NATURAL COSMETICS FROM SOUTH AFRICAN WETLAND PLANTS

Prof Namrita Lall

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Natural Cosmetics from South African Wetland Plants

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by

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EXECUTIVE SUMMARY

Background and rationale

Aquatic plants are found in ecosystems all over the world, ranging from swamps and rivers to wetlands and ocean shores. Aquatic plants can produce phytochemicals, protecting them from environmental stresses. These phytochemicals have been used in drug development to treat human diseases for centuries. However, minimal research has been undertaken to explore the potential of indigenous South African aquatic plants and their phytochemicals for the treatment of various diseases.

Aims and objectives

This project aimed to investigate the potential of indigenous South African aquatic plants that are traditionally used as well as some previously unknown ones, for the treatment of:

- Acne
- Skin hyperpigmentation
- Wrinkles
- Periodontal diseases
- Tuberculosis
- Cancer

Furthermore, the project aimed to develop novel alternative treatments from aquatic plants which accumulate lead and which can be commercialised, benefiting the South African economy and indigenous knowledge holders.

Methodology

A serial microtiter dilution assay was used to measure the minimum inhibitory concentration (MIC) values for plant extracts using PrestoBlue™ as the growth indicator. The antibacterial activity of the samples was determined against *Cutibacterium acnes*, *Prevotella intermedia*, *Streptococcus mutans*, *Mycobacterium tuberculosis* and *Mycobacterium smegmatis.*

The minimum biofilm formation inhibition concentration (MBFIC) of the lead samples were tested against the biofilm formation of *M. smegmatis*.

Isolation studies were carried out on ET and PS that were identified as lead samples against the enzymes associated with wrinkle formation (elastase) and pigmentation (tyrosinase). GC-MS analysis was used to identify potentially active compounds in the plants.

The cytotoxicity and anticancer activity of the samples were tested against HaCaT, MEL-1, MCF-7 and HeLa cell lines.

The neat ethanol extracts were sent in their undiluted form for irritancy studies after which they were formulated in a cream formulation and underwent *in vivo* efficacy studies at Future Cosmetics for 28 days.

Results and discussion

CAA, TC, BR and GP demonstrated moderate antibacterial activity against *Cutibacterium acnes* at minimum inhibitory concentrations (MIC) of 250 µg/ml and 125 µg/ml, respectively.

Of those examined, JL was the most effective inhibitor of tyrosinase with a fifty percent inhibitory concentration (IC_{50}) of 31.64 ± 6.91 µg/ml while CS, CAA, ET, GP, PS and CM followed with moderate inhibitory activity with IC_{50} values ranging from 64.26 ± 5.12 µg/ml to 75.03 ± 4.98 µg/ml. Two isolated fractions of PS displayed moderate tyrosinase inhibitory activity with IC_{50} values of 68.77 ± 2.80 µg/ml and 79.82 ± 3.40 µg/ml. The GC-MS analysis revealed the major constituents to be (9Z)-9-Octadecenamide or 2, 4, 5-trimethoxybenzaldehyde and 4-(4-Nitrophenyl)-2-butanol, which could be responsible for the tyrosinase inhibition observed. No improvement in the tyrosinase inhibitory activity was seen when the lead samples were tested in combination. Clinical studies confirmed that JL was effective in promoting skin even-tone after 14 to 28 days of consecutive use, twice a day.

Excellent inhibition of elastase was observed for ET and PS that were comparable to the positive control ursolic acid, a known inhibitor of elastase. Clinical studies confirmed ET and PS were effective in reducing the appearance of wrinkles after 14 to 28 days of consecutive use twice a day.

The ethanolic extracts of BE and BR inhibited *S. mutans* at MIC values of 1.6 mg/ml and 3.1 mg/ml respectively.

Three of the plant extracts, CAA, PM and TC showed antibacterial activity against *M. tuberculosis*. CAA and PM were found to target biofilm formation of *M. smegmatis*. BR displayed noteworthy anti-biofilm activity with a minimum biofilm formation inhibitory concentration (MBFIC) of 62.5 µg/ml.

None of the samples displayed significant anti-cancer activity. The samples were confirmed to be non-toxic on human keratinocytes (HaCaT) with IC_{50} values above 100 µg/ml.

The lead aquatic plants were successfully cultivated at Botanica Natural Products.

Key findings

- JL, ET and PS passed clinical studies and can be considered for commercialisation.
- BE and BR displayed noteworthy activity against periodontal bacteria, *S. mutans*
- BR displayed noteworthy biofilm inhibitory activity against *M. smegmatis*

Conclusion

Of the twenty-seven indigenous aquatic plants investigated, three of the plants, PS, JL and ET, have been confirmed as holding potential for the treatment of various skin disorders.

BE and BR displayed potential against periodontal diseases and tuberculosis.

Future recommendations

- *In vivo* clinical studies for oral care could be explored in future projects for BE and BR
- Further studies could investigate the potential of BR targeting antibiotic resistance associated with tuberculosis
- Further studies are required to upscale PS and JL to viable products that can be commercialised with communities and indigenous knowledge holders benefiting from the research conducted.

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1. INTRODUCTION

In South Africa, traditional medicine is the preferred form of primary health care for approximately 70% of the population (Macaskill, 2010). Each year 19 500 tons of medicinal plants (Macaskill, 2010) are used in the treatment of illnesses affecting South Africans and, while terrestrial ecosystems are abundant and rich in diversity, the lack of ethnobotanical studies done on wetland ecosystems (Zhang et al., 2014) have impacted the number of potential resources that South Africans could be exploiting.

According to Macaskill (2010), wetlands support a great diversity of plant species, some of which have for generations been traditionally used by communities as a source of medicine, food and building materials (Zhang et al., 2014), proving that these plants can be applied to a variety of aspects of everyday life.

Aquatic plants and wetlands can be described as plants that have an association with an environment that has an abundance of water and vegetation itself (Tomlinson, n.d.). This wet habitat may range from open water to regions where the soil is just seasonally water-logged and the aquatic plants may be found either in, on or under the water, with specific adaptations that allow them to survive in each condition (Tomlinson, n.d.). These unique adaptations have developed over time in aquatic plants through an evolutionary process as they are believed to be phylogenetic descendants of terrestrial plants (Bornette & Puijalon, 2009).

Many aquatic plants share their environment, not just with other plants, but with microorganisms and wildlife as well and, like all plants, have the ability to produce phytochemicals to help them survive, grow and compete in such habitats. According to history, phytochemicals and drug development have been proven to be the medicinally important constituents of plants that are used to treat human ailments due to their ability to initiate physiological effects (Bhowmik et al., 2013). Many studies have been conducted on the bioactive compounds of aquatic plants found worldwide, but there has been little documentation undertaken on South African aquatic plants.

The current research project focused on the potential of indigenous South African aquatic plants for the treatment of skin disorders that include acne vulgaris, skin hyperpigmentation and wrinkles. Furthermore, the potential of these plants to target

periodontal diseases, tuberculosis and cancer was investigated, with a view to conceivably developing novel alternative treatments.

1.1 Skin disorders

1.1.1 Acne vulgaris

One of the most common human diseases of the skin is acne vulgaris caused by *Cutibacterium acnes*, effecting 85% of teenagers and 11% of adults (White, 1998; Fitz-Gibbon et al., 2013). Acne is found only in humans and is a condition that uniquely affects the pilosebaceous unit (hair follicles) of the face, back and chest, which are associated with oil glands (Williams et al., 2012). It can regress spontaneously after puberty however. Factors that promote its development include increased sebum production, ductal cornification (open and closed comedones), alterations in the microbial flora and inflammation (Jappe, 2003; Bhatia & Maisonneuve, 2004; Degitz et al., 2007). These factors cause the appearance of excessive grease on the skin (seborrhoea), non-inflammatory lesions (open and closed comedones), inflammatory lesions (papules and pustules) and scarring that can range in severity (Williams et al., 2012).

Current treatments

Systemic antibiotics administered for the treatment of acne vulgaris have both antimicrobial and anti-inflammatory properties as they can reduce the population of *C. acnes* within follicles resulting in the inhibition of bacterial-induced inflammatory cytokine production (Haider & Shaw, 2004).

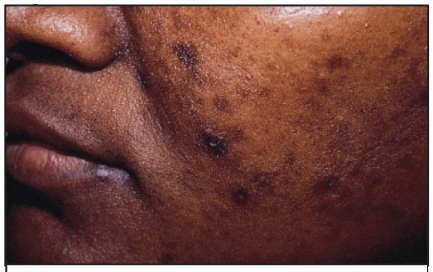


Figure 1:
Hidden inflammation in a darker-skinned acne patient with prominent post-inflammatory hyperpigmented spots
(White, 1998)

One of the most commonly prescribed and used antibiotics in the treatment of acne vulgaris is tetracycline. Tetracycline reduces the leukocyte chemotaxis as well as bacterial lipase activity (Haider & Shaw, 2004). Tetracyclines are effective antibiotics as their mechanism of action primarily relies on the compound binding to the bacterial ribosomes, preventing the possibility of the amino acyl-tRNA from binding to the acceptor site, inevitably inhibiting protein synthesis (Griffon et al., 2011). This ultimately reduces the population of *C. acnes* and decreases the production of sebum free fatty acids and extracellular lipases (Sapadin & Fleischmajer,

2006). However, the emergence of resistant strains of *C. acnes* has resulted in tetracycline treatments becoming ineffective.

1.1.2 Skin hyperpigmentation

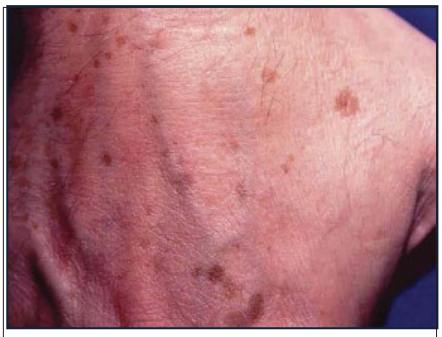


Figure 2: Solar lentigos on the hand (Ortonne et al., 2006)

The diversity of skin colour across the globe ranges from very pale skin to very dark; but, across this array of colours, there is a desire for uniform and even skin tone (Ortonne & Bissett, 2008). Problems with the pigmentary system include the overproduction of melanin resulting in hyperpigmentation (darker patches of skin) disorders, the result either of post-inflammatory, solar lentigos or melasma.

Acne lesions, ingrown hairs, scratches and insect bites are among the common activities that can cause the skin to become inflamed, resulting in post-inflammatory hyperpigmentation (PIH). Pigmentary disorders result due to the production of reactive oxygen species which stimulates melanin-producing cells (Figure 1).

Solar lentigos are hyperpigmented spots on the skin, better known as age spots and are a result of chronic exposure of the skin to ultraviolet (UV) light, resulting in a permanent genetic increase in the mRNA of melanogenesis-related proteins (Figure 2) (Ortonne & Bissett, 2008).

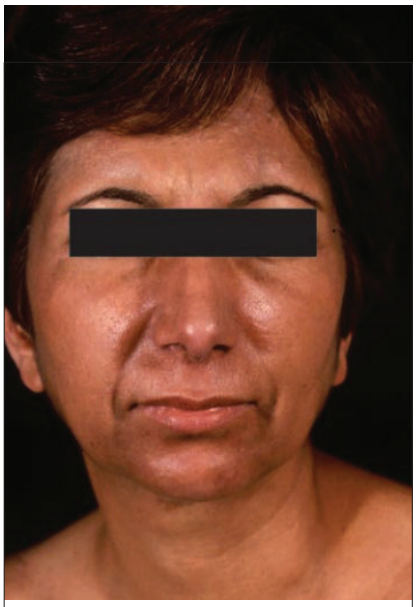


Figure 3: Melasma on the face (Ingber, 2009)

Melasma presents itself as symmetrical lesions on the facial skin and is a result of sun exposure, hormone therapy, pregnancy and the use of certain drugs and medications (Figure 3) (Pearl & Grimes, 1995).

The enzyme involved in all cases of skin hyperpigmentation is tyrosinase.

Tyrosinase

Tyrosinase is a copper-containing glycoprotein that functions as the rate-limiting enzyme within the pathway of melanogenesis. Since tyrosinase is the first enzyme in this process, by targeting its degradation or inhibition, there could be a drastic decline in the abundance of melanin, resulting in a more evened skin tone; hence, it is the common target when alleviating cutaneous hyperpigmentation (Ando et al., 2007).

Current treatments

The desire for an even skin tone has resulted in many treatments that are currently available. Traditional depigmenting agents, including hydroquinone, corticosteroids and kojic acid, although highly effective, have many adverse side effects and safety issues ranging from ochronosis, atrophy, carcinogenesis and other local or systemic effects. The emergence of such issues has provided an opportunity for researchers to develop new products, which have fewer side effects, to address pigmentation problems; thus, the search for novel natural and botanical extracts (Zhu & Gao, 2008).

1.1.3 Wrinkles

Ageing can be defined as the accumulation of diverse deleterious changes occurring in cells and tissues with advancing age that is responsible for the increased risk of disease and poor health (Harman, 2003). It is a multifactorial and very complex process evoking many theories to explain it (Kowald & Kirkwood 1996; Weinert & Timiras, 2003).

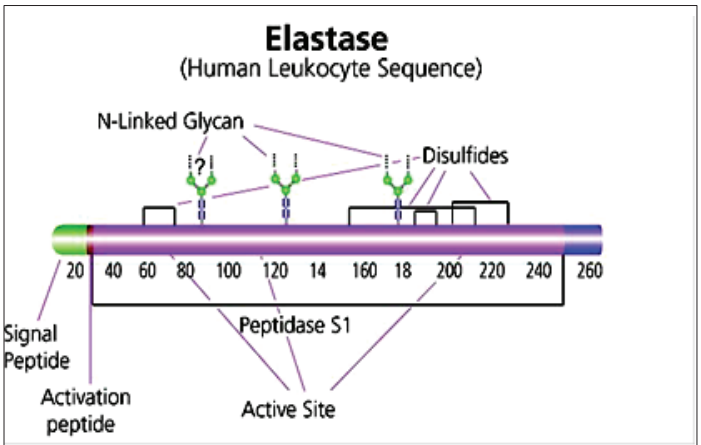


Figure 4: Basic structure of Human Leukocyte Elastase used in the present study indicating the active site and approximate locations of N-linked glycans and disulfide bonds of the enzyme in its active form (Sigma-Aldrich, 2015).

The dermal layer of the skin contains the structural components of the skin, which provide it with its structural integrity and elasticity. One of these structural components, elastin, is an insoluble elastic fibrous protein which forms the main component of animal connective tissues and tendons (Yan et al., 1994). Elastin is an

essential part of various human body tissues, including the arteries, skin and lungs, which depend upon elasticity, (Daamen et al., 2007; Fulop et al., 2012). The presence of elastin within these tissues is the attribute that affords them the ability to stretch and recoil and is therefore, the main contributor to skin elasticity and firmness (Daamen et al., 2007; Yuan & Walsh, 2006). The resulting problem is that the skin exhibits drastically declined levels of elastin with ageing, which in turn results in a loss of strength and flexibility, materialising as visible wrinkles (Ndlovu et al., 2013).

The flexibility and strength of elastin is increasingly diminished over time as the activity of elastase, the key degrader of elastin, is enhanced with age (Figure 4) (Nar et al., 2001). In addition to this, as time progresses, elastin is thought to be produced at a slower rate. The exclusivity of the elastase-elastin interaction and the decreased quality of the elastin network as a consequence of this interaction has resulted in a recent increase of interest in degradative enzymes, such as elastase, for its role in skin ageing (Maity et al., 2011; Jabs, 2012).

Elastase is naturally required by the skin to degrade dysfunctional proteins that remain within the extracellular matrix; for instance, after a wounding event so that they can be eliminated for repair to occur. This problem is further compounded by the fact that the number of epidermal stem cells (responsible for allowing the skin to differentiate and regenerate itself) decline with age and with this, the skin's ability to regenerate important structural components, such as elastin and collagen, also declines (Jabs, 2012; Nar et al., 2001). For that reason, inhibitors capable of inhibiting the elastase enzyme can be used in the successful treatment of wrinkles to fight the appearance of ageing.

1.2 Periodontal care

Periodontal diseases are included in the list of major health problems that humans face, with dental caries and periodontitis being among the most important and preventable infectious diseases (Nguyen & Martin, 2008). Oral care influences the general quality of life and poor oral health can be linked to more serious conditions including coronary heart disease (Dorn et al., 1999).

Streptococcus mutans is a gram-positive, anaerobic bacterium that lives within the mouth and can metabolise a variety of carbohydrates resulting in an acidic environment that becomes the leading cause of tooth decay and dental caries (Palombo, 2011).

Prevotella intermedia is a black pigmented, anaerobic, Gram-negative oral pathogen (Dorn et al., 1998). These pathogens are found living within the periodontal pockets in between human teeth where they co-exist with other oral microorganisms forming part of the human oral microbiota (Marcotte & Lavoie, 1998) however, when *P. intermedia* is found at higher bacterial populations than the other oral bacteria, the associated symptomatic diseases are periodontitis and acute necrotising ulcerative gingivitis (Haffajee & Socransky, 1994).

1.3 Tuberculosis

Tuberculosis (TB) is an infectious disease of the lungs and is a serious health problem globally, but particularly in developing nations. It is a bacterial disease caused by an organism known as *Mycobacterium tuberculosis*, and is contagious; easily spread through the air from one infected individual to another person (Figure 5). However, people infected with TB are not equally infectious; generally, people with TB of the throat or lungs are more contagious than others. Bacteria within the droplets expelled during coughing, sneezing and talking are released into the air, causing the spread of TB when they find a host.

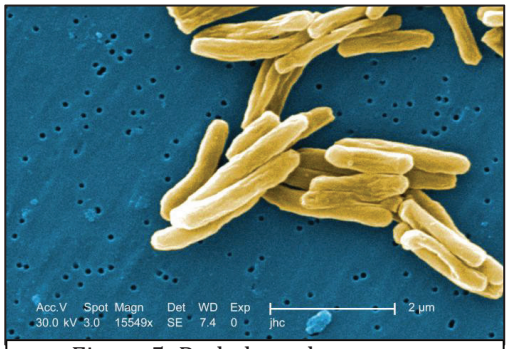


Figure 5: Rod-shaped structures of *Mycobacterium tuberculosis* under a scanning electron microscope (Todar, 2012)

TB is categorised in two forms: latent and active. Latent infections refer to the presence of a small population of *M. tuberculosis* bacteria within the body. However, this condition does not produce any symptoms due to the high response of the immune system (Amberson, 1938). It is believed that around one-third of the global population has latent TB. This form of TB can occur through the early restriction of the bacteria within the lungs; however, without proper treatment or having a compromised immune system due to HIV infections, it can progress to the stage of TB disease (Gomez & McKinney, 2004). Active infections are so characterised when the bacteria has the ability to cause the manifestation of symptoms and the condition can be transmitted from the host.

In 2015, there were an estimated 10.4 million new incidents of TB worldwide, of which 1.2 million (11%) were people already living with HIV. It was found that six countries accounted for 60% of the new cases worldwide, with South Africa being one of them. There is a direct need for major advances in global TB prevention as the rate of decline year to year is only 1.5%, while deaths total over a million individuals annually, resulting in TB remaining as one of the top 10 causes of death, worldwide (Figure 6) (WHO, 2015).

Figure 6: Global statistics of tuberculosis from 2015 (WHO, 2015)

1.4 Cancer

According to the United States Food and Drug Administration, a herbal remedy that claims to cure or prevent a human disease is considered a drug. In such case, a New Drug Application (NDA) needs to be filed with supporting scientific information, stating the biological activity in humans and the safety of the remedy. Therefore, it is necessary to verify the biological activity of the plant samples' general cytotoxicity by evaluating their cytotoxic effects against a normal cell line (Antignac et al., 2011; Kamatou, 2006).

Plants contain secondary metabolites as a natural defence mechanism against external factors. Although there are several secondary metabolites that have essential nutritional value, such as ascorbic acid, carotenoids and other vitamins, there are also compounds that may be toxic, such as alkaloids, cyanides, indoles, isothiocyanates or phenols (Antignac et al., 2011).

Cytotoxicity can be defined as the alteration of cellular morphology, failure of the cells to attach to surfaces, changes in the rate of cell growth, cell death and cell disintegration when the cells are exposed to a toxic agent (Horváth, 1980; Miret et al., 2006). Toxicity tests are carried out *in vitro* to determine the toxic effects of the tested compound or extract on a wide range of human and mouse cell lines. *In vitro* cytotoxicity tests are conducted to minimise animal toxicity testing (AltTox, 2016). Identification of cytotoxicity mainly occurs when cells undergo apoptosis and necrosis during exposure to a toxic agent (Miret et al., 2006).

Cancer cell lines investigated in the present study are as follows:

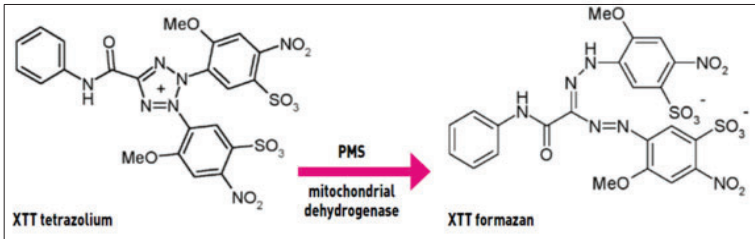


Figure 7: Schematic representation for the reduction of XTT tetrazolium (2, 3-Bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) to XTT

1.4.1 Skin melanoma (MEL-1)

Melanoma is a type of skin cancer which affects the skin and its cells. Melanoma is regarded as one of the most aggressive forms of cancer as it can metastasize (spread) to various locations on the body including, but not limited to the brain, lungs, liver and bones (Melnikova & Ananthaswamy, 2005). The World Health Organisation estimated that approximately 55 000 cancer-related deaths were due to melanoma in 2015 worldwide (World Health Organisation, 2015).

1.4.2 Breast cancer (MCF-7)

Breast cancer starts in the ducts and walls of the breast. It affects both men and women worldwide. In the UK alone, about 55,200 people are annually diagnosed with breast cancer. Around one in eight women and one in 870 men is at risk to develop breast cancer (Cancer Research UK, 2017).

1.4.3 Cervical cancer (HeLa)

Cervical cancer occurs when the cells in the cervix multiply uncontrollably. Cervical cancer accounts for approximately 9% of female cancer deaths each year. It most typically occurs in women below the age of 45 and is often caused by the human papillomavirus (HPV). According to the UK Cancer Research Institute, one in ten females are diagnosed with cervical cancer and each year, approximately 273,000 of deaths in the UK are attributed to this devastating disease (Cancer Research UK, 2005).

The XTT assay is based on measuring the number of viable cells after they have been exposed to various treatments. The cleavage of XTT tetrazolium salt that has a yellow colour to XTT formazan that is intensely red in colour, is monitored (Figure 7). This reduction of XTT tetrazolium to formazan can only occur in living cells, in the presence of mitochondrial dehydrogenase enzymes. However, when the cells are exposed to various treatments this results in membrane disruption and the enzyme is inactivated. Therefore,

the amount of formazan salt produced is directly proportional to the number of viable cells present (Roche, 2007).

1.5 Plant background

Table 1: Traditional use and biological activity of selected indigenous South African aquatic plants

Plant species	Traditional use	Biological activity	Reference
BE	Toothache & headaches	DCM: Methanol extract displayed activity against *Streptococcus mutans* at 1.6 mg/ml	Akhalwaya et al., 2018
BR	None reported	None reported	
CAA	None reported	None reported	
CB	Traditionally used to treat leprosy. The whole plant has been used medicinally to treat sore throats, burns, irritation of the eyes and stomach and infantile thrush. In southern Africa, the plant is used to combat infertility. In India and the Philippines the leaves and stems are used as a nutritional source for people and livestock,	Seeds tested against *Mycobacterium smegmatis*, mild inhibition was observed	Bagchi et al., 1998; Mbazima et al., 2008; van der Burg, 2004
CC	In the Zulu communities of South Africa, this genus is used widely to treat bodily swelling and problems relating to the urinary tract.	The leaves, bulbs and roots of CC were tested for their ability to inhibit acetylcholinesterase using a microtiter plate assay, the bulbs showed the greatest percentage inhibition of 68% at 0.1 mg/ml.	Elgorashi et al., 2001; Jäger et al., 2004.
CP	The corm is made into a decoction by Zulu, Xhosa and Sotho communities living in KwaZulu-Natal, South Africa, and is used to treat dysentery and diarrhoea.	None reported	McGaw et al., 2000

Plant species	Traditional use	Biological activity	Reference
CM	The rhizomes and bulbs of the related species is used to treat respiratory issues by traditional communities in KwaZulu-Natal	A study of the extracts of a related species showed a variety of effects including anti-inflammatory, anti-microbial, analgesic and anti-depressant activity	Ahmad et al., 2012; Chinsembu, 2015
CS			
CBr	Zulu communities in South Africa use the bulbs to treat intestinal worms such as tapeworm and roundworm.	None reported	Gerstner, 1939
ET	Not medicinally utilised but this plant, due to its structure, makes a good thatching material for roofs.	None reported	Harborne, 1979; Harborne et al., 1985
EZ	The seeds are used to treat asthma while the leaves are made into a tea for relief of tuberculosis. Powdered bark is used as a treatment for rheumatism while a tea of the bark is used for blood disorders.	The aqueous extract of the leaves showed a ratio of 0.9 inhibition when compared to that of the positive control, neomycin, against *Staphylococcus aureus* using the disc diffusion assay. The ethyl acetate, ethanol and aqueous extracts of the leaves did not show any inhibition towards the other microbes tested (*Staphylococcus aureus, Staphylococcus epidermis, Bacillus subtilis, Micrococcus luteus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*) The extracts of the leaves showed between 65% and 82% (ethanol) 17% and 90% (ethyl acetate) cyclooxygenase inhibition at concentrations of 50 and 500 µg/ml, respectively.	van Rensburg, 1982; Hennessy, 1991; Pillay et al., 2001

Plant species	Traditional use	Biological activity	Reference
ER	In China, the whole plant is used to treat wounds and ulcers by making a decoction, while in India it is used as a cooling medicine for gonorrhoea and has, for many centuries, been used as a treatment for skin wounds	The water, methanol and ether extracts of the related species showed no antibacterial activity against *Mycobacterium phlei* but did against *Staphylococcus aureus*	Jain & Srivastava, 2005; Mannan et al., 2008; Sen, 1980
GP	The roots are traditionally used in a remedy for pimples, skin eruptions, wounds, gonorrhoea, syphilis and urinary tract infections	Acetone and water extracts of the root were inactive against *Mycobacterium tuberculosis.* Minimum inhibitory concentration (MIC) against clinically important bacterial strains were reported at 39 µg/ml (*Staphylococcus aureus*); 9.8 µg/ml (*Staphylococcus epidermis*); 18 µg/ml (*Bacillus cereus*); 39 µg/ml (*Enterococcus faecalis*). Contains the compound venusol which is effective against breast cancer	Drewes et al., 2005; Lall & Meyer, 1999; Mathibe et al., 2016; Watt & Breyer-Brandwijk, 1962; Steenkamp et al., 2004
JL	The rhizomes of most related species are used to treat many ailments relating to the intestines, in Lesotho communities.	The antimicrobial compound is isolated from the aerial portions of related species. The antibacterial activity of the isolated compound was tested against the bacteria, *Staphylococcus aureus* and *Candida albicans*, results showed that the efficacy of the pure compound to provide antibacterial properties increased when illuminated by ultraviolet-A radiation. The compound, isolated from a relative showed inhibition against *Mycobacterium phlei* at 900 and 1800 ppm and no inhibition against *Mycobacterium smegmatis*.	Chapatwala et al., 1981; Duke, 2007; Hanawa et al., 2002; Khare, 2008; Moteetee & Van Wyk, 2011

Plant species	Traditional use	Biological activity	Reference
KP	None reported	None reported	
MS	A juice prepared from the leaves of related species is applied to the skin as a treatment for snakebites and traditionally used as a diuretic (urine production stimulant) and febrifuge (fever reducing agent). According to the ancient *Materia Medica* of China, the whole plant of related species is said to have anti-inflammatory and detoxifying properties	None reported	Duke & Ayensu, 1985; Stuart, 1911
ML	Traditionally used internally for the treatment of pulmonary and urinary tract infections. Externally used to relieve swelling and treat sores and minor wounds of the skin. The leaves and stems can be added to boiling water to release a vapour that can be inhaled to relieve nasal and bronchial congestion. It is also traditionally used to treat pulmonary tuberculosis and whooping cough. Used as a treatment for colic, menstrual disorders, indigestion, flatulence, headaches, fever and colds	Possible activity against *Mycobacterium smegmatis* using the methanol extracts in the agar diffusion method	Sökmen et al., 2000; Van der Walt, 2004; Watt & Breyer-Brandwijk, 1962
NC	Traditionally used as a poultice for healing wounds.	Ethyl acetate extracts of a related species were evaluated for antimicrobial activity against 10 clinically important bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Enterococcus faecalis, Xanthomonas campestris, Streptococcus mutans,*	Ammani & Kumar, 2012; Mabona & van Vuuren, 2013

Plant species	Traditional use	Biological activity	Reference
		Lactobacillus casei, Lactobacillus acidophilus) using the agar well diffusion method. The ethyl acetate leaf extracts were active against all the investigated bacterial strains.	
NT	During hunting accidents, an emollient plaster from a related species was made using the stems, leaves and flowers. The emollient plaster is applied to the wounds to help with extracting small shot fragments	Related species has shown antibacterial properties	Chowdhury, 2012; Fern, 2014.a
PM	None reported	None reported	
PS	The leaves and roots of the species are pounded and applied to syphilitic sores and skin infections to reduce swelling.	The tyrosinase inhibitory effects of a related species of flower extracts were tested by in order to develop a new agent for skin whitening. The extract showed inhibitory activity on mushroom tyrosinase with an IC_{50} value of 70.8 ± 2.2 µg/ml. Possible presence of leukaemia preventing compound, apigenin as well as the immune-inhibitory compound of lymphocyte proliferation, calycopterin	Fern, 2014.b; Hussein et al., 2012
PL	Used in traditional herbal remedies for its anti-microbial activity. The leaves of related species can be made into a poultice and used externally for the inflammation associated	Related species have shown inhibition of *Mycobacterium phlei*.	Grieve, 2012; Samuelsen, 2000; Watt & Breyer-Brandwijk, 1962

Plant species	Traditional use	Biological activity	Reference
	with insect bites, minor sores, boils and rashes caused by poison ivy. When taken as a tea, tincture or syrup, the leaves of related species are known to help with respiratory tract problems – relieving coughing and bronchitis as it acts as an expectorant (loosens/thins/liquefies mucous to enable it to be brought up and other material from the lungs, bronchi and trachea). Related species have been used in traditional herbal remedies due to its astringent, anti-toxic, anti-histamine, demulcent (reduces pain and inflammation in membranes), styptic (blood coagulant agent) and diuretic properties.		
SK	Arrow poison (toxic), dropsy, scabies and wound healing.	None reported	Notten, 2001
SF	None reported	None reported	
TL	The Khoi-San communities of South Africa traditionally infuse the herb with milk. The milk is then given to patients to treat intestinal worms, fever, influenza, high blood pressure, tuberculosis. The plant is also used mainly as a culinary herb to add flavour to food or recreationally to strengthen the taste of tobacco.	None reported	Watt & Breyer-Brandwijk, 1962

Plant species	Traditional use	Biological activity	Reference
TC	Female flower extract of the relative species had wound healing accelerating activity. The narrow leaves are used as thatch for mats and baskets while the seeds are used as pillow stuffing. A decoction of the rhizomes is used to treat venereal diseases, bleeding, diarrhoea, swelling and urinary problems. During labour the decoction can either be taken orally or applied externally to promote the removal of the placenta and strengthen uterine contractions to ensure an easy delivery. Related species are also taken to promote fertility in women, enhance male potency and libido and improve circulation	The methanol extracts did not show any inhibition or toxicity against *Mycobacterium smegmatis* at the highest concentration tested (2500 µg/ml)	Akkol et al., 2011; Hutchings et al., 1996; Nielsen et al., 2012; Watt & Breyer-Brandwijk, 1962; Voigt & Porter, 2007
ZA	The washed leaves are heated and used as a dressing for wounds, boils, minor burns, insect bites and sores. Patients suffering from gout or rheumatism also use the warmed leaves as poultice to reduce the pain. Traditional communities located in the Cape, powder the rhizome and use it as a poultice for inflamed wounds. The plant can be boiled and eaten by mixing it with honey or syrup as a treatment for asthma and bronchitis; it can also be gargled for the relief of sore throats. The plant must be boiled or cooked in some way as the raw plant material causes swelling of the throat due to the presence of microscopic calcium oxalate crystals	The leaves and roots are said to possess antibacterial properties. The methanol extracts of the leaves and stems showed no inhibition or toxicity against *Mycobacterium smegmatis* at the highest concentration tested (2500 µg/ml)	Nielsen et al., 2012; Roberts, 1990; Rood, 2008; Watt & Breyer-Brandwijk, 1962; Wink & van Wyk, 2008

2. MATERIAL AND METHODS

2.1 Plant collection and identification

Various plant parts (related to traditional use, impact on sustainability and quantity of available material) of twenty seven different plant species were harvested from the botanical gardens located at the Hatfield Campus and the LC de Villiers Sports Campus of the University of Pretoria, South Africa. Plant species that were unavailable at the University were purchased from Wild Flower Nursery (Hartbeespoort, South Africa). Voucher specimens were prepared for each species and were housed in the H.G.W.J. Schweickerdt Herbarium at the Department of Plant and Soil Sciences, University of Pretoria**.** Table 1 depicts the plant species collected, their reported traditional use and identified biological activity, while Table 2 indicates the parts of the plants used and the collection sites.

2.2 Preparation of plant extracts

The plant materials collected from the areas surrounding the water were surface rinsed and frozen at minus 80°C for 3 days. Thereafter, the materials were freeze-dried and subsequently ground to a fine powder using a 2 mm IKA grinder (MF 10.1 Head 2870900). The ground plant material was weighed to allow for a 1:5 ratio of plant material to 100% ethanol. The plant species collected directly from the water were blended with ethanol in the same ratios after surface rinsing. Both mixtures were then placed on a shaker (Labcon Shaker 3086U) and left to shake for 7 days. The ethanolic extraction liquid was separated from the ground/blended plant material using a filter (Whatman® No.1 filter paper) and vacuum pump. The ethanol was evaporated from the sample using a rotary vapour (BUCHI® Rotavapor B-480) apparatus. The resulting extract was placed in the -4°C freezer to be used for subsequent experiments.

2.3 Skin disorders & periodontal health

2.3.1 Antibacterial microtiter dilution assay

A serial microtiter dilution assay was used to measure the minimum inhibitory concentration (MIC) values for plant extracts using PrestoBlue™ as the growth indicator (Eloff, 1998; Lall et al**.**, 2013). Pure cultures were obtained by streaking a bead on sterile TS nutrient agar (*P. intermedia*) and BHI agar (*S. mutans* and C*. acnes*). Agar used for oral

pathogens were supplemented with sucrose for additional nutrients. The plates were then incubated at 37°C. Bacterial subcultures were prepared 48 hours (oral pathogens) and 72 hours (*C. acnes*) respectively, prior to the experiment. The plant extracts were dissolved in 10% dimethyl sulfoxide (DMSO) (to a concentration of 20 mg/ml and 2 mg/ml for oral pathogens and *C. acnes*, respectively*)*. Seven-fold serial dilutions (100 µl) of each plant extract were prepared in sterile 96-well microtiter plates in triplicates. The final concentrations of the extracts ranged from 10-0.08 mg/ml (oral pathogens) and 500-3.91 µg/ml (*C. acnes*). Positive controls included chlorhexidine gluconate (concentration ranged from 12.5-3.8 x 10^{-4} mg/ml for oral pathogens) and tetracycline (100-0.78 µg/ml for *C. acnes*). Bacterial subcultures were then inoculated in respective nutrient broths to a density of 3×10^8 colony forming units per ml (CFU/ml) which corresponds to a 1 McFarland Standard (oral pathogens) and 1.5×10^8 CFU/ml which corresponded to a 0.5 McFarland Standard (*C. acnes*) whereby 100 µl of the bacterial suspension was added to the plates. After 24 hours (oral pathogens) and 72 hours (*C. acnes*) incubation at 37°C in anaerobic conditions, 20 µl PrestoBlue was added. After 1 hour the MIC was determined as the lowest concentration whereby no colour change was observed.

2.3.2 Colourimetric tyrosinase inhibition assay

The colourimetric tyrosinase inhibition assay was performed according to the method described by Mapunya et al. (2011). The plant extracts were dissolved in 100 µl DMSO to a 20 mg/ml stock solution. The stock solution was diluted with 50 mM potassium phosphate buffer (pH 6.5) to 600 µg/ml in a 24-well plate. In 96-well microtiter plates placed on ice, 70 µl of varying concentrations of each plant sample was added to 30 µl of tyrosinase enzyme (48 units/ml in phosphate buffer pH 6.5) in triplicate. After 5 minutes of incubation on ice, 110 µl of the substrate (2 mM L-tyrosine) were added to all the wells. The final concentrations of each plant extract and positive control (kojic acid) was 200-1.5 µg/ml. The optical density of each microtiter plate was measured over a period of 30 minutes at a wavelength of 492 nm using Bio-Tek® PowerWave™ XS multi-well plate reader (KC Junior) (A.D.P., Weltevreden Park, South Africa). The fifty percent inhibitory concentration (IC_{50}) was determined by analysing the resulting data using the GraphPad Prism© 4.0 software (San Diego, California, USA). The combinational study was performed in the same manner. However, a ratio of 1:1 of sample 1 and sample 2 was

used. Seven plant samples accounted for sample 1 and sample 2 (CAA, CM, CS, ET, GP, JL and PS). These samples were selected based on their inhibitory activity towards tyrosinase in the initial evaluation. They were then combined in a 1:1 ratio with one another, resulting in twenty-one different combinations to assess their combinational effects and interactions. The interactions were assessed by determining the sum fractional inhibitory concentration (∑FIC) index (Equation 1).

$$\mathrm{FIC}_{(a)} = \frac{\mathrm{IC}_{50(a\ in\ combination\ with\ b)}}{\mathrm{IC}_{50(a\ independantly)}}$$

$$\mathrm{FIC}_{(b)} = \frac{\mathrm{IC}_{50(b\ in\ combination\ with\ a)}}{\mathrm{IC}_{50(b\ independantly)}}$$

$$\sum \mathrm{FIC} = \mathrm{FIC}_{(a)} + \mathrm{FIC}_{(b)}$$

Equation 1: The sum fractional inhibitory concentration (∑FIC) index equation used to calculate the interaction between two samples (a and b) in a 1:1 ratio and the resulting interaction (van Vuuren & Viljoen, 2011; de Rapper et al., 2012)

2.3.3 Anti-elastase activity

The ability of four extracts to inhibit porcine pancreatic elastase (PPE) (Sigma) was determined by measuring the release of p-nitroaniline from N-Succinyl-Ala-Ala-Ala-p-nitroanilide spectrophotometrically at 405 nm according to the method of Bieth et al. (1974) with modifications,

The reaction mixture contained 100 mM Tris buffer (pH 8.0), 0.5 M HCl and the test sample serially diluted to yield six concentrations (250-7.8 µg/mL), to which 20 µL PPE (5 mM) was added and incubated for 15 minutes followed by the addition of N-Succinyl-Ala-Ala-Ala-p-nitroanilide (4 mM). The change in the absorbance of the reaction mixture was measured for 15 minutes from which the rate was obtained. One unit of elastolytic activity is defined as the release of 1 µM of p-nitroaniline/min. An IC_{50}, or concentration at which 50% of the enzyme activity is inhibited, was calculated using GraphPad Prism 4 software.

2.4 Tuberculosis

2.4.1 Antimycobacterial activity

Mycobacterium smegmatis is a fast-growing and non-pathogenic species of mycobacterium. It is most commonly used as a model in the physiology of mycobacteria, as it has relevance to the pathogenic species *Mycobacterium tuberculosis*.

M. tuberculosis (H37Rv)

M. tuberculosis was prepared two weeks before the commencement of the MABA assay. The bacterium was sub-cultured and incubated at 37 ºC using Middlebrook 7H9 broth that was supplemented with glycerol, PANTA and OADC enrichment for 21 days. The inoculum was prepared in sterile 7H9 medium adjusted to a 0.5 McFarland standard (1.5 x 108 CFU/ml). This concentration was further diluted to 1:20 ratio and used throughout the experiment. The MIC of the extracts was determined according to the method of Franzblau et al. (1998) with slight modifications. The ethanol extracts were dissolved in 20% DMSO in sterile Middlebrook 7H9 media to obtain a stock solution of 200 mg/ml. A 2-fold serial dilution of each extract was made with the 7H9 medium to yield volumes of 100 µl/well and final concentrations ranging from 3.13-1000 µg/ml. Isoniazid (INH), at final concentrations ranging from 0.03-4.0 µg/ml served as the positive control. The inoculum (100 µl) was added to each well to make up the final volume of 200 µl. Control wells, without the tested extracts, and a 5% DMSO solvent control, were also included in the assay. The outer wells contained 200 µl sterile distilled water to compensate for evaporation. The plates were sealed and incubated at 37 ºC for 5 days. Forty microliters of 1:1 Alamar Blue reagent: 10% Tween 80 was added to the medium control wells, and re-incubated at 37 ºC for 24 hours. After observing a colour change, Alamar blue reagent was added to the rest of the test wells and incubated at 37 ºC for an additional 24 hours. A colour change from blue to pink was indicative of bacterial growth.

Minimum biofilm formation inhibition concentration (MBFIC)

The MBFIC method was carried out as described by Oosthuizen et al. (2019). One hundred microliters of a 1.5 × 106 CFU/mL *Mycobacterium smegmatis* (MC2 155) inoculum were added into the wells of a polystyrene 96-well microplate (7H9 broth media supplemented with 2% glycerol omitting Tween80). Serially diluted samples

(100 µl) ranging from 7.8 to 1000 µg/ml were added to yield a final assay volume of 200 µl. Ciprofloxacin (10 µg/ml) was used as the positive control. Control wells without plant extracts as well as a solvent control (DMSO, 2.5%) was added. The plates were incubated at 30°C for 4 days under stationary conditions which induce biofilm formation. The minimum biofilm formation inhibitory concentration (MBFIC) was visually determined by identifying the lowest concentration where no biofilm formation could be observed.

2.5 Cancer

2.5.1 Cytotoxicity

Cell culture

The Human Keratinocyte (HaCaT) and Human Malignant Melanoma (MEL-1) cell lines were maintained in culture flasks containing Dulbecco's Modified Eagle's Medium supplemented with 1% antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin and 250 µg/ml fungizone) and 10% heat-inactivated FBS). The cells were cultured to confluency in a humidified incubator set at 5% CO_2 and 37°C and sub-cultured. Detachment was achieved through treatment with trypsin-EDTA (0.25% trypsin containing 0.01% EDTA) for 10 minutes followed by the addition of supplemented media to inhibit the reaction.

Cell counting

In order to obtain the desired amount of cells per assay, the cells were counted. The cells were detached from the service of confluent flasks through trypsinization. The detachment of the cells was inhibited after 10 minutes by the addition of complete media. The cells were then centrifuged in a falcon tube at 980rpm for 5 minutes to obtain a pellet. The supernatant was discarded and the pellet resuspended in approximately 2-5 ml complete medium. In a trypan blue solution, the cells were suspended in a 1:10 dilution (10 µl of cells in 90 µl of trypan blue solution). In the chambers of a hemacytometer, about 10 µl of this mixture was transferred after the cells were counted with a hand-held tally under a microscope.

The concentration of cells per ml was calculated as follows (Momtaz et al., 2008):

$$\text{Cell concentration(cell suspension)} = \#\text{cells counted per square x 10 x 10 000}$$
$$= \text{cells per ml}$$

$$\text{Cell number (cell suspension)} = \text{Cell concentration x Volume of cell suspended}$$

$$\text{Volume of cell suspension} = \frac{\text{Cell concentration wanted x Volume wanted}}{\text{Concentration of cells in suspension}}$$

In vitro cytotoxicity test

The Cell Proliferation Kit II was used to determine the cytotoxicity of the ethanol extracts through the XTT colourimetric assay. In the wells of a 96-well plate, 100 µl of cell suspension with a concentration of $1x10^5$ cells/ml were plated in triplicates as shown in figure 8. The cells were left to attach, and the plates incubated at 37 ºC with 5% CO_2 for 24 hours. Extracts were prepared to a stock solution of 20 mg/ml and added to the plates with a starting concentration of 400 µg/ml to 1.563 µg/ml (Figure 8). The negative control was 2% DMSO and the positive control Actinomycin D with a concentration ranging from 0.5-0.004 µg/ml. A reference plate was prepared that contained the medium and samples and no cells, this was done in duplicate (Figure 9). The plates were incubated for 72 hours at 37 ºC in 5% CO_2, humidified atmosphere (Momtaz et al., 2008).

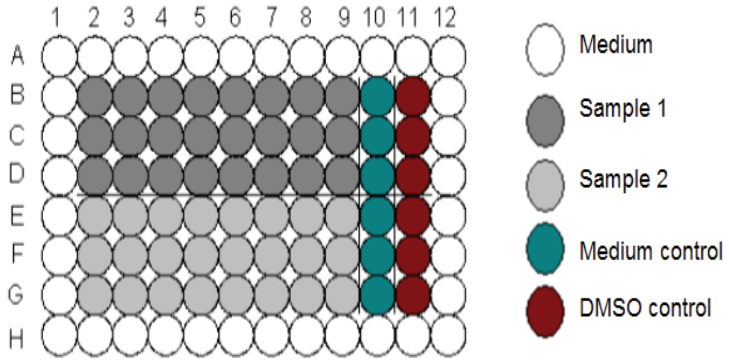


Figure 8: Layout of samples in 96-well plate. Dark grey (A-D): Sample 1. Light grey (E-G): Sample 2. Green (row 10): Medium control. Red (row 11): DMSO control.

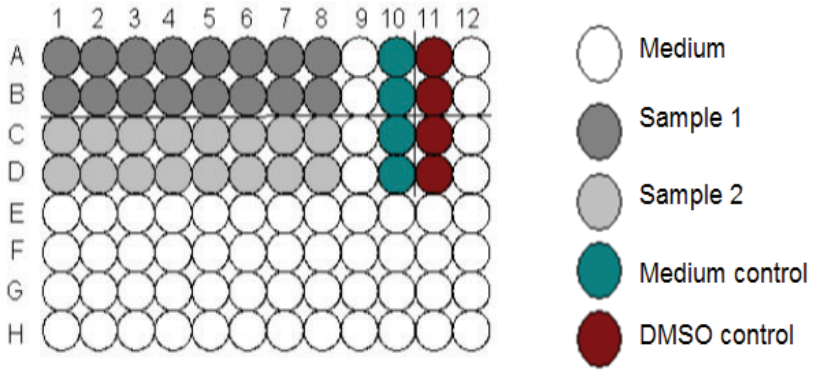


Figure 9: Layout for blanks in 96-well plate. Dark grey (A-D): Sample 1 colour control. Light grey (E-G): Sample 2 colour control. Green (row 10): Medium control. Red (row 11): DMSO control.

After incubation 50 µl of XTT reagent (1 mg/ml XTT with 0.383 mg/ml PMS) was added to a final concentration of 0.3 mg/ml. The cells were further incubated for 2 hours. This resulted in a colour change and the absorbance was determined at a wavelength of 490 nm with the Bio-Tek® PowerWave™ XS multi-well plate reader (A.D.P., Weltevreden Park, South Africa) and KC Junior software (Highland Park, Winooski, Vermont, USA). The concentration at which 50% of the cells are killed (IC_{50}) value was calculated by using Graph Pad Prism 4 program (Version 4 Graph Pad Software, San Diego, CA, USA) (Momtaz et al., 2008).

2.6 Clinical studies

2.6.1 Irritancy

Twenty (20) subjects between the ages of 21 and 65 were recruited. Only nineteen (19) subjects completed the study up to T72hours. The procedure of patch testing was explained to them verbally and each subject signed a form of consent and medical history. Personal details and each subject's assessment of their own skin type were recorded. Visual assessments of the test sub-sites were made at 24, 48 and 72 hours. The controls and products were applied to the inner forearm at 0 hours. The Finn Chambers® covered the patch areas for the first 24 hours after which they were removed and the subject could clean the area by any normal means. Colour photographs were taken at each time interval to serve as a recording of the observations. The controls and product were applied to the inner forearm according to a rotating position sequence in order to avoid position and recording bias. The following rating system was used to classify the reactions:

0 = No response

0.5 = Minimal/doubtful response

1 = Mild erythema, spotty or diffuse

2 = Definite erythema, uniform redness, itching or burning response. Swelling may occur

3 = Strong and severe uniform redness, swelling or spreading beyond the area of the disc may occur

4 = Fiery redness, oedema, papules, bullae

2.6.2 Pigmentation

The study was carried out under controlled conditions with the following mean values:
Temperature Range: (°C) 20.00 to 23.90
Humidity Range (%RH) 35.50 to 43.80

The procedure of testing was explained to them verbally and a form of consent and medical history was signed by each subject. Personal details and each subject's assessment of their own skin type were recorded. Colour photographs were taken at each time interval to serve as a recording of the study conducted. All subjects were instructed to rest for twenty (20) minutes before any testing was performed. The designated left and right test sub-sites were cleansed with a standard eye make-up remover and allowed to air dry for three (3) minutes. The designated left and right test sub-sites were demarcated with a surgical marker as per test substance sequence. The temperature and relative humidity were recorded during the time of the study and noted down. At least three (3) measurements were taken at each test sub-site at every time interval and saved to the instrumental software and converted to Excel for further calculations and statistical evaluation. Between each reading, the instrumental projection tube was gently cleaned with a tissue and alcohol. A study technician trained the test subjects in applying the test products evenly to the test sites, according to the test substance sequence. Test subjects were instructed to apply the test substances twice a day as per application instructions. Subjects were restricted from using any topical products or medication not approved by the study coordinator for the duration of the study. Subjects were informed when to return to the testing facility for each time interval evaluation. All subjects were instructed to rest for twenty (20) minutes before any testing was conducted.

2.6.3 Anti-wrinkle

In order to evaluate the efficacy of PS and ET as wrinkle reducing agents, a double-blinded *in vivo* instrumental study was conducted. Twenty-six (26) Caucasian females between the ages of 25 and 65 with Fitzpatrick skin photo-types I-III were recruited and accepted. Two subjects were excluded from the test panel based on the inclusion and exclusion criteria as they did not attend follow up consultations. The procedure of testing was explained to them verbally and a form of consent and medical history was signed by each subject. Personal details and each subject's assessment of their own skin type were recorded. The study was carried out under controlled conditions with the following mean values: Temperature Range: (°C) 19.90 to 22.00, Humidity Range (%RH) 40.00 to 46.30. All subjects were instructed to rest for twenty (20) minutes before any testing was performed. The designated left and right test sub-sites were cleansed with alcohol and allowed to air dry for three (3) minutes. Applicants were required to apply 0.6mg of the test and placebo formulations twice a day for the duration of the study as per application instructions and were prohibited from using any topical products or medication not approved by the study co-ordinator for the duration of the study. The designated left and right test sub-sites were demarcated with surgical marker as per test substance sequence. At least three (3) measurements were taken at each test sub-site at every time interval and saved to the instrumental software and converted to Excel for further calculations and statistical evaluation. A study technician trained the test subjects in applying the test products evenly to the test sites, according to the test substance sequence. Evaluations were conducted using the Visioscan® VC98 device on the baseline (day 0), day 14 and day 28. Colour photographs were taken at each time interval of the designated test sites to serve as a recording of the study conducted. Subjects were informed when to return to the testing facility for each time interval evaluation. The subjects complied with the rules and specifications of the study and all results recorded were noted down and used in the calculations. The instrumental Visioscan® VC98 was gently sterilised with a tissue and alcohol, before and after use on all test subjects.

2.6.4 Data Analysis

The Visioscan® calculates values that quantitatively relates to smoothness, scaliness, wrinkles and the like, from an eight (8) bit greyscale captured image, which defines intensity reflected from a subject. The change in the parameter of captured images is

calculated and used as an overall indication in the change of wrinkle reduction intensity. Readings will be taken on each designated spot on each test site to serve as the specific greyscale measurement component of the study. A sample can be defined as a subset of the whole population which is investigated by a researcher and whose characteristics will be generalised to the entire population. A sampling plan can be described as a design, scheme of action or procedure that specifies how the participants are to be selected in a study. A distinction is made between probability and non-probability sampling. In this study, use was made of a non-probability sampling method. A non-probability sampling method can be described as the selection of a population element to be part of the sample-based in some part on the judgment of the researcher. A sample of convenience was used in this study. The objectives were to determine whether significant differences existed between the test product and control regarding the parameter tested.
The following statistical data analysis procedures were used:

2.6.5 Descriptive analyses

Descriptive statistics are primarily aimed at describing the data.

2.6.6 Inferential statistics:

Test hypotheses about differences in two (2) populations based on measurements made on the test substance on human skin subjects.

Statistically significant differences between variables are indicated by a significance value p. If the value of p is equal to or less than 0.05, it indicates that there is a statistically significant difference, at the 5% level of confidence.

2.7 Column chromatography

2.7.1 PS

Having obtained good antityrosinase activity by PS, this sample was further investigated in order to identify its bioactive compounds and their molecular docking potential against tyrosinase. Dried ethanolic extract (10.26 g) of PS was applied to a 10 x 70 cm silica column of gradient hexane and dichloromethane (DCM) of increasing polarity (0% to 100% DCM) as the eluent. One hundred and fifty-three fractions were collected as follows: fractions 1-5 (100% hexane); fractions 6-18 (95% hexane and 5% DCM); fractions 19-21 (90% hexane and 10% DCM); fractions 22-70 (75% hexane and 25%

DCM); fractions 71-147 (50% hexane and 50% DCM); fractions 148-190 (25% hexane and 75% DCM); fractions 190-200 (100% DCM). Fractions were analysed using thin-layer chromatography (TLC) with the respective solvent ratios as eluents. The TLC plates were developed using vanillin (7.5 g) and sulphuric acid (5 ml) in 250 ml ethanol, the plates were then heat dried. Fractions with similar TLC profiles and presence of compounds were pooled together resulting in seventeen (17) major fractions. The combined fractions (F1-F17) were tested for tyrosinase inhibition.

2.7.2 ET

The crude ethanolic extract was completely dissolved in water and exhaustively extracted by consecutive liquid/liquid partition with ethyl acetate (750 ml) and saturated butanol (750 ml) using a separating funnel. The ethyl acetate, saturated butanol and aqueous fractions were evaporated to obtain three fractions.

The water fraction identified to have the highest elastase activity was selected for column chromatography.

The water fraction was subjected to silica gel column chromatography using hexane and ethyl acetate (EtOAc)/methanol mixtures of increasing polarity (0 to 50%) In total, 250 fractions were collected and combined into 6 sub-fractions.

2.8 Gas chromatography-mass spectrometry (GC-MS)

2.8.1 PS and ET

The plant extracts were sent to the Chemistry Department of the University of Pretoria for GC-MS analysis.

2.8.2 PS fractions

GC-MS analysis of the two active major fractions against tyrosinase (F14 and F15 with IC_{50} values of 68.77 ± 2.796 µg/ml and 79.82 ± 3.404 µg/ml, were respectively, both dissolved in 2 ml distilled ethanol) were obtained through the use of a LECO Pegasus® 4D GC-TOFMS (LECO Africa (Pty) Ltd., Kempton Park, South Africa) equipped with a Rxi-5SilMS column (30 m × 0.25 mm ID film thickness × 0.2 µm) (Restek, Bellefonte, PA, USA). GC-MS spectroscopic detection was carried out while operating in electron ionisation mode (EI+) at 70 eV. Ultra-high purity helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min. Mass transfer line, injector and ion-source

temperatures were set at 280°C, 250°C and 230°C, respectively. The oven temperature was programmed at 40°C (hold for 3 min) with an increase of 10°C/min to 300°C (hold for 5 min). A split ratio of 10:1 was employed with a scan-interval of 10 spectra per second and fragments from 40 to 550 Da was programmed. The relative percentage of the chemical constituents in the fractions were expressed as percentage by peak area normalisation. The peaks obtained were compared with those in the National Institute of Standards and Technology (NIST) library.

2.9 Cultivation system design

Curator of the Manie van der Schyf Botanical Garden of the University of Pretoria, Mr Jason Sampson, was consulted on designing a cultivation system for the selected plant species. A waterlogging system was decided on due to the ease of cultivation in rural areas. Raised beds of 20cm high borders were created using large precast concrete bricks fixed in place with cement (or timber poles or trenches dug into the ground can be used) and lined with black PVC plastic of >500 microns thick. This lining was attained from Aquatan (Pty) Ltd in Kempton Park, Gauteng. These shallow ponds were filled with a medium of equal parts red clay topsoil, compost and fertilizer with organic substances, such as bone meal and manure. Propagules were planted directly into this soil for rapid growth and regular harvesting. This ultimately maintained waterlogged conditions necessary for all the species described above except for ET which was grown under general field conditions with sufficient moisture.

Propagation took place in small, growth medium filled pots and was kept moist under shaded conditions. JL and PS were propagated by directly rooting cuttings in prepared beds, although, preferably, severed shoots are bound together in bunches and rooted in tubs of water under shaded conditions. CS was rapidly multiplied by rooting the inflorescences of flowering mother plants, either by floating in water or in pots kept moist.

3. RESULTS AND DISCUSSION

Based on the literature review conducted: twenty seven South African wetland plant species were selected for this study, their collection site and plant parts that were harvested are indicated in Table 2.

Table 2: The different freshwater aquatic and wetland plant species collected for the present study, the area and the plant parts that were collected and the PRU numbers of each species

Plant species Abb.	Part	PRU numbers	Location
BE	Leaves	NA	Rainwater Harvesting Garden 2019
BR	Leaves	NA	Rainwater Harvesting Garden 2019
CAA	Leaves and inflorescence	122250	Rainwater Harvesting Garden March 2016
CB	Whole plant	121874	UP – Manie van der Schijff Botanical Garden
CBr	Leaves	123074	Wildflower Nursery October 2016
CC	Leaves	NA	Plant Science Complex Pond October 2016
CP	Leaves	NA	Wildflower Nursery October 2016
CM	Stems and inflorescence	122256	Rainwater Harvesting Garden March 2016
CS	Stems and inflorescence	122258	Rainwater Harvesting Garden March 2016
ET	Stems	122257	Rainwater Harvesting Garden March 2016

Plant species Abb.	Part	PRU numbers	Location
ER	Stems	121875	UP – Manie van der Schijff Botanical Garden
EZ	Leaves	123065	Wildflower Nursery October 2016
GP	Leaves and stems	122254	Rainwater Harvesting Garden March 2016
JL	Whole plant	122255	Manie van der Schijff Botanical Garden March 2016
KP	Leaves	122251	Wildflower Nursery October 2016
MS	Leaves and stems	122251	Rainwater Harvesting Garden March 2016
ML	Leaves, stems and inflorescence	121876	UP – Manie van der Schijff Botanical Garden
NC	Leaves and stems	122252	Rainwater Harvesting Garden March 2016
NT	Leaves, stems and flowers	122253	Rainwater Harvesting Garden March 2016
PM	Leaves and stems	123068	Wildflower Nursery October 2016
PS	Leaves, stems and inflorescence	122180	LC de Villiers Wetland Restoration May 2016
PL	Leaves, stems and inflorescence	122181	LC de Villiers Wetland Restoration May 2016

Plant species Abb.	Part	PRU numbers	Location
SK	Leaves	123069	Wildflower Nursery October 2016
SF	Leaves	123070	Wildflower Nursery October 2016
TL	Leaves	123071	Wildflower Nursery October 2016
TC	Rhizomes and lower stems	121877	UP – Manie van der Schijff Botanical Garden
ZA	Leaves	121878	UP – Manie van der Schijff Botanical Garden

NA: Not available

3.1 Skin disorders

3.1.1 Antibacterial activity against *C. acnes* (acne vulgaris)

CAA, TC, BR and *GP* demonstrated antibacterial activity against *Cutibacterium acnes* at 250 µg/ml and 125 µg/ml, respectively**,** when compared to the positive control tetracycline with an observed MIC of 1.56 µg/ml (Table 3).

Table 3: Anti-bacterial activity of aquatic plant ethanolic extracts against *Cutibacterium acnes*. Minimum inhibitory concentration (MIC)

Samples	MIC (µg/ml)
Tetracycline	1.56
BE	NA
BR	125
CAA	250
CB	NA
CBr	NA
CC	NA
CM	NA
CP	NA
CS	NA

ET	NA
ER	NA
EZ	NA
GP	125
JL	NA
KP	NA
Samples	**MIC (µg/ml)**
ML	NA
MS	NA
NC	NA
NT	NA
PM	NA
PS	NA
PL	NA
SF	NA
SK	NA
TC	250
TL	NA
ZA	NA

NA not active at the highest concentration tested (500 µg/ml)

3.1.2 Anti-tyrosinase activity

The ability of each plant extract to inhibit the activity of tyrosinase varied depending on species (Table 4). However, a few plant species exhibited noteworthy results and in some cases, this is the first time that these species are being reported to be effective inhibitors of the tyrosinase enzyme. JL was the most effective inhibitor of tyrosinase with an IC_{50} value of 31.64 ± 6.91 µg/ml while CS, CAA, ET, GP, PS and CM followed with moderate inhibitory activity of IC_{50} values double those of JL (ranging from 64.26 ± 5.12 to 75.03 ± 4.98 µg/ml).

Table 4: Tyrosinase fifty percent inhibitory concentration (IC_{50}) of aquatic plant extracts and PS major fractions

Samples	**Tyrosinase IC_{50}±SD (µg/ml)**
Kojic acid	2.52 ± 2.84
BE	NA
BR	NA
CAA	64.71 ± 5.36

CB	NA
CBr	NA
CC	NA
CM	75.03 ± 4.98
CP	NA
CS	64.26 ± 5.12
Samples	**Tyrosinase IC_{50}±SD (µg/ml)**
ET	65.26 ± 8.38
ER	NA
EZ	NA
GP	70.43 ± 3.59
JL	31.64 ± 6.91
KP	NA
MS	NA
ML	53.63 ± 1.35
NC	NA
NT	NA
PM	NA
PS	71.59 ± 4.23
PL	NA
SF	NA
SK	NA
TC	NA
TL	NA
ZA	NA
PS: F1-F12	NA
PS: F13	177.65 ± 6.77
PS: F14	68.77 ± 2.80
PS: F15	79.82 ± 3.40
PS: F16-F17	NA

NA not active at the highest concentration tested (200 µg/ml)

3.1.3 Anti-Tyrosinase activity: combinational studies

Seven plant species in twenty-one different combinations were analysed for any combinational effects and interactive properties. The IC_{50} values and ΣFICs of each of the combinations for tyrosinase inhibition are presented in Table 5. The plant samples tested in combination were individually selected due to their moderate-to-significant inhibitory activities; these species included JL, CS, CAA, ET, GP, PS and CM. The results, found to be

mostly non-interactive or indifferent effects on each of the samples together, were noted (having a ΣFIC value between 1.00 and 4.00). However, an interaction worth highlighting is the combination in a 1:1 ratio of GP and JL. The ΣFIC value obtained suggested that although effective alone, together their ΣFIC value was greater than 4.00 (4.12), indicating that the plant extracts had an antagonistic effect on one another.

The lowest ΣFIC observed was for the combinations between CAA and CS as well as ET and GP with ΣFIC of 1.28 and 1.29, respectively. Taking into account the IC_{50} values for each of these combinations, it is interesting to note that in combination, these samples also represented the lowest IC_{50} values of all combinations tested (41.15 ± 7.177 µg/ml and 43.44 ± 3.708 µg/ml, respectively). Other combinations worth mentioning include CAA and JL as well as CM and JL, obtaining IC_{50} values of 46.49 ± 5.298 µg/ml and 44.92 ± 2.753 µg/ml, respectively. In all these combinations, the resulting IC_{50} values were approximately 0.6 times lower than those obtained for the individual components (Table 5).

Table 5: Combinational tyrosinase inhibitory activity of the bioactive plant extracts

Samples	**IC_{50} (µg/ml) ± SD**	**$FIC_a + FIC_b$**	**(∑FIC) Index**	**Interaction**
Kojic acid	2.91 ± 1.27	-	-	-
CAA[a]:CM[b]	100.0 ± 3.36	1.56 + 1.33	2.89	Indifferent
CAA[a]:CS[b]	41.15 ± 7.18	0.64 + 0.64	1.28	Indifferent
CAA[a]:ET[b]	51.78 ± 2.89	0.80 + 0.79	1.59	Indifferent
CAA[a]:GP[b]	114.10 ± 2.21	1.76 + 1.62	2.38	Indifferent
CAA[a]:JL[b]	46.49 ± 5.30	0.72 + 1.47	2.19	Indifferent
CAA[a]:PS[b]	71.63 ± 5.33	1.11 + 1.00	2.11	Indifferent
CM[a]:CS[b]	53.0 ± 4.72	0.71 + 0.82	1.53	Indifferent
CM[a]:ET[b]	51.85 ± 1.95	0.69 + 0.79	1.48	Indifferent
CM[a]:GP[b]	100.1 ± 4.96	1.33 + 1.42	2.75	Indifferent
CM[a]:JL[b]	44.92 ± 2.75	0.60 + 1.42	2.02	Indifferent
CM[a]:PS[b]	82.49 ± 6.65	1.10 + 1.15	2.25	Indifferent
CS[a]:ET[b]	59.74 ± 3.20	0.93 + 0.92	1.85	Indifferent
CS[a]:GP[b]	66.92 ± 6.12	1.04 + 0.95	1.99	Indifferent
CS[a]:JL[b]	73.19 ± 4.97	1.14 + 2.31	3.45	Indifferent
CS[a]:PS[b]	57.36 ± 6.01	0.89 + 0.80	1.69	Indifferent
ET[a]:GP[b]	43.44 ± 3.71	0.67 + 0.62	1.29	Indifferent
ET[a]:JL[b]	76.81 ± 5.93	1.18 + 2.43	3.61	Indifferent
ET[a]:PS[b]	101.3 ± 5.97	1.55 + 1.42	2.97	Indifferent
GP[a]:JL[b]	89.96 ± 5.44	1.28 + 2.84	4.12	Antagonistic
GP[a]:PS[b]	67.31 ± 5.61	0.96 +0.94	1.90	Indifferent
JL[a]:PS[b]	79.4 ± 4.52	2.51 + 1.11	3.62	Indifferent

[a] sample 1
[b] sample 2

3.1.4 Anti-elastase activity

CB, BE and BR all had IC_{50} values above 100 µg/ml while PL had no activity at the highest concentration tested of 250 µg/ml. Excellent inhibition of elastase was exhibited by ET, ER and PS that were comparable to ursolic acid, a known inhibitor of elastase (Table 6). The water fraction ET had an elastase inhibitory activity, with an IC_{50} value of 34.4 µg/ml (Table 6).

Six pooled fractions isolated from the ET water fraction displayed no elastase inhibition at the highest tested concentration of 250 µg/ml. Therefore the compounds present in ET have a combined effect against the elastase enzyme (Table 6).

Table 6: The fifty percent inhibitory concentration (IC_{50} µg/ml) values of the plant extracts against the elastase enzyme

Sample	IC_{50}±SD (µg/ml)
Ursolic acid	2.96±1.23
BE	116.2±5.1
BR	162.4±5.74
CB	148.45±1.91
ET	13.5 ± 1.5
ER	518.7±1.53
PS	29.5±3.4
PL	NA
ET water fraction	34.4±3.5
Fraction 1	NA
Fraction 2	NA
Fraction 3	NA
Fraction 4	NA
Fraction 5	NA
Fraction 6	NA

NA not active at the highest concentration tested (250 µg/ml)

3.2 Periodontal diseases

3.2.1 Antibacterial activity against *P. intermedia* and *S. mutans* (periodontal diseases)

The ethanolic extracts of BE and BR inhibited *S. mutans* at MIC values of 1.6 mg/ml and 3.1 mg/ml, respectively. At the highest concentrations tested (10 mg/ml) none of the other plant extracts were able to inhibit the growth of either of the oral pathogens (*P. intermedia* and *S. mutans*). Chlorhexidine was observed to have an MIC of 0.78 mg/ml and 0.39 mg/ml against *P. intermedia* and *S. mutans*, respectively (Table 7).

Table 7: The minimum inhibitory concentration (MIC: µg/ml) of the plant extracts against *Prevotella intermedia* and *Streptococcus mutans*

Samples	***P. intermedia* MIC (mg/ml)**	***S. mutans* MIC (mg/ml)**
Chlorohexidine	0.78	0.39
BE	Not tested	1.6
BR	Not tested	3.1
CAA	NA[a]	NA[a]
CB	NA[b]	NA[b]
CBr	NA[a]	NA[a]
CC	NA[a]	NA[a]
CM	NA[a]	NA[a]
CP	NA[a]	NA[a]
CS	NA[a]	NA[a]
ET	NA[a]	NA[a]
ER	NA[b]	NA[b]
Ez	NA[a]	NA[a]
GP	NA[a]	NA[a]
JL	NA[a]	NA[a]
KP	NA[a]	NA[a]
ML	NA[b]	NA[b]
MS	NA[a]	NA[a]
NC	NA[a]	NA[a]
NT	NA[a]	NA[a]
PM	NA[a]	NA[a]
PS	NA[a]	NA[a]
PL	NA[a]	NA[a]
SF	NA[a]	NA[a]
SK	NA[a]	NA[a]
TC	NA[b]	NA[b]
TL	NA[a]	NA[a]
ZA	NA[b]	NA[b]

NA[a] not active at the highest concentration tested (10 mg/ml)
NA[b] not active at the highest concentration tested (25 mg/ml)

3.3 Tuberculosis

3.3.1 Antimycobacterial activity

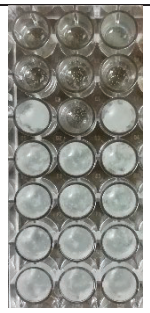
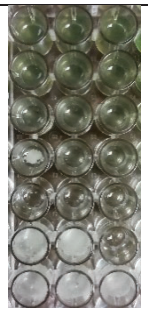
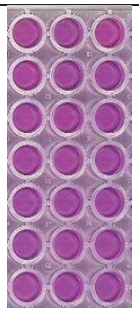
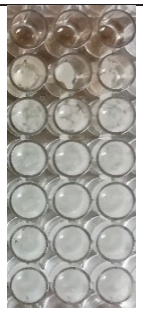
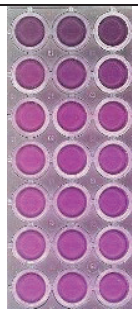
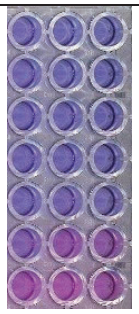
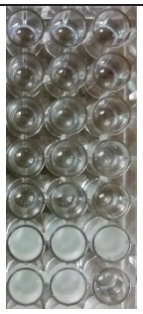
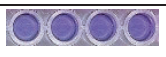
At the tested concentrations, just three of the plant extracts (CAA, PM and TS) showed antibacterial activity against *Mycobacterium tuberculosis*. The anti-biofilm activity of the lead samples was determined: BR, *CAA* and *PM* were found to target biofilm formation of *M. smegmatis* (Figure 10 and Table 8). *BR* displayed noteworthy anti-biofilm activity with an MBFIC of 62.5 µg/ml.

Table 8: The minimum inhibitory concentration (MIC: µg/ml) and minimum biofilm formation inhibitory concentration (MBFIC) of the plant extracts against *Mycobacterium tuberculosis*[b] and *Mycobacterium smegmatis*[a]

Samples	MIC (µg/ml)	MFBIC (µg/ml)
Isoniazid[b]	0.15	0.31
Ciprofloxacin	0.31	0.13
BE	NA[a]	1000
BR	NA[a]	62.5
CAA	1000	1000
CB	NA[b]	
CBr	NA[b]	
CC	NA[b]	
CM	NA[b]	
CP	NA[b]	
CS	NA[b]	
ER	NA[b]	
ET	NA[b]	
EZ	NA[b]	
GP	NA[b]	
JL	NA[b]	
KP	NA[b]	
ML	NA[b]	
MS	NA[b]	
NC	NA[b]	
NT	NA[b]	
PL	NA[b]	
PM	1000	500

Samples	MIC (µg/ml)	MFBIC (µg/ml)
PS	NA[b]	
SF	NA[b]	
SK	NA[b]	
TC	1000	1000
TL	NA[b]	
ZA	NA[b]	

NA[a] not active at the highest concentration tested (1000 µg/ml) against *Mycobacterium smegmatis*
NA[b] not active at the highest concentration tested (1000 µg/ml) against *Mycobacterium tuberculosis*

Plant	MIC	MBFIC	Plant	MIC	MBFIC
BE			PM		
BR			TC		
CAA			Cip[e]		
Med			Bac		

Cip[e]: Ciprofloxacin
MIC: Minimum inhibitory concentration
MBFIC: Minimum biofilm formation inhibitory concentration

Figure 10: Antimycobacterial and antibiofilm activity on *Mycobacterium smegmatis*

3.4 Anticancer and cytotoxicity

The IC_{50} indicates the concentration at which 50% of the cells are killed. This value is expressed in µg/ml. An IC_{50} from 0-50 µg/ml indicates the sample to be toxic *in vitro,* a noteworthy result against cancer cells. An IC_{50} from 50-100 µg/ml indicates the sample to be moderately toxic *in vitro*. An IC_{50} >100 µg/ml indicates the sample to be non-toxic *in vitro*.

Table 9: The concentration of the sample at which fifty percent of the cell growth is inhibited (IC_{50}; µg/ml) of the plant extracts for human keratinocyte (HaCaT) and human malignant melanoma (MEL-1), breast cancer (MCF-7) and cervical cancer (HeLa) cell lines

Sample	**IC_{50}±SD (HaCaT) (µg/ml)**	**IC_{50}±SD (MEL-1) (µg/ml)**	**IC_{50}±SD (MCF-7) (µg/ml)**	**IC_{50}±SD (HeLa) (µg/ml)**
Actinomycin D	<0.005	<0.005	<0.005	<0.005
BE	259±27.29	-	-	NI
BR	231.4±11.16	-	NI	NI
CAA	NI	140.05±1.34	NI	NI
CB	NI	NI	NI	186.05±1.77
CBr	NI	NI	92.13±14.52	NI
CP	NI	NI	102.3±8.54	NI
CC	205.05±16.33	207.3±9.09	389.75±14.50	NI
CS	NI	181.3±1.13	167.05±2.05	NI
CM	NI	185.65±2.05	135.85±11.67	NI
ER	NI	NI	384.7±47.71	NI
ET	102.15±1.77	NI	NI	NI
EZ	NI	NI	NI	NI
GP	NI	207.1±2.91	189.3±4.3	NI
JL	NI	104.1±5.01	NI	NI
KP	NI	NI	NI	NI
ML	217.4±4.49	NI	196.75±4.45	NI
MS	NI	108.1±2.55	251.25±5.35	NI
NT	NI	201.35±0.35	NI	NI
NC	199.1±7.01	NI	NI	NI
PM	NI	NI	NI	NI
PS	NI	NI	127.3±13.28	NI
PL	NI	NI	115.6±8.04	NI
SF	NI	NI	NI	NI
SK	NI	NI	NI	NI

Sample	IC_{50}±SD (HaCaT) (µg/ml)	IC_{50}±SD (MEL-1) (µg/ml)	IC_{50}±SD (MCF-7) (µg/ml)	IC_{50}±SD (HeLa) (µg/ml)
TC	NI	NI	247.8±17.63	NI
TL	NI	NI	NI	NI
ZA	NI	478.6±14.67	NI	99.70±4.82

NI: No inhibition at the highest concentration tested of 400 µg/ml

All the samples were confirmed to be non-toxic for human keratinocytes (HaCaT) and could potentially be applied to the skin without adverse effects. CBr and ZA were confirmed to be moderately toxic towards the HeLa and MCF-7 cell lines, respectively (Table 9).

3.5 GC-MS

3.5.1 JL GC-MS

No literature was found that reported on these compounds relating to pigmentation or irritancy. (See Annexure A). The GC-MS analysis of JL plant extract showed the presence of 106 compounds that could contribute to the medicinal property of this plant. The area percentage was obtained, the area under the peaks is proportional to the number of compounds that are present in the plant sample. Six compounds had a high area percentage, these are: 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester (9.0501%), 1-Docosene (5.7523%), Pyrimidine-2,4,6-trione, 1-butyl-5-[(2-piperazin-1-yl-ethylamino)methylene] (3.3294%), Acrolein, dimethyl acetal (3.3294%), Butanedioic acid, dimethyl ester (3.0935%) and Hexanoic acid, 2-ethyl-, methyl ester (3.052%).

3.5.2 PS GC-MS

As in the case of JL GC-MS, no literature was found that has reported on the compounds, PS GC-MS, relating to wrinkle reduction (See Annexure B). The GC-MS analysis of *PS* plant extract showed the presence of 84 compounds that could contribute to the medicinal property of this plant. Compounds with an area percentage above 1% included 3-(5-Methylfuryl)-N-furamidopropionamide (1.00%), 3-Acetoxytridecane (1.10%), 1-(7-Hydroxy-1,6,6-trimethyl-10-oxatricyclo[5.2.1.0(2,4)]dec-9-yl)ethanone (1.80%), Neophytadiene (1.07), Neophytadiene (1.95%), Undecanoic acid, ethyl ester (1.21%), 1,10-Undecadiene (1.64%), Squalene (1.10%), Eicosane (1.10%), á-Sitosterol (1.17%), á-

Amyrin (1.44%), 1-Iodo-2-methylundecane (1.74%), Benzothiazole (2.80%), Decane, 2,4-dimethyl- (2.78%), 2,3-Dioxabicyclo[2.2.1]heptane, 1-methyl- (2.40%), 2,5-Dimethyl-2-(2-tetrahydrofuryl)tetrahydrofuran (2.14%), 2,2-Dimethyl-4-octenal (2.50%), Adipic acid, cyclobutyl isohexyl ester (2.50%), 9,12-Octadecadienoic acid, methyl ester, (E,E)- (2.50%), Stigmasterol (2.34%), Urs-12-en-3-ol, acetate, (3á)- (2.20%), Lupeol (2.20%), Acetate, 4-hydroxy-3-methyl-2-butenyl- (3.9%), Pentanoic acid, 2-methyl-, anhydride (3.61%), Neophytadiene (3.16%), n-Hexadecanoic acid (3.60%), cis-3-Butyl-4-vinyl-cyclopentene (3.69%), Octadecanoic acid (3.03%), á-Sitosterol (8.01%).

PS fractions GC-MS

Two major fractions F14 and F15 of *PS* was sent for GC-MS analysis based on their moderate tyrosinase inhibitory activity. The major constituents of F14 included: 1,7-Dimethyl-5-phenyltricyclo[4.1.0.$0^{2,7}$]hept-3-ene (52.66%); (9Z)-9-Octadecenamide (8.52%); 2,4,5-trimethoxybenzaldehyde (7.88%); (2R)-2-Amino-3-methyl-1-butanol (5.30%) and Dodecanamide (5.28%). While, the major constituents of F15 included: Benzenepropanol, à-methyl-á-nitro-, (R*,R*)-(.+.)- (24.34%); (9Z)-9-Octadecenamide (10.42%); 1,7-Dimethyl-5-phenyltricyclo[4.1.0.$0^{2,7}$]hept-3-ene (8.04%) and Dodecanamide (7.72%). The rest of the constituents in each fraction were recorded as being present below 5%. The composition determined for each fraction corresponds to 97.97% (F14) and 89.89% (F15) of the entire GC-MS chromatogram.

3.6 Clinical studies

Irritancy test

Patch testing is a reliable means to determine the potential irritancy of products, including natural products. By conducting the investigation as a in a standard means with the necessary controls, it could be deduced whether or not the test sample is in fact an irritant.

The classification of results is interpreted as follows:
Non-irritant =Mean Score (Average plus standard deviation) falls below that of negative control test substances performed better or similar to that of demineralised water.

Mild Irritant =Mean Score (Average plus standard deviation) falls above that of negative control, but was lower than that of the positive control. A percentage increase in compatibility potential relative to negative control is given.

Irritant =Mean Score (Average plus standard deviation) falls above that of positive control, test substances performed worse than the positive control.

JL was confirmed to be a non-irritant compared to the positive control sodium laureth sulfate, while PS was found to be a mild irritant (mean score > than demineralised water), despite no subjects exhibiting adverse reactions after 48 hours. Since PS and ET were tested neat, the mild irritant activity might not have an effect when tested in a formulation. There was no report of skin irritation during human clinical studies when tested in a formulation (Table 10).

3.6.1 Pigmentation

In vivo studies confirmed that JL at 10% in a cream formulation with 10% niacinamide was effective in increasing the lightness of dark spots; thus decreasing the pigmentation level from day fourteen (D14) up until day twenty-eight (D28) of consecutive use (twice a day) when compared to the control (Table 11-12).

Table 10: JL, ET and PS *in vivo* skin irritancy patch test

Sample	Average Value	Mean score	Number of subjects with reactions after 48 hrs	Skin Compatibility Potential % (TP-NC)/(PC-NC)	Skin Compatibility
Positive control	0.93	1.47	12	100.00	Irritant
JL	0.16	0.33	0	-2.00	Non-irritant
Negative control –	0.13	0.30	0	0.00	Non-irritant
Positive control	0.59	1.25	8	100	Irritant
PS	0.23	0.48	0	18.66	Mild irritant
Negative control –	0.13	0.3	0	0	Non-irritant
Positive control	0.59	1.25	8	100	Irritant
ET	0.12	0.33 0	0	3.06	Mild Irritant
Negative control –	0.13	0.3	0	0	Non-irritant

Table 11: Comparison of Chromameter values for JL and control sites for the increase in the lightness of identified dark spots on the face

Time interval	JL	Control	p-value
Baseline (BL) **Standard deviation**	37.24 4.35	36.68 4.20	0.102
Difference (Day 14 – BL) **Standard deviation**	1.96 1.66	0.85 0.92	0.00
Difference (Day 28 – BL) **Standard deviation**	1.94 1.83	0.78 1.52	0.001

Table 12: Comparison of Chromameter values for JL and control sites for the increase the lightness of uneven skin tone on the face

Time interval	JL	Control	p-value
Baseline (BL) **Standard deviation**	36.44 4.20	36.99 4.84	0.286
Difference (Day 14 – BL) **Standard deviation**	1.84 1.70	0.74 1.36	0.007
Difference (Day 28 – BL) **Standard deviation**	1.67 1.67	0.52 1.53	0.004

3.6.2 ET and PS anti-wrinkle

All data parameters were descriptively analysed by ordering and summarising of data by means of tabulation and the calculation of descriptive measures. A Wilcoxon Signed Rank Sum Test was performed to compare the treated and untreated control sites at baseline (BL, day 0) which found a statistical significant difference on a 5% level of confidence, which indicated the test product sites as being significantly smaller than the control sites. A t-Test was performed to determine the treatment effect by comparing the average difference values at day 14 (D14) of the treated and control test-sites, which found a statistical significant difference on a 5% level of confidence, indicating the test product sites to be significantly smaller than the control sites. A Wilcoxon Signed Rank Sum Test was performed to determine the treatment effect by comparing the average difference values at day 28 (D28) of the treated and control test-sites, which found a statistical significant difference on a 5% level of confidence, also revealing the test product sites to

be significantly smaller than the control sites. It can therefore be concluded that the test product was effective in decreasing the wrinkle depth after twenty-eight days (D28) of consecutive use (twice a day) when compared to a placebo control which evidenced a statistical significant difference on a 5% level of confidence (Tables 13 and 14).

Table 13: Comparison of VISIOSCAN® VC98 values PS and control sites for wrinkle reduction

Time interval	PS	Control	p-value
Baseline	311.71	279.94	0.009
Day 14-baseline	48.04	73.85	0.000
Day 28-baseline	45.83	71.21	0.003

Table 14: Comparison of VISIOSCAN® VC98 values ET and control sites for wrinkle reduction

Time interval	ET	Control	p-value
Baseline	760.82	632.47	0.019
Day 14-baseline	-117.75	21.08	0.021
Day 28-baseline	-161.63	-28.44	0.000

3.7 Cultivation and harvesting

Botanica Natural Products (Figure 11) is a farm based in Limpopo Province where medicinal plants are grown for natural products. They are involved in several community-based projects to uplift the community they serve. These projects include:

- Job creation
- Workshops that teach the community about farming practices and entrepreneurship
- School projects: teaching students how to cultivate medicinal plants

Figure 11: Botanica Natural Products. Photo from Botanica website

Botanica Natural Products agreed to use a portion of their land for the waterlogging system to cultivate the identified aquatic and wetland plants. This has resulted in job creation through the preparation and maintenance of the waterlogging system. The community has gained experience in growing medicinal plants that could potentially result in a community-based business. The freshwater aquatic and wetland plants

selected for further cultivation studies based on their outstanding biological activities are currently grown at Botanica Natural Products (Table 15 and Figures 12-14).

Table 15: Selected aquatic and wetland plants based on their biological activity for cultivation and harvesting at Botanica Natural Products.

Plants selected	**Biological activity**	**Type of aquatic**	**Original harvesting location**	**Plant part collected**
CM	Moderate tyrosinase inhibitory activity	Emergent	UP – Rainwater Harvesting Garden	Stems and inflorescence
CS	Moderate tyrosinase inhibitory activity	Emergent	UP – Rainwater Harvesting Garden	Stems and inflorescence
ET	Lead sample: elastase inhibitory activity	Riparian	UP – Rainwater Harvesting Garden	Stems and inflorescence
JL	Lead sample: tyrosinase inhibitory activity	Riparian	UP – Manie van der Schijff Botanical Garden	Whole plant
PS	Lead sample: elastase inhibitory activity	Emergent	UP – LC de Villiers Sports campus	Leaves, stems and inflorescence

Figure 12: Preparation of the waterlogging system at Botanica. Photo taken by Zane Coles

Figure 13: Lead fresh water aquatic plants growing at Botanica. Photo taken by Zane Coles

Figure 14: Maintenance of the waterlogging system at Botanica. Photo taken by Zane Coles

4. CONCLUSION AND FUTURE RECOMMENDATIONS

From the twenty-seven indigenous aquatic plants investigated, three of the plants, PS, JL and ET have been confirmed as having potential for the treatment of various skin disorders.

PS major fractions (F14 and F15) displayed noteworthy tyrosinase inhibitory potential. GC-MS analysis revealed the major constituents to be (9Z)-9-Octadecenamide or 2, 4, 5-trimethoxybenzaldehyde (F14) and 4-(4-Nitrophenyl)-2-butanol (F15), that could be responsible for the tyrosinase inhibition observed.

PS and ET displayed potential as an elastase inhibitor *in vitro*. *In vivo,* PS and ET were found to reduce the appearance of wrinkles after twenty-eight days of consecutive use twice a day.

In vitro, JL was identified as the lead plant for the inhibition of tyrosinase. JL was confirmed to be a non-irritant on human volunteers. Clinical studies confirmed that JL was effective to decrease irregular skin tone over 14 to 28 days of consecutive use twice a day.

Further studies are required to upscale ET, PS and JL to viable products that can be commercialised, with communities and indigenous knowledge holders benefiting from the research conducted.

BE and BR, only introduced later in the project, are traditionally used for treating toothache. *In vitro*, BE and BR displayed noteworthy antibacterial activity against *S. mutans* with an MIC of 1.6 mg/ml and 3.1 mg/ml respectively compared to the positive control Chlorohexidine with an MIC of 0.39 mg/ml. *In vivo* clinical studies for oral care could be explored in future projects for BE and BR.

Five samples were identified to have antimycobacterial and anti-biofilm activity against *M. tuberculosis* and *M. smegmatis* respectively. BE, CAA, PM and TC displayed some antimycobacterial and biofilm inhibitory activity. BR displayed noteworthy anti-biofilm activity against *M. smegmatis* biofilms, serving as a model for tuberculosis biofilm studies. Further studies could investigate the potential of BR targeting antibiotic resistance associated with tuberculosis.

The identified lead plants were successfully planted at Botanica Natural Products.

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ANNEXURE A

JL GC-MS

JL			
Peak #	Name	Area	Area %
1	Hexanoic acid, methyl ester	10908906	1.1741
2	Methane, trimethoxy-	7186908	0.77353
3	Propanedioic acid, dimethyl ester	6862063	0.73857
4	à-Amino-2,5-dihydro-5-methyl-2-furanaceticacid	554819	0.059715
5	4-Propoxy-2-butanone	554819	0.059715
6	Orthoformic acid, tri-sec-butyl ester	0	0
7	Allyl(methoxy)dimethylsilane	161767	0.017411
8	2(3H)-Furanone, dihydro-5-methyl-	8603556	0.926
9	Thiazole, tetrahydro-	943892	0.10159
10	Benzene, 1-ethyl-2-methyl-	149919	0.016136
11	Butyric acid, 4-isopropoxy-, methyl ester	24389222	2.625
12	Acetamide, N,N'-ethylenebis(N-nitro-	24346770	2.6205
13	Hexanal dimethyl acetal	24368080	2.6227
14	3-Octanone	15510605	1.6694
15	Acetoxyacetic acid, 2-dimethylaminoethyl ester	15483583	1.6665
16	Pentanoic acid, 4-oxo-, methyl ester	15483583	1.6665
17	Decane	5644344	0.6075
18	Cyclohexane, 1,1-dimethoxy-	75687	0.008146
19	Benzene, 1,3-dichloro-	2443025	0.26294
20	Methane, trimethoxy-	282112	0.030364

21	Benzene, 1-ethyl-4-methyl-	95020	0.010227
22	Heptanoic acid, methyl ester	16509121	1.7769
23	Hexanethioic acid, S-ethyl ester	16509121	1.7769
24	Methane, trimethoxy-	3672183	0.39524
25	1-Nonen-3-ol	3901835	0.41996
26	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	27612186	2.9719
27	Butanedioic acid, dimethyl ester	28742170	3.0935
28	Hexanoic acid, 2-ethyl-, methyl ester	28356320	3.052
29	2-Furanol, tetrahydro-2,3-dimethyl-, trans-	10806107	1.1631
30	2-Cyclohexen-1-one, 3-methyl-	6115386	0.6582
31	Acetophenone	1443066	0.15532
32	5-Undecanone	4763055	0.51265
33	Heptane, 1,1-dimethoxy-	6532618	0.70311
34	Acrolein, dimethyl acetal	30933930	3.3294
35	Pyrimidine-2,4,6-trione, 1-butyl-5-[(2-piperazin-1-yl-ethylamino)methylene]-	30933930	3.3294
36	Decane	4795584	0.51615
37	Benzaldehyde dimethyl acetal	572539	0.061623
38	2,5-Hexanedione	4121714	0.44362
39	Octanoic acid, methyl ester	5162574	0.55565
40	Pentanedioic acid, dimethyl ester	11176655	1.2029
41	Octanal dimethyl acetal	12055428	1.2975
42	Octanal dimethyl acetal	1466188	0.15781
43	Pentanoic acid, 5,5-dimethoxy-, methyl ester	8745453	0.94128
44	Spiro(1,3-dioxolane)-2,3'-(5'-androsten-16'-ol), TMS derivative	751176	0.080849
45	Benzothiazole	1483298	0.15965

46	Acetate, 4-hydroxy-3-methyl-2-butenyl-	11725818	1.2621
47	Nonanoic acid, methyl ester	4481002	0.48229
48	Dodecane, 1,1-dimethoxy-	3415213	0.36758
49	Heptanoic acid, 7,7-dimethoxy-	6464689	0.6958
50	2-Octanol, 8,8-dimethoxy-	1436251	0.15458
51	Decanoic acid, methyl ester	2535897	0.27294
52	Cyclopentanone ethylene ketal	1495227	0.16093
53	Decanoic acid, methyl ester	2662507	0.28657
54	2,3-Dimethyl-5-oxohexanethioic acid, S-t-butyl ester	3376791	0.36345
55	Methane, trimethoxy-	2288802	0.24634
56	Dodecane, 1,1-dimethoxy-	1117682	0.1203
57	Octan-2-one, 3,6-dimethyl-	6185728	0.66577
58	Hexanoic acid, methyl ester	3017892	0.32482
59	2,3-Octanedione	11036808	1.1879
60	2,5-Dimethyl-2-(2-tetrahydrofuryl)tetrahydrofuran	13200526	1.4208
61	Dodecane, 1,1-dimethoxy-	2465439	0.26536
62	2,2-Dimethyl-4-octenal	5502504	0.59224
63	Ethanamine, 2,2'-oxybis-	4750672	0.51132
64	2-Propen-1-ol, 2-bromo-, acetate	20525891	2.2092
65	Octanoic acid, 6,6-dimethoxy-, methyl ester	2292595	0.24675
66	Undecanoic acid, 10-methyl-, methyl ester	12778592	1.3754
67	p-Nitrophenyl hexanoate	24146090	2.5989
68	Dodecane, 1,1-dimethoxy-	1317366	0.14179
69	Hexadecane, 1,1-dimethoxy-	4323993	0.46539
70	Diethyl Phthalate	1580269	0.17009
71	Glycine	1141942	0.12291

72	Dodecane, 1,1-dimethoxy-	3530676	0.38001
73	Nonanoic acid, methyl ester	1274209	0.13714
74	Tridecanedioic acid, dimethyl ester	5163644	0.55577
75	Glycine	632544	0.068081
76	Glycine	1241321	0.1336
77	Dodecane, 1,1-dimethoxy-	6407587	0.68965
78	Decanoic acid, methyl ester	5054111	0.54398
79	Dodecane, 1,1-dimethoxy-	2725849	0.29338
80	Dodecane, 1,1-dimethoxy-	6587739	0.70904
81	Undecanoic acid, methyl ester	5648352	0.60793
82	Isopropyl myristate	5648352	0.60793
83	Phthalic acid, hexyl 2-methoxyethyl ester	742917	0.079961
84	Phthalic acid, butyl undecyl ester	4133436	0.44488
85	Dodecane, 1,1-dimethoxy-	4867546	0.5239
86	Dodecane, 1,1-dimethoxy-	6007158	0.64655
87	Tridecanoic acid, methyl ester	30635456	3.2973
88	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	12336612	1.3278
89	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	84084461	9.0501
90	n-Hexadecanoic acid	4257718	0.45826
91	Dodecane, 1,1-dimethoxy-	4532683	0.48785
92	Dodecane, 1,1-dimethoxy-	7220928	0.77719
93	Decanoic acid, 2-methyl-	4014903	0.43213
94	1,3-Octanediol	1683944	0.18124
95	Dodecane, 1,1-dimethoxy-	2921354	0.31443
96	Hexadecanoic acid, 15-methyl-, methyl ester	7584949	0.81637
97	Acetic acid, dimethoxy-, methyl ester	3220988	0.34668

98	Hexadecanoic acid, 15-methyl-, methyl ester	3071107	0.33054
99	Octadecane, 1,1-dimethoxy-	4196041	0.45162
100	Benzyl butyl phthalate	16610456	1.7878
101	Hexadecanoic acid, 15-methyl-, methyl ester	4702143	0.50609
102	Hexanedioic acid, bis(2-ethylhexyl) ester	3845619	0.41391
103	Nonanal dimethyl acetal	4849422	0.52195
104	1-Docosene	53444560	5.7523
105	Diisooctyl phthalate	25516408	2.7463
106	dl-à-Tocopherol	4233076	0.45561
Total	Total	9.29E+08	100

ANNEXURE B

PS GC-MS

PS			
Peak #	Name	Area	Area %
1	Benzene, 1-methoxy-4-methyl-	0	0
2	Cyclopentane, 1-acetyl-1,2-epoxy-	1502647	0.16537
3	Lilac aldehyde A	6333739	0.69705
4	2(3H)-Furanone, dihydro-5-methyl-	6333739	0.69705
5	Lilac aldehyde A	1810649	0.19927
6	3,4-Diacetylfurazan	5160306	0.56791
7	1-Hexanol, 2-(hydroxymethyl)-	4266574	0.46955
8	Acetate, 4-hydroxy-3-methyl-2-butenyl-	1229246	0.13528
9	Decane	158422	0.01743
10	4-Nonanone, 7-ethyl-	2683937	0.29538
11	1,2-Butanediol	228471	0.02514
12	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	5801857	0.63852
13	2-Cyclohexen-1-one, 6-(1-hydroxy-1-methylethyl)-3-	1681997	0.18511
14	Bicyclo[3.1.1]heptan-2-one, 3,6,6-trimethyl-	5384554	0.59259
15	2-Hexanone, 6-bromo-	5800780	0.6384
16	Cyclopentanol, 2-methyl-, acetate, cis-	6015664	0.66205
17	Silane, tetraethenyl-	3858802	0.42468
18	5-Decen-3-one, 9-hydroxy-2,2,9-trimethyl	4474804	0.49247
19	2-Methyl-4-octenal	7184928	0.79073
20	3-(5-Methylfuryl)-N-furamidopropionamide	9108307	1.0024
21	2-Butanone, 3,4-epoxy-3-ethyl-	7409231	0.81541
22	3-Acetoxytridecane	9960540	1.0962
23	Undecane	8146602	0.89656

24	Hexanoic acid, anhydride	2329738	0.2564
25	Benzothiazole	25435431	2.7993
26	Decane, 2,4-dimethyl-	25262662	2.7802
27	Acetate, 4-hydroxy-3-methyl-2-butenyl-	35436945	3.9
28	1-Hexene, 3,4,5-trimethyl-	7063567	0.77737
29	2-Heptene, 5-ethyl-2,4-dimethyl-	4396163	0.48381
30	6,7-Dodecanedione	4566297	0.50254
31	2,3-Dioxabicyclo[2.2.1]heptane, 1-methyl-	21778139	2.3968
32	2-Pentenoic acid, 2-methoxy-3-methyl-, methyl ester	7891244	0.86846
33	Hexadecane	4962845	0.54618
34	1-(7-Hydroxy-1,6,6-trimethyl-10-	16381040	1.8028
35	p-Nitrophenyl hexanoate	3665630	0.40342
36	2,5-Dimethyl-2-(2-tetrahydrofuryl)tetrahydrofuran	19451755	2.1407
37	8-Nonen-2-one	2828730	0.31131
38	2,6-Heptadien-1-ol, 2,4-dimethyl-	8627686	0.94951
39	1,3-Dioxolane-2-propanoic acid, 2,4-dimethyl-, ethyl	4347606	0.47847
40	2,2-Dimethyl-4-octenal	22753277	2.5041
41	Adipic acid, cyclobutyl isohexyl ester	22753277	2.5041
42	Pentanoic acid, 2-methyl-, anhydride	32783734	3.608
43	Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl-	1782941	0.19622
44	2,2-Dimethyl-4-octenal	7994907	0.87987
45	Hexadecane	4414578	0.48584
46	Ledol	8366919	0.92081
47	4H-Imidazol-4-one, 2-amino-1,5-dihydro-	4710764	0.51844
48	Pyrrolidin-2-one, 5-heptyl-	1785041	0.19645
49	Neophytadiene	28741979	3.1632
50	Neophytadiene	9744695	1.0724
51	Neophytadiene	17680953	1.9459

52	Phthalic acid, butyl hex-3-yl ester	2806842	0.3089
53	n-Hexadecanoic acid	32746737	3.6039
54	Undecanoic acid, ethyl ester	10986180	1.2091
55	Phytol	7859730	0.86499
56	1,10-Undecadiene	14873498	1.6369
57	cis-3-Butyl-4-vinyl-cyclopentene	33541134	3.6913
58	9,12-Octadecadienoic acid, methyl ester, (E,E)-	22670935	2.495
59	6-Butyl-1,4-cycloheptadiene	8507697	0.9363
60	Octadecanoic acid	27555499	3.0326
61	Hexadecanoic acid, butyl ester	8641080	0.95098
62	Octadecanoic acid, ethyl ester	1268561	0.13961
63	Hexanedioic acid, bis(2-ethylhexyl) ester	5819914	0.6405
64	Heptacosane	4273053	0.47027
65	Eicosane	6213110	0.68378
66	Phthalic acid, di(6-methylhept-2-yl) ester	532764	0.05863
67	Tetracosane	6928288	0.76248
68	Heptacosane	8268041	0.90993
69	Squalene	9998429	1.1004
70	Eicosane	9998429	1.1004
71	2-methyloctacosane	6328402	0.69646
72	Heptacosane	8521309	0.9378
73	ç-Tocopherol	4307823	0.47409
74	dl-à-Tocopherol	4957454	0.54559
75	Carbonic acid, eicosyl vinyl ester	7752895	0.85323
76	á-Sitosterol	15555445	1.7119
77	Stigmasterol	21225416	2.3359
78	Heptacosane	6591860	0.72546
79	á-Sitosterol	72831982	8.0154

80	á-Amyrin	13109520	1.4428
81	Urs-12-en-3-ol, acetate, (3á)-	19961205	2.1968
82	Lupeol	19961205	2.1968
83	1-Iodo-2-methylundecane	15846271	1.7439
84	Heptacosane	5732389	0.63087
Total	Total	908647434	100

ANNEXURE C

Archiving of data

Technology Transfer

WORKSHOPS

Year	Description	Number of participants
2018	Traditional health practitioner workshop	45

CONFERENCES

Year	Description	Title/Reference
2017	Women in Water Empowerment Programme	Szuman, K, Lall, N. Building a supportable economy from sustainable ecology: The beneficial value of wetlands. Women in Water 2017. Presentation
2018	44th Annual Conference of the South African Association of Botanists	Fibrich B, Kishore N, Madikizela B, Gao X, Puri A, Banga A, Lall N. "Mother Nature's remedies to reduce the appearance of wrinkles. 44th Conference of the South African Association of Botanists. Pretoria, South Africa. Presentation (Winner: Best PhD presentation)
2018	National Wetlands Indaba Conference	Fibrich, BD, Lall, N, Madikizela, B. The Wonders of Wetland Plants for Ageing Skin. National Wetlands Indaba Conference 2018. Poster Radebe, PG, Fibrich, BD, Madikizela, B, Lall, N. Fighting aging through elastase inhibition using South African wetland plants. National Wetlands Indaba Conference 2018. Poster (Winner: Best poster)
2019	45th Annual Conference of the South African Association of Botanists	Fibrich, BD, Lall, N. A wrinkle in time: The potential of fermented, gold nano particles and ethanolic extracts of *Persicaria senegalensis* on the maintenance of the dermal matrisome in photoaging skin.

		Department of Plant and Soil Sciences Post-Graduate Symposium, 2019. Plenary
2019	The Society of Cosmetic Chemists South Africa (COSCHEM)	Fibrich, BD, Lall, N. A wrinkle in time: The potential of fermented gold nano particles and ethanolic extracts of *Persicaria senegalensis* the maintenance of the dermal matrisome in photoaging skin. COSCHEM, 2018. Presentation
2019	Department of Plant and Soil Sciences Post-graduate Symposium	Radebe, PG, Lall, N. Anti-wrinkle potential of South African wetland plants focusing on skin elasticity and hydration. Department of Plant and Soil Sciences Post-graduate Symposium 2019. Presentation Fibrich, BD, Lall, N. A wrinkle in time: The potential of fermented, gold nano particles and ethanolic extracts of *Persicaria senegalensis* on the maintenance of the dermal matrisome in photoaging skin. Department of Plant and Soil Sciences Post-graduate Symposium 2019. Presentation (Winner: Best PhD presentation)

ARCHIVING OF DATA

BOOKS IN PROGRESS

Title	Number of chapters & pages	Editor	Publisher	Publishing date
The potential of aquatic plants of the world for pharmaceutical and cosmetic applications.	6 Chapters; 500 pages	N. Lall	Taylor & Francis	2020

MANUSCRIPTS IN PROGRESS

Makhosaya, Z., Mnengi, D., Nakin, N.V., Lall, N. Questionnaire based Report on Public Awareness of Use and Conservation of Wetland Plants in the Rural Areas of Eastern Cape, South Africa.
Radebe, P.G., Fibrich, B.D., Twilley, D., De Canha, M., Ooshuizen, C.B., Verma, S., Lall, N. Reversing the effect of skin aging using Elegia tectorum (L.F) Moline& H.P. Linder

Szuman, K., De Canha, M., Lall, N. The potential of South African wetland plants as effective inhibitors of tyrosinase. Journal of Applied Research on Medicinal and Aromatic Plants. To be submitted 15 December 2019

DISSERTATIONS

Year	Student	Title	Degree	Supervisors
2019	Zesipho Makhosaya	Uses of wetland plant biodiversity in the O.R Tambo district municipality, Eastern Cape, South Africa	MSc	Ms D Mnengi Dr NV Nakin & Prof. N Lall
2019	Princess Gugulethu Radebe	Reversing the effect of skin aging using Elegia tectorum (L.F) Moline& H.P. Linder	MSc	Prof N. Lall
2018	Karina Szuman	The therapeutic and medicinal potential of South African freshwater aquatic and wetland plants	MSc	Prof N. Lall
2017	Princess Gugulethu Radebe	Fighting aging through elastase inhibition using South African wetland plants	BSc (Hons)	Prof N. Lall

ARCHIVING OF SAMPLES

The plant extracts have been filed in the plant extract library in the Department of Plant and Soil Sciences: Medicinal Plants Research Group. Herbarium specimens have been filed in the H.G.W.J. Schweickerdt Herbarium at the University of Pretoria.

ANNEXURE D

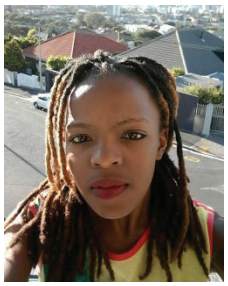
Capacity building

During the past 5 years, the following students have obtained their degrees and/or currently involved in doing them:

Karina Szuman

University of Pretoria

BSc Biotechnology (2014); BSc (Hons) Medicinal Plant Sciences (2015); MSc Medicinal Plant Sciences (2017)



Dikonketso Bodiba

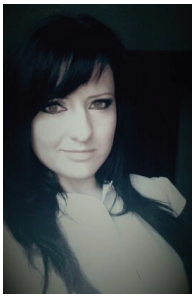
University of Pretoria

BSc Microbiology (2014); BSc (Hons) Medicinal Plant Sciences (2015); MSc Medicinal Plant Sciences (2017); PhD Medicinal Plant Sciences (current)

Princess Gugulethu Radebe

University of Pretoria

BSc Biotechnology (2016); BSc Hons Medicinal Plant Sciences (2017); MSc Medicinal Plant Sciences (current)



Bianca Fibrich

University of Pretoria

BSc Microbiology (2014); BSc (Hons) Medicinal Plant Sciences (2015); MSc Medicinal Plant Sciences (2017); PhD Medicinal Plant Sciences (current)

Marco De Canha

University of Pretoria

MSc Medicinal Plant Sciences (2014); PhD Medicinal Plant Sciences (2019); Postdoc Fellow Medicinal Plant Sciences (current)

Dr Carel Oosthuizen

University of Pretoria

MSc Medicinal Plant Sciences (2014); PhD Medicinal Plant Sciences (2018); Postdoc Fellow Medicinal Plant Sciences (current)

Zesipho Makhosaya

Walter Sisulu University

University of Pretoria will be hosting Zesipho from 7 to 10 May 2018, to learn cell culture techniques and anti-cancer bio-assays.

Dr Danielle Twilley

University of Pretoria

MSc Medicinal Plant Sciences (2014); PhD Medicinal Plant Sciences (2018); Postdoc Fellow Medicinal Plant Sciences (current)

Prof. Namrita Lall

University of Pretoria

NRF/DST Chair: Plant Health Products from IKS