South African medicinal plant extracts used in the treatment of fungal infections.

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KEYWORDS

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ABSTRACT

South African medicinal plant extracts used in the treatment of fungal infections.

The incidence of fungal skin infections is increasing at an alarming rate, especially in people with underlying immunosuppressive conditions. Currently, the most used methods of treatment are in the azole group of synthetic treatments. A major problem with these synthetic treatments' is the increase in azole-resistant strains spreading worldwide. Additionally, an estimated 65–80% of all fungal infections are biofilm related, with biofilms found in *Candida* species such as *Candida albicans*, *Candida dubliniensis*, *Candida glabrata* and *Candida tropicalis* which infect the skin.

There is an increased demand for alternative treatments that could be antifungals and medicinal plants could be the potential solution, as some plants possess antifungal properties. The overall aim of this study is to investigate the potential effect of South African medicinal plants used for the treatment of fungal infections against pathogens of dermatological relevance. The objectives were 1) to conduct a comprehensive literature search to identify indigenous medicinal plant extracts used to treat fungal skin infections, 2) to perform a cross-sectional study using an interview-based questionnaire to investigate the knowledge and practices of Cape bush doctors and validate their use of selected medicinal plants for treating dermatophyte infections in the Cape Metropole communities and 3) to investigate the *in vitro* bioactivity of selected plant extracts identified from the literature search and interviews.

Fifteen medicinal plants were found using an in-depth literature search and all 15 plants were validated during the interviews as plants commonly used for fungal skin infections. *Agathosma betulina* was reported to have fungicidal and fungistatic

activities against fungal skin infections. *Aloe ferox* and *Bulbine frutescens* were also the most used by bush doctors for fungal skin infections. Therefore, these three extracts were selected for further investigation in this study. The minimal inhibitory concentration (MIC) of the extracts was evaluated against four different skin-related *Candida* species namely *C. tropicalis*, *C. albicans*, *C. dubliniensis* and *C. glabrata*. The optimum concentration for cell proliferation was between 200-400 µg/mL for *B. frutescens* and 200 µg/mL for both *A. ferox* and *A. betulina* extracts, while *Candida* spp. inhibition was observed at a concentration of 200 µg/mL. This suggests that these extracts can be used at the 200 µg/mL dosage as antifungals against the *Candida* species while also aiding with the growth of epidermal cells at the same concentration. The present study highlighted that these medicinal plant species possess antifungal activity against *Candida* pathogens and have promising potential to be used for pharmaceutical product development.

Key Words: *Candida*, Fungal infections, *Agathosma betulina*, *Aloe ferox*, *Bulbine frutescens*

DECLARATION

I declare that "*South African medicinal plant extracts used in the treatment of fungal infections.*" is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources that I have used or quoted have been indicated and acknowledged by complete references.

Banele Michelle Ndlovu……  ……August 2023

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CHAPTER 1

General Introduction: Summary of thesis

1.1 Overview of the study

Available information on medicinal plants used in the treatment of fungal skin infections is fragmentary and widely dispersed in literature (van Wyk, 2008). Although there is information on some ethnic groups such as the Khoi-San, and their use of South African medicinal plants to treat various ailments, information on how they use these plants to treat fungal skin infections remains poorly recorded (Philander et al., 2014; van Wyk & Moteetee, 2019). Medicinal plants such as *Aloe ferox*, *Bulbine frutescens* and *Agathosma betulina* have been used traditionally and have antimicrobial activities against various bacteria as well as fungal spores and strains including *Candida* species (Fajinmi et al. 2019; Kambiz & Afolayan, 2008; Moolla et al., 2007; Rachuonyo, et al. 2016; Viljoen et al., 2006). Therefore, these medicinal plants used by the Khoi-San need to be validated or screened for their antifungal activities against candidiasis as there is little scientific basis for their use. This includes examining the ethnobotanical use of these plants and determining the efficacy of plants with known antifungal, preventative, or inhibitory properties (Erhabor et al., 2019; Kostadinova et al., 2016; van Wyk, 2008; 2011; van Wyk & Moteetee, 2019). There is also a need to conduct *in vitro* assays as preliminary standardised tests to provide data on useful medicinal plants that can protect and support the epidermal cells to treat or prevent fungal skin infections. This will provide a reasonable foundation for how these medicinal plants could interact with the skin and whether they have cytotoxic or proliferative properties. Determining the minimum inhibitory concentration (MIC) is the most frequently used endpoint to determine antimicrobial activity against

the relevant fungal pathogens (van Vuuren & Holl, 2017). Thus, this study will determine the MIC values of these plants against *Candida albicans*, *C. dubliniensis*, *C. glabrata* and *C. tropicalis*.

1.2 Problem statement and aim of the study.

In recent years, fungal skin infections have increased in incidence with a 20-25% prevalence (Rai et al., 2017). In South Africa this incidence is because fungal skin infections are associated with the human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS). This is attributed to South Africa being at the epicenter of HIV/AIDS with approximately 80% of immunocompromised patients suffering from fungal infections (Madzinga et al., 2018; Maema et al., 2020). Fungal skin infections have been associated with increased stress, anxiety, depression, and increased diagnoses of suicidal behaviour, especially in adolescent patients (Madzinga et al., 2018). Concerningly, the treatment methods for fungal skin infections are becoming more difficult especially because fungal pathogens have developed resistance to the standard azole therapy and other synthetic treatment methods (Maema et al., 2020). Reports have shown that medicinal plants could be a potentially promising solution as they have been used as ingredients for antimicrobial agents for many years, including antifungal agents (Ndhlala et al., 2011; van Vuuren & Holl, 2017; van Wyk & van Staden, 2002).

A few studies on South African medicinal plants and their potential antifungal properties have been done with most reports on plants found in the KwaZulu Natal, Limpopo and Eastern Cape provinces (Afolayan et al., 2014; Maema et al., 2020; Shikwambana & Mahlo, 2020). Although, the Western Cape (WC) province is home to the diverse Cape floristic region, housing various plant species, data on the use of

these plants as potential antifungal ingredients are sparse and even more so with regards to their use on skin models (Philander et al., 2014; van Vuuren & Holl, 2017). It is worth exploring the antifungal and dermatological properties of these medicinal plants as most of them are already used in skin care product formulations such as lotions, soaps, scrubs, and cleansers. These products include Ingram's camphor, Palm oil and Shea butter (Athar & Nasir, 2005; Stallings & Lupo, 2009; Surber & Kottner, 2017).

A. ferox, *B. frutescens* and *A. betulina* are three indigenous plant species found in the Cape floristic region in the WC. Several studies have employed these plants as antimicrobial agents with antifungal properties against prevalent dermatophyte pathogens showing that plants exhibit either fungistatic or fungicidal properties against *Trichophyton* and *Microsporum* species (Fajinmi et al., 2019; Ghuman et al., 2016; Hokken et al., 2019). Kühbacher et al., (2017) have reported on the effects of these species on *Candida albicans* and non-albicans *Candida* such as *Candida tropicalis*, *C. dubliniensis* and *C. glabrata*. However, to our knowledge, the use of these plants as an alternative means to fight against dermatophyte infections by providing protection, support, and strength to the barrier function of the epidermis has not been fully explored. There is also a need to determine the effect that the use of these plant species could have on normal epidermal cells, as a possible means to improve and strengthen the normal epidermal barrier instead of directly treating fungal skin infections.

1.3 Aims and objectives of the study.

The overall aim of this study is to investigate the potential effect of South African medicinal plants used for the treatment of fungal infections against pathogens of

dermatological relevance. This could lead to the development of new antifungal treatments using indigenous plants as key ingredients to combat fungal skin infections. The objectives of this study are:

a) To conduct a comprehensive literature review to identify indigenous medicinal plant extracts used to treat fungal skin infections and to compile an inventory thereof.

b) To perform a cross-sectional study using an interview-based questionnaire to investigate the knowledge and practices of Cape bush doctors and validate their use of selected medicinal plants for treating dermatophyte infections in the Cape Metropole communities. The questionnaire will be divided into four, 1) demographic information, 2) the overall knowledge of the bush doctors regarding skin infections, 3) the practices of the bush doctors along with the medicinal plants they used when treating fungal skin infections, and 4) validation of 15 medicinal plants obtained from a comprehensive review

c) To investigate the *in vitro* bioactivity of selected plant extracts identified from the literature search and interviews. This includes a) to determine the proliferative and cytotoxic effects of three plant extracts on human epidermal cells and b) to evaluate the susceptibility of *Candida* species to plant extracts by using broth micro-dilutions.

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CHAPTER 2

Literature review

2.1 Skin disorders and infections

The human skin is a complex system which functions as a physical barrier. It consists of a multitude of mechanisms and substances that work together to prevent the entry of microorganisms (antimicrobial barrier), as well as the movement of water and electrolytes (permeability barrier) (Braff et al., 2005; Feingold, 2012). The skin is a dynamic organ housing a wide variety of microbes, including bacteria and fungi (Jo et al., 2016). Thus, disruptions to the skin barrier particularly those that inoculate pathogens into the dermis, cause or exacerbate skin infections (Belkaid & Tamoutounour, 2016). Understanding fungal mycobiome on the skin is very important due to the close relationship between commensal and pathogenic fungi. These include *Malassezia*, *Candida* and dermatophytes, which can cause superficial fungal skin infections such as seborrheic, dermatitis and dermatophytosis once they penetrate the skin (Jo et al., 2016).

2.2 Epidemiology of fungal skin infections

Many studies have reported that superficial fungal infections are responsible for a broad spectrum of diseases in humans (Bitew, 2018; Jensen et al., 2007; Kim et al., 2015; Mehrmal et al., 2020). Skin and subcutaneous diseases are reportedly prevalent in 26.79% of the world's population. Amongst these skin diseases, dermatophyte infection cases had the highest growth of 0.23% between 2012 and 2017 (Mehrmal et al., 2020). The World Health Organisation (WHO) predicts that the prevalence of dermatophytes is quickly approaching 20% of the world's population

and its incidence continues to increase with time (Kim et al., 2015). A more recent study examining the global burden of diseases reported that fungal skin infections are the 4th most common cause of illnesses globally (Narang et al., 2019). However, there are still very few data present on the global incidence of dermatophytes due to a lack of regular national surveillance systems, no obligatory reporting of fungal skin diseases, poor diagnostic tests performances and well-designed published studies (Bongomin et al., 2017).

2.3 Dermatophyte infections and barrier function of the skin

2.3.1 Permeability barrier

Dermatophytes are highly specialized pathogenic fungi primarily affecting the stratum corneum layer of the epidermis of the skin (Jensen et al., 2007; Murat et al., 2013). The stratum corneum is the outermost epidermal layer. It is responsible for the permeability and microbial barrier functions of the skin. The cutaneous permeability barrier is formed from the extracellular lipids, cholesterol, free fatty acids and corneocytes during epidermal differentiation of the skin (Jensen et al., 2007). Its main function is to prevent the movement of water and electrolytes (Feingold, 2012). The permeability barrier may also act as an antimicrobial shield that prevents microbial penetration into the deeper epidermis (Oren et al., 2003). Epidermal differentiation is crucial for the integrity of the permeability barrier. Thus, impairment or disruption of the permeability barrier leads to changes in the epidermal proliferation and differentiation. Disruptions can be due to open wounds or burns, resulting in the loss of the protective epithelial layer, thus leading to opportunistic fungal skin infections involving *Candida albicans* (Mabona & van Vuuren, 2013; Tabassum & Hamdani, 2014). Changes in epidermal proliferation disrupt epidermal differentiation because

there is less time for proper differentiation during exaggerated cell renewal (Murat et al., 2013). The permeability barrier is involved in the microbial barrier as it co-regulates the expression of microbial peptides in the epidermis. Consequently, the disruption of the permeability barrier stimulates antimicrobial peptide production (Feingold, 2012).

2.3.2 Antimicrobial barrier and keratinocytes

The antimicrobial barrier is the second line of defence beyond the permeability barrier (Jensen et al., 2007). The antimicrobial barrier is mediated by lipids, free fatty acids and antimicrobial peptides (Feingold, 2012). Antimicrobial peptides play a defensive role in many organisms from insects to humans, due to their broad-spectrum activity against bacteria, virus and fungal infections (Murat et al., 2013). Antimicrobial peptides are synthesised by resident skin cells (keratinocytes) and recruit inflammatory cells (neutrophils) when there is infection or injury to the skin at sites of potential microbial entry and provide a soluble barrier that acts as an inhibition to infection (Braff et al., 2005; Murat et al., 2013). There are many antimicrobial peptides found in the epidermis, but the best studied in mammalian skin are human beta-defensin 2 (hBD-2) and human cathelicidins (LL-37) (Jensen et al., 2007; Murat et al., 2013). Expression of hBD-2 by the keratinocytes has demonstrated antifungal activity against *Malassezia furfur*, *Trichophyton rubrum* and *Candida albicans* infections, as well as against bacterial superinfections (tinea is colonized by a bacteria) and inflamed psoriatic lesions (Jensen et al., 2007; Murat et al., 2013). During inflammatory conditions, LL-37 is deposited at the site of injury by both keratinocytes and neutrophils (Braff et al., 2005). According to Murat et al. (2013), LL-37 protein expression in the skin increases in patients with tinea corporis, lesions of *Trichophyton inguinalis* as well as during psoriasis, lupus erythematosus contact dermatitis. Additionally,

understanding antimicrobial and permeability barrier can lead to new avenues and approaches in treatment dermatological infections such as those caused by dermatophytes (Murat et al., 2013).

2.4 Dermatophyte infections of the stratum corneum

A major cause of dermatophyte infections in both developed and developing countries is the practice of braiding which exposes the stratum corneum of the scalp to fungal spores, as well as the application of oils, which may adhere to and retain spores increasing the risk of spread (Ameen, 2010). During the early stages of infection, the yeast fungi changes into its hyphae form to penetrate epithelial and endothelial cell which causes both a disturbance in and damage to the extracellular space in the stratum corneum (Liu et al., 2019). This affects the barrier function by disrupting epidermal proliferation and differentiation (Jensen et al., 2007). The result of this is dermatophytosis, the most contagious infection affecting the outermost keratinized layers of the skin and its appendages (Bitew, 2018; Verma & Gaffen, 2019). It causes erythema and scaling of the skin and affects both humans and other mammals (Jensen et al., 2007; Verma & Gaffen, 2019). Dermatophytosis has been described as a disease of worldwide importance and public health problem in many parts of the world particularly developing countries (Bitew, 2018). If left untreated, dermatophytosis can cause long-lasting infections that may seriously impair a person's quality of life (Verma & Gaffen, 2019). Superficial dermatophytosis results in disturbed skin barrier function, reduced stratum, corneum hydration, changes in epidermal proliferation and differentiation (Murat et al., 2013). Although dermatophyte infections are mostly limited to the skin, they may disseminate into deep tissue and inner organs

including the central nervous system, thus becoming severe and life-threatening particularly in immunocompromised individuals (Rippke et al., 2018).

2.5 Prevalence, incidence, and risk factors of fungal skin infections

Dermatophytes are fungal agents that developed a dependency on human and animal infections for survival as the infection can spread from person-to-person (anthropophilic), animal to person (zoophilic) or soil to person (geophilic) (Kim et al., 2015). The distributions of dermatophytes and the causative agents are likely to vary from place to place. This is attributable to the ever-changing patterns of migration, tourism, socio-economic conditions, and increasing number of populations across the world. Thus, obtaining the overall incidence and prevalence of dermatophytes is challenging (Bongomin et al., 2017; Kim et al., 2015). There are reports on dermatophytes thought to be endemic to Africa and Asia that are now increasing in frequency in developed countries because of migration (Ameen, 2010). Other factors increasing the prevalence and incidence of dermatophytes include high population densities and poor sanitary conditions (Bongomin et al., 2017).

The severity of dermatophyte infections ranges from asymptomatic-mild mucocutaneous infections to potentially life-threatening systemic infections (Bongomin et al., 2017; Rippke et al., 2018). Over 80% of patients could be saved from developing a life-threatening fungal skin infection, but early detection, recognition and management of such infections could be a challenge (Bongomin et al., 2017). Especially, because some antifungal treatment can be expensive, toxic, and not equally available in all countries. Patients in some developing and rural settings have little access to dermatologists. Hence, most fungal skin infections are misdiagnosed as secondary inflammatory diseases such as syphilis and annular psoriasis having the

ability to mimic dermatophyte infections (Bongomin et al., 2017; Mehrmal et al., 2020; Yadgar et al., 2017). Misdiagnosis can lead to incorrect therapy, worsening of symptoms, additional skin and soft tissue conditions (Yadgar et al., 2017). Fungal skin infections progress to life-threatening illnesses due to resistance to antifungal treatment which can result in severe side effects, increased medical fees and social cost if not treated quickly during the earlier stages (Kim et al., 2015).

There is an increased number of people having severe dermatophyte infections which later progresses to death in both developed and developing countries worldwide. Immunosuppressive diseases such as the HIV pandemic, tuberculosis (TB) and diabetics are a major cause of this increase in incidence (Bongomin et al., 2017; Rippke et al., 2018). South Africa has the largest HIV pandemic, the highest incidence of TB and has the most economic inequity (poverty) in the world. Consequently, dermatophyte infections are widespread throughout the country (Schwartz et al., 2019). All these conditions on their own or in combination can predispose people to fungal skin infections.

2.6 Synthetic treatment of dermatophytes, mode of action and resistance

When treating dermatophyte infections, the main target of most antifungals is the suppression of hyphae growth and the formation of biofilms of the fungi (Liu et al., 2019). The current treatment methods for dermatophyte infections include the azole group synthetic drugs such as ketoconazole, itraconazole and terbinafine (Gowhar et al., 2015). These antifungals act mainly by exploiting ergosterol, a membrane sterol found in fungi. Ergosterol serves as a bio-regulator of membrane fluidity, asymmetry and membrane integrity in fungal cells (Ghannoum & Rice, 1999). The azole antifungals act to reduce ergosterol synthesis as well as the inhibition of fungal

cytochrome P450 enzymes (Dhamgaye et al., 2014; Gowhar et al., 2015). The inhibition of the cytochrome P450 enzymes leads to the depletion of ergosterol, resulting in the formation of a plasma membrane with altered structure and function (Ghannoum & Rice, 1999). Other available synthetic antifungals interfere with the causative agents' cell wall synthesis and others cause aqueous pore formation in the fungi ensuring the leakage of crucial cellular components and subsequent cell death of various strains (Dhamgaye et al., 2014).

The current available synthetic antifungals have undesirable side effects and are ineffective against new or re-emerging fungal strains (Da et al., 2019). Furthermore, most developing countries do not have access to proper technological facilities or people to develop these synthetic chemical treatments (Erhabor et al., 2019a). Another major problem faced when treating patients suffering from dermatophytes is that the infection is amenable to self-treatment. This leads to the unsupervised and often misleading use of over the counter antifungals resulting in resistance (Da et al., 2019). Antifungal resistance is a major public health threat experienced by both humans and animals (Erhabor et al., 2019). Most fungal species are intrinsically resistant to antifungals and have the ability to resist standard antifungal treatment by forming biofilms which contain hyphae (Liu et al., 2019). Resistance to azoles is also caused by the prolonged use of antifungal drugs which leads to the emergence of multidrug-resistant strains posing additional clinical problems (Dhamgaye et al., 2014). Incorrect use of the treatment blocks the azoles mode of action and changes their target or ability to penetrate cells. Additionally, synthetic antifungals have adverse effects which include renal damage, abnormalities of the liver and/or gastrointestinal problems (Gowhar et al., 2015). Other reports have shown that several antifungal synthetic drugs not only present problems of resistance but also have side effects,

including allergies (Salhi et al., 2019). There is a major need to find antifungal treatments with low toxicity, a broad spectrum of activity and new modes of action (Liu et al., 2019).

2.7 Medicinal plants as an alternative treatment method.

In recent years, it has been documented that traditional medicinal remedies have created great interest as an alternative to synthetic drugs. Medicinal plants are thought to be more accessible and relatively cost-effective herbal medicines (Erhabor et al., 2019; Jamshidi-Kia et al., 2018; Salhi et al., 2019). Studies have reported that medicinal plants have bioactive secondary metabolites which are a vital source of antimicrobials. Herbal mixtures contain multiple and complex compounds offering a promising solution to multi-drug resistance (Erhabor et al., 2019; Ndhlala, et al., 2011). There is an increased interest in the discovery and commercial development of medicinal plant treatments. This indicates that medicinal plants could potentially have significant benefits in the world health system as well as generating national income especially in developing countries (Erhabor et al., 2019).

2.8 Historical background of medicinal plants

Herbal medicine is the science of using plants for their medicinal value and this science has been widely used by various people throughout history (Dias et al., 2012; Jamshidi-Kia et al., 2018; van Wyk, 2008; van Wyk & van Staden, 2002). To prevent, relieve or treat illnesses through leaves, flowers, stems, berries and roots, which are all able to produce a variety of chemical substances which can act upon the body (Mativandlela et al., 2009). According to Jamshidi-Kia et al. (2018), it is estimated that over 50% of the available drugs are somehow derived from medicinal plants. Moreover, most prescription medication used nowadays contains at least one active

ingredient from plant materials or extracts or a chemical which is synthesized to simulate actions of natural plant compounds. The most well-known example is the anti-inflammatory agent acetylsalicylic acid (aspirin), derived from salicin which was originally isolated from the white willow bark tree, *Salix alba Laudanum* (Dias et al., 2012). Morphine is the most commercially important alkaloid isolated from *Papaver somniferum* Laudanum, commonly known as opium poppy. In 1870, for the first time, crude morphine was boiled in acetic anhydride resulting in diacetylmorphine (heroin) which is readily converted to codeine, a painkiller. In present times, morphine is used as a standard to measure new synthetic analgesic drugs (Dias et al., 2012; Mativandlela et al., 2009). The drug quinine, isolated from the bark of *Cinchona succirubra* has been used for centuries to treat malaria, fever, indigestion, mouth and throat diseases. It is notably known as an anti-malarial drug (Maldonado et al., 2017).

2.9 Modern medicine from medicinal plants

Africa is a continent richly endowed with thousands of diverse and endemic plant species. These plant species contribute to nearly 25% of the world trade in biodiversity (Moyo et al., 2011). Many of the continent's plants resources are used in forestry, agriculture, horticulture and medicine (Mativandlela et al., 2009). African medicinal plants have a long history of use and supposed safety. These medicinal plants can be utilized in the treatment of various diseases and are part of African traditional medicine. African traditional medicine is the oldest medicinal system with various plant-based treatments being used or being discovered (Erhabor et al., 2019). A few examples of such products include vincristine and vinblastine used as antimitotic treatments. Vincristine is particularly used as a chemotherapeutic agent which comes from the Madagascar periwinkle (Berg & Parsons, 2015). Another example is rooibos

with is known for its various antioxidant activities and is most notably used as a refreshment drink and health tea beverage (Da Rocha et al., 2001; Mahomoodally, 2013). Due to historical and cultural reasons, especially in most of the developing countries in Africa, there are 70-80% of people who depend on these traditional medicines and plant-based preparations for the treatment of infectious diseases (Mahwasane et al., 2013; Salhi et al., 2019). Despite the availability of modern medicine in some communities, herbal medicine is still often preferred due to its efficacy and cost-effectiveness (Anyanwu & Okoye, 2017). More than 80% of the African population seek medical attention from traditional healers for health issues (Narang et al., 2019). The traditional healers use medicinal plants to treat a variety of illnesses such as chest pains, tuberculosis (TB), diarrhoea and arthritis (Cragg & Newman, 2013). Most of the knowledge of the rich botanical African and cultural diversity was passed down from generation to generation (Mahwasane et al., 2013). This form of oral-traditional knowledge dates back thousands of years and has left a legacy of indigenous knowledge. However, due to cultural changes, a major disadvantage of oral-traditional knowledge is the lack of proper documentation or recording systems causing a risk that the information can be lost to future generations (van Wyk & Moteetee, 2019).

2.10 Herbal extracts and dermatophyte infections research

2.10.1 Conventional medicine vs synthetic medicines

As part of cosmetology, phyto-cosmetics involve the use of plant species for cosmetic purposes. However, herbal remedies are not limited to the beautification of the skin but can be also used for therapeutics focused on various skin diseases (Ndhlovu et al., 2019). Due to the large scale of resistance and adverse reactions of commercially

available drugs, there is an increased used of herbal extracts (Gowhar et al., 2015). Many conventional medicines are originally derived from plants, with about 33% of traditional remedies currently used for dermatological disorders compared to only 1-3% of synthetic drugs. The reason for the increased use of herbal extracts for treatment of dermatophytes is because of their ability to stop bleeding and accelerate the healing processes (Salhi et al., 2019). Current antifungal agents used such as echinocandins and amphotericin B are developed from natural resources. Other natural products found in herbal extracts including peptides, saponins, flavonoids and essential oils can also be useful treatment options because they possess antifungal properties (Liu et al., 2019).

2.10.2 Mode of action of herbal extracts

The mode of action of most herbal compounds when treating dermatophytes includes, interfering with mitogen-activated protein kinase signalling and cell wall integrity pathway of fungal strains (Lordani et al., 2018). Some herbal extracts increase the generation of reactive oxygen species leading to fungal strain cell necrosis. These mechanisms are independent of any of the known multi-drug resistant mechanisms (Dhamgaye et al., 2014). A recent study reported that herbal extracts can inhibit efflux pumps caused by *Candida albicans* and other microbes that cause an increase in toxic substances in the extracellular milieu and contributes to cellular drug resistance (Da et al., 2019). Numerous studies have reported on the use of medicinal plants that exert antifungal activities. These various extracts have the ability to increase membrane permeability of fungi and disrupt the fungal cell membrane formation preventing the development of hyphae (Afolayan et al., 2002; Da et al., 2019; Dhamgaye et al., 2014; Liu et al., 2019; Mabona et al., 2013; Thibane et al., 2019). Additionally, the extracts

can cause serious damage to the ultrastructure of the fungi including membrane structures of the cell wall, cytoplasmic, nucleus and mitochondrial membranes (Da et al., 2019; Dhamgaye et al., 2014; Liu et al., 2019).

2.10.3 Herbal extracts used in the treatment of dermatophyte infections

A few examples of antifungal extracts include one found in Kenya for instance, an aqueous extract from the leaves of *Schizozygia coffaeoides* used for washing ringworm infected skin, and root extracts mixed with coconut oil used for the treatment of skin sores (Hu et al., 2015). Evidence from a study by Hu et al. (2015), indicates that herbal medicines benefit barrier function in both normal and diseased skin. The study suggests that herbal extracts such as Hesperidin and Apigenin could increase keratinocyte differentiation, lipid production, lamellar body formation and antimicrobial peptide. The upregulation of antimicrobial peptide expression using herbal extracts could improve the barrier function, this could be beneficial in finding new antifungal therapies (van Wyk & van Staden, 2002). Ethnopharmacology testing of the biological activity of the crude extract and isolated compounds has received considerable attention (van Wyk, 2008). Advances in laboratory techniques have strengthened interest in the field and scientific validation of traditional medicines into the treatment regimens of conventional health systems (Erhabor et al., 2019).

2.11 Treatment of dermatophytes with South African medicinal plants research

South Africa possesses a unique and diverse botanical heritage, with major ethnic plant groups such as Zulus, Xhosas, Khoi, Ndebele, and Swazi possessing strong indigenous plant knowledge (van Wyk, 2008). South Africa is characterized by a population known for its excessive reliance on its plant resources for food, medicine,

cultural and spiritual demands (Moyo et al., 2011). At least 70% of South African locals are believed to consult one of more than 20 000 traditional healers in the country (Erhabor et al., 2019). Largely because there are over 30 000 indigenous South African plant species, of which over 3 000 species are used therapeutically (Ndhlovu et al., 2019). Traditional healers often combine various plants parts or even different plant species to make herbal mixtures (Ndhlala et al., 2011). These medicinal plants mixtures have properties including antimicrobial activities, antiparasitic, anti-inflammatory and antioxidant properties (Erhabor et al., 2019).

2.12 South African medicinal plants with antimicrobial properties

2.12.1 *Agathosma betulina*

Agathosma betulina is an essential oil-bearing pant belonging to the citrus or rue family Rutaceae. The plant is typically of the fynbos found in the Western Cape province and particularly abundant on sunny hillsides and the rocky sandstone slopes in the mountainous areas in the Cape (Moolla & Viljoen, 2008). It is called “round-leaf buchu” because it is a fragrant plant that could be dried and powdered (Viljoen & Moolla, 2007). *A. betulina* is a resprouting, broad-leaved, fragrant shrub that grows to a height of 2 m. The leaves are strongly aromatic, and the oil is golden in colour, with a strong-sweetish, peppermint-like odour. The flowers are large, star-shaped, five petalled, usually solitary, axillary, and white to purplish pink in colour. The traditional uses of the plant include: as an antispasmodic; an antipyretic; a liniment; a cough remedy; for the relief of rheumatism, gout and bruises (Moolla & Viljoen, 2008). The hydodistilled essential oils of this plant reportedly have antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Candida albicans* with (MIC values ranging between 2 and 4 mg/mL (Moolla et al., 2007; Viljoen et al., 2006). A

study by Fajinmi et al. (2019), the essential oil volatiles of *A. betulina* at 40 µl were able to cause the destruction of hyphae and spores of *Trichophyton rubrum* as well as the inhibition/reduction of *Trichophyton mentagrophytes*.

2.12.2 *Aloe ferox*

Aloe ferox locally referred to as the “Cape aloe” or “bitter aloe” is a single-stemmed plant belonging to the Asphodelaceae family (Chen et al., 2012). It is commonly found in the Cape coastal regions of South Africa and grows to a length of 3m and has thick, fleshy bitter leaves which are arranged in rosettes, while its orange-red flowers stand approximately 1m above the leaves (Chen et al., 2012; Shackleton & Gambiza, 2007). The plant is traditionally used as a laxative, in the treatment of arthritis and inflammation by direct application to the affected area (Chen et al., 2012; Froldi et al., 2019). The extracts of the plants have reportedly exhibited inhibitory responses on the growth of *C. albicans* and *Neisseria gonorrhoea* (Kambiz & Afolayan, 2008). The Aloe gels contains compounds such as aloin which showed strong antimicrobial activity against a wide range of Gram-negative and Gram-positive bacterial and fungal strains (Zapata et al., 2013). In the study by Liu et al. (2015), aloin was reported to protect human skin fibroblast cells against heat stress-induced damage. The aloin did not display any cytotoxic activity in cell culture at up to 1 mg/mL (Froldi et al., 2019).

2.12.3 *Bulbine frutescens*

Bulbine frutescens is a fast-growing succulent perennial plant species also belonging to the Asphodeloideae family. It is a southern African plant widely spread throughout the Northern, Western and Eastern Capes of South Africa (Coopoosamy, 2011; Pather et al., 2011). It has rows of green fleshy leaves and a greyish stem with a petaled star-shaped flower that is yellow-orange in colour. *B. frutescens* have been reportedly used

by traditional healers to treat rashes, sores, blisters and burns (Pather et al., 2011). The stems and roots of the plant contain compounds such as antraquinones such as chrysophanol and knipholone and the sap of the plant contains glycoproteins called aloctin. These compounds can play a role in the wound healing process. The leaf extracts of the plant reportedly inhibit gram positive bacterial growth such as *Bacillus subtilis*, *Micrococcus kristinae* and *S. aereus* (Coopoosamy, 2011). A study by Rachuonyo, et al. (2016) also reported that B. frutescens demonstrated antifungal activities against *C. albicans*. The *in vivo* wound healing properties of the leaf gel extracts were also identified in pigs (Pather et al., 2011).

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CHAPTER 3

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Does South Africa hold the key to the development of alternative treatments for resistant dermatophyte infections? A review

3.1 Introduction

Dermatophyte infections are becoming very prevalent in humans (Zida et al., 2017). These fungal skin infections are sometimes persistent and potentially contagious, affecting 20-25% of the world's population ranging from neonates to the elderly (Havlickova et al., 2008; Mabona and van Vuuren, 2013; Tabassum and Hamdani, 2014; Rai et al., 2017). Naturally, in healthy individuals with no underlying diseases, fungal skin infections can be effectively eradicated by immune system components such as macrophages and antifungal agents (Hokken et al., 2019). However, in persons with underlying immunosuppressive conditions such as HIV/AIDS, haematological malignancies and transplant recipients, the incidence of dermatophyte infections continue to increase (Carrillo-Munoz et al., 2006; Coopoosamy and Naidoo, 2011a; Rai et al., 2017; Bitew, 2018; Sharif et al., 2018). This is due mostly to the treatment and management of fungal skin infection medication being noticeably limited by problems of safety. Most compounds that are effective against fungi are also toxic to mammalian cells because of the shared eukaryotic cellular structures (Hokken et al., 2019). Some antifungal agents reportedly cannot penetrate the site of infection

and poor patient compliance play a role in the increased cases of dermatophyte infections as well as the increase in antifungal resistance (Khosravi et al., 2013).

Current treatment methods for dermatophyte infections include amphotericin B and echinocandins but the most used are the azole group of synthetic treatments such as ketoconazole, itraconazole and terbinafine (Gowhar et al., 2015). Reports show that the azole drugs are not entirely selective when binding to the active site of the pathogen and can also inhibit mammalian cytochrome 450 enzymes, which are responsible for the metabolism of various substances including drug-drug interactions. This is very problematic especially in immunocompromised patients already receiving other medications which could result in drug-drug interactions (Wiederhold, 2018). Some of the antifungal agents in the azole group have been reported to cause severe acute and chronic toxicities such as renal function impairment (Carrillo-Munoz et al., 2006). According to Tariq et al. (2019), ketoconazole, when used against dermatophytes such as *Trichophyton* species, has caused unpleasant side-effects including nausea and abdominal pain. This toxicity limits its therapeutic use in most cases.

A major problem medical communities face in treating dermatophyte infections is the increase in azole-resistant strains spreading worldwide (Zida et al., 2017). According to Hokken et al. (2019) the increasing rate of fungal skin infections is likely caused by strains that are resistant to commonly used antifungals, making it difficult to treat their diseases and is consequently accompanied by high-mortality rates. High levels of primary resistance to terbinafine have been reported against *Trichophyton rubrum* fungal isolates (Khosravi et al., 2013). Some fungal strains are resistant to antifungal

agents through overexpression of biofilm-specific efflux pumps that give them the ability to excrete antifungal compounds (Hokken et al., 2019).

Biofilms have been reported to contribute to the elevation of drug resistance because they are notoriously difficult to eliminate and are a source of many recalcitrant infections (Jabra-Rizk et al., 2004; Robbins et al., 2011; Desai et al., 2014). Fungal biofilms are complex surface-associated cell populations that are embedded in an extracellular matrix and can attach to biotic (mucosal or tissue surfaces) or abiotic surfaces (e.g., catheters or prostheses) which serve as substrates for biofilm growth (Fanning and Mitchell, 2012; Desai et al., 2014; van Acker et al., 2014). Sessile cells are adherent cells found in a biofilm. These cells are phenotypically and physiologically different from non-adherent (planktonic) cells and require much higher concentrations of antifungal drugs to be destroyed. This is a major cause of biofilm-related antifungal resistance as an estimated 65-80% of all fungal infections are biofilm-related (van Acker et al., 2014). Some factors contributing to biofilm cells becoming more antifungal resistant include, biofilm structural complexity, presence of extracellular matrix, metabolic heterogeneity, and biofilm-associated up-regulation of efflux pump genes (JabraRizk et al., 2004; Fanning and Mitchell, 2012; Desai et al., 2014). The biofilm extracellular matrix is one of the most influential factors promoting antifungal resistance. The matrix can prevent access of antifungals to cells embedded in the biofilm by slowing the diffusion rate or specifically binding antifungals extracellularly (Desai et al., 2014; Hokken et al., 2019). There is a major need for antifungal treatment that specifically targets biofilm-associated infections.

Limited availability of most antifungal agents used, and the increasing number of treatment failures have motivated the current search for therapeutic alternatives, which

have the potential to work effectively as antifungal agents (Khosravi et al., 2013). The ideal antifungal agents should have a broad spectrum of fungicidal activity, be selective and non-toxic to host cells as well as the ability to bypass drug-drug interactions. Such drug-drug interactions have been reported in South Africa where more than 90% of HIV-infected patients develop opportunistic fungal skin diseases at some stage of the viral infection and must take multiple medications simultaneously (Afolayan et al., 2014). Research on active constituents of traditional medicines is a strong possible solution as alternative antifungal agents. The use of herbal medicine has widely increased over the years with over 4.5 billion people (64% of the world's population) known to use herbal medicine (Khosravi et al., 2013). About 27 million people in the South African population rely on medicinal plants to treat a broad range of infections (Abdullahi, 2011; Coopoosamy and Naidoo, 2011b; Ghuman and Coopoosamy, 2011). This is because the country has a diversity of indigenous medicinal plants with dermatological therapeutic relevance (Manning and Goldblatt, 2012; Raimondo, 2015). The research for and use of plant dermatological treatments have increased in recent years (Rashidi et al., 2014; Endo et al., 2015; Fajinmi et al., 2017). Scientists are seeking medicinal plant species that could be developed into medicines for various skin diseases including fungal skin infections (Mintah et al., 2019). Information on the use of medicinal plants used in the treatment of dermatophyte infections is lacking. A systematic review can be implemented to address this gap in knowledge by identifying, appraising, and summarizing all relevant studies on the topic with a reproducible methodology (Greenhalgh, 1997; Uman, 2011). Hence, this study aimed to review existing literature using a systematic approach to identify studies concerning the use of indigenous South African medicinal plants used to treat fungal skin infections.

3.2 Method

3.1.2 Data collection

A literature search was performed regarding the use of indigenous South African medicinal plants on the skin to treat fungal diseases. It was noted that there is a lack of *in vivo* studies currently found in this research area. Thus, the search was limited to *in vitro* studies regardless of language or publication status (published, unpublished, in the press and progress). Computerized literature searches were performed on Medline, Scopus, GoogleScholar, Medline EBSCOhost and Science Direct databases. Also, the Global Electronic Thesis and Dissertations (ETD) and South African National ETD were searched for grey literature. For the retrieval of publications in the databases, the researchers conducted a specific search to define the maximum Medical Subject Headings (MeSH) terms that were related to the research goal. The key terms were “Plant (MeSH)” And “Skin (MeSH)” And “Fungal (MeSH)”. In addition to these keywords, all plants from the South African Pharmacopoeia Monograph project were included in the searches (Mukinda and Eagles, 2010). Each plant was searched individually and in conjunction with the MeSH terms. Further papers were retrieved from reference lists of review articles. Due to the limited number and diversity of studies, including the difficulty to prove comparisons between studies, a meta-analysis was not conducted.

3.2.2 Study selection

Studies were considered eligible for this review based on the following inclusion criteria: publications that described the use of medicinal plant species (alone or with any combination of South African herbs) indigenous to South Africa to treat fungal skin infections. This included studies with no language restrictions and date limitations.

Studies that were non-South African plants (not endemic to South Africa, and imported plants) were excluded, viral and bacterial studies were also excluded. For studies that included the different types of antimicrobial testing (bacterial, viral and fungal), only the fungal sections were analysed, while the bacterial and viral sections were omitted.

The inclusion of data was assessed by all authors. Discrepancies and disagreements were resolved by consensus among the researchers in several meetings. The first phase of this study was the retrieval of articles based on titles and abstracts of potentially relevant studies in each database. In the second phase, the full PDF articles were downloaded and assessed for eligibility. The reference list of review articles was also evaluated in search of other publications of interest not retrieved in the database search in phase 1.

3.3 Results and Discussion

In recent years, treatments used against fungal pathogens have exhibited several side effects since compounds that are effective against fungi are toxic to mammalian cells (Khosravi et al., 2013; Hokken et al., 2019). The growing interest for either incorporating or replacing current antifungal agents with herbal plants extracts is due to the emergence of azole and echinocandin resistant fungal pathogens (Hokken et al., 2019). Therefore, acknowledging that various South African indigenous medicinal plants and their derivatives can make a significant contribution to the development of alternative antifungals for the treatment of dermatophyte infections is important (Coopoosamy and Naidoo, 2011a; Ghuman and Coopoosamy, 2011) Therefore, this review of existing literature for these medicinal plants with antifungal activity is essential.

In the present study, a total of 67 abstracts were identified from electronic searches. The search identified 62 articles, with 0 from Pubmed, 5 From Medline, 8 From Scopus, 29 From Google Scholar, 8 from Science Direct and 7 from the Global and South African ETD. An additional 5 articles were retrieved from reference lists of review articles. After the removal of duplicates, as well as screening from relevant titles and abstracts. a total of 49 articles underwent a full-text review and 10 articles met the inclusion and exclusion criteria.

Table 3.1 summarises the results of the 15 medicinal plant species found during the investigation. Here, the botanical names of the species are arranged in alphabetical order along with their common name, family, the region in South Africa where it is found, and the parts used. The medicinal plant species were widely distributed in 8 South African provinces (excluding North West). Majority of the 15 plant species

belonged to families located within or in some parts of the Eastern and Western Cape provinces. This could be attributed to the diversity of plants found in the Cape Floristic Region within the 2 provinces. The region hosts almost 20% of all flora on the African continent (Afolayan et al., 2002; Manning and Goldblatt, 2012). According to the South African National Biodiversity Institute (SANBI), South Africa has over 20 000 plant species of which over 2 000 plant species are recorded as used locally for medicinal purposes (van Wyk, 2011; Raimondo, 2015; Victor et al., 2015).

Table 3.1: Summary of the 15 medicinal plant species used to treat fungal skin infections.

Botanical name	Common name	Family	Region	Part used	References
1. *Agathosma betulina* (Berg.) Pillans	Buchu (Afr)	Rutaceae (Citrus)	WC & EC	Leaves	(Fajinmi et al., 2019)
2. *Aloe arborescens* Mill.	Krantz aloe (Eng), kransaalwyn (Afr), inkalane or umhlabana (Zulu)	Xanthorrhoeaceae	WC, NC, EC, KZN & MP	Leaves	(Ghuman et al., 2016)
3. *Aloe aristate* (Haw.) Boatwr. & J.C. Manning	Bearded Aloe (Eng), or Hardy Aloe (Eng)	Xanthorrhoeaceae	WC, NC, EC, KZN & MP	Leaves	(Ghuman et al., 2016)
4. *Aloe ferox* Mill.	Bitter aloe, (Eng); bitteraalwyn, (Afr); iNhlaba (Zulu)	Xanthorrhoeaceae	WC, NC, EC, KZN & MP	Leaves	(Afolayan et al., 2002; Ghuman et al., 2016)

5. *Bulbine frutescens* (L.) Willd.	Snake flower, cat's tail, (Eng), balsam kopieva (Afr), geelkatstert (Afr)	Xanthorrhoeaceae	WC, NC, EC, KZN & MP	Leaves, stem & roots	(Ghuman et al., 2016)
6. *Bulbine natalensis* var. *curvata* Van Jaarsv*.*	Ibhucu (Zulu), Rooiwortel (Afr)	Xanthorrhoeaceae	WC, NC, EC, KZN & MP	Leaves, bulbs & roots	(Ghuman et al., 2016)
7. *Coleonema album* (Thunb.) Bartl. & J.C. Wendl.	White confetti bush (Eng), assbossie (Afr)	Rutaceae	WC & EC	Leaves	(Esterhuizen et al., 2006; Fajinmi, 2016; Fajinmi et al., 2017; Fajinmi et al., 2019)
8.*Coleonema pupulchellum* I. Williams	Confetti buchu (Afr)	Rutaceae	WC & EC	Leaves	(Fajinmi, 2016; Fajinmi et al., 2017)
9.*Elytropappus rhinocerotis* (L.f.) Less.	Renosterbos (Afr)	Asteraceae	EC & WC	Stem	(Hulley et al., 2019)

10. *Eucomis autumnalis* (Mill.) Chitt	Pineapple flower (Eng); wildepynappel, (Afr); umathunga (Zulu)	Asparagaceae	EC, LMP, Gua, FS & MP	Leaves, stem & bulbs	(Ghuman et al., 2016)
11. *Haworthiopsis limifolia* (Marloth) G.D.Rowley (= *Haworthia limifolia* Marloth)	Fairy Washboard (Eng)	Xanthorrhoeaceae	WC, NC, EC, KZN & MP	Leaves, stem & roots	(Naidoo and Coopoosamy, 2011; Ghuman et al., 2016)
12.*Hypericum aethiopicum* Thunb. subsp.	Unsukumbili (Zulu)	Hypericaceae	KZN	Leaves, stem & roots	(Ghuman et al., 2016)
13. *Merwilla plumbea* (Lindl.) Speta	Wild squill, (Eng); blouberglelie (Afr)	Hypericaceae	EC, KZN & FS	Leaves, bulbs & roots	(Ghuman et al., 2016)

14. *Tetradenia riparia* (Hochst.) Codd	Iboza (Zulu); watersalie (Afr); ginger bush (Eng)	Lamiaceae	KZN, MP & NC	Leaves & stem	(Coopoosamy and Naidoo, 2011a; Endo et al., 2015; Ghuman et al., 2016)
15.*Zantedeschia aethiopica* (L.) Spreng.	Calla lily (Eng); arum lily (Eng)	Araceae	WC, LMP, NC & EC, KZN & MP	Stem & bulb/rhizome	(Ghuman et al., 2016)

Afr, Afrikaans; EC, Eastern Cape; Eng, English; FS, Free State; Gau, Gauteng; KZN, Kwa-Zulu Natal; LMP, Limpopo; MP, Mpumalanga; NC, Northern Cape; WC, Western Cape

Table 3.2 shows the antifungal properties of the 15 medicinal plants found. The table includes 13 dermatophyte causing fungal species namely, *Trichophyton mentagophyte, Trichophyton rubrum*, *Candida albicans, Microsporum gypseum*, *Aspergillus flavus*, *Aspergillus glaucus*, *Candida tropicalis, Alternaria alternata, Aspergillus niger*, *Mucor hiemalis*, *Cladosporum cucumerinum*, *Penicillium notatum* and *Schizophyllum commune*. Overall *T*. *rubrum* and *T*. *mentagophytes* were amongst the most common fungi isolated from the skin in most studies (Table 3.2). A review of the studies indicated that some medicinal plant extracts were used to treat more than one fungal skin infection. The present study revealed a gap in research knowledge in that all the studies found only screened the various ways the extracts and their active constituents inhibited or prevented the growth of dermatophyte infections using *in vitro* methods. However, none of the studies evaluated the plant extract efficacies *in vivo* models. This highlights the need for more studies that can assess the effects of these South African plant extracts *in vivo*. Further research is needed to fill this major gap in knowledge.

The crude extracts from the endemic and indigenous plants in most of the studies cited, employed different solvents such as hexane, methanol, ethyl acetate, water, chloroform, and dichloromethane. The plant parts used were also distinct such as leaves stem, roots, and bulbs/rhizomes while some of the extracts were hydrodistilled to obtain essential oils (EO). Essential oil volatiles are secondary metabolites present in various plant parts such as buds, flowers, barks amongst other parts (Suroowan et al., 2019). Medicinal plants selected in the present study could be administered in various ways. One study reported that the extracts could be applied topically (directly rubbed onto infected areas of the skin) when hydrodistilled to obtain EO (Fajinmi et al., 2019). The extracts from *Elytroppapus rhinocerotis* could be burnt and inhaled or

the plant leaves could be directly applied to the skin (Hulley et al., 2019). Extracts from *Haworthia limifolia* reportedly could be taken orally through chewing or brewed as a tea (Coopoosamy and Naidoo, 2011b; Naidoo and Coopoosamy, 2011). The preparation methods mentioned in the studies included aqueous infusion (submerging in water for a specified period) or decoction (boiling in water or any other solvent) (Coopoosamy and Naidoo, 2011b). Most studies used organic solvents as a means of preparation.

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Table 3.2: Antifungal properties of the 15 medicinal plants from South Africa found in the review.

Type of extracts	Active constituents	Fungal species	Test results	Main effectivity	Observations	References
Essential oils	Limonene,menthone, isomenthone, pinene, myrecene,cineole, terpinen-4-ol, germacrene, spathulenol, viridiflorol, silphiperfol-6-en-5-one, caryophyllene & trans-β-ocimene	*T. rubrum, T. mentagrophytes & M. gypseum*	MIC values: 0.195, 0.391, 0.049 mg/mL	Fungistatic, reduction of fungal growth. Fungal growth index 2.3% Fungicidal effects	Alteration & destruction of fungal hyphae & spores. Extracts more effective on *Trichophyton, mentagrophytes*. Limonene was the most abundant volatile.	(Fajinmi, 2016; Fajinmi et al., 2017; Fajinmi et al., 2019; Hulley et al., 2019)

Ethanol & aqueous	N/A	*A. flavus* & *A. glaucus*	Zone of inhibition: very high inhibition	Fungistatic	Ethanol leaf extracts had higher growth inhibition than aqueous extracts	(Coopoosamy and Naidoo, 2011a)
Ethanol & aqueous	N/A	*C. albicans* & *C. tropicalis*	Zone of inhibition: very high inhibition	Fungistatic	The ethanolic stems & leaves were more effective in growth inhibition than aqueous extracts	(Esterhuizen et al., 2006; Fajinmi, 2016; Naidoo and Coopoosamy, 2011; Coopoosamy and Naidoo, 2011a

Ethanol & aqueous	*N/A*	*T. rubrum*	Zone of inhibition: medium inhibition	Fungistatic	Ethanol extracts were more effective than aqueous extracts	Endo et al., 2015; Fajinmi, 2016; Fajinmi et al., 2017; Fajinmi et al., 2019)
Hydroalcoholic	N/A	*T. rubrum, T. mentagrophytes & M. gypseum*	MIC values: 0.0625-0.25 mg/mL, 0.0019 mg/mL, 0.0156 mg/mL	Fungistatic,	Strong inhibition and irregular growth pattern inhibiting fungal growth and causing alterations in hyphae.	(Endo et al., 2015; Hulley et al., 2019)

Acetone & ethanol	Coumarin aglycones	*C. albicans* & *C.cucumerinum*	MIC values:0.039-0.299 mg/mL Bioautographic screen & zone of inhibition: high inhibition	Fungistatic	Inhibition of spore germination & mycelial growth	(Esterhuizen et al., 2006)
Acetone	N/A	*A. alternata, M. hiemalis* & *S. commune*	Zone of inhibition: 100% inhibition	Fungicidal	Extracts have effective antimycotic activity	(Afolayan et al., 2002)

Acetone	N/A	*A. niger* and *P. notatum*	Zone of inhibition: above 50% inhibition	Fungistatic	Extracts have a relative effective antimycotic activity	(Afolayan et al., 2002)
Hexane, methanol,chloroform, DCM, acetone	Flavonoids, tannins, proanthocyanidin	*T. rubrum, T. mentagrophytes M. gypseum, C. albicans & C. tropicalis.*	MIC values: 0.63 & 0.31 mg/mL	Fungistatic 90-100% survival rate after 24 hours	Chloro & DCM extracts were most effective. Extracts generally lack a cytotoxic effect	(Ghuman et al., 2016)

A. alternata, Alternaria alternate; *A.flavu, Aspergillus flavus*; *A. glaucus, Aspergillus glaucus*; *A. niger*, *Aspergillus niger*; *C. albicans, Candida albicans*; *C. tropicalis, Candida tropicalis*; *C. cucumerinum, Cladosporium cucumerinum*; Chloro, chloroform; DCM, dichloromethane; MIC, Miminum Inhibitory Concentrations; *M. gypseum*, *Microsporum gypseum*; *M. hiemalis, Mucor hiemalis*; N/A, Not available; *P.notatum*, *Penicillium notatum*; *S. commune, Schizophyllum commune*; *T. mentagrophytes, Trichophyton, mentagrophytes*; *T. rubrum, Trichophyton rubrum*

Compared to the current antifungal treatments available, the South African medicinal plants showed good antifungal activity. The antifungal activity of a compound is currently characterised *in vitro* by the determination of the minimum inhibitory concentration (MIC) or minimum fungicidal concentration (MFC). The phytochemical's *in vitro* activity is considered significant in MIC values lower or between 0.1 mg/mL - 0.625 mg/mL (Zida et al., 2017). Most of the extracts showed notable MIC results with the most effective isolated from the Asteraceae family (*E. rhinocerotis*). The extracts from *E. rhinocerotis* were reported to have the lowest MIC values (0.0098 and 0.0350 mg/mL) ever recorded for a natural product against *T. mentagrophytes* (Hulley et al., 2019). While extracts from *Coleomena pulchellum* were found to exhibit great antifungal activity with low MIC values of 0.195, 0.139 and 0.049 mg/mL against *T. rubrum, T. mentagophyte* and *M. gypseum* respectively (Fajinmi et al., 2017). Extracts isolated from species belonging to the Xanthorrhoeaceae family, *Aloe ferox* and *Aloe arborescens* also had low MIC values 0.31 mg/mL and 0.63 mg/mL. Both plant extracts were found to be effective in treating ringworms (Ghuman et al., 2016). Extracts from *Tetradenia riparia* also had antifungal activity against dermatophytes with MIC values of 0.25 and 0.625 mg/mL (Endo et al., 2015). Lastly, the extracts from *Hypericum aethiopicum* exhibited good antifungal activity with the MIC value of 0.63 mg/mL (Ghuman et al., 2016). The low MIC values provide important information to promote the research and production of alternative ingredients for antifungal products.

A few of the extracts from *A. ferox, A. arborescens* and *H. aethiopicum* showed promising safety levels when investigated for toxicity. This display of non-toxicity suggests that the extracts potentially have fungal selectivity which is very important as most synthetic antifungals are non-selective between host cells and fungal cells (Hokken et al., 2019). A few of the plant extracts from *A. ferox*, *Agathosma betulina*

and *Coleonema album* displayed fungicidal activities by inhibiting 100% of fungal growth. However, most of the extracts only investigated fungistatic activities by inhibiting or preventing further growth of the fungal strains and further research would be beneficial to demonstrate any fungicidal activities as well.

Most fungal species are intrinsically resistant to antifungals and can resist standard synthetic antifungal treatment through the formation of biofilms. The main mechanisms of action of biofilms are to protect the fungi against drug-induced oxidative stress, drug efflux and shielding by the biofilm extracellular matrix (van Acker et al., 2014; Liu et al., 2019). Some fungal pathogens form biofilms comprised of yeast and hyphal cells embedded in the matrix. *Candida albicans* biofilms are comprised of primarily yeast-form and hyphal cells, both of which are required for biofilm formation (Fanning and Mitchell, 2012). According to Desai et al. (2014) *C. albicans,* hyphae play a critical role in biofilm formation though adherence and metabolite enhancement. An intact hyphal morphogenesis pathway is critical for the accumulation of full biofilm biomes. Extracts from *T. riparia* as well as *A. betulina* and *C. album* EO have been reported to target hyphae by causing morphological alterations to hyphae and inhibiting hyphal formation, thus preventing biofilm formation (Moolla and Viljoen, 2008; Endo et al., 2015; Fajinmi et al., 2019). These extracts could be useful in preventing the formation of biofilms as another study has also reported on the strong ability of A. *betulina* and *C. album* EO volatiles to inhibit biofilm formation and decreasing biofilm activity, thereby favouring their use as antifungal agents (Fajinmi et al., 2019).

A wide range of antifungal drugs are sequestered by beta-glucans present in the cell wall of biofilm cells and surrounding extracellular matrix. The extracellular matrix accumulates as the biofilms mature, and the number of drugs that reach the sessile

cells will be too low to exert an antimicrobial effect (Desai et al., 2014; van Acker et al., 2014). Medicinal plant extracts from *T. riparia* are reportedly effective against biofilm formation because of their affinity for extracellular matrix beta-glucans (Zida et al., 2017). The extracts could cause degradation of matrix beta-glucans which impaired biofilm-associated resistance mechanisms (Desai et al., 2014). *T. riparia* extracts could potentially hold a key in eliminating the matrix's component protecting biofilms, which is useful in the treatment of biofilm-related dermatophyte infections. Natural compounds extracted from *T. riparia*, *A. betulina*, and *C. album* species may potentially be useful for the treatment of biofilm-related dermatophyte infections.

The present study highlights the properties possessed by herbal extracts from plants such as *C. album* that include the disruption of the fungal cell membrane and the ability to cause pore formation in the fungal plasma membrane. This is important as most fungal pathogens have several resistant mechanisms such as efflux pumps that transport antifungals out of the cell (Hokken et al., 2019). The results of this review also highlighted the importance of *A. betulina*, *C. album* and *C. pulchellum* EO volatiles and their constituents that had significant antifungal activities against skin diseases of fungal origin. These activities included inhibition of fungal growth and changes in the morphology of fungal strains by disrupting hyphal formation. According to Fajinmi et al. (2017) EO are known to have several properties which are of great importance to skincare health. These properties include skin barrier function and stimulation of cell regenerations. Other reports have shown that EO volatiles have significant antifungal activities, that include the disruption of the fungal cell walls and phytoplasma membranes through a permeabilization process that leads to the disintegration of mitochondrial membranes. EO volatiles also inhibit synthesis of DNA, RNA, and

polysaccharides in fungal pathogenic cells (Zida et al., 2017; Fajinmi et al., 2019; Tariq et al., 2019).

Essential oil volatiles may be beneficial in skin treatments as their chemical structures are hydrophobic and resemble the intracellular hydrophobic molecules of the skin epidermis (Feingold, 2012; Fajinmi et al., 2019). Important for water and electrolyte movement and known as the natural moisturising factors required for skin hydration (Tabassum and Hamdani, 2014; Hu et al., 2015). Currently, EO are being introduced to produce creams, lotions, soaps, body washes and ointments which are direct topical applications to skin (Feingold, 2012; Fajinmi et al., 2019). This supports the use of EO as antifungals, although the proper mode of action still needs to be studied.

3.4 Final Considerations

The current study was undertaken to investigate available studies on South African medicinal plants used to treat fungal skin infections. This study was done as a means of finding alternative treatment options to combat problematic fungal skin infections. Antifungals used clinically in the therapy of skin diseases vary in their modes of action which limits their efficacy. This review shows that South African medicinal plants displayed a broad-spectrum of antifungal mechanisms, including cell membrane disruption, inhibition of cell wall formation, dysfunction of fungal mitochondria and inhibition of efflux pumps. It is evident from the literature that there is currently a renewed interest in South African plant-based medicines as antifungal herbal therapies. South Africa has a strong traditional medicinal knowledge system supporting the use of ethnomedicine against skin ailments. However, more scientific studies and validation of the bioactivity of selected medicinal plant species will justify their use and incorporation as natural remedies. This review shows that South Africa harbours medicinal plants with good antifungal activity for use in effectively treating fungal skin infections. They show promise to replace synthetic antifungals associated with increased resistance and toxicity. However, the study also highlighted the need for *in vivo* toxicity studies to be conducted for these medicinal plants to establish safe dose ranges. Addressing these limitations in future studies will contribute to the development, production, and commercialisation of these plants in herbal-based antifungal medicines, cosmetic and skincare products. Harvesting and sale of medicinal plant species supported by scientific proof of efficacy and safety, will also contribute to increased health and income for indigenous communities, especially women who are most likely to invest in the welfare and education of their children.

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CHAPTER 4

Medicinal plants used to treat fungal skin infections by the bush doctors in the Western Cape

4.1 Introduction

The Western Cape (WC) province of South Africa (SA) is the homeland of the diverse Cape Floristic Region (CFR), where over 30 000 plant species are found. Fynbos is the dominant flora in the CFR and accounts for about 10% of the world's vascular plants (Philander et al., 2014). An estimated 129 locally occurring plant species of the various plants in this region are wild harvested for traditional applications. Additionally, approximately 533 medicinal plant species are used in local traditional remedies (Petersen et al., 2015a; Philander et al., 2014). Some of the plant species found in the CFR are used to treat over 30 ailments, including gastrointestinal symptoms, urogenital complaints, cardiovascular diseases, and skin infections (Philander, 2011).

In SA, there are several studies reporting on the medicinal practices of various traditional health practitioners, such as diviners, herbalists, and faith healers. However, in this study, the focus is on bush doctors in the Cape Metropole (Dahlberg & Trygger, 2009; Davids et al., 2014; Gebashe et al., 2019; Zuma et al., 2016). The Rastafari herbalists commonly called bush doctors (*bossiedokters*) are found within the WC; the name originates from the traditional Khoi-San herbalists, who are recognised as the oldest healers in the WC (Philander, 2011; Philander, 2012; Philander et al., 2014). They promote and trade medicinal plants in most towns, city centres and rural areas in the WC (Philander, 2011; Philander et al., 2014).

The people of Khoi-San descent are very knowledgeable, having a long history of medicinal plant usage and making use of plant species such as bitter aloe (*Aloe ferox*), rooibos tea (*Aspalathus linearis*) and honeybush tea (*Cyclopia intermedia*) (De Beer & van Wyk, 2011; De Vynck et al., 2016; Philander, 2011). Most of these plant species are reportedly rich in anti-inflammatory components, such as resveratrol and flavonoids, which have been used for treating various skin wounds, particularly for the reduction and reversal of skin damage (Dlova & Ollengo, 2018). In a study conducted among bush doctors, Philander, (2011) found that they used various plant compounds to treat skin ailments, such as burns, rashes, bruises, bites, acne, eczema, irritated skin, pimples, deep cuts, and dermatophyte infections.

Skin infections typically occur through wounds, which are physical injuries resulting from the opening or breaking of the skin (Kumar et al., 2007). Disruptions to the skin barrier, particularly of inoculating pathogens into the dermis, could cause or exacerbate skin infections such as superficial fungal skin (dermatophyte) infections (Belkaid & Tamoutounour, 2016). In 2020, dermatophyte infections were reported to affect 27% of the world's population, and approximately 19.7% of people with severe dermatophyte infections subsequently lead to death, especially in developing countries (Gnat et al., 2020; Mehrmal et al., 2020). Generally, this is because of an increase in the incidence of immunosuppressive diseases such as HIV/AIDS (human immunodeficiency virus/acquired immunodeficiency syndrome), tuberculosis and diabetes mellitus (Bongomin et al., 2017; Rippke et al., 2018). SA has the highest global incidence of HIV and tuberculosis, consequently, dermatophyte infections are widespread (Schwartz et al., 2019).

The available synthetic antifungals to treat dermatophyte infections have undesirable side effects and are ineffective against new or re-emerging fungal strains. These infections may increase in severity when a wound is left untreated and fungal pathogens enter the epidermis (Da et al., 2019). Because of limitations in resource-constrained countries, synthetic treatments or appropriate pharmacological drugs, such as corticosteroids (prednisone), antibiotics (erythromycin and tetracycline) and antifungal agents (fluconazole and itraconazole) are unavailable (Erhabor et al., 2019). Antifungal resistance is a public health threat experienced by humans and animals, resulting from unsupervised and incorrect usage of over-the-counter antifungals for dermatophyte infections, as they are usually amenable to self-treatment (Da et al., 2019; Erhabor et al., 2019).

Most fungal species are intrinsically resistant to antifungals and can resist standard antifungal treatment by forming biofilms that contain hyphae (Liu et al., 2019). Resistance to antifungals is also caused by the prolonged use of antifungal drugs, leading to an emergence of multidrug-resistant strains, and posing additional clinical problems (Dhamgaye et al., 2014). Also, synthetic antifungals have adverse side effects, including allergies, renal damage, liver abnormalities, or gastrointestinal problems (Gowhar et al., 2015). There is a great need to find antifungal treatments with low toxicity, a broad spectrum of activity, and new modes of action (Liu et al., 2019).

It is of great importance to find adequate treatments that will accelerate the resolution of dermatophyte infections. According to Raina, et al. (2008), the conventional synthetic medical treatment of skin infections includes administering drugs either topically or orally. Plant species such as *Aloe vera*, *Chromoleana odorzota* and *Tridax*

procumbens reportedly possess pro-healing effects by encouraging crucial healing steps. These steps include blood clotting, platelet aggregation, and the acceleration of the healing process by causing an inflammatory response at the site of injury (Kumar et al., 2007; Molazem et al., 2015; Raina, et al., 2008). By promoting these processes, medicinal plants may contribute to treating dermatophyte infections, whereas some extracts can also provide adequate tissue perfusion and nutrition to restore the affected area (Ayyanar & Ignacimuthu, 2009; Davis & Perez, 2009; Kumar et al., 2007; Pereira & Bartolo, 2016; Raina, et al., 2008; Tsala et al., 2013).

The bush doctor's traditional healing practices are fundamentally different from western medicine, as almost all traditional herbalists are trained through word-of-mouth (oral-traditional system) apprenticeship under an elder herbalist (Grierson & Afolayan, 1999; Petersen et al., 2015a). Therefore, it is important to prevent the erosion of this traditional knowledge in SA by preserving a part of the cultural heritage and conserving the information about useful plants. Irrespective of the scientific and anecdotal reports of herbal medicine in the WC, the knowledge and experience of the bush doctors in treating dermatophyte infections with medicinal plants have not been well-documented (Philander, 2011).

This study aimed to document the medicinal plant knowledge and practices of bush doctors in the Cape Metropole communities for treating dermatophyte infections and validate the usage of 15 identified medicinal plants. As a result, an interview-based survey was conducted to 1) explore the knowledge and practices of bush doctors concerning the use of traditional medicine in treating dermatophyte infections and 2) to validate whether the results found in the previous review (Ndlovu et al., 2021) are

consistent with the plants currently used by local practitioners in the Cape Metropole communities for treating dermatophyte infections.

4.2 Methods

4.2.1 Study area

The WC province is located between 34° 0' S and 20°0' E, covering a total land area of 129 449 km^2 (Wilkinson, 2000). The interviews were conducted in the Cape Metropole area, a Category A municipality with an area of 2 446 km^2 found within the southern peninsula of the WC with a coastline of 294 km, stretching from Gordon's Bay to Atlantis (Goodness, 2018). SA has about 3 380 indigenous plant species, of which 190 species are found solely within the Cape Metropole area (Goodness, 2018; Raimondo et al., 2009).

4.2.2 Research tool

The interview-based questionnaire protocol (*Appendix 2*) was adapted based on a similar study designed by Hughes et al. (2015). The study instrument was divided into four sections, 1) demographic information, 2) the overall knowledge of the bush doctors regarding skin infections, 3) the practices of the bush doctors along with the medicinal plants they used when treating fungal skin infections, and 4) validation of 15 medicinal plants obtained from a comprehensive review. The review study was based on a literature search of *in vitro* studies on South African medicinal plants used to treat dermatophyte infections (Ndlovu et al., 2021).

4.2.3 Population and sampling

Participants self-identifying as practising bush doctors were recruited using a snowball sampling method, where well-known healers around the Cape Metropole were approached initially, who then referred new participants (Heckathorn, 2011). The new participants were subsequently asked if they could also refer other participants. The

inclusion criteria for participant selection required a knowledgeable bush doctor to reside in the Cape Metropole area for at least six months and have knowledge of WC medicinal plants used in treating skin infections. Thirty-two referrals were approached to participate in the study, of which 20 referrals were interviewed as they met the inclusion criteria and were willing and available to participate. Bush doctors who had not been leaving in the Cape Metropole for at least 6 months, had no knowledge of Cape medicinal plants and participants younger than 18 years old were excluded.

4.2.4 Data collection

The study was approved by the Biomedical Research Ethics Committee (BMREC) at the University of the Western Cape (Reference number: BM19/7/15) (*Appendix 3).* Data collection occurred between November 2019 and September 2021. Appointments to conduct the interviews were first made with participants through email or text messaging. The participants had the option to choose between face-to-face meetings at a location of their preference or through online video conferencing platforms such as Zoom or Google Meet.

A pilot study was conducted for instrument validation and appropriateness by interviewing two bush doctors who were excluded from the final cohort of participants. The researcher asked the bush doctors questions using the questionnaire in their preferred language (English or Afrikaans) and the interviews were audio-recorded for subsequent transcription verbatim.

Prior to the interview, the research interviewer provided each participant with an information leaflet detailing the reasons for this study and why they were chosen to participate, the study methods, potential risks and benefits of the study as well as their rights as participants. Participants were also be informed verbally of their rights as

participants and the freedom to withdraw at any point in time without prejudice. The research interviewer then advised the participants that should they wish to participate they are required to complete an informed consent document. Participants of the study were assured of their confidentiality and anonymity. To ensure privacy of participants, no individually identifiable information about the participant was shared with others. Participants were assigned an identifying number and this number was used for all data capturing purposes. Furthermore, files were transferred from the collection device (tape recorder) and saved on a secure drive that was only accessible to the researchers working on the project. The audio transcript files did not contain the names of the participants, instead dates were used as descriptors. A separate “key” was created as a Word document that matches the file with the participant on a secure separate drive. When saving survey data, the first names and last names are removed. There were no costs involved or remuneration for participation in this study.

4.2.5 Data analysis

Information was captured in Microsoft Excel and analysed using IBM SPSS ver.29.0 software (SPSS, Chicago, IL, USA). The descriptive statistical data were processed using univariate methods. Mean, median and variance were calculated to assess the distribution of each quantitative variable. These data were summarised by frequency distribution tables and summary statistics showing percentages for different categories of the interview-based questionnaire responses. Pearson’s correlation coefficient tests to assess the relationships between categorical variables were used where applicable. The obtained ethnobotanical data was analysed using frequency of citation (FC) according to Taïbi et al. (2021) where the sum of participants that cite a use for the medicinal plant was calculated.

4.3 Results

4.3.1 Demographic characteristics

Twenty participants were included in the analysis, and their characteristics are presented in Table 4.1. Most were male (90%) and had secondary or tertiary education (35%), whereas 85% self-identified as bush doctors while 3% identified as either independent health practitioners or traditional herbalists. Chronologically, most participants were between ages 31 and 40 years (30%), resided in the Cape Metropole region since birth (85%), and 45% had been practising between 21 and 30 years. Interestingly, the data showed a good to moderate correlation (R^2=0.64) between age and length of practising, and R^2=0.65 between the length of residence and practising (data not shown). A high correlation (R^2=0.891) between the age and length of residence was also observed (data not shown).

Table 4.1: Demographic characteristics of participants

Characteristics	Frequency (Percentages)
Gender	
Male	18 (90%)
Female	2 (10%)
Education	
Primary	2 (10%)
Secondary	8 (40%)
Tertiary	8 (40%)
Unknown/Missing	2 (10%)
Total	20 (100%)
Type of practitioner	
Bush Doctor	17 (85%)
Other	3 (15%)
Total	20 (100%)
Age Group (Years)	
21-30	5 (25%)
31-40	6 (30%)
41-50	5 (25%)
51-60	3 (15%)
> 61	1 (5%)
Total	20 (100%)
Length Of Residence (Years)	
Since Birth	17 (85%)
5-10	1 (5%)
10-20	1 (5%)
>20	1 (5%)
Unknown/Missing	-
Total	20 (100%)
Length of Practising (Years)	
1-5	4 (20%)
6-10	5 (25%)
11-20	3 (15%)
21-30	8 (45%)
Unknown/Missing	-
Total	20 100%)

4.3.2 Knowledge and practices of the bush doctors

The bush doctors who treated their patients for different dermatophyte infections using medicinal plants are illustrated in Figure 4.1. Reportedly, 78% of them treated patients for *tinea capitis* (scalp ringworm and hair shafts), and 67% for both *tinea corporis* (ringworm affecting arms, trunk and legs) and *tinea pedis* (athlete's foot). Less than 30% of the participants treated patients for *tinea barbae* (facial hair follicles or bearded individuals) and 22% for *candidiasis* (diaper rash).

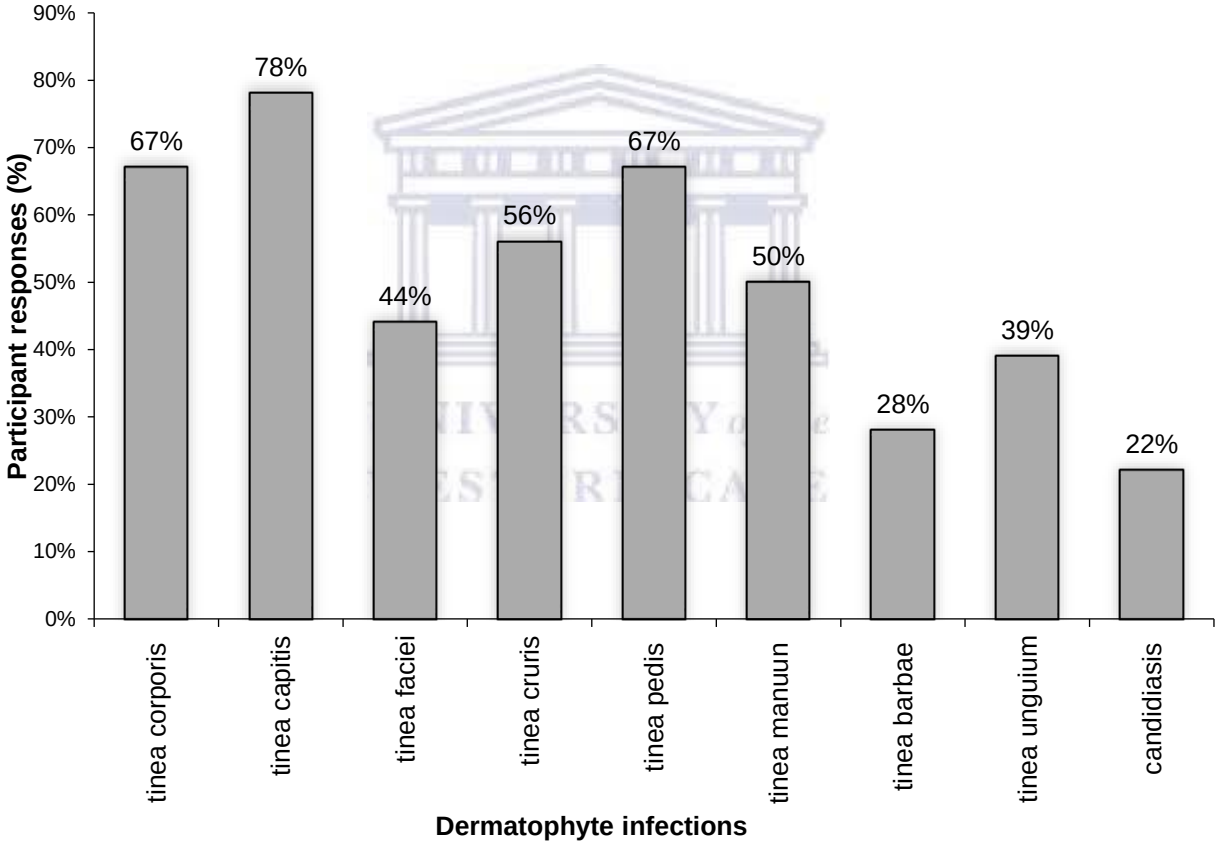

Figure 4.1. Dermatophyte infections commonly treated by the bush doctors.

The various administration methods used by the bush doctors are depicted in Figure 4.2. The most frequently used modes of administration were oral intake (41%), which

included drinking teas or mixing the extracts with food, and topical application (31%), where the plant species were either applied directly to the skin infection or mixed with lotions and creams. Only 25% used bathing as an application method, which included washing the body with various plant infusions or steaming the infected area. Very few bush doctors (3%) mentioned using inhalations**.**

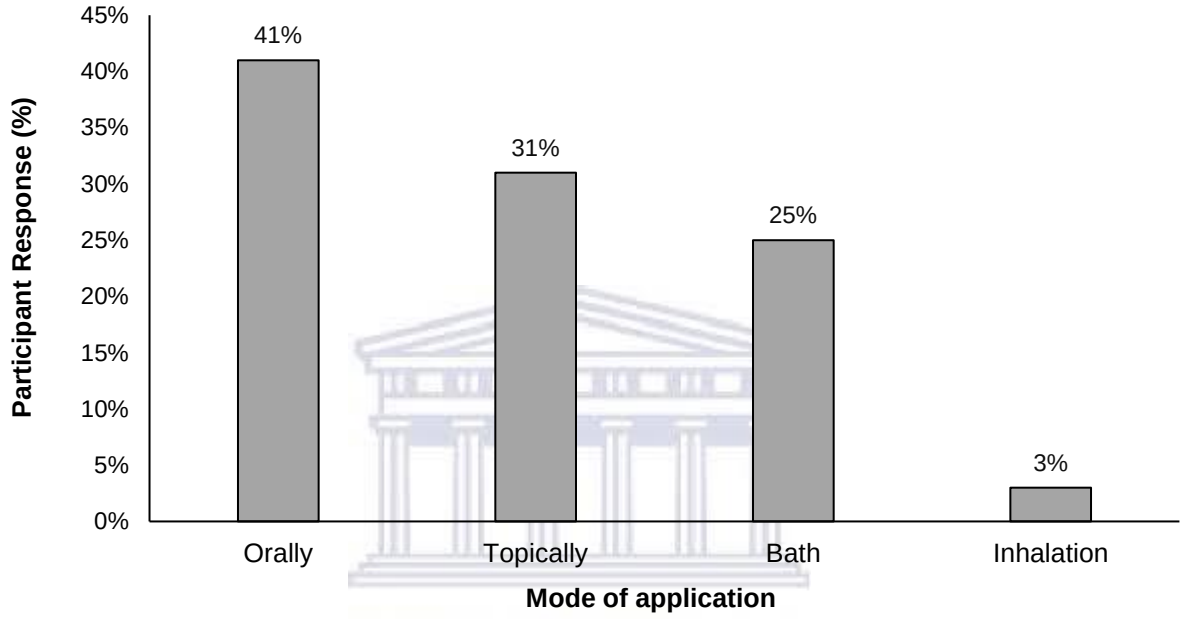

Figure 4.2. Mode of application of medicinal plants in treating dermatophyte infections.

4.3.3 Validation of plants

The results of the review study previously conducted Ndlovu et al. (2021) on the *in vitro* studies identified 15 South African medicinal plants possessing antifungal activity against dermatophyte infections, with some of the plants being considered for product development. These included *Agathosma betulina*, *Aloe arborescens*, *Aloe aristata*, *A. ferox*, *Bulbine frutescens*, *Bulbine natalensis*, *Coleonema album*, *Coleonema pulchellum*, *Elytropappus rhinocerotis*, *Eucomis autumnalis*, *Haworthia limifolia*, *Merwilla plumbea*, *Tetradenia riparia* and *Zantedeschia aethiopica*.

The participants also validated all 15 plants for their usage in treating dermatophyte infections such as athlete's foot, jock itch, ringworms and diaper rash. Most of them (80%) validated the use of *A*. *aristata* and *A. ferox*, with 75% and 65% validating using *E. autumnalis*, *A. betulina* and *A. arborescens*, respectively. Of the participants, 40% confirmed using *E. rhinocerotis*, with 35% having used *Z. aethiopica* and *B. natalensis*. The use of *H. limifolia* for dermatophyte infections was corroborated by 30% of the participants. *B. frutescens* and *C. pulchellum* were validated by 25% of the participants, with 20% validating the use of *C. album* and *T. riparia*. Only 10% confirmed using *M. plumbea* in treating dermatophyte infections.

4.3.4 Medicinal plants used in treating dermatophyte infections.

In Table 4.2, 20 additional medicinal plants used by bush doctors in the Cape Metropole to treat dermatophyte infections are given. These additional medicinal plant species are listed by their botanical names according to their family, common name, frequency of citation, location in SA, and the type of dermatophyte infection treated. The most identified plant species belonged to the Asteraceae, Amaryllidaceae, Lamiaceae and Melianthaceae families. The results of the FC showed that the most cited medicinal plant species to treat dermatophyte infections were *Melianthus major* (FC = 7), *Boophone disticha* (FC = 6), *Tulbaghia violacea* (FC = 6), *Haemanthus coccineus* (FC = 5), *Salvia africana-caerulea* (FC = 5) and *Lobostemon montanus* (FC = 5).

Table 4.2: Medicinal plants used for treating dermatophyte infections by the bush doctors in the Cape Metropole region.

Family	Scientific name	Local name	Frequency of citation (FC)	Location in South Africa	Type of dermatophyte infection
Melianthaceae	*Melianthus major* L.	Kruidjie-roer-my-nie	7	NC, WC and EC	*Tinea pedis*, *tinea capitis*, *tinea corporis*, *candidiasis* and *tinea cruris*
	Mentha longifolia L.	Ballerja	2	Gauteng, KZN and FS	*Tinea pedis*, *tinea capitis*, *tinea corporis* and *tinea cruris*
Amaryllidaceae	*Boophone disticha* (L.f.) Herb.	Incwadi	6	EC, Free State (FS), Gauteng, KZN, Limpopo, Mpumalanga and WC	*Tinea capitis* and *tinea corporis*

	Tulbaghia violacea Harv.	Wilde knoffel	6	EC, KZN and Limpopo	*Tinea pedis*, *tinea cruris*, *tinea capitis*, *tinea corporis*, *tinea manuum* and *tinea barbae*
	Haemanthus coccineus L.	bobbejaansool	5	EC and WC	*Tinea pedis*, *tinea capitis*, *tinea corporis* and *tinea unguium*
Lamiaceae	*Salvia africana* L. (= *Salvia africana-caerulea* L.)	Blousalie	5	NC and WC	*Tinea pedis*, *tinea capitis*, *tinea corporis*, *tinea cruris*, *tinea unguium* and *candidiasis*
	Leonotis leonurus (L.) R.Br.	Wild dagga	3	EC, FS, Gauteng, KZN, Limpopo, Mpumalanga, Northwest (NW), NC and WC	*Tinea pedis*, *tinea capitis*, *tinea corporis* and *candidiasis*

Boraginaceae	*Lobostemon montanus* H. Buek	Agtdaegeneesbos	5	EC, FS, Gauteng, KZN, Limpopo, Mpumalanga, NW, NC and WC	*Tinea pedis*, *tinea cruris tinea capitis*, *tinea corporis* and c*andidiasis*
Hypoxidaceae	*Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall.	African potato	3	EC, FS, KZN, Mpumalanga, Gauteng and Limpopo	*Tinea pedis*, *tinea capitis*, *tinea corporis*, *tinea barbae* and *tinea unguium*
Asteraceae	*Helichrysum petiolare* Hilliard & B.L. Burtt	Imphepho	3	KwaZulu-Natal (KZN), WC and EC	*Tinea pedis*, *tinea corporis* and *tinea manuum*
	Artemisia afra Jacq. ex Willd.	Wilde-als	1	Gauteng, Limpopo, WC and KZN	*Tinea pedis*, *tinea capitis*, *tinea corporis*, *tinea manuum*, *tinea barbae, candidiasis*, *tinea unguium*, *tinea faciei* and *tinea cruris*

	Pteronia incana (Burm.) DC.	Asbossie	1	EC, NC and WC	*Tinea pedis, tinea cruris*
Balanophoraceae	*Sarcophyte sanguinea* Sparrm. subsp. *sanguinea*	Umavumbuka	1	EC, KZN, Limpopo and Mpumalanga	*Tinea pedis*, *tinea barbae*, *tinea corporis* and *tinea capitis*
Fabaceae	*Liparia splendens* (Burm.f.) Bos & De Wit subsp. *splendens*	Bergdahlia	1	WC	*Tinea capitis* and *tinea corporis*
Geraniaceae	*Pelargonium betulinum* (L.) L'Herit.	Kanferblaar	1	WC	*Tinea corporis* and *tinea manuum*

Meliaceae	*Ekebergia capensis* Sparrm.	Cape ash	1	WC, EC, KZN, Mpumalanga and Limpopo	*Tinea capitis* and *tinea corporis*
Oleaceae	*Olea europaea* subsp. *africana* (Mill.) P.S.Green	Wild olive	1	EC, FS, Gauteng, KZN, Limpopo, Mpumalanga, NW, NC and WC	*Tinea capitis* and *tinea corporis*
Strelitziaceae	*Strelitzia reginae* Banks subsp. *reginae*	Kraanvoëlblom	1	EC and KZN	*Tinea capitis* and *tinea corporis*
Velloziaceae	*Xerophyta retinervis* Baker	Bobbejaanstert	1	Gauteng, KZN, Limpopo, Mpumalanga and NW	*Tinea capitis*, *tinea corporis*, *tinea pedis* and *tinea barbae*

Xanthorrhoeaceae	*Aloe microstigma* Salm-Dyck	Karoo-aalwyn	1	Northern Cape (NC), Western Cape (WC) and Eastern Cape (EC)	*Tinea pedis* and *tinea manuum*

4.4 Discussion

The present study showed that medicinal plants still play a significant role in meeting the healthcare needs of the people in the WC communities. Most of the participants in the study were male, which is consistent with a study by Philander, (2012), who showed that 98% of the participants were also male bush doctors in WC. A notable correlation was seen between the age of the participants and their length of residence suggesting that the older, more experienced bush doctors had more knowledge of the WC medicinal plants. A similar study also determined that older people have greater knowledge of medicinal plants and their uses due to their prolonged contact with them (Tugume et al., 2016).

The most common dermatophyte infections that bush doctors treated were *tinea capitis* and *tinea pedis*. Marais and Osuch, (2017) also reported that *tinea capitis* is very common in SA, especially among children (with >1 000 000 cases per annum), and *tinea pedis* had a prevalence rate of 41%. In addition, Schwartz et al. (2019) found that an estimated 3.2 million South Africans were affected by a fungal disease each year, especially in immunosuppressed individuals.

Most dermatophyte antifungals are topically administered conventional drugs, with limited number of cellular targets and are associated with side effects. Additionally, dermatophyte infections such as *tinea capitis* and *tinea unguim* usually require systemic treatments (Gnat et al., 2020). Dermatophyte infections are also routinely observed in patients with depressed cellular immunity, such as diabetes mellitus, a leading cause of natural death in the WC (Davids et al., 2016). In the present study, it

was observed that bush doctors frequently administered medicinal plants orally as they believe in targeting the possible underlying conditions and not just the dermatophyte infection, which may solely be a symptom. According to a study by Hainer, (2003) oral treatment is the most preferred especially for dermatophyte infections such *as tinea capitis,* and *tinea barbae*. Topical application was another popular mode of administration which includes combining plant species with different types of vehicle formats, such as lotions and creams and applying it directly to the skin infection. A similar study reported that topical application for a dermatophyte infection is usually preferred as opposed to systemic treatment, as the treatment is applied directly to the site of infection enhancing the efficacy of treatment, and decreasing systemic side effects (Akhtar et al., 2015).

The main plants validated by the bush doctors were *A. ferox*, *A. aristata* and, *A. betulina*. These results correlate with our previous findings in which literature showed that extracts from *A. ferox* and *A. betulina* were among the most investigated and had displayed fungicidal activities by inhibiting 100% of dermatophytic growth (Ndlovu et al., 2021). In their studies, Afolayan et al. (2014) and Omoruyi et al. (2012) also found that *A. ferox* could be used to treat eczema, skin irritation, burns, ringworms, and boils among the Eastern Cape (EC) communities. Furthermore, another study showed that *A. aristata* was also used to cure skin rashes, burns and wounds (Coopoosamy & Naidoo, 2012). Thus, there is a correlation between the plant species used in WC bush doctor communities and those being investigated for their antifungal properties.

Although the bush doctors identified most of the 15 medicinal plants as those they would prescribe to their patients, they highlighted that there were various other plants they had used that were not included in the list obtained from our previous study. They

documented 20 additional plant species, mostly belonging to the Asteraceae, Amaryllidaceae, Lamiaceae and Melianthaceae families, of which have antifungal properties as reported in various studies (Afolayan et al., 2014; Krstin et al., 2018; Mabona et al., 2013; Madike et al., 2017; Singh et al., 2020).

Medicinal plants such as *M. major*, *B. disticha*, *T. violacea* and *S. africana-caerulea* were among the most cited additional plants used by the participants against various dermatophyte infections. Studies have corroborated the cosmetic uses of these plants with various reports on their wound healing properties (Asong et al., 2019; Chingwaru et al., 2019; Mwinga et al., 2019). Interestingly, *Aloe microstigma* and *Olea europaea* subsp. *africana* have reportedly been used for dermatophyte infections, such as ringworms and foot fungus (Nortje & van Wyk, 2015; van Vuuren et al., 2019). Other plants such as *M*. *longifolia*, *Leonotis leonurus*, *Artemisia afra* and *Hypoxis hemerocallidea* have also reportedly been used to treat various skin ailments (Asong et al., 2019; Mwinga et al., 2019; Nortje & van Wyk, 2015). However, most of the additional plant species need to be investigated further for their anti-dermatophyte activities.

The Cape bush doctors notably do not only rely on medicinal plants indigenous to the WC region. They also used plants such as *T. violacea*, *H. hemerocallidea*, *Xerophyta retinervis*, *Strelitzia reginae* and *Sarcophyte sanguinea* found in several other provinces. Possibly this is attributed to the high levels of provincial migration, especially between the Eastern and Western Cape provinces. According to Petersen et al. (2015b), the EC traditional health practitioners would harvest, share, and trade their plants in various locations in the WC and with other local practitioners. In this

manner, plant knowledge is shared between the different areas of SA, and the bush doctors would then incorporate these plants into their practices.

4.5 Conclusion

The present study indicated that the Cape Metropole harbours a diversity of plant species, which bush doctors use to treat dermatophyte infections. The medicinal plant species used by the bush doctors are consistent with what is currently being investigated in *in vitro* studies. Plants such as *A. ferox, A. betulina* and *B. frutescens* are reportedly being used to treat dermatophytes by the bush doctors. Additionally, this study set out to validate these medicinal plants; however, it was found that various other plant species possibly had better healing and antifungal properties that are not being investigated to treat dermatophyte infections. Thus, there is still a gap in research on medicinal plant species that require further investigation into their potential benefits. The information provided within the present study provides a foundation upon which further research into the pharmacological benefits of documented plants should be undertaken.

4.5.1 Limitations of the study

The participants in the study were recruited using a snowball sampling method, with the researcher having little control over the sampling method. To find participants, the researcher had to rely on the previous participants who were interviewed. Additionally, the researcher had no guarantee if this was a valid distribution of the population and the sample. Sampling bias was also a consideration, as the initial participants could nominate people they knew well, making it highly possible that these bush doctors shared the same traits and characteristics. Therefore, the sample obtained might have only been a small subgroup of the entire population.

The participant selection was limited to those residing in the Cape Metropole region, while those in other areas and provinces, who had potentially better access to plant

material, were not included. Finally, in this study, the communication and language barriers were other limitations because not all bush doctors had access to computers or capable phones for online video conferencing; however, face-to-face meetings were also feasible. Although this study had some limitations, however, the results found may be used as baseline data for future studies.

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CHAPTER 5

The *in vitro* bioactivity of selected South African plants on human skin cells and their antifungal potential of dermatological relevance.

5.1 Introduction

The incidence of fungal infections is increasing at an alarming rate, presenting an enormous challenge to healthcare professionals. Superficial mycoses such as candidiasis are skin diseases with a great prevalence affecting between 20-25% of people globally (Gnat et al., 2021). Cutaneous candidiasis is the fungal infection of the skin, hair, and nails caused by *Candida* species that are opportunistic infections occurring in immunosuppressive diseases as predisposing factors (Declara et al., 2022). Over 30 *Candida* species reportedly cause infections in humans, with *Candida albicans* being the most represented species as it forms a part of the skin microbiome (Kühbacher et al., 2017). Other non-albicans species such as *Candida tropicalis*, *Candida dubliniensis*, and *Candida glabrata* have also been isolated from the skin of persons with underlying immunosuppressive conditions such as HIV/AIDS and patients undergoing chemotherapy (Espinosa-Hernández et al., 2020).

Fluconazole is one of the most used drugs for the treatment of candidiasis. However, fluconazole only has fungistatic activity against species such as *C. albicans* (Lu et al., 2021). A major problem medical communities face in treating fungal infections is the increase in azole-resistant strains spreading worldwide. High levels of primary resistance to fluconazole have been reported against fungal isolates of *C. albicans*, *C. glabrata* and *C. tropicalis* (Jin et al., 2018; Lu et al., 2021). The ideal antifungal agents

should have a broad spectrum of fungicidal activity and be selective to host cells (Pereira, 2021; Wong et al., 2014).

Medicinal plants have been used for the treatment of skin conditions for many years, with ingredients from plant extracts forming the largest categories of cosmetic skincare formulae (Gediya et al., 2011; Sharma et al., 2003; Wilkinson & Brown, 1999). South Africa (SA) has a rich diversity of plant species with about 117 reportedly used traditionally to treat various skin disorders including acne, boils, ringworm, sores, and wounds (Lall & Kishore, 2014). Van Wyk (2008) reported that at least 90 of these South African medicinal plants are being considered for further development and commercialisation in skincare products. SA plants are used in moisturising cosmetic formulations to treat various skin ailments which include the African oil palm (*Elaeis guineensis* Jacq), Baobab (*Adansonia digitata*), Marula (*Sclerocarya birrea*), Sesame (*Sesamum indicum*), Bitter Aloe (*Aloe ferox*), Rooibos tea (*Aspalathus linearis*) and Honeybush tea (*Cyclopia Intermedia*) (Lall & Kishore, 2014). A previous review (Ndlovu et al., 2021) and an ethnobotanical survey identified that plant species such as *Aloe ferox* (*A. ferox*), *Agathosma betulina* (*A. betulina*) and *Bulbine frutescens* (*B. frutescens*) are popular in Western Cape communities for the treatment of fungal skin infections. These three plant species have been chosen for further investigation in this study.

Aloe ferox, commonly known as “bitter aloe”, is a medicinal plant indigenous to the Cape coastal region of SA, occurring in Swellendam in the west and extending to the southern parts of KwaZulu-Natal in the east (Chen et al., 2012; Glen & Hardy, 2000). The ethanolic extracts of *A. ferox* have been reported to treat eczema, pimples, venereal sores and arthritis (Frum & Viljoen, 2006). *Bulbine frutescens*, also known as

the “burn jelly plant”, is widespread throughout parts of the Northern, Western and Eastern Cape regions. *B. frutescens* extract has been reported to promote cutaneous wound healing as well as treat rheumatism, sciatica, and itching (Frum & Viljoen, 2006; Pather & Kramer, 2012; Pather et al., 2011). *Agathosma betulina*, commonly referred to locally as "buchu", is found in the Cape region and its essential oils are reportedly effective in the treatment of *Trichophyton rubrum* and *Trichophyton mentagrophytes* strains which are a common cause of athlete’s foot and ringworm (Fajinmi et al., 2019; Lis-Balchin et al., 2001).

Although there is anecdotal evidence showing that the above-mentioned plants have beneficial dermatological capabilities, there is still a need to scientifically evaluate the susceptibility of *Candida* species and to determine the minimal inhibitory concentration (MIC) dosages of these extracts. Few studies have investigated the extracts’ effect on cell viability, cellular growth rates, and cytotoxicity when exposed to epidermal cells and barrier function. Moreover, safety concerns exist, such as determining the therapeutic concentrations that can be used on human skin without causing any harm. Thus, the screening of *in vitro* proliferation and cytotoxicity of the extracts needs to be performed on selected human cell lines before promoting their use in cosmetic formulations. These procedures can provide safety data for consumers who choose to use natural products (Ribeiro et al., 2015). The present study aimed to determine the cytotoxic and proliferative effects of *A. ferox, A. betulina and B. frutescens* hydroethanolic extracts on normal human epidermal (HaCaT) cells and to evaluate their antifungal activity against different skin-related *Candida* species, namely *C. tropicalis*, *C. albicans*, *C. dubliniensis* and *C. glabrata*.

5.2 Methodology

5.2.1 Plant extracts

The commercially prepared ethanol-water extract (60%) *A. ferox* (50% w/v), *A. betulina* (10% w/v) and *B. frutescens* (50% w/v) were obtained from Parceval Pharmaceuticals, (South Africa). For the cell culture experiments the hydroethanolic extracts were diluted in complete Dulbecco's Modified Eagle's Medium (DMEM) (Cat no. BE12-709F, Lonza, Cape Town, South Africa) to generate 10 mg/mL stock solutions and stored at 4 °C. For the microdilution broth assays the hydroethanolic extracts were diluted in RPMI-1640 medium (Cat no. RPMI-STA, Capricorn Scientific, Ebsdorfergrund, Germany) to generate 10 mg/mL stock solutions and stored at 4 °C this was used for the experiments.

5.2.2 Cell culture assays

5.2.2.1 Cell line

The HaCaT cell line was purchased from Cell Line Service (Eppelheim, Germany). The cells were seeded and propagated in complete DMEM supplemented with 10% heat-inactivated foetal bovine serum (HI-FBS) (Cat no. 12389962, Hyclone, Little Chalfont, UK), antibiotic (penicillin (100 U/mL) and streptomycin (100 mg/mL) (GIBCO, NY, USA) and maintained in a humidified incubator with 5% CO_2 at 37 °C. Treatments were carried out 24 h after plating and cells were subsequently treated with optimised concentrations of 200, 400, 600 and 800 µg/mL of the plant extract for 24, 48 and 72 h.

5.2.2.2 Determination of cell proliferation and viability

The crystal violet (CV) assay was used to determine the effects of the ethanolic extracts on cell proliferation and viability as previously described (Saotome et al., 1989). Cell proliferation is determined via the triarylmethane dye that binds to ribose molecules such as DNA in nuclei. The HaCaT cells were seeded into 96-well tissue test plates (Greiner Bio-one, Kremsmunster, Austria) at a density of 1×10^4 cells/well and allowed to adhere overnight. The cells were treated with the various concentrations of the three plant extracts for 24, 48 and 72 h. Cells without the extract treatments served as a negative control and 1% Triton-X 100 was used as a positive control. At the end of each period, the cell proliferation levels were assessed via CV assay (stock 0.1%: PBS) with 0.2% Triton-X 100 as the solubilizing agent and detected using a microplate spectrophotometer reader (Glomax Multi Detection System, Promega, Wisconsin, USA) at 570 nm.

5.2.2.3 Determination of cytotoxicity

To evaluate the cellular cytotoxicity the lactate dehydrogenase (LDH) assay was used. This assay determines the release of the LDH enzyme which is released into the cell culture when the plasma membrane is damaged (Kumar et al., 2018). LDH activity was measured using an LDH-cytotoxicity kit (Cat no. 11644793001, Roche Diagnostics, Mannheim, Germany) and was performed as per the manufacturer's instructions. Briefly, the HaCaT cells were seeded in 96-well plates at a density of 1×10^4 cells/well. After 24 h, 200, 400, 600 and 800 µg/mL concentrations of the plant extracts were added, and the cells were incubated for 24, 48 and 72 h. At the end of each time point, the LDH activity was measured, and the absorbance was read at 490 nm using a microplate reader (Glomax Multi Detection System, Promega, Madison, USA).

5.2.2.4 Cellular morphological changes

To evaluate the cellular morphological changes occurring after the HaCaT cells were treated with the extracts, a haematoxylin and eosin (H&E) cell stain was conducted as previously described (Vorster et al., 2012). Cells were seeded on heat-sterilised coverslips in 6-well plates at a density of 2.5×10^5 cells per well and allowed to attach overnight. Cells were subsequently exposed to the different concentrations of the three plant extracts and appropriate controls respectively for 24, 48 and 72 h, after which coverslips were fixed with Bouin's fixative (30 min) and 70% ethanol (20 min). Coverslips were rinsed with water, stained with haematoxylin (20 min), rinsed (water and 70% ethanol) and stained with eosin (7 min). Coverslips were then dehydrated stepwise with ethanol (70%, 96%, 100%) and xylene, mounted on microscope slides with Dibutylphthalate Polystyrene Xylene **(**DPX) mountant (Cat no. 44581, Sigma-Aldrich, St. Louis, USA) and dried overnight. Qualitative evaluation (400 × magnification) was conducted using the 360 4i Nikon (Tokyo, Japan) microscope. The ImageJ software (National Institutes of Health (NIH), Bethesda, MD, USA) was used to analyse and process the images for better resolution.

5.2.3 Microdilution assay

5.2.3.1 Fungal strains and preparation

Candida albicans (ATCC 90028), *C. dubliniensis* (NCPF 3949a), *C. glabrata* (ATCC 26512) and *C. tropicalis* (ATCC 950) were obtained as a gift from the Microbial Endogenous Infections Studies (MEnIS) Research Laboratories, Department of Medical Biosciences, University of the Western Cape, South Africa. *Candida* species were inoculated in Sabouraud dextrose agar (SDA) plates incubated for 24 h at 37 ℃

to allow sufficient time for growth. These species were then sub-cultured and maintained on SDA plates for the duration of the experiments.

5.2.3.2 Broth microdilution method

The MIC values of the extracts against susceptible fungi were determined using the broth microdilution method modified from (Eloff, 1998) and in accordance with the CLSI guidelines (Nascente et al., 2009). Briefly, the test organisms were standardised to match the 0.5 McFarland standard. From this a standardised fungal stock prepared from a 1:100 dilution with sterile RPMI broth to give a final inoculum of approximately 10^6 CFU/mL. The extracts were prepared as previously described in 5.2.1, then a 200 µg/mL starting concentration of each was added to the first wells and serially diluted to yield different test concentrations (0.19 - 200 µg/mL). The diluted fungal culture of 100 µL was added to each well. The antifungal fluconazole (200 µg/mL) (Sigma–Aldrich, Germany) was added as a positive control and a negative control was included to determine if the solvent (ethanol) exhibited any antimicrobial effects. Each microtiter plate was sealed with a sterile adhesive film to prevent evaporative loss of the plant extracts and incubated at 37 °C for 24 h. The solution of p-iodonitrotetrazolium violet (02 mg/mL) (INT) (Cat no. 58030, Sigma–Aldrich, Germany) at 40 µL was added to each well and incubated at 37 °C for 30 min. Following the addition of INT, the development of a pinkish colour (visualized by the naked eye) indicated microbial growth since viable fungi can convert INT to red formazan through reduction reactions. The wells that remained clear/yellowish indicated inhibition of fungal growth. The MIC was recorded as the lowest concentration of the extract that prevented the appearance of visible growth of the microorganism after 24 h of incubation (Penduka et al., 2011). The study was done in triplicate on alternate days.

5.2.4 Data analysis

Statistical data processing was performed using SPSS 29.0 software (SPSS, Chicago, IL, USA). The data were presented as mean and standard error of mean from triplicate measurements. One-way analysis of variance (ANOVA) and Student *t*-test were used to evaluate statistical significance of differences among groups. The median inhibitory concentration IC_{50} values were calculated from linear regression plots using Microsoft excel software (Microsoft Corporation, Redmond, WA, USA). Statistical significance was accepted at the level of $p < 0.05$.

5.3 Results

5.3.1 Effects of the different plant extracts on cell viability and proliferation.

The hydroethanolic extracts from *A. ferox* (Figure 5.1A) showed a significant ($p < 0.05$) increase of 16% in cell proliferation at the lower dosage of 200 µg/mL after 48 h and a 20% decrease in proliferation at higher concentrations (600 and 800 µg/mL) after longer exposure (72 h) to the extract compared to the negative control. The hydroethanolic extracts from *B. frutescens* (Figure 5.1B) displayed a significant ($p < 0.05$) decrease of 40% in cell proliferation at higher concentrations (800 µg/mL) after longer exposure (72 h). However, cells exposed to lower concentrations (200 and 400 µg/mL) displayed a significant increase. The HaCaT cells exposed to B. frutescens at 400 µg/mL concentration had a 17% significant ($p < 0.05$) increase in cell proliferation after 24 h compared to the control (Figure 5.1B). The hydroethanolic extracts from *A. betulina* (Figure 5.1C) exhibited a significant ($p < 0.05$) time- and dose-dependent reduction on HaCaT cells. After 72 h, a significant 24% decrease in cell proliferation was noted after 600 and 800 µg/mL compared to the control. The results showed that the *A. betulina* extracts exhibited no significant change in the cell proliferation at lower dosages (200 - 600 µg/mL) after 24 and 48 h of treatment. A significant ($p < 0.05$) decrease in cell proliferation was observed in cells exposed to the positive control (1% Triton-X 100) at all exposure times.

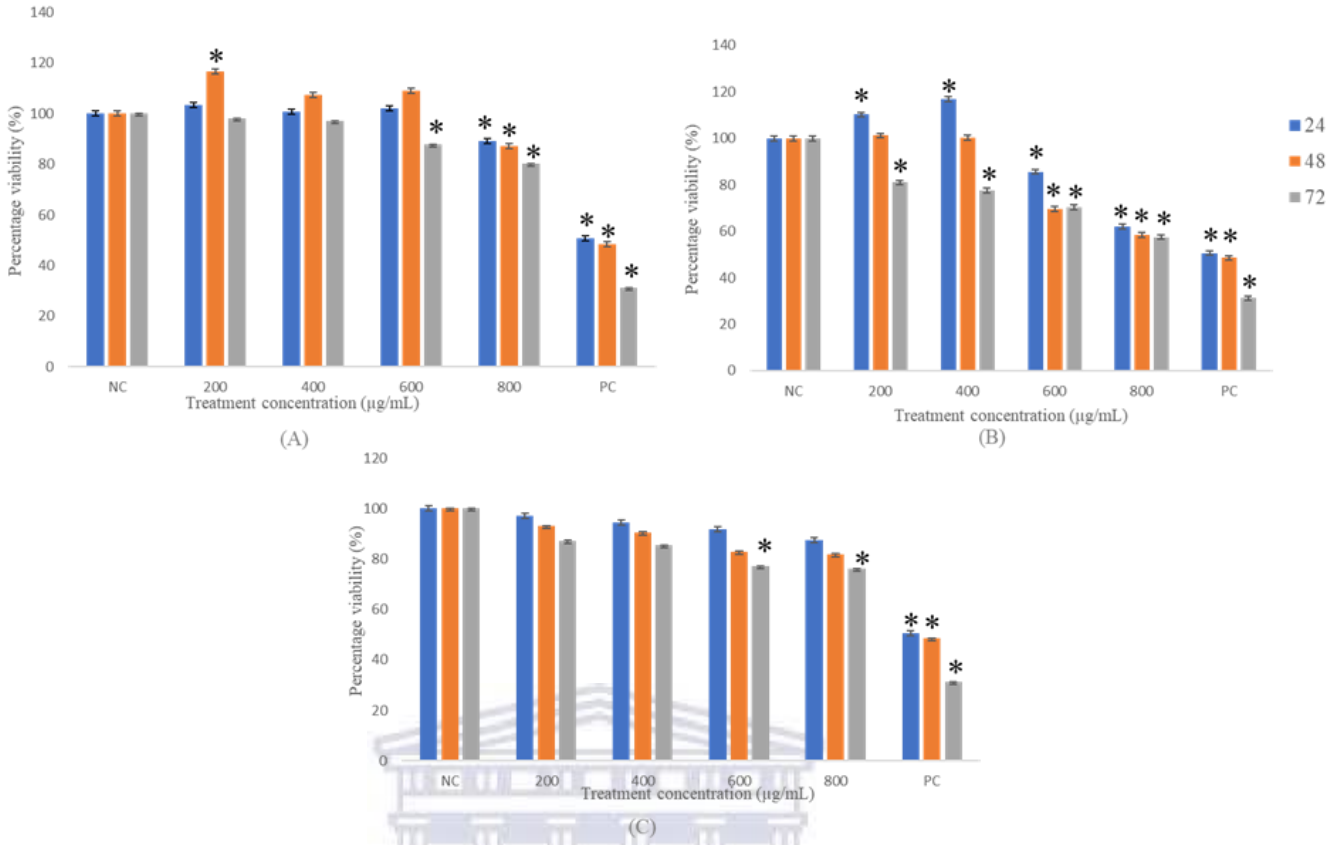

Figure 5.1. Cell viability of normal skin (HaCaT) cells treated with 200; 400; 600 and 800 µg/mL concentrations of *Aloe ferox* (A), *Bulbine frutescens* (B) and *Agathosma betulina* (C) hydroethanolic extracts for 24, 48 and 72 h determined using a crystal violet assay. NC: negative control - untreated cells with media; PC: positive control - cells treated with 1% Triton-X 100. Data represent the mean ± SEM, n = 3, where * indicates statistical significance at level $p < 0.05$ compared to the negative control.

Table 5.1 shows the IC_{50} values for HaCaT cells after exposure to the different plant extracts. It is notable that *A. ferox, A. betulina* and *B. frutescens* were able to reduce the cell viability of HaCAT cells with IC_{50} values of 599.89 µg/mL, 768.21 µg/mL, and 771.06 µg/mL after longer exposure (72 h), respectively. The results indicated that a reduction in cell viability occurred after longer exposure (72 h) of the three extracts to the HaCaT cell line. All three extracts displayed negligible cytotoxic effects on the cells after 24 and 48 h exposure.

Table 5.1: Cell reduction and selectivity of the hydroethanolic extracts from *Aloe ferox*, *Agathosma betulina* and *Bulbine frutescens* at different exposure times.

Reduction in cell viability (IC_{50}value) (µg/mL)			
Plant extracts	**Exposure times (h)**		
	24 h	**48 h**	**72 h**
Aloe ferox	4 035.5	728.42	599.89
Agathosma betulina	4 204.08	2 803.97	768.21
Bulbine frutescens	957.291	845.5	771.06

5.3.2 Potential cytotoxic effects of the selected plant extracts.

The results obtained from the LDH assay on the HaCaT cells are displayed in Figure 2 (A, B and C). These results confirmed that all three selected plant extracts exhibited negligible cytotoxicity towards the HaCaT cells after exposure to the various dosages at all time points compared to the negative control. The only significant ($p < 0.05$) increase in cytotoxicity was observed in the positive control at all exposure times.

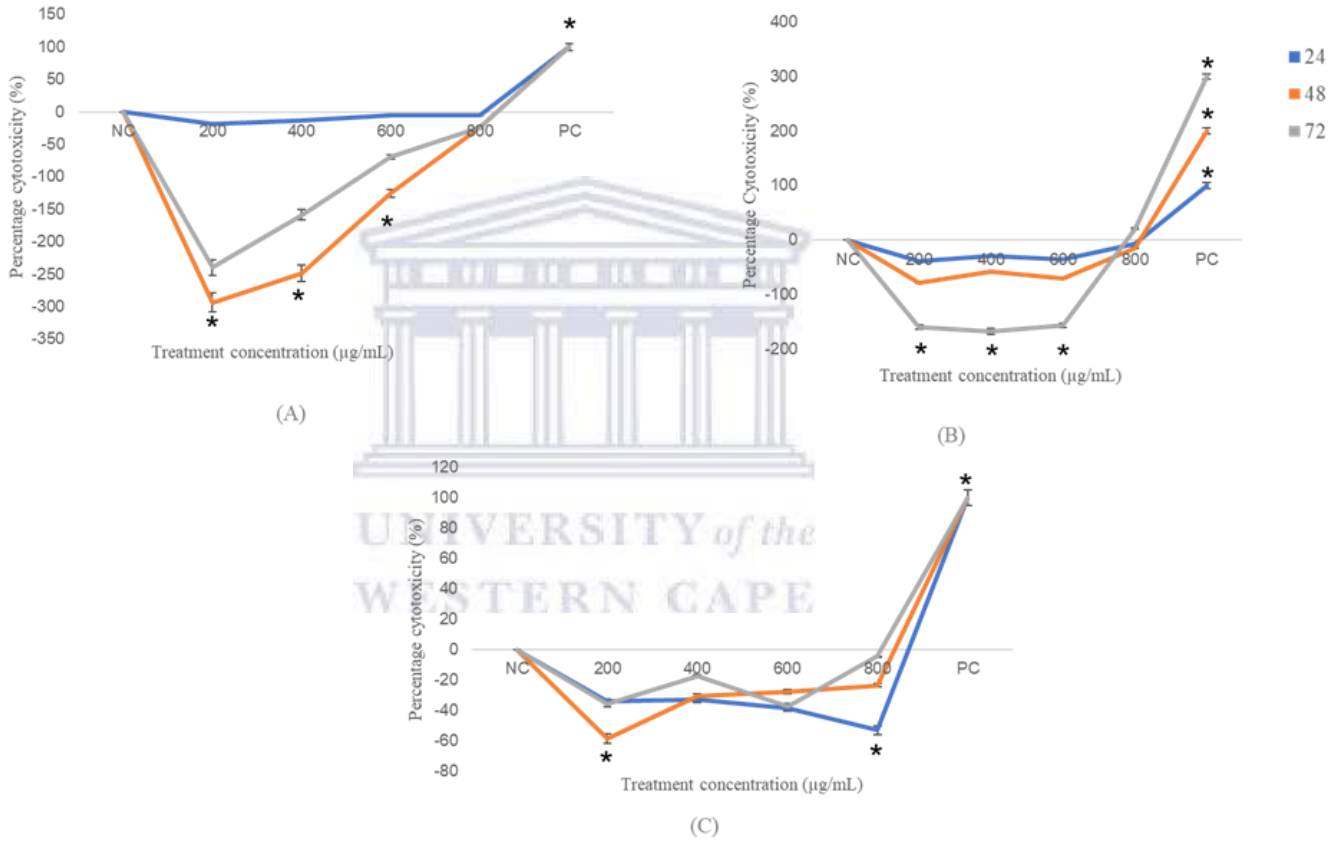

Figure 5.2. Cell cytotoxicity determined using the lactate dehydrogenase (LDH) assay. HaCaT cells were treated with 200; 400; 600 and 800 µg/mL concentrations of *Aloe ferox* (A), *Bulbine frutescens* (B) and *Agathosma betulina* (C) hydroethanolic extracts for 24, 48 and 72 h. NC: negative control - untreated cells with media; PC: positive control - cells treated with 1% Triton-X-100. Data represent the mean ± SEM, n = 3, where * indicates statistical significance at level $p < 0.05$ compared to the negative control.

5.3.3 Effects of the plant extracts on cell morphology

The H&E stain combines two dyes haematoxylin and eosin to stain various tissue elements for easy observation. Haematoxylin is a basic blue dye which stains basophilic structures such as chromatin and ribosomes, while acidic eosin counterstains elements such as cytoplasm, red blood cells, collagen, and muscle fibres (Fischer et al 2008). Qualitative analysis by H&E staining revealed little to no morphological changes in response to the three extracts (*A. ferox*, *A. betulina* and *B. frutescens*) after exposure to the HaCaT cell line at the various concentrations (Figure 5.3). The results of the H&E staining further validated the data obtained from the CV and LDH assays in that the figures displayed mitotically active cells with intact nuclei and cytoplasmic properties across these concentrations and exposure times. Apoptotic characteristics (hypercondensed chromatin, membrane blebbing) were only observed in samples of the positive control after 24, 48 and 72 h.

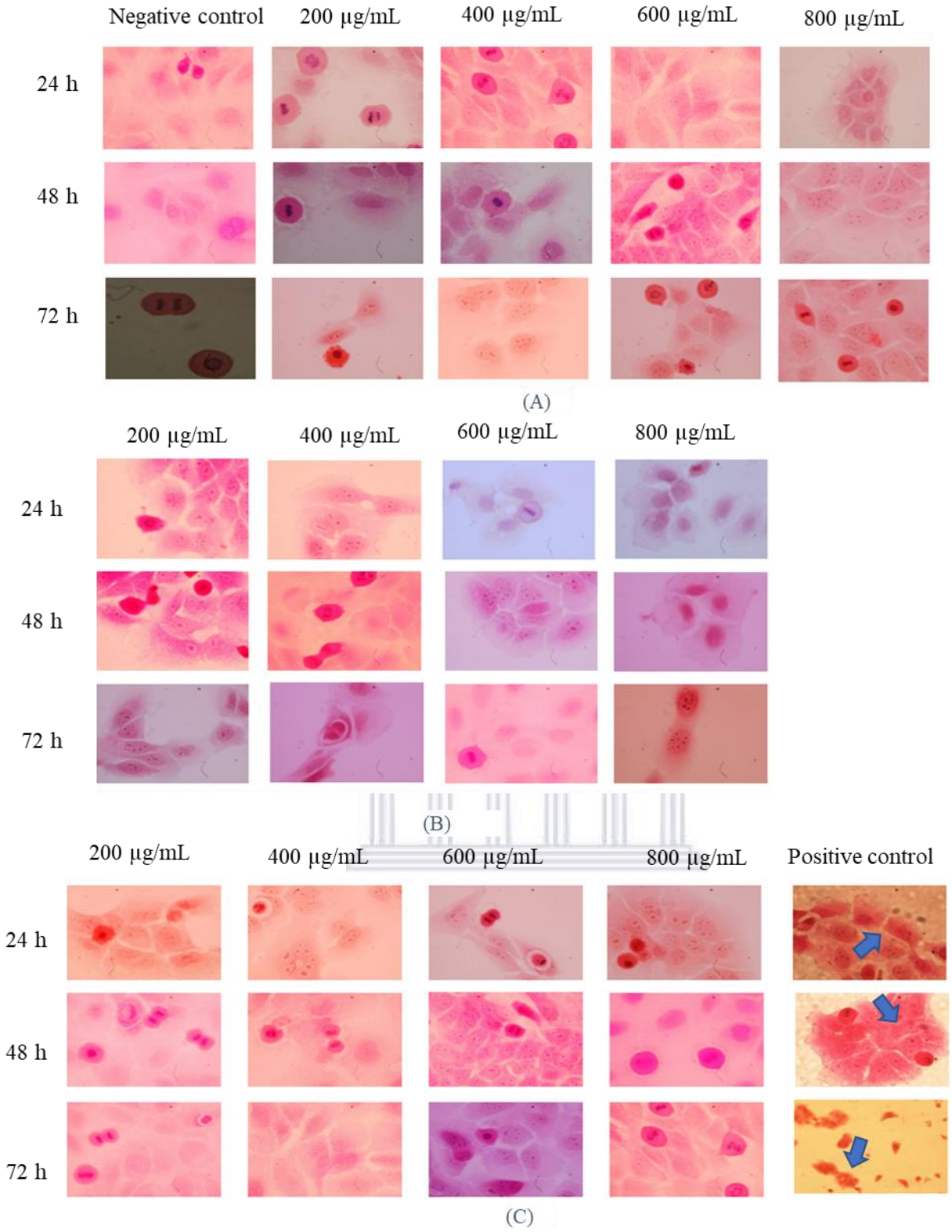

Figure 5.3. Cellular morphology of the HaCaT cells after treatment with 200; 400; 600 and 800 µg/mL concentrations of *Aloe ferox* (A), *Bulbine frutescens* (B) and *Agathosma betulina* (C) hydroethanolic extracts. After treatment, the cells were stained with H&E and the photographs taken at 40x. Arrows show irregularly shaped cells.

5.3.4 Microdilution assays

Figure 5.4 (A and B) displays the microdilutions of the fungal growth after exposure to the different concentrations of the selected plant extracts. Table 5.2 annotates the MIC results showing that the hydroethanolic extracts had a broad spectrum of antifungal activity as they were able to inhibit the growth of the tested fungal strains between the ranges of 12.5 - 200 µg/mL. The extracts of *A. betulina* displayed antifungal activity against *C. tropicalis* and *C. albicans,* with MIC values of 12.5 µg/mL and 50 µg/mL (Figure 5.4A), respectively. *Aloe ferox* extracts exhibited some antifungal activity against all the fungal strains tested, with MIC values of 200 µg/mL (Figure 5.4B). The *B. frutescens* extracts showed antifungal activity against all the fungal strains with most activity found when tested against *C. dubliniensis* with an MIC value of 100 µg/mL (Figure 5.4B).

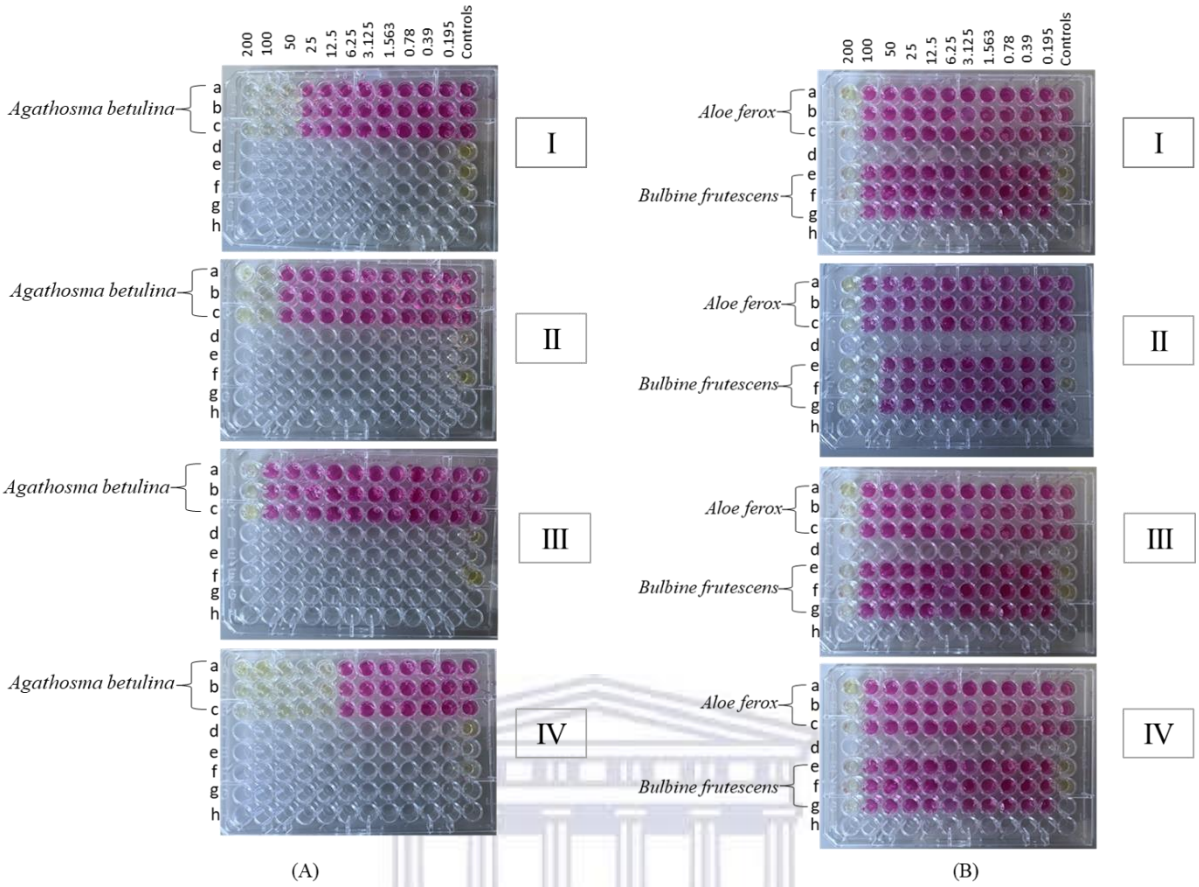

Figure 5.4. Concentrations (µg/mL) versus yeast growth (pink colour). *Agathosma betulina* (A), *Aloe ferox* (B) and *Bulbine frutescens* (B) hydroethanolic extract against *Candida albicans* (I), *C. dubliniensis* (II), *C. glabrata* (III) and *C. tropicalis* (IV). The control wells a-f: 0.5 McFarland standard, *Candida* species in RPMI, ethanol (solvent), fluconazole, saline and RPMI media.

Table 5.2: Determination of the antifungal activity of *Agathosma betulina*, *Aloe ferox* and *Bulbine frutescens* hydroethanolic extracts using a micro-dilution bioassay.

***Candida* species**	**Plant extracts (µg/mL)**		
	Agathosma betulina	*Aloe ferox*	*Bulbine frutescens*
Candida albicans	50	200	200
Candida dubliniensis	100	200	100
Candida glabrata	200	200	200
Candida tropicalis	12.5	200	200

5.4 Discussion

The *A. ferox*, *B. frutescens* and *A. betulina* extracts have been used in traditional medicine to treat various ailments, including skin diseases. Reports on the anecdotal evidence for the traditional use of these extracts found that they have been used as traditional wound healing agents as well as for cosmetic and antibiotic applications (Mabona & van Vuuren, 2013; Watt & Breyer-Bradwyjk 1962; Pather et al 2011). If these medicinal plant extracts are to be considered as antifungal agents, it is important that they are cytotoxic to the fungal strain while being selective to the host cells. The present study reports the concentrations of extracts with a proliferative or non-cytotoxic effect on normal skin cells, while also indicating the concentration of the extracts that was cytotoxic to fungi.

The results for cell proliferation showed time-dose responses suggesting that the lower the dosage and the shorter the exposure time, the more effective the extract. Both *A. ferox and B. frutescens* had an increase in proliferation at the lower concentrations (200 µg/mL and 400 µg/mL) and a decreased proliferation at higher concentrations (600 µg/mL and 800 µg/mL). *Agathosma betulina* only exhibited a decrease in cell viability after longer exposure with negligible changes observed in treatment concentrations after 24 h. These results suggest that the optimum concentration for cell proliferation was between 200 - 400 µg/mL for *B*. *frutescens* and 200 µg/mL for both *A. ferox* and *A. betulina* hydroethanolic extracts.

A compound is considered non-toxic when cell viability higher than 80% is obtained. A weak cytotoxicity is associated with 80 - 60% cell viability, moderate cytotoxicity is linked to 60 - 40% viability and a cell viability lower than 40% indicates cytotoxicity

(Lopez-Garcia et al., 2014). To further validate that these extracts were not cytotoxic to the normal skin cells an LDH cell cytotoxicity assay was performed. The results obtained from the assay confirmed that overall, the three selected ethanolic plant extracts displayed weak to non-cytotoxic properties. These extracts also had insignificant cytotoxic effects with low IC_{50} results found *A. ferox* (IC_{50} 599.89 µg/mL), *B. frutescens* (IC_{50} 768.21 µg/mL) and *A. betulina* (IC_{50} 771.06 µg/mL). Therefore, these extract dosages could potentially be considered safe to use on human skin.

The results coincide with those of previous studies, such as the one conducted by Fox et al,. (2017) which showed that *A. ferox* whole-leaf and gel materials caused insignificant toxicity on HaCaT cells rendering the plant safe to use on skin. Similar reports found that *A. ferox* and *A. betulina* extracts could also selectively inhibit microbial growth on the skin with the extracts having no dermal toxicity or side effects (Mahomoodally, 2013; van Vuuren, 2008). Bringmann et al., (2002) also reported that *B. frutescens* constituents such as knipholore did not exhibit any cytotoxic effects on normal mammalian cells. The results suggest that these extracts could potentially be alternative agents that would mitigate the inadvertent cytotoxicity of other antimicrobial and skin treatment agents to healthy skin cells.

To further elucidate the proliferative effects and non-cytotoxic effects of the extracts, it was important to investigate the potential morphological changes that occurred in the HaCaT cells upon exposure to the extracts. The H&E stain was performed to view the changes in cellular integrity and to monitor the potential induction of cell death (apoptosis) (Vorster et al., 2012). However, the direct mechanism of apoptosis was not fully explored in this study and can be viewed as a limitation. The H&E stain proved that all three extracts did not display any cytotoxic effects on the cells as the cellular

structural integrity remained intact and unaffected after exposure. This suggests that the plant extracts are not harmful to the cells at any of the tested concentrations, but could potentially cause cells to proliferate, a useful property if the extracts are to be used as antifungal agents (Agyare et al., 2016).

The three extracts exhibited antifungal activity against the fungal strains tested. The antifungal properties of the plant extracts were investigated as it is imperative that the extracts were not toxic to host cells and selectively target fungi. According to an antimicrobial classification by Eloff, (2021) MIC results < 0.02 mg/mL showed remarkable activity, between 0.021 - 0.04 mg/mL showed excellent activity, very good activity was between 0.41 - 0.08 mg/mL, and good activity between 0.081 - 0.16 mg/mL. Average activity was between 0.161 - 0.32 mg/mL and weak activity > 0.32 mg/mL. Accordingly, the results of the present study found that *A. betulina* displayed excellent activity against *C. tropicalis* and very good activity against *C. albicans*. *Bulbine frutescens* displayed good activity against *C. dubliniensis* while *A. ferox* showed average activity against all four *Candida* species.

Very few studies have been conducted on the antifungal properties of these extracts against non-albicans *Candida* species. According to Sharma et al. (2019), some bioactive compounds isolated from medicinal plants play a vital role in modern medicine and are very potent drugs. *Bulbine frutescens* showed effective activity against the *Candida* strains, and this may be due to the phytochemicals present in the plant such as flavonoids, tannins, saponins and alkaloids. These are known to have antifungal effects and are toxic to most microbes (Chapagain et al., 2007; Rachuonyo, et al., 2016). Research has shown that *A. ferox* has compounds such as aloin which exhibited antimicrobial activity (MIC: 5 mg/mL) against *C. albicans* (Kambiz &

Afolayan, 2008). *Agathosma betulina* demonstrated potential antifungal activity with MIC values of 12 and 50 µg/mL against the *C. tropicalis* and *C. albicans* respectively. These results are similar to work conducted by Hübsch et al. (2014) on essential oils of *A. betulina* which showed a strong antifungal activity (MIC: 2 mg/mL) against *C. albicans*. *Agathosma betulina* has coumarin derivatives which reportedly have antimicrobial properties (Moolla et al., 2007). These three plant extracts could potentially be used as rich sources of antifungal compounds.

The optimum concentration for skin cell proliferation was determined to be approximately 200 µg/mL for the three plant extracts. The extracts were able to inhibit the visible growth of the different skin-related *Candida* species at concentrations of 200 µg/mL. These results suggest that the extracts could be specific in their mechanism of targeting fungal skin infections while being non-toxic and causing healthy cells to proliferate at the same concentration. However, further studies to determine the effects of these extracts on fungal infected skin cells need to be explored as this did not fall within the scope of this study. Overall, the results of the present study suggest that extracts from *A. ferox*, *B. frutescens* and *A. betulina* have the potential to be used as natural ingredients against cutaneous candidiasis.

5.5 Conclusion

The present study highlighted that the three hydroethanolic medicinal plant species investigated possess antifungal activity against *Candida* pathogens and have promising potential to be used for pharmaceutical product development. Effective antimicrobial activities were observed in the three extracts of the South African plants. The MIC results suggests that these plants are potentially safe for use as antifungals in traditional medicine, however further *in vitro* and *in vivo* studies where skin cells are infected with the various *Candida* species should be conducted. The results reported here might be used for further laboratory and clinical assays of the active compounds present in these plants.

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CHAPTER 6

Final Discussion and Conclusion

In Chapters 1 and 2, literature revealed that fungal skin infections have increased in incidence and prevalence in South Africa because of their association with the HIV/ AIDS and TB. Limited availability of most antifungal agents used, and the increasing number of treatment failures motivated the current search for therapeutic alternatives, which have the potential to work effectively as antifungal agents. It was worth exploring the antifungal and dermatological properties of medicinal plants as most of them are currently used in skin care product formulations such as lotions, soaps, scrubs, and cleansers.

In Chapter 3, an in-depth literature search on South African medicinal plants that could be used to treat fungal skin infections found a list of fifteen medicinal plants that have antifungal properties. Few research studies had been done on the anti-dermatophyte activities of plants such as *A. betulina*, *A. ferox* and *B. frutescens*. These plants species have the potential to be developed as antifungal agents as they could offer safe alternatives to synthetic antifungals currently used, particularly since they are less toxic, readily available, and are supported by indigenous and traditional knowledge systems.

In Chapter 4, to verify and gain further knowledge on the fifteen plants identified through literature searches, interviews were conducted among the Cape bush doctors in the Western Cape communities. The bush doctors confirmed the use of the 15 plants with most of them corroborating the use of *A. betulina* and *A. ferox* as plant species they used to treat dermatophyte infections. This is significant as it revealed

that the literature is consistent with what is currently used within the community. In addition, the bush doctors reported a further 20 plant species that had antifungal properties and were effective alternatives. This shows that there is limited research in this field and the need to further explore all these plant species remains.

In Chapter 5, an *in vitro* bioactivity investigation of the hydroethanolic extracts of *A. betulina*, *A. ferox* and *B. frutescens* selected from the literature search and interviews was performed. The study investigated the proliferative and cytotoxic effects of the extracts on human epidermal cells. Broth micro-dilutions were also performed to evaluate the susceptibility of *Candida* species to the extracts. This was to ensure the extracts were cytotoxic to the fungal strain while being selective to the host cells. The extracts were able to inhibit the visible growth of the different skin-related *Candida* species such as *C. albicans*, *C. dubliniensis*, *C. glabrata* and *C. tropicalis* at concentrations at 200 µg/mL. The extracts had proliferative effects and showed little cytotoxicity upon exposure to the HaCaT epidermal cells at 200 µg/mL concentrations. These results suggest that the extracts could be specific in their mechanism of targeting fungal skin infections while being non-toxic to healthy cells. This is necessary information for the plant extracts as possible leads for drug development. *A. betulina*, *A. ferox* and *B. frutescens* could potentially be supplementary agents that would mitigate the inadvertent cytotoxicity of antifungal agents to healthy skin cells. Future studies could include the detection of apoptotic markers to confirm whether the plant extracts do not induce cell apoptosis and *in vivo* studies, where the anti-*Candida* properties of the extracts against infected skin cells are investigated.

Limitations in the study included the participant selection being only limited to the bush doctor community in the Cape Metropole region. During the interviews communication

and language barriers were limited as not all participants had access to computers or capable phones for video conferencing. For the *in vitro* study, the direct mechanism of potential cell death (apoptosis) was not fully explored. Additionally, the determination of the effects of *A. betulina*, *A. ferox* and *B. frutescens* on fungal infected skin cells *in vivo* did not fall within the scope of the main objectives of this study.

Despite the limitations, the results found in this study can be used as baseline data for future research. This study has provided more information to the scientific community pertaining to alternative treatments for fungal skin infections. Similar studies can also explore the synergistic properties of the various plants and experiments focusing on the properties found in the secondary metabolites of the plant extracts. It would be beneficial to include the use of the additional plant extracts which were described in both the literature search (Chapter 3) and the surveys (Chapter 4) for their use in the treatment of fungal skin infections.

APPENDIX 1

Journal article

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Review article

Does South Africa hold the key to the development of alternative treatments for resistant dermatophyte infections? A review

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ABSTRACT

Background: Dermatophyte infections are becoming increasingly prevalent in humans. Limited availability of antifungal agents and the increasing number of treatment failures have motivated research for therapeutic alternatives, which can be developed as potential antifungal agents. Medicinal plant extracts have been proposed as alternative treatments and scientists are seeking medicinal plant species that could be developed into medicines for various skin diseases including fungal skin infections. South Africa has a rich diversity of indigenous medicinal plants with dermatological therapeutic relevance.

Aim: This study aimed to review research that have documented the use of medicinal plants indigenous to South Africa in the treatment of fungal skin infections with a view to identify plants with the potential for further therapeutic development.

Methods: Computerized literature searches were performed on databases MEDLINE, SCOPUS, GOOGLE SCHOLAR, MEDLINE EBSCOHOST, SCIENCE DIRECT, Global Electronic Thesis and Dissertations (ETD) and South African National ETD.

Results: Fifteen medicinal extracts were identified as having significant antifungal activity. This is of significant importance as most fungal pathogens are developing resistance to conventional synthetic antifungal agents.

Conclusion: This review identified a diversity of South African medicinal plant species which have the potential to be developed for the treatment of fungal skin infections. These medicinal plants offer a safe alternative to synthetic antifungals currently used, particularly since they are less toxic, readily available, and are supported by indigenous and traditional knowledge systems.

1. Introduction

Dermatophyte infections are becoming very prevalent in humans (Zida et al., 2017). These fungal skin infections are sometimes persistent and potentially contagious, affecting 20–25% of the world's population ranging from neonates to the elderly (Havlickova et al., 2008; Mabona and Van Vuuren, 2013; Tabassum and Hamdani, 2014; Rai et al., 2017). Naturally, in healthy individuals with no underlying diseases, fungal skin infections can be effectively eradicated by components of the immune system such as macrophages, as well as antifungal agents (Hokken et al., 2019). However, in persons with underlying immunosuppressive conditions such as HIV/AIDS, haematological malignancies and transplant recipients, the incidence of dermatophyte infections continue to increase (Carrillo-Munoz et al., 2006; Coopoosamy and Naidoo, 2011a; Rai et al., 2017; Bitew, 2018; Sharif et al., 2018). This is due mostly to the treatment and management of fungal skin infection medication

Abbreviations: Afr, Afrikaans; *A. alternata*, *Alternaria alternate*; *A. flavu*, *Aspergillus flavus*; *A. niger*, *Aspergillus niger*; *A. betulina*, *Agathosma betulina*; *A. ferox*, *Aloe ferox*; *A. arborescens*, *Aloe arborescens*; *A. aristata*, *Aloe aristata*; *A. glaucus*, *Aspergillus glaucus*; *C. albicans*, *Candida albicans*; *C. tropicalis*, *Candida tropicalis*; *C. cucumerinum*, *Cladosporium cucumerinum*; *C. album*, *Coleonema album*; *C. pulchellum*, *Coleonema pulchellum*; Chloro, chloroform; DCM, dichloromethane; EC, Eastern Cape; ETD, Electronic Thesis and Dissertation; *E. rhinocerotis*, *Elytropappus rhinocerotis*; Eng, English; EOs, essential oils; FS, Free State; Gau, Gauteng; *H. limifolia*, *Haworthia limifolia*; HIV, Human Immunodiffecieny Virus; KZN, KwaZulu-Natal; LMP, Limpopo; *M. gypseum*, *Microsporum gypseum*; MFC, minimum fungicidal concentration; MIC, minimun inhibitory concentration; MP, Mpumalanga; NC, Northern Cape; *P. notatum*, *Penicillium notatum*; *S. commune*, *Schizophyllum commune*; SANBI, South African National Biodiversity Institute; *T. riparia*, *Tetradenia riparia*; *T. mentagrophyte*, *Trichophyton, mentagrophytes*; *T. rubrum*, *Trichophyton rubrum*; WC, Western Cape.

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being noticeably limited by safety issues. Most compounds that are effective against fungi are also toxic to mammalian cells because of the shared eukaryotic cellular structures (Hokken et al., 2019). Some antifungal agents reportedly cannot penetrate the site of infection and poor patient compliance play a role in the increased cases of dermatophyte infections as well as the increase in antifungal resistance (Khosravi et al., 2013).

Current treatment methods of dermatophyte infections include amphotericin B and echinocandins but the most commonly used methods are in the azole group of synthetic treatments such as ketoconazole, itraconazole and terbinafine (Gowhar et al., 2015). Reports show that the azole drugs are not entirely selective when binding to the active site of the pathogen and can also inhibit mammalian cytochrome 450 enzymes, which are responsible for the metabolism of various substances including drug-drug interactions. This is very problematic especially in immunocompromised patients already receiving other medications which could result in drug-drug interactions (Wiederhold, 2018). Some of the antifungal agents in the azole group have been reported to cause severe acute and chronic toxicities such as renal function impairment (Carrillo-Munoz et al., 2006). According to Tariq et al. (2019), ketoconazole, when used against dermatophytes such as *Trichophyton* species, has caused unpleasant side-effects including nausea and abdominal pain. This toxicity limits its therapeutic use in most cases.

A major problem medical communities face in treating dermatophyte infections is the increase in azole-resistant strains which are spreading around the world (Zida et al., 2017). According to Hokken et al. (2019) the increasing rate of fungal skin infections is likely caused by strains that are resistant to commonly used antifungals. This makes it difficult to treat infections and is consequently accompanied by high-mortality rates. High levels of primary resistance to terbinafine have been reported against *Trichophyton rubrum* fungal isolates (Khosravi et al., 2013). Some fungal strains are resistant to antifungal agents through the overexpression of biofilm specific efflux pumps that give them the ability to excrete antifungal compounds (Hokken et al., 2019).

Biofilms have been reported to contribute to the elevation of drug resistance because they are notoriously difficult to eliminate and are a source of many recalcitrant infections (Jabra-Rizk et al., 2004; Robbins et al., 2011; Desai et al., 2014). Fungal biofilms are complex surface-associated cell populations that are embedded in an extracellular matrix and are able to attach to biotic (mucosal or tissue surfaces) or abiotic surfaces (*e.g.* catheters or prostheses) which serve as substrates for biofilm growth (Fanning and Mitchell, 2012; Desai et al., 2014; Van Acker et al., 2014). Sessile cells are adherent cells found in a biofilm. These cells are phenotypically and physiologically different from non-adherent (planktonic) cells and require much higher concentrations of antifungal drugs to be destroyed. This is a major cause of biofilm-related antifungal resistance as an estimated 65–80% of all fungal infections are biofilm related (Van Acker et al., 2014). Some factors contributing to biofilm cells becoming more antifungal resistant include: biofilm structural complexity, presence of extracellular matrix, metabolic heterogeneity and biofilm-associated up-regulation of efflux pump genes (JabraRizk et al., 2004; Fanning and Mitchell, 2012; Desai et al., 2014). The biofilm extracellular matrix is one of the most influential factors promoting antifungal resistance. The matrix can prevent access of antifungals to cells embedded in the biofilm by slowing the diffusion rate or specifically binding antifungals extracellularly (Desai et al., 2014; Hokken et al., 2019). There is a major need for antifungal treatment that specifically targets biofilm-associated infections.

Limited availability of most antifungal agents used, and the increasing number of treatment failures have motivated the current search for therapeutic alternatives, which have the potential to work effectively as antifungal agents (Khosravi et al., 2013). Ideal antifungal agents should have a broad spectrum of fungicidal activity, be selective and non-toxic to host cells, and be able to by-pass drug-drug interactions. Such drug-drug interactions have been reported in South Africa where more than 90% of HIV-infected patients develop opportunistic fungal skin diseases at some stage of the viral infection and have to take multiple medications simultaneously (Afolayan et al., 2014). Research on the active constituents of traditional medicines is a strong possible solution as alternative antifungal agents. The use of herbal medicine has widely increased over the years with over 4.5 billion people (64% of the world's population) known to use herbal medicine (Khosravi et al., 2013). About 27 million people of the South African population rely on medicinal plants to treat a broad range of infections (Abdullahi, 2011; Coopoosamy and Naidoo, 2011b; Ghuman and Coopoosamy, 2011). This is because the country has a diversity of indigenous medicinal plants with dermatological therapeutic relevance (Manning and Goldblatt, 2012; Raimondo, 2015). The research and use of dermatological treatments obtained from plants have increased in recent years (Rashidi et al., 2014; Endo et al., 2015; Fajinmi et al., 2017). Scientists are seeking medicinal plant species that could be developed into medicines for various skin diseases including fungal skin infections (Mintah et al., 2019). Information on the use of medicinal plants used in the treatment of dermatophyte infections is lacking. A systematic review can be implemented to address this gap in knowledge by identifying, appraising and summarizing all relevant studies on the particular topic with a reproducible methodology (Greenhalgh, 1997; Uman, 2011). Hence, this study aims to review existing literature using a systematic approach to identify studies concerning the use of indigenous South African medicinal plants used to treat fungal skin infections.

1. Method

1.1. Data collection

A literature search was carried out in regard to the use of indigenous South African medicinal plants on the skin to treat fungal diseases. It was noted that there is a lack of *in vivo* studies currently found in this research area. Thus, the search was limited to *in vitro* studies regardless of language or publication status (published, unpublished, in the press and progress). Computerized literature searches were performed on MEDLINE, SCOPUS, GOOGLE SCHOLAR, MEDLINE EBSCOHOST and SCIENCE DIRECT databases. Also, the Global Electronic Thesis and Dissertations (ETD) and South African National ETD were searched for grey literature. For the retrieval of publications in the databases, the researchers conducted a specific search to define the maximum Medical Subject Headings (MeSH) terms that were related to the research goal. The key terms were "Plant (MeSH)" and "Skin (MeSH)" and "Fungal (MeSH)". In addition to these keywords, all plants from the South African Pharmacopoeia Monograph project were included in the searches (Mukinda and Eagles, 2010). Each plant was searched individually and in conjunction with the aforementioned MeSH terms. Further papers were retrieved from reference lists of review articles. Due to the limited number and diversity of studies, including the difficulty to prove comparisons between studies, a meta-analysis was not conducted.

1.2. Study selection

Studies were considered eligible for this review based on the following inclusion criteria: publications that described the use of medicinal plant species (alone or with any combination of South African herbs) indigenous to South Africa to treat fungal skin infections. This included studies with no language restrictions and date limitations. Studies that were non-South African plants (*i.e.* not endemic to South Africa and imported plants) were excluded, viral and bacterial studies were also excluded. For studies that included the different types of antimicrobial testing (bacterial, viral and fungal), only the fungal sections were analysed, while the bacterial and viral sections were omitted.

The inclusion of data was assessed by all authors. Discrepancies and disagreements were resolved by consensus among the researchers in several meetings. The first phase of this study was the retrieval of articles

based on titles and abstracts of potentially relevant studies in each database. In the second phase, the full PDF articles were downloaded and assessed for eligibility. The reference list of review articles was also evaluated in search of other publications of interest not retrieved in the database search in phase 1.

1. Results and discussion

In recent years, treatments used against fungal pathogens have exhibited several side effects since compounds that are effective against fungi are toxic to mammalian cells (Khosravi et al., 2013; Hokken et al., 2019). The growing interest for either incorporating or replacing current antifungal agents with herbal plants extracts is due to the emergence of azole and echinocandin resistant fungal pathogens (Hokken et al., 2019). Therefore, acknowledging that various South African indigenous medicinal plants and their derivatives can make a significant contribution to the development of alternative antifungals for the treatment of dermatophyte infections is important (Coopoosamy and Naidoo, 2011a; Ghuman and Coopoosamy, 2011). Therefore, this review of existing literature for these medicinal plants with antifungal activity is essential. In the present study, a total of 67 abstracts were identified from electronic searches. The search identified 62 articles, with 0 from PUBMED, 5 from MEDLINE, 8 from SCOPUS, 29 from GOOGLE SCHOLAR, 8 from SCIENCE DIRECT and 7 from the GLOBAL and SOUTH AFRICAN ETD. An additional 5 articles were retrieved from reference lists of review articles. After the removal of duplicates, as well as screening from relevant titles and abstracts a total of 49 articles underwent a full-text review and 10 articles met the inclusion and exclusion criteria.

Table 1 summarises the results of the 15 medicinal plant species found during the investigation. Here, the botanical names of the species are arranged in alphabetical order along with their common name, family, the region in South Africa where it is found and the parts used. The medicinal plant species were widely distributed in 8 South African provinces (excluding the North west). The majority of the 15 plant species belonged to families located within or partially in some parts of the Eastern and Western Cape provinces. This could be attributed to the diversity of plants found in the Cape Floristic Region within the two provinces. The region hosts almost 20% of all flora on the African continent (Afolayan et al., 2002; Manning and Goldblatt, 2012). Ac- cording to the South African National Biodiversity Institute (SANBI), South Africa has over 20,000 plant species of which over 2000 plant species are recorded as used locally for medicinal purposes (Van Wyk, 2011; Raimondo, 2015; Victor et al., 2015).

Table 2 shows the antifungal properties of the medicinal plants found. The table includes 13 dermatophyte-causing fungal species namely; *Trichophyton mentagophyte*, *Trichophyton rubrum*, *Candida albicans*, *Microsporum gypseum*, *Aspergillus flavus*, *Aspergillus glaucus*, *Candida tropicalis*, *Alternaria alternata*, *Aspergillus niger*, *Mucor hiemalis*, *Cladosporum cucumerinum*, *Penicillium notatum* and *Schizophyllum commune*. Overall *T. rubrum* and *T. mentagophytes* were amongst the most common fungi found in the majority of the studies (Table 2). A review of the studies indicated that some medicinal plant extracts were used to treat more than one fungal skin infection. The present study revealed a gap in research knowledge: all the studies only screened the various ways the extracts and their active constituents inhibited or prevented the growth of dermatophyte infections using *in vitro* methods. However, none of the studies evaluated the plant extract efficacies using *in vivo* models. This highlights the need for more studies that can assess the effects of these South African plant extracts *in vivo* and further research is needed to fill this major gap in knowledge.

Most of the studies employed the crude extracts in different solvents such as hexane, methanol, ethyl acetate, water, chloroform and dichloromethane. Leaves stem, roots and bulbs/rhizomes were the plant parts studied and some of the extracts were hydrodistilled to obtain essential oils (EOs). Essential oil volatiles are secondary metabolites present in plant parts such as buds, flowers, and barks (Suroowan et al., 2019). Medicinal plants were administered in various ways. One study

Table 1
Summary of the 15 medicinal plant species used to treat fungal skin infections.

Botanical name	Common name	Family	Region	Part used	References
1. *Agathosma betulina*	Buchu (Afr)	Rutaceae (Citrus)	WC & EC	Leaves	(Fajinmi et al., 2019)
2. *Aloe arborescens*	Krantz aloe (Eng), kransaalwyn (Afr), inkalane or umhlabana (Zulu)	Xanthorrhoeaceae	WC, NC, EC, KZN & MP	Leaves	(Ghuman et al., 2016)
3. *Aloe aristata*	Bearded Aloe (Eng), or Hardy Aloe(Eng)	Xanthorrhoeaceae	WC, NC, EC, KZN & MP	Leaves	(Ghuman et al., 2016)
4. *Aloe ferox*	Bitter aloe, (Eng); bitteraalwyn, (Afr); iNhlaba (Zulu)	Xanthorrhoeaceae	WC, NC, EC, KZN & MP	Leaves	(Afolayan et al., 2002; Ghuman et al., 2016)
5. *Bulbine frutescens*	Snake flower, cat's tail, (Eng), balsam kopieva (Afr), geelkatstert (Afr)	Xanthorrhoeaceae	WC, NC, EC, KZN & MP	Leaves, stem & roots	(Ghuman et al., 2016)
6. *Bulbine natalensis*	Ibhucu (Zulu), Rooiwortel (Afr)	Xanthorrhoeaceae	WC, NC, EC, KZN & MP	Leaves, bulbs & roots	(Ghuman et al., 2016)
7. *Coleonema album*	White confetti bush (Eng), assbossie (Afr)	Rutaceae	WC & EC	Leaves	(Esterhuizen et al., 2006; Fajinmi, 2016; Fajinmi et al., 2017, 2019)
8. *Coleonema pulchellum*	Confetti buchu (Afr)	Rutaceae	WC & EC	Leaves	(Fajinmi, 2016; Fajinmi et al., 2017)
9. *Elytropappus rhinocerotis*	Renosterbos (Afr)	Asteraceae	EC & WC	Stem	(Hulley et al., 2019)
10. *Eucomis autumnalis*	Pineapple flower (Eng); wildepynappel, (Afr); umathunga (Zulu)	Asparagaceae	EC, LMP, Gua, FS & MP	Leaves, stem & bulbs	(Ghuman et al., 2016)
11. *Haworthia limifolia*	Fairy Washboard (Eng)	Xanthorrhoeaceae	WC, NC, EC, KZN & MP	Leaves, stem & roots	(Naidoo and Coopoosamy, 2011; Ghuman et al., 2016)
12. *Hypericum aethiopicum*	Unsukumbili (Zulu)	Hypericaceae	KZN	Leaves, stem & roots	(Ghuman et al., 2016)
13. *Merwilla plumbea*	Wild squill, (Eng); blouberglelie (Afr)	Hypericaceae	EC, KZN & FS	Leaves, bulbs & roots	(Ghuman et al., 2016)

Table 2
Antifungal properties of the 15 medicinal plants from South Africa found in the review.

Type of extracts	Active constituents	Fungal species	Test results	Main effectivity	Observations
Essential oils	Limonene,menthone, isomenthone, pinene, myrecene,cineole, terpinen-4-ol, germacrene, spathulenol, viridiflorol, silphiperfol-6-en-5-one, caryophyllene & trans-β-ocimene	*T. rubrum*, *T. mentagrophytes* & *M. gypseum*	MIC values: 0.195,0.391, 0.049 mg/mL Zone of inhibition: very	Fungistatic, reduction of fungal growth. Fungal growth index 2.3% fungicidal effects	Alteration & destruction of fungal hyphae & spores. Extracts more effective on *Trichophyton*, *mentagrophytes*. Limonene was the most abundant volatile.
Ethanol & aqueous	N/A	*A. flavus* & *A. glaucus*	high inhibition	Fungistatic	Ethanol leaf extracts had higher growth inhibition than aqueous extracts
		C. albicans &	Zone of inhibition: very		The ethanolic stems & leaves were
Ethanol & aqueous	N/A	*C. tropicalis*	high inhibition	Fungistatic	more effective in growth inhibition than aqueous extracts
Ethanol & aqueous	*N/A*	*T. rubrum*	Zone of inhibition: medium inhibition	Fungistatic	Ethanol extracts were more effective than aqueous extracts
Hydroalcoholic	N/A	*T. rubrum*, *T. mentagrophytes* & *M. gypseum*	MIC values: 0.0625−0.25 mg/mL, 0.0019 mg/mL, 0.0156 mg/mL	Fungistatic,	Strong inhibition and irregular growth pattern inhibiting fungal growth and causing alterations in hyphae.
Acetone & ethanol	Coumarin aglycones	*C. albicans* & *C. cucumerinum*	MIC values:0.039−0.299 mg/mL Bioautographic screen & zone of inhibition: high inhibition	Fungistatic	Inhibition of spore germination & mycelial growth
Acetone	N/A	*A. alternata*, *M. hiemalis* & *S. commune*	Zone of inhibition: 100% inhibition	Fungicidal	Extracts have effective antimycotic activity
Acetone	N/A	*A. niger* and *P. notatum*	Zone of inhibition: above 50% inhibition	Fungistatic	Extracts have a relative effective antimycotic activity
Hexane, methanol, chloro, DCM, acetone	Flavonoids, tannins, proanthocyanidin	*T. rubrum*, *T. mentagrophytes M. gypseum*, *C. albicans* & *C. tropicalis.*	MIC values: 0.63 & 0.31 mg/mL	Fungistatic 90−100% survival rate after 24 h	Chloro & DCM extracts were most effective. Extracts generally lack a cytotoxic effect

A. alternata, *Alternaria alternate*; *A.flavu*, *Aspergillus flavus*; *A. glaucus*, *Aspergillus glaucus*; *A. niger*, *Aspergillus niger*; *C. albicans*, *Candida albicans*; *C. tropicalis*, *Candida tropicalis*; *C. cucumerinum*, *Cladosporium cucumerinum*; Chloro, chloroform; DCM, dichloromethane; MIC, Miminum Inhibitory Concentrations; *M. gypseum*, *Microsporum gypseum*; *M. hiemalis*, *Mucor hiemalis*; N/A, Not available; *P.notatum*, *Penicillium notatum*; *S. commune*, *Schizophyllum commune*; *T. mentagrophytes*, *Trichophyton, mentagrophytes*; *T. rubrum*, *Trichophyton rubrum.*

reported that when EOs are extracted through hydrodistillation, they can then be applied topically (directly rubbed onto infected areas of the skin) (Fajinmi et al., 2019). It was reported that the extracts from *Elytroppapus rhinocerotis* could be burnt and inhaled or the leaves could be applied directly to the skin (Hulley et al., 2019). Extracts from *Haworthia limifolia* were reported to be taken orally through chewing or brewed as a tea (Coopoosamy and Naidoo, 2011b; Naidoo and Coopoosamy, 2011). The preparation methods mentioned in the studies included aqueous infusion (submerging in water for a specified period) or decoction (boiling in water or any other solvent) (Coopoosamy and Naidoo, 2011b). Most studies used organic solvents as a means of preparation.

When compared to the current antifungal treatments available, the South African medicinal plants showed good antifungal activity. This was evaluated in *in vitro* studies by determining the minimum inhibitory concentration (MIC) or minimum fungicidal concentration (MFC). The phytochemical's *in vitro* activity is considered significant if MIC values are found to be lower or between 0.1 mg/mL - 0.625 mg/mL (Zida et al., 2017). Most of the extracts showed notable MIC results with the most effective isolated from the Asteraceae family (*E. rhinocerotis*). The extracts from *E. rhinocerotis* were reported to have the lowest MIC values (0.0098 and 0.0350 mg/mL) ever recorded for a natural product against *T. mentagrophytes* (Hulley et al., 2019). While extracts from *Coleomena pulchellum* were found to exhibit great antifungal activity with low MIC values of 0.195, 0.139 and 0.049 mg/mL against *T. rubrum*, *T. mentagophyte* and *M. gypseum* respectively (Fajinmi et al., 2017). Extracts isolated from species belonging to the Xanthorrhoeaceae family, *Aloe ferox* and *Aloe arborescens* also had low MIC values of 0.31 mg/mL and 0.63 mg/mL. Both plant extracts were found to be effective in treating ringworm (Ghuman et al., 2016). Extracts from *Tetradenia riparia* also had antifungal activity against dermatophytes with MIC values of 0.25 and 0.625 mg/mL (Endo et al., 2015). Finally, extracts from *Hypericum*

A. betulina and *C. album* EOs to inhibit biofilm formation and decrease biofilm activity. (Fajinmi et al., 2019). These extracts could be useful as antifungal agents to prevent the formation of biofilms.

A wide range of antifungal drugs are sequestered by beta-glucans which are present in the cell wall of biofilm cells and the surrounding extracellular matrix. The extracellular matrix accumulates as the biofilms mature, and the amount of drugs that reach the sessile cells becomes too low to exert an antimicrobial effect (Desai et al., 2014; Van Acker et al., 2014). Medicinal plant extracts from *T. riparia* are reportedly effective against biofilm formation because of their affinity for extracellular matrix beta-glucans (Zida et al., 2017). The extracts have the ability to cause the degradation of matrix beta-glucans which impaired biofilm-associated resistance mechanisms (Desai et al., 2014). *T. riparia* extracts could be important in eliminating the matrix's component of protective biofilms, which is useful in the treatment of biofilm-related dermatophyte infections. Natural compounds extracted from *T. riparia*, *A. betulina*, and *C. album* species may potentially be useful in the treatment of biofilm-related dermatophyte infections.

The present study shows that some herbal extracts possess antifungal properties. These properties include the disruption of the fungal cell membrane and the ability of the extracts to cause pore formation in the fungal plasma membrane. This is important as most fungal pathogens have several mechanisms of resistance such as efflux pumps that transport antifungals out of the cell (Hokken et al., 2019). The results of this review also highlighted the importance of *A. betulina*, *C. album* and *C. pulchellum* EOs and their constituents which had significant antifungal activities against fungal skin diseases. These activities included the inhibition of fungal growth and changes in the morphology of fungal strains by disrupting hyphal formation. According to Fajinmi et al. (2017) EOs are known to have several properties which are of great importance to skincare health. These properties include the enhancement of the skin barrier function, the stimulation of cell regeneration, EOs also have both a high efficiency and low drug fungal resistance (Fajinmi et al., 2017; Nazzaro et al., 2017). Other reports have shown that EOs have significant antifungal activities, which include the disruption of fungal cell walls and phytoplasma membranes through a permeabilization process that leads to the disintegration of mitochondrial membranes. EOs also inhibit the synthesis of DNA, RNA and polysaccharides in fungal pathogenic cells (Zida et al., 2017; Fajinmi et al., 2019; Tariq et al., 2019).

Essential oils may be beneficial in skin treatments as their chemical structures are hydrophobic and resemble the intracellular hydrophobic molecules of the skin epidermis (Feingold, 2012; Fajinmi et al., 2019). Currently, EOs are being used to produce creams, lotions, soaps, body washes and ointments (Feingold, 2012; Fajinmi et al., 2019). This supports the use of EOs as antifungals, although the proper mode of action still needs to be studied. However, it is of great importance that the EO concentrations are within the toxicity range for mammalian cells (Nazzaro et al., 2017). According to Orchard and van Vuuren (2017) if high concentrations of EOs are used to treat skin ailments, they are known to produce adverse effects such as allergic contact dermatitis, skin irritation, or photosensitization.

1. Final considerations

The current study was undertaken to investigate available studies on South African medicinal plants used to treat fungal skin infections. This study was carried out as a means of finding alternative treatment options to combat problematic fungal infections of the skin. Antifungals used clinically for skin diseases vary in their modes of action which limits their efficacy. This review shows that South African medicinal plants displayed a broad-spectrum of antifungal mechanisms, including cell membrane disruption, inhibition of cell wall formation, dysfunction of fungal mitochondria and the inhibition of efflux pumps. It is evident from the literature that there is currently a renewed interest in South African plant-based medicines as antifungal herbal therapies. South Africa has a strong system of traditional medicinal knowledge which supports the use of ethnomedicine for skin ailments. However, more scientific studies and bioactivity validation of selected medicinal plant species will justify their use and incorporation as natural remedies. This review shows that South Africa harbours medicinal plants with good antifungal activity for use in effectively treating fungal skin infections. They show promise to replace synthetic antifungals which are associated with increased resistance and toxicity. However, the study also highlighted the need for *in vivo* toxicity studies to establish safe dose ranges. Addressing these limitations in future studies will contribute to the development, production and commercialisation of these plants in herbal-based antifungal medicines, cosmetic and skincare products.

Contributions

F.R, C.A and J.F designed the study. B.N conducted the searches. All authors were involved in the data extraction, quality assessment and narrative of included studies. All the authors contributed equally in preparing the manuscript. All authors have approved the final article.

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Ethical approval

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix 2

Interview questionnaire

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FACULTY OF NATURAL SCIENCES

DEPARTMENT OF MEDICAL BIOSCIENCES

Interview-based questionnaire

For official purposes only
Participant Number:
Date of interview:
Name of interviewer:
Questionnaire no:
Informed consent: Yes / No

Section A: Participants demographic profile

1. Age: _____
2. Gender: Male ☐ Female ☐
3. Highest level of formal education completed:

Primary ☐ Secondary ☐ Tertiary ☐ No education ☐

Other: Please specify _____

4. How long have you been staying in the Cape Metropole area?

<1yr ☐ 1-5yrs ☐ 5-10yrs ☐

Other: Please specify _____

5. User status:

Herbalist ☐ Traditional healer ☐ Bush doctor ☐

Other: Please specify _____

6. What is your speciality?

Please specify _____

Section B: Validation of South African medicinal plant use.

Please indicate if you are not familiar with these names and a picture of the plant will be shown

1. From the list below please indicate which of the plants do you use to treat wounds and fungal skin infections.

☐ Buchu *(Agathosma betulina)*

http://www.flowervalley.co.za/en/species/agathosma-betulina-2/

☐ Kransaalwyn *(Aloe arborescens)*

http://pza.sanbi.org/aloe-arborescens

☐ *Aloe aristata*

https://worldofsucculents.com/aristaloe-aristata-lace-aloe/

☐ Bitteraalwyn *(Aloe ferox)*

http://pza.sanbi.org/aloe-ferox

☐ Geelkatstert *(Bulbine frutescens)*

http://pza.sanbi.org/bulbine-frutescens

☐ Rooiwortel *(Bulbine natalensis)*

https://wildflowernursery.co.za/indigenous-plant-database/bulbine-natalensis/

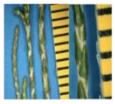
☐ Asbossie *(Coleonema album)*

http://pza.sanbi.org/coleonema-album

☐ Confetti buchu (*Coleonema pulchellum)*

http://pza.sanbi.org/c/coleonema-pulchellum

☐ Renosterbos *(Elytropappus rhinocerotis)*



http://pza.sanbi.org/dicerothamnus-rhinocerotis

☐ Wildepynappel *(Eucomis autumnalis)*

http://pza.sanbi.org/eucomis-autumnalis

☐ *Haworthia limifolia*

http://pza.sanbi.org/haworthiopsis

☐ Blouberglelie *(Merwilla plumbea)*

http://pza.sanbi.org/merwilla-plumbea

☐ Watersalie *(Tetradenia riparia)*

http://pza.sanbi.org/tetradenia-riparia

☐ Arum lily (Zantedeschia aethiopica)

http://pza.sanbi.org/zantedeschia-aethiopica

2) Preparation and use of the medicinal plants (for each of the plants selected in the list above questions a-c will be asked).

a) In what form is the plant administered?

Tea ☐ Powder ☐ Tincture ☐ Tablet/ capsule ☐ Ointment ☐

Other: Please specify _____

b) Do you combine any of the plants listed? Yes ☐ No ☐

If yes, please specify _____

Section C: Knowledge regarding skin conditions and the treatments used by Cape Bush doctors.

1. How did you gain medicinal plant knowledge?

Grandparent(s) ☐ Parent/ guardian ☐ Other relative (specify) _____

Other: Please specify _____

2. Please indicate if you have used medicinal plants to treat any of the following conditions

Wound healing: Abrasion (skin scrapes) ☐ Burns (Thermal, chemical, or electrical) ☐ Lacerations (deep cut or tearing of the skin) ☐ Punctures (wounds deep enough to damage internal organs) ☐ Crush injuries (complete tearing away of the skin caused by violent accidents) ☐ Surgical wounds (cut or incision in the skin usually made by a scalpel during surgery) ☐ Blisters (fluid-filled pockets under the skin) ☐ Skin ulcers (ulceration of the skin e.g. Diabetic Foot Ulcer) ☐

Other: Please specify _____

3. Please indicate if you have used medicinal plants to treat any of the fungal skin infections listed below:

Athletes foot (fungus grows on the feet) ☐ Jock itch (red and itchy rash often affects the skin in the groin and inner thighs) ☐ Ringworm (a red, itchy, circular rash with clearer skin in the middle) ☐ Oral thrush (raised, creamy white, sore patches in the mouth or tongue) ☐ Diaper rash (red and inflamed rash in the diaper area that could crack open and lead to severe bleeding) ☐ Other: Please specify _____

4. Please specify if there are any other skin conditions that you have treated using medicinal plants not mentioned previously

5. Are there any prohibitions involved in the usage of medicinal plants for the treatment of skin conditions?

Yes ☐ No ☐

6. If yes, please specify (E.g. not for use by pregnant women, or children):

_____

Section D: Practices on the use of medicinal plants for wound healing and treatment fungal skin infections.

1. How long have you been a Cape Bush doctor?

<1 yr. ☐ 1-5yrs ☐ 5-10yrs ☐

Other: Please specify _____

2. Which plants do you use to treat fungal skin infections listed below:

Fungal skin infection	Plant (s) used
Athlete's foot Yeast infection Ringworm Nail fungus Oral thrush	
Diaper rash	
Other:	

3. From the plants mentioned in questions 2 above questions a-g will be asked.

a) How long do you harvest your plants?

Monthly ☐ Seasonally ☐ Yearly ☐

Other, please specify_____

b) Is there any specification on time and season to collect the plants?

Monthly ☐ Seasonally ☐ Yearly ☐

Other, please specify_____

c) How do you prepare the herbal remedies?

Tea ☐ Powder ☐ Tincture ☐ Tablet/ capsule ☐ Ointment ☐ Other: Please specify _____

d) Do you combine multiple plants?

Yes ☐ No ☐

e) If yes, please specify: _____

f) In what way do your patients take the treatments?

Orally (Drinking/chewing) ☐ Inhalation ☐ Topically (apply on skin) ☐ Bath Other:

Please specify _____

g) How long is the treatment duration?

<1 week ☐ 1-2weeks ☐ 6 weeks ☐

Other: Please specify _____

1. Which plants would you used to treat the wound related conditions listed below:

Wound type	Plant (s) used
Abrasion (skin scrapes against a rough or hard surface, road rash)	
Burns (caused by fires or flames, hot liquids or steam, contact with a hot object or agents like grease or tar, chemicals, or electricity.)	
Lacerations (deep cut or tearing of the skin, dog or snake bites bee stings,)	
Puncture (wound caused by objects such as needles, nails sometimes bullet wounds and are deep enough to damage internal organs)	
Crush injuries (complete tearing away of skin and can be caused by violent accidents; body-crushing, explosions)	
Blisters (fluid-filled pockets under the skin)	
Skin ulcers (ulceration of the skin e.g. Diabetic Foot Ulcer, genital ulcer)	
Surgical wounds (cut or incision in the skin that is usually made by a scalpel during surgery. Seroma and hematoma; a fluid or blood-filled area that develops under the skin)	
Other:	

2. From the plants mentioned in questions 4 above questions a-g will be asked.

a) How long do you harvest your plants?

Monthly ☐ Seasonally ☐ Yearly ☐

Other, please specify_____

b) Is there any specification on time and season to collect the plants?

Monthly ☐ Seasonally ☐ Yearly ☐

Other, please specify_____

a) How do you prepare the herbal remedies?

Tea ☐ Powder ☐ Tincture ☐ Tablet/ capsule ☐ Ointment ☐ Other:

Please specify _____

b) Do you combine multiple plants?

Yes ☐ No ☐

c) If yes, please specify: _____

d) In what way do your patients take the treatments?

Orally (Drinking/chewing) ☐ Inhalation ☐ Topically (apply on skin) ☐ Bath Other:

Please specify _____

e) How long is the treatment duration?

<1 week ☐ 1-2weeks ☐ 6 weeks ☐

Other: Please specify _____

f) Anything else you wish to add?

Appendix 3

Ethical clearance

UNIVERSITY of the WESTERN CAPE

50 YEARS of hope, action & knowledge

12 November 2020

Ms B Ndlovu, Dr F Rahiman & Prof J Klaasen
Medical Biosciences
Faculty of Natural Sciences

Ethics Reference Number: BM19/7/15

Project Title: Knowledge, practices and validation of medicinal plants used for skin-related conditions in the Western Cape region of South Africa.

Approval Period: 24 July 2020 – 24 July 2023

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above mentioned research project.

Any amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report annually by 30 November for theduration of the project.

Permission to conduct the study must be submitted to BMREC for record-keeping.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer
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NHREC Registration Number: BMREC-130416-050

Appendix 4

Conferences and publications related to this study.

Publications

• **Ndlovu, B**., Africa, C., Klaasen, J. and Rahiman, F., (2021). Does South Africa hold the key to the development of alternative treatments for resistant dermatophyte infections? A review. Journal of Herbal Medicine, p.100474. Doi: https://doi.org/10.1016/j.hermed.2021.100474

• **Ndlovu, B**., De Kock, M., Klaasen, J. and Rahiman, F., (2021). In Vitro Comparison of the Anti-Proliferative Effects of *Galenia africana* on Human Skin Cell Lines. Scientia Pharmaceutica, 89(1), p.12. Doi: https://doi.org/10.3390/scipharm89010012

Conference presentations:

• **Ndlovu B**, Klaasen, J. and Rahiman, F., (2019). 22nd IPUF conference. A preliminary study investigating the effects of *Galenia africana* on HaCaT epidermal cells. Indigenous Plant Use Forum, Limpopo.

• **Ndlovu B.**, Klaasen, J. and Rahiman, F., (2022). 22nd IPUF conference. Medicinal plants used in wound healing among the western cape bush doctors. Indigenous Plant Use Forum.