full.

FUTURE COSMETICS CC
From Concept to Product

Table 5: Comparison of objective sensory (physical count, BLACKHEADS) values for FCAG008/4354 compared to FCAG008/AqC test sites:
Mean
(± Standard Deviation)

Time Interval	FCAG008/4354	Table Ref.	FCAG008/AqC	Table Ref.	(Diff) p-Value	Table Ref.
BASELINE	5.25 (± 6.72)	F17	2.00 (± 0.71)	F19	Not applicable	Not applicable
D14 - BASELINE	-1.75 (± 6.01)	F17	0.50 (± 2.83)	F19	Not applicable	Not applicable
D28 - BASELINE	-3.00 (± 5.66)	F18	-0.50 (± 1.41)	F20	Not applicable	Not applicable

Conclusions:

Objective sensory (physical count, BLACKHEADS)

It can therefore indicate that the Test Product (FCAG008/4354) could be effective in reducing the blackhead count after fourteen (14) to twenty-eight (28) days; should this study be conducted in full.

Figure 9.8: *In vivo* papule and blackhead count (passed for 14/28 days) results for 70% ethanol extract of *Leucosidea sericea* from Future Cosmetics