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Evaluation of medicinal potential of *Boophone disticha (L.f.)* Herb. used by the indigenous people in the Raymond Mhlaba Municipality Eastern Cape

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Evaluation of medicinal potential of *Boophone disticha* (L.f.) Herb. used by the indigenous people in the Raymond Mhlaba Municipality Eastern Cape

By

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FACULTY OF SCIENCE AND AGRICULTURE

UNIVERSITY OF FORT HARE

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DECLARATION

I, Yamkela Gwayi, declare that this dissertation, submitted to the University of Fort Hare for a Master's degree in botany at the Faculty of Science and Agriculture, is my own work and has never been submitted to any other institution for any academic degree.

I declare that I have followed the rules and guidelines on references and citations in scientific literature. I also confirm that all the origins of materials used in this dissertation have been thoroughly identified and correctly checked. Again, I declare that I am completely aware of the policy of plagiarism of the University of Fort Hare and I have taken every precaution to comply with the regulations of the University.

Name: Yamkela Gwayi Signature: …

…….

We confirm that the above work was performed by the above-mentioned candidate under our supervision.

Dr Buyisile Mayekiso

Signature: …… Date: ……

Prof LV Buwa-Komoreng

Signature: …… Date: ……

DEDICATION

I dedicate this dissertation to all the people that never stopped believing in me.

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ETHICAL APPROVAL

The study involved the use of plants and was carried out following the approval of the University of Fort Hare's Ethics Committee with reference number: MAY011SGWA01.

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GENERAL ABSTRACT

Boophone disticha (L.f.) Herb. is a bulbous plant native to southern African areas of the continent and spreads to tropical Africa. The plant has been noted in literature for its high toxicity and has a long lineage of its use in traditional medicine for the treatment of various diseases. Due to high demand in the conventional trade market, the plant's conservation status has been shown to be declining. The plant has been reported to cause human poisoning, which can lead to death, but the plant is still administered by an indigenous community to treat various diseases. This study investigated the therapeutic potential of *B. disticha* used by the people of Raymond Mhlaba municipality to show and verify its use in traditional medicine and how it can be used as a possible drug ingredient. This study was also set out to investigate the anti-mycobacterial activity of the plant for the first time for the *B. disticha* growing in the Eastern Cape region of South Africa

The phytochemical analysis of *B. disticha* was carried out on four different plant parts (i.e., roots, leaves, bulb inner and outer scales) and the plant was extracted using methanol and distilled water. The qualitative phytochemical analysis displayed the presence of tannins, flavonoids, phenols and alkaloids in all plant parts for both extraction solvents. Anthraquinones were absent in all the plant extracts. The detected phytochemicals (tannins, flavonoids, phenols and alkaloids) were quantified. The roots had the highest phytochemical content for methanol extract for each phytochemical tested [439.67±1.53 mg/g (QE) flavonoids, 2414.67±1.53 mg/g (GAE) phenols and 527.33±2.08 mg/g (GAE) alkaloids] compared to all the plant parts. Methanol extracts from *B. disticha* revealed highest phenolic contents (2414.67±1.53 mg/g GAE) for the roots, 1395.33±2.52 mg/g (GAE) for the bulb inner scales, 1560±1 mg/g (GAE) for the leaves and 1550.7±18.9 mg/g (GAE) for the bulb outer scales). The total flavonoid content of the bulb outer scales aqueous extract was not detected. The presence of significant

amounts of phytochemical compounds indicates that *B. disticha* has a higher medicinal value and can be extensively investigated to extract bioactive ingredients that are useful to the society, and that could be sold for higher production than using synthetic drugs with side effects.

The antimicrobial activity of *B. disticha* extracts (methanol and distilled water) was evaluated using micro-dilution bioassay in 96-well micro-plates against nine disease-causing bacterial strains (5 Gram-negative and 4 Gram-positive) and three fungal isolates. The results revealed that methanol and aqueous extract of *B. disticha* demonstrated very good activity, with Gram-positive strains being more sensitive than Gram-negative ones. *Boophone disticha* aqueous extracts displayed the best activity against *Staphylococcus aureus* with MIC and MBC values ranging from 0.39 to 0.78 mg/ml. The methanolic extract of *B. disticha* leaves and outer scales of bulb displayed good activity against *Klebsiella pneumoniae* at MIC 0.78 mg/ml. The methanolic extract of *B. disticha* bulb outer scales also displayed good inhibition against *Proteus vulgaris* at MIC 0.78 mg/ml, with the root extract exhibiting activity against *Shigella flexineri* (MIC value of 0.078 mg/ml). Concerning antifungal activity, *B. disticha* extracts showed very poor inhibition properties against the fungal isolates.

Anti-mycobacterium potential of *B. disticha* extracts (methanol and distilled water) was evaluated using micro-dilution bioassay in 96-well microtiter plates. The plant parts tested were the roots, bulb inner scales, leaves and the bulb outer scales. The highest activity against *Mycobacterium tuberculosis* was observed with the root methanol extract at MIC 0.78 mg/ml.

The anti-inflammatory properties of *B. disticha* were investigated using the 5-lypoxygenase (5-LOX) assay. The overall anti-inflammatory activity results for the *B. disticha* extracts were poor; at low concentrations, the plant displayed negative results. The leaves methanol extracts did show little activity at 0.4 mg/ml.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background of study

Plants are the most common source of food and medicine, and play an important role in global health (Jamshidi-Kia, 2018). Sofowora et al. (2013) defines a medicinal plant as any plant that contains compounds which may be used for diagnostic purposes in one of its plant parts. These compounds are also precursors for the recovery of valuable drugs. Medicinal plants serve a purpose in the treatment or healing of specific illnesses and diseases that are known to affect human health (Schulz et al., 2001). Such plants have been established as a global basis for a variety of vital benefits to human health, social and economic structures (Geldenhuys and Mitchell, 2006). Mulaudzi et al. (2011) further noted that these medicinal plants are considered as valuable sources of ingredients which are the primary starting points for the manufacture of drugs in either pharmacopoeia, conventional herbal remedies or drug synthesis. There has been a significant renewed interest in historically used medicinal plants, with several international and local projects actively exploring Southern Africa's botanical resources with the goal of testing indigenous plants for pharmacologically active compounds (Calixto, 2000).

The origin of the use of medicinal plants by man was innate, as it is the case with animals (Stojanoski, 1999). The widespread use of homeopathic medicinal products in the medical industry has been linked to the availability of natural medicinal products (Parekh, 2007). Botanical remedies are universal. There are different disease hypotheses and different systems used in medicine in every culture and era (Parekh, 2007). The World Health Organization (WHO) said that approximately 80% of the world's inhabitants rely on herbal medicine as their

primary source of health care and conventional medical practice (Bandaranayake, 2006). The tradition of using plants or biologically active compounds to cure diseases is a therapeutic modality that has stood the test of time (Gilani, 2017). Maroyi (2011) reported that a large fraction of the inhabitants in many developing countries depend heavily on conventional practitioners and homeopaths to meet their primary health needs. The indigenous people of South Africa have relied heavily on herbal medicine for all aspects of their health care services for centuries (Grieson and Afolyan, 1999). Traditional medicine has a very deep rooted and rich cultural heritage in South Africa, as in other African countries (Buwa-Komoreng et al., 2019). It is estimated that approximately 12 to 15 million South Africans will continue to use traditional methods of care of as many as 700 natural plant species (Afolayan and Meyer 1997).

Recently, there has been a cultural recovery among developed countries towards more natural healing methods (Barnes et al., 2003), and most Westerners turn to different and complimentary pharmaceutical products (Makunga et al., 2005). Improved use of herbal medicines or phyto-medicines has resulted in increased focus on innovation based on certain preparations (Makunga et al., 2005).

Boophone disticha is of considerable ethno-botanical interest as a hallucinogen in traditional medicine and is in high demand with the indigenous people and traditional healers (Golding, 2002). Due to the over exploitation the of *B. disticha*, the conservation status of the plant was listed as 'declining' [www.redlist.sanbi.org (accessed 28/06/16)] and because of this it needs to be cultivated. As it is a perennial that requires several years to establish and become harvestable, a system has to be developed whereby many plants can be grown in a shorter period of time. This will ensure the production of bulbs that can be used by traditional healers and in so doing, reduce large scale collection from the wild (Cheeseman, 2013). Gadaga et al. (2010) reported that *B. disticha* possessed a very high level of toxicity that mainly affects the central nervous system, however at low dosage its components have possible healing benefits

and maybe a top contender in the discovery of new drugs. Thus, it is important that more detailed research investigations are carried out in order to improve understanding of the pharmacological effects of extracts of this herb.

1.2 Justification of study

Boophone disticha is generally considered to be an exceptionally toxic plant but is considered one of the most widely traded and helpful medicinal plants (Cheesman, 2013). It has been revealed that the plant's bulbs resulted in dreadful human poisoning after medicinal administration (du Plooy et al., 2001). As toxic and lethal, *B. disticha* is known to have been used as an antiseptic and pain-relieving dressing by Xhosa people after circumcision (Nair and van Staden, 2014). Furthermore, some of the indigenous tribes (Sothos, Xhosas and Zulus) use bulb extracts and infusions to treat various diseases, including burns, injuries, cultural nutritional application, swelling, anxiety and psychosis (Nair and van Staden, 2014).

Boophone disticha's status was classified as 'declining' (Williams et al., 2013) and such results were expected considering the scale of its exploitation in the traditional commercial sector. *Boophone disticha* is the only plant in the South African Amaryllidaceae that is recognised as a traditional medicine in the treatment of tuberculosis (Watt and BreyerBrandwijk, 1962), although no pharmacological trials were conducted to confirm this property. Information on the use of the plant to treat fungal infections is scarce in the literature (Viladomat et al., 1997). Despite this, *B. disticha* is not specified for such purposes, while some species, including *Amaryllis belladonna*, *Crinum macowanii* and *Crinum moorei*, exhibited antifungal or anti-yeast activity (Cheeseman, 2013).

Therefore, this study scientifically investigated different medicinal values of *B. disticha*. This will help decide which part of the plant best validates all the documented traditional uses in

line with the investigated assays. This study also helps bridge the gap in literature in an attempt to validate the claims stipulated in traditional medicine that *B. disticha* is used in treating tuberculosis.

1.3 Aim of the study

The aim of this study was to assess, prove and record the pharmacological importance of *B. disticha* that is regarded as an extremely toxic plant and how it can be used as an alternate source of medicine.

1.4 Specific objectives

The following objectives were specified:

- To quantitatively and qualitatively evaluate the phytochemical constituents of *B. disticha*.
- To investigate the antibacterial and antifungal potentials of *B. disticha.*
- To screen for the anti-mycobacterium potential of *B. disticha* in the fight against tuberculosis.
- To determine the anti-inflammatory properties of *B. disticha*.

1.5 Dissertation outline

This dissertation is composed of discrete chapters prepared for manuscript in different peer reviews accredited journals. Chapter 1 is the introduction; it gives background information of the study and also outlines the aims and objectives of the study. Chapter 2 stipulates the literature review on the importance of medicinal plants around the world, Africa and South

Africa. A detailed information on the medicinal herb of interest, *B. disticha*, is laid out and divided into subsections discussing the botanical description, morphology and distribution of the medicinal herb. An overview on the traditional medicinal uses and pharmacological uses of the plant are documented i.e., phyto-chemistry, anti-inflammatory and antimicrobial activity assessment, validity and reliability of the tested plant are also on the literature review. Chapter 3 reports on evaluation of qualitative and quantitative phytochemicals found in of *B. disticha.* Evaluation of the phytochemicals gives an indication of its ethno-medicinal values. The effects of different solvents on the different plant parts were screened to find out which solvent and plant part is best for the extraction of the phytochemicals. Chapter 4 reports the antimicrobial activity, by evaluating the antifungal and antibacterial activities of *B. disticha* against some human pathogenic organisms. Furthermore, an evaluation of the plant extracts as possible anti-mycobacterial agents is reported in Chapter 5. Chapter 6 is an evaluation on anti-inflammatory activity of *B. disticha*, in an attempt to validate one of its ethno-pharmacological uses. Chapter 7 is the general conclusion and recommendations for the study.

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CHAPTER TWO

LITERATURE REVIEW

2.1. Medicinal plant use in the world

Over the last few years, more and more people have chosen homeopathic remedies or products to enhance their quality of life, either alone or in conjunction with others. Herbs are making a comeback and herbal "renaissance" is happening globally (Pan et al., 2014). Traditional medicine has indeed been used as basic health care for vulnerable people in emerging countries, but also in countries where mainstream medicine is dominant in the health care facility (Oyebode et al., 2016). Almost half of the world's inhabitants depend on herbal plants for their primary health care (Mahomoodally, 2013). Mamedov (2012) estimated that 35-75% of the world's 250 000 plant species are used worldwide, with 14-28% used as medicinal products. Furthermore, Mamedov (2012) reported that, more than 50 significant therapeutic drugs have recently come from tropical crops in worldwide markets.

Ethno-botany has been used as a method to classify herbs of medicinal value for the manufacturing of drugs prescribed in developed countries (Ballick and Cox, 1997). WHO (2003) stated that comprehensive research on herbal medicine has been documented and gained prominence in all areas of the developing world, and awareness has spread in the developed world. Herbal therapy has been used at least once by more than 50% of the population in Europe, North America and other developed countries around the world. Approximately 60% of the population in Hong Kong used herbalist facilities (Farag et al., 1989). Herbal preparations represented 30 – 50% of total Chinese medicinal intake. About 75% of people in San Francisco, South Africa and London have used herbal therapy to alleviate the effects of Human Immunodeficiency Virus / Acquired Immunodeficiency Syndrome (HIV / AIDS)

(Halberstein, 2005). Approximately 70% of the population in Canada and 90% in Germany have used herbal medication, with the number of medical doctors who obtained training in the use of natural remedy medicine nearly increased to 10800 between 1995 and 2000 in Germany (WHO, 2003). In France and Australia, approximately 49% of the population use herbal medicine (Abdullahi, 2011). About 158 million adults have been reported to use complementary medicine in the United States (WHO, 2003). Approximately USD 17 billion was spent on herbal remedies in the United States in 2000 and USD 230 million spent annually in the United Kingdom on alternative medicine. The worldwide market for herbal medicine now stands at over USD 60 billion annually and the trend is rising rapidly (WHO, 2003).

2.2 Medicinal plant use in Africa

Traditional African medicine is the oldest of all therapeutic technologies, and perhaps the most assorted. Africa is regarded as the cradle of humanity with a wealthy biological and cultural diversity marked by regional healing practise distinctions (Mahomoodally, 2013). Before the colonial period and the Europeans' advent of mainstream medicine, millions of Africans depended exclusively on herbal medicine as the only accessible health care scheme in rural and urban societies (Abdullahi, 2011). The difficulty or failure to access modern health care owing to the price or inadequacy of health care suppliers and the absence of efficient Western medical treatment for certain illnesses such as malaria and HIV/AIDS, which are global diseases but have the worst affected Africa, could have caused ongoing interest in herbal therapy in the African health care (Mahomoodally, 2013).

Africa has an enormous variety of approximately 40 to 45 000 plant species, about 50 000 of which have been utilized in the African Traditional Medicine System (Mahomoodally, 2013).

Research has shown that African medicinal plants accumulate significant secondary metabolites in order to be able to resist the typical tropical ultraviolent rays of the sun and also to prevent various pathogenic assaults. Hence, these plants show more ability to amass chemoprotective biocompounds to survive in harsh environments than species from the northern hemisphere. A study undertaken by Abegaz et al. (2004) on different species of *Dorstenia* showed that only species from the tropical rainforest in Central Africa had more biological activities than related ones from outside the tropics (Manach et al., 2004; Mahomoodally, 2013). Although attitudes or emotions to herbal medicine in Africa have been simultaneous and contradictory (Bello, 2006; Abdullahi, 2011), herbalism is still practiced even in modern Africa after ages of its existence without much-reported negative impacts (Okigbo and Mmeka, 2006).

In Africa, there is a growing demand for herbal therapy for the management of rheumatic and neurological complaints (Carpentier et al., 1995). According to WHO (2003), up to 80% of African populations rely on herbal medication for their health care needs. Chirdan et al. (2008) reported the use of herbal remedies at home as the first option of therapy in Ghana, Mali, Zambia and Nigeria for about 60% of kids with elevated fever as a consequence of malaria. Convulsion also known as "*degedege*" in Tanzania has been extensively managed and treated with plant products, and over 70% of Ghanaians rely on phytotherapy (Abdullahi, 2011). In Nigeria, some patients suffering from hypertension integrated herbs into their conventional treatments (Amira and Okubadejo, 2007).

Apart from the insufficient technical requirements and quality control norms for the use of herbal medicinal systems in Africa, the rapid loss of natural habitats of some medicinal plant species as a consequence of anthropogenic activities and the rapid erosion of traditional knowledge is also a pressing problem (Mahomoodally, 2013). In the African continent, the greatest rate of deforestation is noted and, despite its privileged biodiversity, the continent has

only a few drugs sold to its credit worldwide (Atawodi, 2005). Scientific validation, documentation and sustainable use of African medicinal plants are therefore needed.

2.3 Medicinal plant use in South Africa

South Africa has a substantial lineage of traditional medicinal plants anchoring around 300 000 flowering plants, of which about 2 100 are geophytes (Louw et al., 2000). This constitutes approximately 10% of the higher plant species globally (van Wyk and Gericke, 2000). South Africa is the third most bio-diverse country in the world and for centuries employed the help of indigenous medicinal plants (Street and Prinsloo, 2012). Trading in medicinal plants is a significant component of South Africa's regional economy, trading more than 700 plant species (Dold and Cocks, 2002). The cultivation of medicinal plants has become a source of rural self-employment, generating income for rural poor people. It is predicted that 27 million natives in South Africa are using natural herbs (Ngwenya et al., 2019) and therefore demand exceeds supply.

Minimum attention has been given to the survival of medicinal plants in southern Africa until the late 1980s. However, due to destructive harvesting and the rapid increase in illicit trade in medicinal plants, the management of these natural plant resources became a matter of urgency (Zchocke et al., 2000). About two-thirds of medicinal plant species in use are harvested from the wild, resulting in declining populations, loss of genetic diversity, local extinction and degradation of habitat (Ngwenya et al., 2019). On this basis, Canter et al. (2005) reported that between 4 000 and 10 000 medicinal species around the world could now be endangered. Dold and Cocks (2002) carried out a survey on the trade in medicinal plants in Eastern Cape Province that identified a minimum of 166 plant species providing 525 tons (476272 kg) of plant material valued at R27 million per year. About 27 million black South Africans use herbal medicines

for a variety of ailments (Abdullahi, 2011). *Boophone disticha* is one such plant species that is integrated in herbal medicine.

2.3.1 *Boophone disticha* (L.f.) Herb.

Boophone disticha belongs to the Amaryllidaceae family, which is highly represented in the Southern African region and feeds at least 250 of the world's approximately 850 species (Meerow and Snijman, 1998). *Boophone distcha* is a native African bulbous tropical and subtropical flowering plant (van Wyk et al., 1997). The plant is generally referred to in Southern African areas as the century plant, toxic bulb, sore eye flower; *gibfol*, *seeroogblom*, *kopseerblom*, *Boesmangif* and *losespook* in Afrikaans; *khutsana-yamaha* and *motlatsitsa* in Sesotho; *incube* and *siphahluka* in Swati and finally *incotho*, *incwadi*, *isishwadi* and also *ibhade* in isiXhosa and isiZulu (Willams et al., 2017).

Boophone is a genus that is made up of two species, *B. disticha* and *B. haemanthoides* (F.M.) Leight (Meerow and Snijman, 1998). There are four types of spelling for the Amaryllidaceous genera *Boophone* by William Herbert, namely *Boophone*, *Buphane*, *Boophane* and *Buphone*, but most taxonomists have accepted that *Boophone* is correct (Archer et al., 2001). *Boophone distcha* was previously called *Buphane toxicaria*, *Haemanthus toxicarius*, *Amaryllis disticha*, *Brunsvigia toxicaria* and *B. toxicarius* (Huttleston, 1960; Archer et al., 2001; Willams, 2012). The genus etymology is a direct warning from the Greek 'bous' = bull, and 'phontes' = killer of, that eating the plant could be deadly to animals (Arche et al., 2001).

2.3.1.1 Distribution and morphology

Boophone species are widely distributed throughout southern Africa to tropical Africa (van Wyk et al., 1997). *Boophone disticha* is the most common and variable member, occurring primarily in the areas of summer rainfall, whereas *B. haemanthoides* was discovered primarily in Namaqualand and South Africa's Western Cape, in regions with winter rainfall. *Boophone disticha* is generally found in open grasslands, but it can grow in most areas where soils are well drained and enough sunlight is present (van Wyk et al., 1997).

The bulb has symmetrically arranged broad leaves that are placed in a peculiar way. The bulb was often referred to as a candelabra bulb because of the arrangement of the leaves (Watt and Breyer-Brandwijk, 1962). Like most plants, this plant blossoms during the spring season and its leaves grow to 45 cm in length and 5 cm in width during the spring and summer seasons. A bulb, which is fully grown at an average height, is estimated to have a diameter of 10-15 cm and is partially exposed above the ground. The bulb is covered with a variety of papery scales that cover the fleshy section. The rounded inflorescence has many attractive pink flowers all at the same distance from the primary stem. The dispersal of the seed of the plant is accomplished when the flower dries out and the wind breaks it out into its surroundings (van Wyk et al., 1997). *B. disticha* does not flower regularly and will not flower for 2 years after repositioning (van Wyk et al., 1997).

Figure 2.1: *B. disticha* showing its bulb and leaves.

Figure 2.2: *B. disticha* showing dense umbel of flowers (Cheeseman, 2013).

2.3.1.2 Medicinal uses

In the literature, *B. disticha* has been quoted for its extensive use in the treatment of multiple disorders. The plant bulbs are the richest alkaloid source, particularly the scale epidermis (Frohne and Pfänder, 2004). Mucilage-filled rap hide cells are also said to contain a big quantity of alkaloids that are produced in all areas of the plant (Frohne and Pfänder, 2004), which is why *B. disticha* is used for treating ailments by a large number of nations.

The application of *B disticha* in medicine has been known for many decades among the native South Africans (Watt and Breyer-Brandwijk, 1962). The hunter-gatherers and herders (Khoi / San) used the plant as both an arrow and a sedative. Some Nguni and Sotho tribes use concoction, extracts, and bulb infusions to treat many illnesses, including burns, wounds, pain, nausea, depression, routine medical disorders, and psychotic symptoms (Hutchings et al., 1996). The most influential use of the plant by these people is its narcotic impact; for example, daze in newly circumcised initiates, sedation of individuals with serious mental illness, and hallucinatory impact during divination practices (De Smet, 1996; van Wyk et al., 2002).

Though highly toxic, *B. disticha* is one of the most commonly used medicinal bulbs in Southern Africa. The fleshy inner bulb scales are boiled and used as a hot compress for oedema treatment (van Wyk et al., 2002). Bulb infusions are prescribed to adults with nausea, abdominal pain, exhaustion, extreme chest pain, constant bladder pain and eye issues by mouth or as enemas. The bulb is also used to treat swollen ulcers and to relieve urticaria, as well as to treat cancer (Watt and Breyer-Brandwijk, 1962). The plant is used in cultural South African Basotho rituals; for example, at the beginning of the initiation period, Basotho boys receive food blended with the bulb and other ingredients (Philander, 2011). This was done in order to fill them with the qualities of their ancestors and with the intension of making men out of them. The indications of intoxication were seen as a signal that their bodies had joined the spirit of manhood.

The bulb is used in Mozambique for magical reasons like "assisting the soccer team win". Manyika people grow *B. disticha* as a blessing to fend off bad dreams, bring forth great luck and rain outside their shelter (Neuwinger, 1994). The *B. disticha* leaves are removed and worn as ornamental body decorations to create fringes. The plant is commonly used in traditional remedies in Zimbabwe and throughout the area to treat various diseases, including boils, burns and agitation (Gelfand et al., 1952). Moisturized scales are applied to boils, ruptured wounds and abscesses to alleviate pain and remove pus (Watt and Breyer-Brandwijk, 1962). Unfortunately, its psychoactive characteristics have contributed to *B. disticha* gradually being used for leisure activities, particularly by young people in Zimbabwe and throughout the region (Acuda and Eide, 1994). This activity was reported to cause toxicity in some fatal cases (Gelfand & Mitchell, 1952; Laing, 1979; Gelfand et al., 1985; du Plooy et al., 2001). After medicinal execution, bulbs have been confirmed to have caused severe and deadly human poisoning (du Plooy et al., 2001).

Both species of *Boophone* have recently been introduced to cultivation (Bryan, 2002). While *B. haemanthoides* are extremely decorative, *B. disticha*, the most prevalent species, is worthy of account due to its fan-shaped foliage and big flower umbels. *Boophone disticha* is extremely decorative and can be cultivated on a sunny veranda in the garden or planted in big containers.

Table 2.1: Summary of the ethno-pharmalogical uses of by several South African ethnic groups, adapted from Nair and van Staden (2013), in the practice of traditional medicine.

Group of use	Characterization of traditional use	Reference
Cultural and dietary	• Bulbs used as an external treatment for initiation bruises by Sotho and Xhosa boys; • The leaves are trimmed to form fringes worn as stylish body accessories • Hunter gatherers and herders of the Cape utilized the bulb as an arrow and dart poisoning	Hutchings et al. (1996), Nair et al. (2013), Viladomat et al. (1997), Watt and Breyer-Brandwijk (1962), Hutchings et al. (1996) Bisset (1989), De Smet (1996, 1998), Neuwinger (1994),

	• Sotho shepherd boys used curled-out bulbs before tin cutlery to steam the milk	Van Wyk et al. (2002, 2005), Watt and Breyer-Brandwijk (1962)
	• After piercing, the bulb scales were used to dress Zulu people's earlobes	Watt and Breyer-Brandwijk (1962)
	• Early European settlers placed bulbs under mattresses to relieve insomnia	Hutchings et al. (1996)
	• Nibbled dried leaves used to treat substance dependence in the Cape	Van Wyk et al. (2002)
		Philander (2011)
		Hutchings et al. (1996)
Welfare	• Dry bulb scales were used to recover body strength.	Verzár and Petri (1987)
	• Water from fresh bulbs has been drunk to improve sexual endurance	Verzár and Petri (1987)
	• Bulbs used to enhance mental vitality	Risa et al. (2004a, 2004b), Stafford et al. (2008)
Personal injury	• Most South African ethnic groups (as well as early European Cape colonizers) use bulbs for skin irritation, bruises, burns, cuts, wounds, boils and swelling. Treatment is thought to relieve pain and remove pus.	Cheesman et al. (2012) Grierson and Afolayan (1999) Hutchings et al. (1996) Mabona and Van Vuuren (2013) Van Wyk et al. (2002, 2005) Watt and Breyer-Brandwijk (1962)
Gastrointestinal	• The leaves are consumed as an inherent cleanser.	Philander (2011)
	• Bulb decoctions are used as antidiarrheal and respiratory depressants.	Hutchings et al. (1996)
	• Fresh root and bulb decoction used for constipation	Hutchings et al. (1996) Wintola and Afolayan (2010)
Urinary	• Bulb mixture used to detoxify the uterus and bladder	Hutchings et al. (1996)
		Philander (2011)
		Viladomat et al. (1997)
		Hutchings et al. (1996)
		Philander (2011), Viladomat et al. (1997)
Circulatory	• Bulbs used for the purification of blood	Hutchings et al. (1996)
	• Leaves used to reduce bleeding	Philander (2011), Viladomat et al. (1997)
Respiratory	• Zulu and San people use papery outer scales to manage asthma	De Beer and Van Wyk (2011), van Wyk et al. (2008) and
	• Bulbs used to relieve dyspnoea	Watt and Breyer-Brandwijk (1962)
	• Zulu women roll snuff on a piece of dried bulb scale to improve the efficacy of the snuff	
Muscular	• Decoction of the bulb used for muscular discomfort and tension	Viladomat et al. (1997), Watt and Breyer-Brandwijk (1962)
	• Zulu patients with 'inkwatshu' are administered oral bulb decoction, marked with calf and finger muscle cramping and toe tightness.	Hutchings et al. (1996), Viladomat et al. (1997)

Neurological	• Roots are charred, grinded and the powder added to the paralysed region	Verzár and Petri (1987)
	• For Touws River hysteria, an early European remedy required lying on a mattress packed with bulb scales	Watt and Breyer-Brandwijk (1962)
	• Bulbs from the Umtentweni region of the south coast of KwaZulu-Natal are required for the management of 'fufunya'	Nielsen et al. (2004), Watt and Breyer-Brandwijk (1962)
	• Bulb derivatives are prescribed orally for stress-related diseases in Sotho, Xhosa and Zulu.	Nyazema (1986), Pedersen et al. (2008), Sobiecki (2008)
	• Bulb infusions drunk for the relief of various mental disorders, including clinical depression;	Hutchings and Van Staden (1994)
	• Extracts of the bulb used for age-related alzheimer's disease	Neergaard et al. (2009), Pedersen et al. (2008), Sandager et al. (2005), Stafford et al. (2008)
		Risa et al. (2004a, 2011b), Stafford et al. (2008)
Inflammatory conditions	• Bulbs that have been softened contain milk used by early Europeans in Lyndenburg to treat distinct inflammatory conditions.	Hutchings et al. (1996), Botha et al. (2005), Van Wyk et al. (2002, 2005),
	• The bulbs are used both for the management and treatment wounds and for ocular diseases.	Verzár and Petri (1987), Watt and Breyer-Brandwijk (1962)
	• Weak bulb decoctions administered orally or via enema to Zulu adults for stomache, headache, chest and bladder pain	Hutchings et al. (1996), Viladomat et al. (1997)
	• The Manyika apply bulb scales locally for relief from urticarial	Watt and Breyer-Brandwijk (1962)
	• Dried leaves moistened with milk or oil is used to treat skin diseases, varicose ulcers and phlebitis	Brandwijk (1962)
	• Fresh leaves applied by Zulu to check bleeding	Hutchings et al. (1996), Viladomat et al. (1997), Watt and
		Breyer-Brandwijk (1962
Cancer	• Bulb extract indicated for this purpose	Botha et al. (2005)
Malaria	• Extract of bulbs demonstrated to be good in the treatment of malaria	Watt and Breyer-Brandwijk (1962)
Tuberculosis	• Umtentweni native dwellers in Kwa Zulu Natal are known to use bulb infusion to cure tuberculosis	Watt and Breyer-Brandwijk (1962)

2.4 Phytochemistry of *Boophone*

In the Amaryllidaceae family, many classes of bioactive compounds such as chalcones, flavonoids, lectins, lignans, peptide terpenoids and isoquinoline alkaloids have given the group special chemical characteristics (Bastida et al., 2006). *Boophone disticha* and additional species of the Amaryllidaceae family are known to produce alkaloids that are extremely toxic (Nair et al., 2013). Since then, the early hunters have poisoned their arrows with bulb extracts for years.

(van Wyk et al., 2002). Due to the historical heritage of the traditional medicinal use of the plant in South Africa, it is not surprising that the plant was one of the first Amaryllidaceae species to be evaluated globally for alkaloid compounds (Tutin, 1911a, b; Lewin, 1912a, b; Tutin, 1913). *Boophone disticha* is also a hub for bioactive compound operations in the Southern African region. Tutin (1911a, b) carried out an initial phytochemical analysis of the plant where lycorin 2 was present with three other unidentified alkaloids. Following this, Lewin (1912a, b) separated a compound loosely referred to as "haemanthine," which Tutin (1913) retained as a blend. A number of compounds, such as furaldehyde, acetovanillone, chelidonic acid, copper, laevulose, pentatriacontane, aphytosterol, ipuranol and fatty acids, were at first known to be present in *B. disticha* (Tutin, 1911a) and its alkaloid contents have become increasingly important due to its remarkable biological properties. Nair and van Staden (2014) reported that the most broad phytochemical study carried out on the plant was that of Hauth and Stauffacher (1961) who described the presence of 11 alkaloids in ethanolic bulb extracts, including buphanidrine13 (19.4%), undulatine14 (18.6%), buphanisine 15 (16.9%), buphanamine 11 (14.1%), nerbowdine 12 (11.1%), crinine 3 (7.2%), distichamine 16 (5.4%), crinamidine 17 (1.2%), acetylnerbowdine 18 (0.6%), lycorine 2 (0.4%) and buphacetine (0.3%). Viladomat et al. (1997) and Nair et al. (2013) confirmed most of these compounds by physical and spectroscopic means, buphacetine's identity continues a mystery to this day. Interestingly, Nair et al. (2012 b) also identified buphanidrine (46.9%), buphanisine (23.9%), crinine and distichamine (7.3% and 21.9%) in *B. haemanthoides.*

Boophone disticha's latest phytochemical inquiry resulted in the isolation of 6-hydroxycrinamine 19 from the bulb methanol extract at a concentration of 0.01 percent dry weight (Adewusi et al., 2012). It is apparent from these collective outcomes that the alkaloids produced by *B. disticha* are all Amaryllidaceae alkaloids' crinane series, with the exception of lycorine 2. In addition, 6-hydroxycrinamine 19 was the only recognized compound in the α-

crinane sub-series (Adewusi et al., 2012), while the other compounds were characteristic of β-crinane alkaloids (Hauth and Stauffacher, 1961; Sandager et al., 2005; Neergaard et al., 2009; Cheesman et al., 2012).

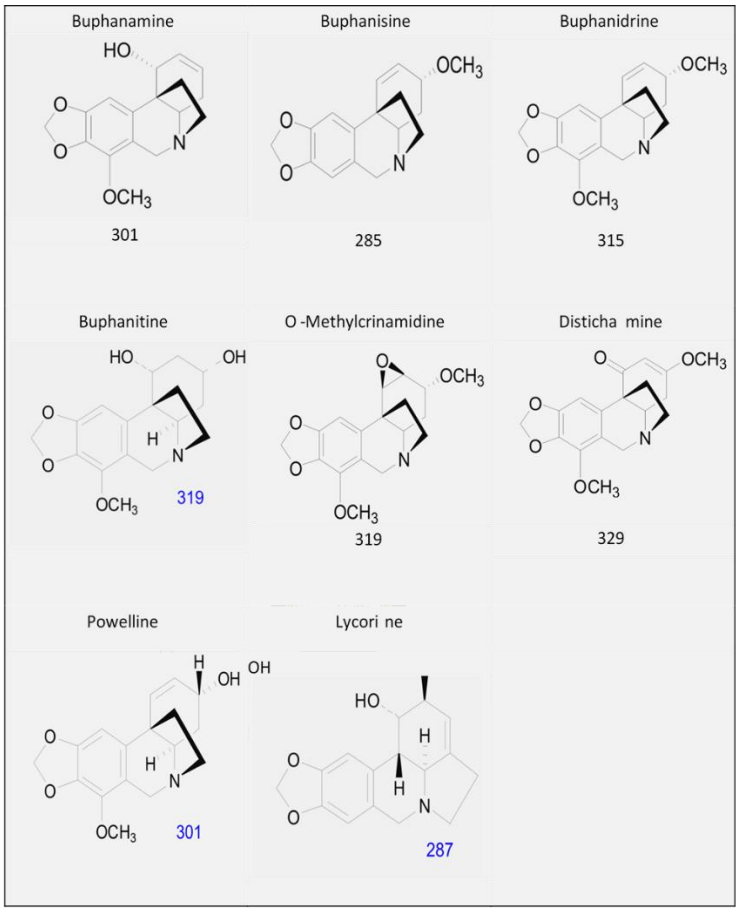

Figure 2.3: Structures of some of the major alkaloids derived from the *B. disticha* bulb. The nominal mass of each structure is shown below each structure (adapted from Steenkamp, 2005).

2.5 Pharmacological uses of *Boophone disticha*

2.5.1 Anti-inflammatory

In South Africa, *B. disticha* is extensively used to treat 'inflammation-related disorders', 'wounds and infections' and 'diseases affecting the central nervous system' (Philander, 2011; Nair and van Staden, 2014). *Boophone disticha* has been reported to be used in the management of inflammatory diseases, bacterial infections, cancer rehabilitation and mummification (van Wyk, 2008). Botha et al. (2005) performed an *in vitro* analysis on *B. disticha* extracts for the production of adenosine triphosphate (ATP) in purified human neutrophils and neutrophil inhibition of the release of superoxide to confirm the so-called / unproven effect of the plant on the immune system and inflammatory reactions. The research findings demonstrated a substantial boost in the manufacturing of superoxide by the neutrophils, which provided an explanation for its traditional use in relieving rheumatic pain, muscle sprains, and other inflammatory diseases. Verification of *B. disticha*'s use for inflammation-related conditions also came from inhibition research of cyclo-oxygenase (COX), in which the plant's ethanolic bulb extracts exhibited (Jäger et al., 1996). Citoglu et al. (1998) reported that lycorine 2 is reported to exhibit anti-inflammatory properties for the activity of specific chemical extractives as demonstrated by the carrageenan-induced paw oedema test in rodents. A study by Elgorashi et al. (2003) showed mild anti-inflammatory activity as determined by COX inhibition tested on *B. disticha* known alkaloid components.

2.5.2. Biological activity

Boophone disticha is a common representative of South African Amaryllidaceae used in the management of lacerations and infections (Rabe and van Staden, 1997). Several experiments have been performed to test the plant's effectiveness against bacterial pathogenicity (Cheesman et al., 2012). In a study conducted by Heyman et al. (2009), the bulb's ethanol extract was active against methicillin sensitive *Staphylococcus aureus*. Additional work on *B. disticha* by Cheesman et al. (2012) showed that bulb extracts were active against two Gram-positive (*Bacillus subtilis* and *S. aureus*) and two Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) strains. Although *B. disticha* is not stated for such reasons, several others have shown antifungal or anti-yeast activity including Amaryllis belladonna, *Crinum macowanii* and *C. moorei* (Viladomat et al., 1997).

Lycorine 2 and vittatine 21 have been recorded with significant activity against *Candida albicans* (MICs of 39 and 31 µg/mL, respectively) (Evidente et al., 2004). Lycorine 2 has been shown to be effective against several bacteria in the operations of individual compounds (Bastida et al., 2006), including poliomyelitis virus at levels as low as 1 µg/mL (Ieven et al., 1982). Studies of the connection between structure and activity involving Herpes simplex virus showed that lycorine exercised its antiviral impact by preventing the activity of DNA polymerase (Ieven et al., 1983). Homolycorine 4, tazettine 5, narciclasine 22 and haemanthamine 23 are other family compounds that have demonstrated antiviral activity in model research (Bastida et al., 2006).

In a report by Watt and Breyer-Brandwijk (1962), *B. disticha* was considered to be used traditionally for the treatment of malaria, although no studies have been conducted to assess the effectiveness of plant extracts against malaria parasite. Several studies have been reported on the toxicity of *B. disticha* and several studies have been conducted to prove and validate its

fatal impact it has on the human life (Galada, 2010). This study focused more on the medicinal potential and pharmacological uses of *B. distcha* and how it can be used as a drug curative in the medical world

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CHAPTER THREE

THE QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *B. DISTICHA*

3.1 Background of study

Medicinal plants or herbs have been reported to possess properties that can impart health benefits for the wellbeing of mankind (Alves et al., 2000). Scientific research has been carried out over the last few years on traditional herbal remedies for a variety of diseases and this has led to the development of new medicines and therapeutic strategies (Shalavadi et al., 2019). Medicinal plants have organic bioactive compounds such as tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids that play an important physiological role in the human body (Edeoga et al., 2005). Phytochemicals are categorized as primary and secondary metabolites. Compounds that are directly associated with the normal development and growth of a plant are referred to as primary metabolites (Geetha and Geetha, 2014). They are common in nature and develop in nearly all species in one form or another e.g., chlorophyll, nucleotides, and amino acids (Geetha and Geetha, 2014). Primary metabolites play an essential role in metabolic processes such as photosynthesis, respiration and nutrient assimilation (Rout and Sahoo, 2015). They are further on used in manufacturing of raw material and as food additives. Smetanska (2008) reported that secondary metabolites are synthesized during the plant's secondary metabolism. Since they have important medicinal properties, they are the fundamental basis for developing many pharmaceutical drugs.

A large variety of medicinal plants are regarded as potential sources for cost effective drugs that are easily accessible and affordable to the general public with less or no side effects (Shalavadi et al., 2019). In an attempt to discover and understand the structures of organic

compounds responsible for plant healing properties, a variety of methodologies have been built to this end (Preethi, 2010).

Plants produce phytochemicals which are a variety of compounds with therapeutic properties that are anticarcinogenic and antimutagenic (El-Sherbiny et al., 2016). Phytochemicals promote plant growth and thus protect plants from pests, damage and add to the colour, fragrance and taste of the plant (Velavan, 2015). The main secondary metabolites include alkaloids, tannins, flavonoids, phloblatannins, saponin and cardiac glycosides (Laveena and Chandra, 2017). Kabera et al. (2014) stated that all secondary metabolites have specialized roles, with saponins possessing antifungal activity, some alkaloids may be helpful for HIV infection, flavonoids have significant anti-cancer effects, and tannins possess antimicrobial activity. *Boophone disticha* is known for its high alkaloid content and the information about other phytochemicals is scarce.

The primary goal of this chapter was to assess the qualitative and quantitative phytochemicals present in the plant.

3.2 Material and methods

3.2.1. Plant collection

The *B. disticha* plant was harvested from Seymour, a town in the Raymond Mhlaba Municipality in the Eastern Cape South Africa, situated at 32° 33' 0" South, 26° 46' 0" East. The plant was identified and certified by herbarium intern Amkelani Bester under the supervision of Professor Cupido at the Giffen Herbarium of the Department of Botany at the University of Fort Hare. The voucher specimens were stored at the herbarium, and the voucher number was UFH 1653.

3.2.2. Extract preparation

The collected plant material was cleaned and the parts of the plant were divided into four distinct parts, i.e. the roots, the inner bulb scales, the leaves and the outer bulb scales. The parts of the plant were washed thoroughly and cut into small pieces. The plant parts were dried in the open ambient temperature and avoided direct sunlight to help deter the denaturation of important and necessary bioactive compounds. The drying period was four weeks. After drying, the plant material was ground with an electrical metal blender. The initial weight for the powered plant material was 115 g for the roots, 135 g for the inner bulb, 120 g for the leaves and 125 g for the outer bulb. Two samples and 500 ml of each solvent (distilled water and methanol) were prepared for each part of the plant. The bottles were then labelled according to the solvent and plant part.

The solution was then left in an orbital shaker for 48 hours and shaken at 155 rpm. To obtain a liquid free of plant residues, the extracts were sieved using a Buchner funnel and filter paper (Whatman no. 1). The methanol extracts were concentrated to total dryness under reduced pressure at 64.7℃ using a rotary evaporator, while the aqueous extract was freeze-dried for 48 hours at 40℃ using a freeze dryer.

3.3. Phytochemical analysis

3.3.1. Qualitative analysis

The following basic techniques were used for the phytochemical screening of selected plant parts to evaluate the presence of phenols, tannins, alkaloids, saponins, flavonoids, steroids, terpenoids, cardiac glycosides and anthraquinones.

3.3.1.1 Test for phenols

In the test for the presence of phenols, 2 ml of dissolved extract was diluted with 2% of $FeCl_3$ as defined by Harborne (1973). The availability of phenols was suggested by a blue-green or black coloration.

3.3.1.2. Test for tannins

To determine the presence of tannins, Sofowora (1993) reported that 2 ml of plant extracts were boiled in a water bath for 5 minutes. Approximately 3-6 droplets of 0.1% (w / v) of $FeCl_3$ solution were added to each test tube containing an extract, and the presence of tannin was indicated by the formation of brownish-green or blue-black dye.

3.3.1.3. Test for saponins

The nature of saponins was assessed as defined by Harborne (1973) with slight modifications. In a water bath, 2 ml of plant extract was simmered and dissolved in 20 ml of distilled water. Then, 10 ml of the filtered plant extract was mixed with 5 mL of distilled water, thoroughly shaken, and watched for the formation of a consistent, long-lasting foam. The foam was then mixed with three globules of olive oil, shaken, and examined for emulsion formation as a saponin indicator.

3.3.1.4. Test for flavonoids

The procedure described by Sofowara (1993) was used to determine the presence of flavonoids. About 1 ml of the plant sample was diluted with 10 ml of $C_4H_8O_2$ for 3 minutes. The presence

of flavonoids was indicated by a yellow colour pattern after 1 ml of NH_4OH was titrated into the mixture.

3.3.1.5. Test for steroids

Harborne's test (1973) was used to determine the presence of steroids. 2 ml of $C_4H_6O_3$ was mixed with 1 ml of crude extracts and 2 ml of concentrated H_2SO_4 at first. Steroids were noted as present because the colour change from violet to blue.

3.3.1.6. Test for terpenoids

For the determination of terpenoids, plant extracts were dissolved by adding 5 ml of plant extracts to 2 ml of chloroform and carefully adding 3 ml of H_2SO_4 to form a layer. The presence of terpenoids was indicated by the layer's reddish-brown colour (Harborne, 1973).

3.3.1.7. Test for cardiac glycosides

The presence of cardiac glycosides was determined using the procedure described by Trease and Evans (1989), with slight modifications. The extracts were dissolved by diluting 5 ml of the extract with 2 ml of glacial acetic acid containing a drop of $FeCl_3$. The concentration of 1 ml of H_2SO_4 was superimposed. Then the formation of a brown ring at the edge was an indication of the presence of cardenolide signature deoxysugar. As a result, a violet band appeared beneath the brown band, while a green band formed in the acetic acid's thin layer.

3.3.1.8. Test for anthroquinones

The method described by Harborne (1973) was used to test for the presence of anthroquinones. About 5 ml of the extract was simmered with 10 ml of H_2SO_4 and then extracted while it was still hot. 5 ml of chloroform was added to the filtration system and shaken. The chloroform layer was removed with a pipette and pipetted into another test tube, where 1 ml of dilute ammonia was added. The effect of anthroquinones in the resulting solution was demonstrated by a pinkish red colour.

3.3.1.9. Test for alkaloids

The method described by Harborne (1973) was used; during this experiment, about 2 ml of plant extract was mixed with 5 ml of 1% HCl and heated in a water bath between 70°C – 80°C. About 1 ml of the solution was mixed with 3-4 droplets of Mayer's and Dragendroff's reagent. The subsequent murkiness of each of these reagents was taken as an effort to validate the existence of alkaloids in the extract.

3.4. Quantitative analysis

Phytochemicals that were detected in all plant parts, i.e., phenols, tannins, flavonoids and alkaloids, were quantified using standard procedures.

3.4.1. Total Phenol content

The Folin and Ciocalteu method was used to calculate the total phenolic content with slight adjustments as described by Prabhavathi et al. (2016). The extract solution was prepared by dissolving 200 µl of the extract with 800 µl of the Folin and Ciocalteu reagent and an additional

2 ml of 7.5% (w/v) Na_2CO_3. 7ml of distilled water was added to the mixture and the tubes were incubated in the dark for 2 hours. A spectrophotometer was used to determine absorbance at 765 nm. The study was carried out three times. The gallic acid standard / calibration graph formula, y = 0.0004 x + 0.0236 R2 0.9923, was used to estimate the phenol content, which was expressed as mg gallic acid equivalent (GAE)/g from the calculation CV/m; where "C" is the concentration as obtained from the standardization curve equation in mg/ml, "V" is the volume of the extract used in the assay in ml and "m" is the mass of the extract used in the assay in "g".

3.4.2 Total Tannin content

The total tannin content was determined using the Folin and Ciocalteu method modified by Hossain et al. (2019), where the extracts were re-dissolved by diluting 0.1 ml of each extract with 7.5 ml of sterile distilled water. With the use of a pipette, 0.5 ml of the Folin Ciocalteu phenol reagent was added into the test tubes of the diluted extract solution. An additional 1 ml of 35 % of Na_2CO_3 was added to the mixture and balanced using distilled water to 10 ml. The tubes were vortexed and, after 30 minutes, the absorbance was read and recorded at 510 nm. Concentrations were carried out in triplicate. The tannin content was calculated using the calibration curve equation, y = 0.0004 x + 0.0236 R^2 0.9923, and was expressed as mg gallic acid equivalent (GAE)/g using the CV / m formula as defined in the above-mentioned phenolic assay.

3.4.3. Total flavonoid content

The total flavonoid content was determined using the $AlCl_3$ colorimetric method modified by Aiyegroro and Okoh (2010). The standard mixture was prepared by diluting 3 ml of each extract in this test with 0.2 ml of 10% (w / v) of $AlCl_3$, adding an additional 0.2 ml of 1 M of potassium acetate and 5.6 ml of distilled water to the mixture and allowing it to stand at room temperature for 30 minutes. The sample was spectrometrically measured at 420 nm. Quercetin was used as standard (1mg/ml). The analysis was carried out in triplicates. The content of the flavonoid was calculated using the calibration curve equation $y = 0.0027+0.0831$, $R^2 = 0.9976$ and expressed as mg of quercetin equivalent (QE)/g using the CV / m formula as defined in the above-mentioned phenolic assay.

3.4.4. Determination of Alkaloid content

The alkaloid content was determined based on the procedure of Omoruyi et al. (2012). The extracts were re-dissolved by mixing 5 g of each extract with 200 ml of 10% acetic acid in ethanol. The mixture was left to stand for 4 hours. The mixture was filtered and the filtrate concentrated into a water bath to one-fourth of its original amount. Drops of concentrated NH_4OH were administered to the extract before precipitation stopped. It was essential to stabilize the mixture as a whole and to wash and filter the accumulated precipitates with diluted NH_4OH. The collected residuals were dried and weighed. The alkaloid content was calculated using the following formula: % alkaloid = final weight of sample /initial weight of extract × 100.

3.5. Results

3.5.1. Qualitative analysis results

The results for the qualitative analysis of the phytochemicals are represented in Table 3.1. All screened plant extracts tested positive for the presence of, alkaloids, tannins, phenols and flavonoids. Cardiac glycosides were detected in most of the samples except in leaves methanol extract. Saponins were detected in root methanol extract, leaves methanol extract and aqueous extract of the inner scales of the bulb. The extracts prepared from the inner scales of the bulb and aqueous extracts from leaves and roots tested positive for the presence of terpenoids. Steroids were only detected in aqueous extracts. The anthraquinones were absent in all extracts.

Table 3.1: The qualitative phytochemical analysis of *Boophone disticha*

Solvent extract	Plant part	Tannins	Terpenoids	Saponins	Flavonoids	Cardiac glycosides	Alkaloids	Anthraquinones	Steroids	Phenols
Methanol	Root	+	-	+	+	+	+	-	-	+
	Bulb inner scales	+	+	-	+	+	+	-	-	+
	Leaves	+	-	+	+	-	+	-	-	+
Aqueous	Bulb outer scales	+	-	-	+	+	+	-	-	+
	Root	+	+	-	+	+	+	-	+	+
	Bulb inner scales	+	+	+	+	+	+	-	+	+
	Leaves	+	+	+	+	+	+	-	+	+
	Bulb outer scales	+	-	-	+	+	+	-	+	+

The (+) sign means present; (-) means absent.

3.5. 2. Quantitative analysis

The results for the quantitative analysis conducted on *B. disticha* are represented in (Table 3.2). Generally, the methanol extracts displayed higher total contents of the screened phytochemicals.

Total phenol content

In this study, the results of the total phenolic content, calculated from the calibration curve (Figure 3.1), are expressed in mg of gallic acid equivalent per gram (mg/g GAE). The methanolic extracts displayed a higher total phenolic content compared to aqueous extracts (Table 3.2). The highest phenolic content was observed from the methanol root extract (2414.67±1.53mg/g GAE), followed by the methanolic leaves extract (1560±1 mg/g GAE) and methanol bulb outer scales extract (1550.7±18.9 mg/g GAE). Concerning the aqueous extracts, *B. disticha* roots had a higher total phenolic content of 871.33±1.52 mg/g GAE, followed by leaves (549.67±1.53 mg/g GAE) and bulb inner scales (302±37.5 mg/g GAE).

Total tannin content

The total tannin content of the *B. distcha* extracts, calculated from the calibration curve (Figure 3.1), was expressed in mg of gallic acid equivalent per gram (mg/g GAE). The *B. disticha* methanol extracts displayed a higher total tannin content compared to the aqueous extracts, with the methanolic root extract having 527.33±2.08 mg/g GAE tannin content, followed by methanol extracts prepared from leaves (442.67±1.53mg/g GAE) and bulb inner scales (376.33±2.52mg/g GAE). The total tannin content for the aqueous extracts ranged between 35.33±2.52 mg/g GAE and 204.67±1.53 mg/g GAE.

Table 3.2. Total phenolic, tannin and flavonoid contents of *Boophone disticha* four selected plant parts extracted using aqueous and methanol

Solvent extract	Plant part	Phenols (mg/g GAE)	Tannins (mg/g GAE)	Flavonoids (mg/g QE)
Methanol	Root	2414.67±1.53^{a}	527.33±2.08^{a}	439.67±1.53^{a}
	Bulb inner scales	1395.33±2.52^{c}	376.33±2.52^{c}	136±0^{d}
	Leaves	1560±1^{b}	442.67±1.53^{b}	289.67±0.58^{b}
	Bulb outer scales	1550.7±18.9^{b}	47.33±2.08^{f}	103±0^{e}
Aqueous	Root	871.33±1.52^{d}	204.67±1.53^{d}	243.67±1.53^{c}
	Bulb inner scales	302±37.5^{f}	164.67±3.51^{e}	77.67±1.53^{f}
	Leaves	549.67±1.53^{e}	167.33±2.08^{e}	134±2^{d}
	Bulb outer scales	117.33±2.08^{g}	35.33±2.52^{g}	nd

Data expressed as mean ± SD; $n = 3$, values with the same superscripts do not differ significantly ($P < 0.05$), the subscript indicates. ND- not detected

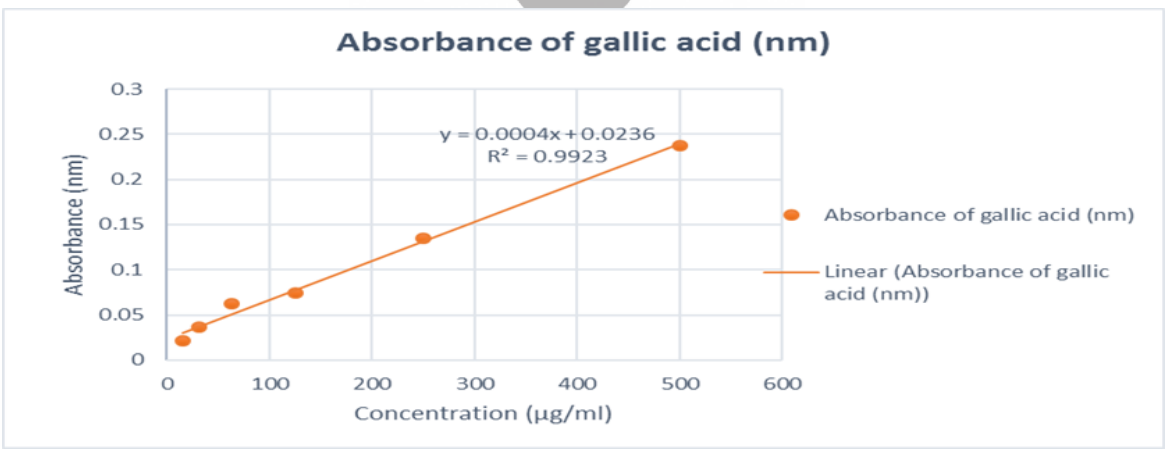

Figure 3.1 Gallic acid standard curve.

All tests were done in triplicate (n=3) used both for the phenol and tannin content

Total flavonoid content

The total flavonoid content of the above-mentioned plant parts, calculated from the calibration curve (Figure 3.2), was expressed in mg of quercetin equivalent per gram (mg/g QE). The higher flavonoid content was recorded from the root methanol extract (439.67±1.53 mg/g QE), followed by methanol leaves (289.67±0.58 mg/m QE), bulb inner scales (136±0 mg/g QE) and bulb outer scales (103±0 mg/g QE) extracts. Concerning water extracts, roots recorded a higher flavonoid content of 243.67±1.53 mg/g QE, with leaves displaying a total content of 134±2 mg/g QE. No flavonoid content was detected with the aqueous extract prepared from bulb outer scales.

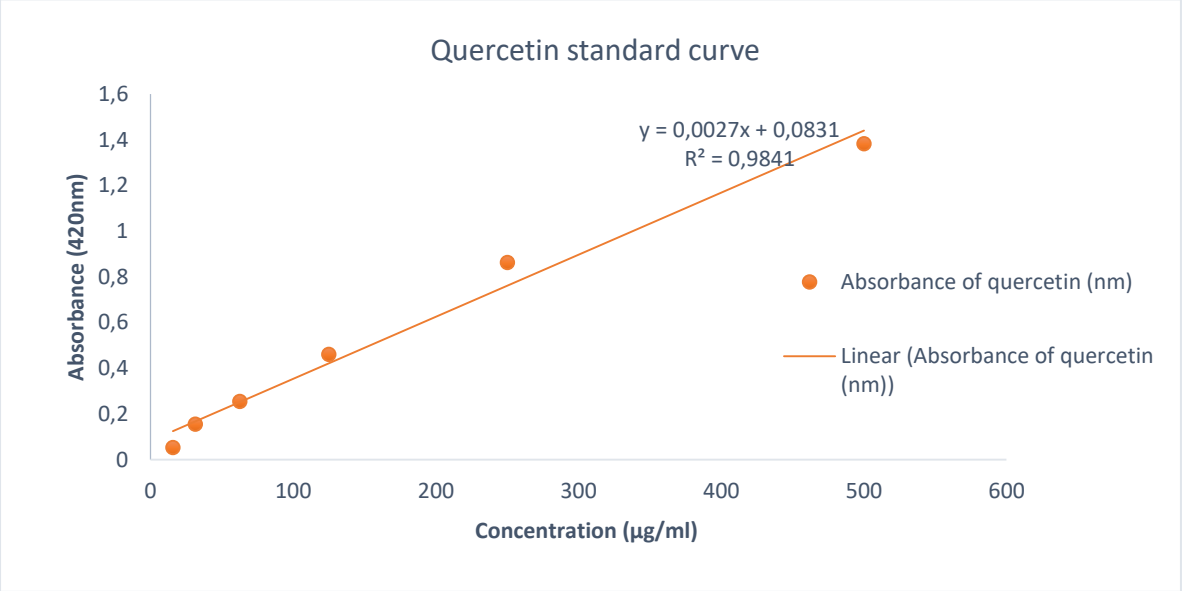

Figure 3.2: Quercetin standard curve

Total alkaloid content

The results for the alkaloid content are represented in Figure 3.3. The methanolic extracts had a higher alkaloid content with the methanolic roots extract displaying a highest alkaloid content of 1956 mg/ml, followed by extracts prepared from the inner bulb scales with an alkaloid

content of 1272 mg/ml and leaves (1114 mg/ml). Concerning the aqueous extract, roots had a higher content of 721 mg/ml.

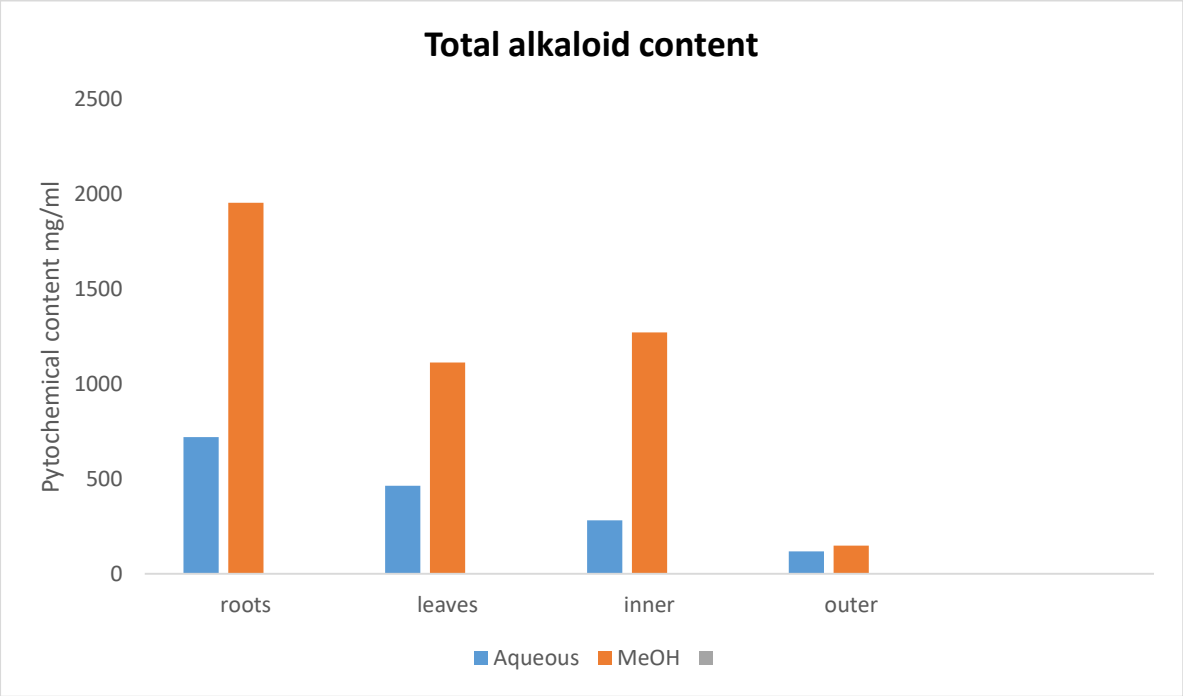

Figure 3.3: The total alkaloid content in different extracts of *B. disticha*

Values are means +-SD, n=3, P<5

3.6 Discussion

The preliminary qualitative phytochemical analysis of different plant parts of *B. disticha* revealed the presence of phenols, tannins, flavonoids, saponins, steroids, terpenoids, alkaloids, and cardiac glycosides. These phytochemicals are thought to possess therapeutic and biological properties (Sofowora, 1993). The presence of alkaloids was expected as presented in the literature (Cheeseman et al., 2012; Ferrer-Serrano et al., 2020; Tonisi et al., 2020). *Boophone disticha* is documented among the first species of Amaryllidaceae to be assessed worldwide for alkaloid components (Tutin, 1911a, b; Lewin, 1912a, b; Tutin, 1913). Alkaloids are the

most common type of secondary plant metabolite, consisting primarily of nitrogen bases synthesized from amino acid building blocks with numerous fundamentalists substituting one or more of the peptide chain's hydrogen atoms, regularly containing oxygen (Omojate et al., 2014). For millennia, alkaloids have been correlated with therapeutic uses, and one of their most significant biochemical activity is cytotoxicity (Pandurangaurthy et al., 2015). A few studies reported the analgesic, antispasmodic, and antibacterial properties of alkaloids (Okwu and Okwu, 2004). The plant acts as a hub for phytochemical activities from the southern region of Africa. Omojate et al. (2014) reported that saponins were responsible for producing an inhibitory effect on inflammation. Saponins are deadly toxic because they cause blood hemorrhage and have been linked to cattle intoxication. They have an unpleasant and sulphurous taste, and they irritate the mucosal membranes (Sodipo et al., 2000). Steroids have been described to have antibacterial properties (Raquel, 2007) and are compounds that are extremely important, owing to their interactions with other substances such as sex hormones (Nobori et al., 1994).

Concerning quantitative phytochemical analysis, the methanolic root extracts displayed the highest phenolic, tannin, flavonoid and alkaloid contents. Methanolic solvents have high polarity; they can extract a diverse range of plant constituents than other solvents (Paulsamy and Jeeshna, 2011; Senguttuvan et al., 2014). In this study, the phenolic compounds extracted were relatively high for all plant parts tested. In the literature, phenolic compounds have been identified as common classes of plant metabolites with biological properties such as anti-apoptosis, anti-aging, anti-carcinogenic, anti-inflammatory, anti-atherosclerosis, cardiovascular defence and endothelial function enhancement, inhibition of angiogenesis and cell proliferation (Han et al., 2007; Nayak and Singh 2007). The higher total phenolic content detected in this study validates the *B. disticha*'s traditional use as documented by Nair and van Staden (2013) and for its role in the treatment of various ailments as documented above.

According to Krings and Berger (2001), a number of research findings indicated that the most prominent antioxidant properties in medicinal plants were in phenolic compounds. Natural antioxidants mainly come from plants in the form of phenolic compounds like flavonoids, phenolic acids, tocopherols etc. (Ali et al., 2008).

In this study, *B. disticha* exhibited a higher tannin content. Tannins are noteworthy because of their styptic and astringent properties (Gupta el al., 2019). They are reported to attach to proline-rich proteins and disturb the protein synthesis (Baron et al., 2019). Doughari (2012) and Omojate et al. (2014) reported that tannins are used as disinfectants and in the treatment of pathogenic diseases such as diarrhoea, with the presence of the phenolic group being responsible for this activity.

The *B. disticha* extracts exhibited a total flavonoid content. Flavonoids are hydroxylated phenolic substances presumed to be generated by plants in response to microbial infection and have been found to be antimicrobials against a broad range of *in vitro* microorganisms (Madduluri et al., 2013). According to Baron et al. (2019), their development is most likely due to their ability to interface with extracellular and dissolvable proteins as well as their engagement with the bacterial cell wall. Flavonoids are also powerful antioxidants with anti-cancer properties (Ravishankar et al., 2013). The substantial flavonoid content in this study, validates the traditional use of *B. disitcha* in wound healing and the treatment of a variety of diseases and conditions by traditional healers from the Eastern Cape province.

Consequently, the results of the present study suggest that the identified phytochemical compounds may be bioactive components and that *B. disticha* is an extremely important reservoir of substantially medicinal bioactive components.

3.7. Conclusion

It is evident that *B. disticha* is responsible for the treatment of various ailments. This is because the phytochemicals identified from this plant have distinctive characteristics that work hand in hand to qualify *B. disticha* as the wonder plant it is renowned to be. The extracts from these plants could be seen as a good source for useful drugs. The phytochemicals present in this study are relevant or agree with the traditional usage of this plant therefore further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Also, additional work is encouraged to clarify the possible mechanism of action of these extracts and at what dosage should the plant be administered as it is extremely toxic and yet of great medicinal value.

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CHAPTER FOUR

ANTIMICROBIAL ACTIVITY OF *B. DISTICHA*

4.1. Background of study

The emergence of new infections, including international antibacterial and antifungal resistance, is becoming a growing public health issue and hence the need for new antimicrobials is of paramount importance (Talbot et al., 2006). Communicable diseases are among the world's top causes of mortality (WHO, 2016). Several transmissible diseases are caused by pathogenic microbes and may exist in as many different species and strains. The discovery of antibiotics proved to be a tool used in the management of microbial infections and significantly improved the efficacy of human health (Laxminarayan et al., 2013). Over the last few decades, however, the dependence and use of antibiotics has contributed to the appearance and transmission of multidrug-resistant strains of a number of microorganism classes (Dantas and Sommer, 2014).

Cheeseman (2013) argued that the development of drug-resistant pathogens was due to overuse and misuse of antibiotics and that an increase in resistance was strongly influenced by an overall increase in the number of antibiotic prescriptions. Rapid development of drug-resistant strains has increased the prevalence of these types of communicable diseases and has contributed to the search for potential antimicrobials from different sources (Maposa et al., 2019). With the emergence of new drug-resistant strains, scientists are making further efforts to discover new and improved antimicrobials (Okeke et al., 2007). Medicinal plants are a good source of various active medicinal compounds (Mukherjee et al., 2010). A significant number of plants has been tested for their antimicrobial activity and various phytochemicals are recommended by experts worldwide (Zhang et al., 2013; Touqeer et al., 2014). Studies on medicinal plants are increasing rapidly not only because they serve as means of developing

new precautionary or curative drugs, but also because they are affordable and considered safer than western drugs (Olajuyigbe and Afolayan, 2012). Thus, the ethno botanical approach was used in this study to screen *B. disticha* for antimicrobial activity. *Boophone disticha* is a common representative of South African Amaryllidaceae used in the treatment of wounds and infections (Rabe and van Staden, 1997).

The aim of this study was to compare the antimicrobial activity of *B. disticha* aqueous and methanolic extract and its fractions on bacterial and fungal isolates.

4.2. Methodology

4.2.1. Antibacterial activity

In this study, nine bacteria known to be responsible for various diseases were used, four Gram-positive, *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus pumilus* (ATCC 14884) and five Gram-negative bacteria, *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (ATCC 13047), *Pseudomonas aeruginosa*, *Shigella flexneri*, and *Proteus vulgaris* (laboratory isolate). These microorganisms were collected from the University of the Free State and stored in agar plates of Mueller Hinton (MH) and rejuvenated for the assay system by growing the aliquot in 2 ml of MH for 24 hours. The condensed bacterial isolates were dissolved by pipetting 1ml of each bacterium into a 99 ml MH broth test tube, this was done to ensure that the bacteria were in the log phase before the experiment began.

The micro-dilution assay using the micro-plate technique was used to measure the minimum inhibitory concentration (MIC) values of antibacterial plant extracts as outlined by Eloff (1998). In 96-well microtiter plates, 100 microliters of sterile water were added to all wells, another 100 microliters of extracts were added to wells on row A. The extracts in well A of the

micro-titre plates were screened at 12.5 mg / ml. With the use of a multi-channel pipette, 100 micro-litres was taken from row A wells to B with a two-fold serial dilution. This was done down the wells to H and the 100 micro-litres was discarded at a concentration of 0.098 mg / ml at this stage. Controls included the antibiotic neomycin, extract-free solutions and extracting solvents. As a marker for bacterial growth p-iodonitrotetrazolium violet (INT) was prepared by making 0.2 mg / ml dissolved in distilled water. A total of 40 µl of INT solution was put on all wells.

The plates were wrapped with parafilm and incubated at 37°C for 30 minutes. The presence of a pinkish/ red colour indicated bacterial growth. Clear wells meant bacterial growth was completely inhibited. The MIC readings were taken as the lowest concentration of the extract that inhibits the growth activity of the bacteria, i.e., a clear well. All extracts were tested in triplicate. The micro-titre plates were further incubated overnight at 37°C for determination of minimum bactericidal concentration (MBC) as described by Balouiri et al. (2016). MBC is the lowest concentration of the plant extract needed to kill 99.9% of the bacteria, where the well remained clear, the bacterial growth was inhibited.

4.2.2. Antifungal activity

Three fungal isolates, *Candida albicans*, *Candida vulgaris* and *Trichophyton mucoides* were obtained from the University of the Free State and preserved on nutrient agar. A broth micro-dilution test, as suggested by Espinel-lngroff and Pfaller, (1995) was performed with minor modifications. The fungal culture was formulated by diluting 400 µl of 24-hour old fungi with 4 ml of sterile saline. The absorbance of the solution was recorded at 530 nm and balanced with sterile saline to match that of the normal McFarland 0.5 standard. From the prepared

fungal culture, a 1:1000 broth dilution was formulated by diluting10 µl of fungal culture with 10 ml of broth.

The aqueous extracts were dissolved in distilled water and the organic solvent extracts were dissolved in dimethyl sulfoxide (DMSO). An amount of 100 µl of broth was added to each well of a 96-well micro-plate. For aqueous extracts, 100µl of the aqueous extract was added to well (A) and serially diluted from (A) by taking 100 µl of water to (B). The above two-fold dilution was repeated down the plate and 100 µl of the last well (H) was discarded. In the case of organic solvent extracts, a total of 25 µl of the extracts was added to the 175 µl broth and serially diluted. This was done in triplicates for each of the extracts. All the wells were then filled with 100 µl of fungal cultures. Amphotericin B was used as a reference for this experiment and the following controls were prepared: broth-only wells, fungal strain without extract and solvent used to dissolve plant extracts.

The micro-plates were incubated at 37°C overnight. As an indicator of fungal growth, 40 µl of 0.2 mg / ml INT dissolved in water was added to the wells and incubated at 37 ° C for 30 min. Where there was no fungal growth, the solution in the well remained clear after incubation with INT. The MIC measurements were recorded as the lowest concentration of the extract that completely inhibited the growth of the fungi tested. All extracts were tested in triplicate. Minimum fungicidal concentration (MFC) was determined as proposed by Balouiri et al. (2016), where MFC is also defined as the lowest concentration of the plant extracts that yields 98%–99.9% killing effect as compared to the initial inoculum.

4.3 Results

Antibacterial activity

The findings of the antibacterial activity of *B. disticha* extracts are presented in Table 4.1. From the results obtained, extracts were considered to be highly active if their MIC values ranged from 0.098 to 0.78 mg/ml, moderate activity with a MIC value of 1.56 mg/ml and weak activity with values above 3.125 mg/ml.

The *B. disticha* aqueous leaves, bulb inner and bulb outer scales displayed the highest activity against *S. aureus* with MIC values ranging between 0.39 and 0.78 mg/ml, while MBC values also ranged from 0.39 to 0.78 mg/ml. Moderate activity (1.56 mg/ml) was observed with *B. disticha* methanol root, leaves and bulb outer scale.

The methanol extract of this plant species against *S. epidermidis* showed moderate results with MIC values of 1.56 mg/ml for these selected plant parts i.e., roots, leaves and bulb outer scales. The bulb inner scales showed poor activity with MIC values of 12.5 mg/ml. The aqueous extract solvent against *S. epidermidis* showed moderate results with the roots having MIC value of 1.56 mg/ml and the bulb inner scales with MIC values of 3.125 mg/ml. The leaves and bulb outer scales had poor activity with MIC 6.25 mg/ml for the leaves and 12.5 mg/ml for the outer scales. The MBC values for all extracts ranged between 12.5 mg/ml and >12.5 mg/ml.

The results obtained for *B. disticha* extracts against *E. faecalis* displayed high activity for the root methanol extract at 0.39 mg/ml. The methanol extract for both the bulb inner and outer scales showed poor activity with MIC values of 6.25 mg/ml. The leaves for the methanol extract showed moderate activity with MIC values of 3.125 mg/ml. The aqueous extract showed extremely poor activity against the bacterium with MIC values of 6.25 mg/ml for the root, bulb inner scales and bulb outer scales. The leaves had MIC value 12.5 mg/ml. The MBC values for all extracts ranged between 12.5 mg/ml and >12.5 mg/ml.

The methanol extract of *B. disticha* leaves and bulb outer scales displayed extremely good inhibitory properties against *K. pneumoniae* with MIC values of 0.78 mg/ml. The roots and the bulb inner scales displayed moderate inhibitory properties with MIC values of 1.56 mg/ml. The aqueous solvent extracts also displayed moderate inhibitory properties with MIC values of 1.56 mg/ml whereas the bulb inner scales together with the bulb outer scales showed poor activity with MIC values of 6.25 mg/ml. The leaves for the aqueous solvent extract displayed extremely poor activity with MIC values of 12.5 mg/ml. The MBC values for all extracts ranged between 12.5 mg/ml and >12. 5mg/ml.

The methanol extract tested against *P. aeruginosa* displayed moderate activity with MIC values of 1.56 mg/ml for the roots and leaves. The bulb inner and outer scales showed poor inhibitory activity with MIC values of 6.25 mg/ml, with the aqueous extract showing moderate activity with MIC values of 1.56 mg/ml. The aqueous root extract also displayed moderate activity with MIC values of 3.125 mg/ml. The bulb inner scales showed poor activity with MIC values of 6.25 mg/ml. The MBC values for all extracts ranged between 12.5 mg/ml and >12.5 mg/ml except for the leaves methanol extract where the MBC was 0.78 mg/ml.

The methanol root extract displayed high activity against *S. flexneri* with an MIC value of 0.78 mg/ml. The extracts prepared from leaves and bulb outer scales displayed moderate activity with MIC values of 1.56 mg/ml and 3.125 mg/ml, respectively. The bulb inner scales displayed poor inhibitory properties with MIC values of 6.25 mg/ml. The aqueous extract against *S. flexneri* displayed poor inhibitory properties for both the root and bulb inner scales with MIC values of 6.25 mg/ml and further extreme poor activity was observed from the leaves and bulb outer scales with MIC values of 12.5 mg/ml. The MBC values for all extracts ranged between 12.5 mg/ml and >12.5 mg/ml.

The methanol roots and leaves extract displayed moderate activity against *B. pumilus* with MIC values of 1.56 mg/ml. The bulb outer and inner scales for the methanol extract solvent showed poor inhibitory properties with MIC values of 6.25 mg/ml. The aqueous extract solvent against *B. pumilus* showed moderate activity for the root extract with MIC values of 3.125 mg/ml. The bulb inner scales extract had poor activity with MIC values of 6.25 mg/ml, whereas the leaves and the bulb outer scales showed extreme poor inhibitory properties with MIC values of 12.5 mg/ml. The MBC values for all extracts ranged between 12.5 mg/ml and >12.5 mg/ml.

The methanol root and bulb outer scales extracts showed extremely good inhibitory properties against *P. vulgaris* at MIC of 0.78 mg/ml. The leaves showed moderate activity with MIC values of 3.125 mg/ml and the bulb inner scales showed poor activity with MIC values of 6.25 mg/ml. The aqueous solvent extracts showed poor activity for both the roots and bulb inner scales with MIC values 6.25 mg/ml. The leaves and the bulb outer scales showed extremely poor activity with MIC values of 12.5 mg/ml. The MBC values for all extracts ranged between 12.5 mg/ml and >12.5 mg/ml.

The methanol solvent extracts against *E. coli* showed moderate activity for the root and leaves with MIC values of 1.56 mg/ml, together with the bulb inner scales with MIC values of 3.125 mg/ml. The bulb outer scales showed poor activity with MIC values of 6.25 mg/ml. The aqueous solvent extract had poor activity for the roots and bulb inner scales with MIC values of 6.25 mg/ml whereas the leaves and the bulb outer scales had extremely poor activity with MIC values of 12.5 mg/ml. The MBC values for all extracts ranged between 12.5 mg/ml and >12.5 mg/ml.

Antifungal activity

For the fungal strains the extracts were considered to be highly active if their MIC values were ranging between 0.39 and 0.78 mg/ml, moderate activity with MIC value of 1.56 mg/ml and poor activity with values above 3.125 mg/ml. The *B. disticha* plant extracts for both the methanol and aqueous solvent showed poor activity against the tested fungal strains i.e., *C. albicans, C. vulgaris* and *T. mucoides* (Table 4.2). All the tested plant parts for both extraction solvents had MIC values ranging from 6.25 mg/ml to 12.5 mg/ml except for the aqueous leaves which had an MIC value of 3.125 mg/ml against *C. albicans.* The MFC values for all the extracts were 25 mg/ml and some above 25 mg/ml.

Table 4.1: Antibacterial activity of *B. disticha* (MIC/MBC values in mg/ml)

Solvent extract	Plant part	Bacterial strains								
		S.a +	*S.e*+	*E.f*+	*K.p*-	*P.a*-	*S.f*-	*B.p*+	*P.v*-	*E.c*-
		MIC/ **MBC** (mg/ml)	MIC/ **MBC** (mg/ml)	MIC/ **MBC** (mg/ml)	MIC/ **MBC** (mg/ml)	MIC/ **MBC** (mg/ml)	MIC/ **MBC** (mg/ml)	MIC/ **MBC** (mg/ml)	MIC/ **MBC** (mg/ml)	MIC/ **MBC** (mg/ml)
Methanol	Root	1.56/>12.5	1.56/12.5	0.39/>12.5	1.56/6.25	1.56/ 6.25	0.78/>12.5	1.56/12.5	0.78/>12.5	1.56/12.5
	Bulb innerscales	3.125/12.5	12.5/12.5	6.25/>12.5	1.56/>12.5	6.25/3.125	6.25/>12.5	6.25/>12.5	6.25/>12.5	3.125/>12.5
	Leaves	1,56/6.25	1.56/>12.5	3.125/6.26	0.78/1.56	1.56/0.78	1.56/6.25	1.56/12.5	3.125/6.25	1.56/12.25
	Bulb Outerscales	1.56/>12.5	1.56/>12.5	6.25/12.5	0.78/>12.5	6.25/>12.5	3.125/>12.5	6.25/>12.5	0.78/>12.25	6.25/12.25
Aqueous	Root	6.25/0.78	1.56/12.5	6.25/12.5	1.56/12.5	3.125/12.5	6.25/12.5	3.125/12.5	6.25/12.5	6.25/12.25
	Bulb inner scales	0.78/0.78	3.125/ 12.5	6.25/12.5	6.25/12.5	6.25/12.5	6.25/12.5	6.25/12.5	6.25/12.5	6.25/12.25
	Leaves	0.78/0.78	12.5/>12.5	12.5/12.25	12.5/>12.5	1.56/>12.5	12.5/ >12.5	12.5/>12.5	12.5/>12.5	12.25/12.25
	Bulb outer scales	0.39/0.39	6.25/>12.5	6.25/>12.5	6.25/>12.5	1.56/>12.5	12.5/>12.5	12.5/>12.5	12.5/>12.5	12.25/>12.25
Neomycin µg/ml (control)		3.125/ **3.125**	0.39/ **0.39**	3.125/ **3.125**	0.78/ **0.78**	12.5/ **12.5**	0.39/ **0.39**	0.78/ **0.78**	3.125/ **3.125**	0.78/ 0.78

S.a = *Staphylococcus aureus*; S.e = *Staphyloccocus epidermidis*; E.f = *Enterococcus faecalis*; K.p = *Klebsiella pneumoniae*; P.a = *Pseudomonas aeruginosa;*

S.f = *Shigella flexneri*; B.p = *Bacillus pumilus*; P.v = *Proteus vulgaris*. E.c = *Escherichia coli*

(+) = Gram-positive; (-) = Gram-negative; (>) = greater than.

Table 4.2: The antifungal activity of *B. disticha* (MIC/ MFC values in mg/ml)

Solvent extract	Plant part	Fungal strains		
		C.a	*C.v*	*T.m*
		MIC/ **MFC**	MIC/ **MFC**	MIC/ **MFC**
Methanol	Roots	25/ >25	6.25/ >25	6.25/>25
	Bulb innerscales	>25/**NA**	12.5/ 25	>25/ **NA**
	Leaves	12.5/>12.5	12.5/ 25	12.5/25
	Bulb outer scales	>25/ **NA**	25/12.5	25/25
Water	Roots	6.25/ 25	6.25/ 12.5	6.25/ 12.5
	Bulb innerscales	12.5/ 25	12.5/ 12.5	12.5/12.5
	Leaves	3.125/ >25	6.25/ >12.5	6.25/>25
	Bulb outer scales	25/ >25	25/ >12.5	25/>25
Streptomycin (µg/ml)		0.39/**0.39**	0.39/**0.39**	0.39/**0.39**

C.a = Candida albicans; C.v = Candida vulgaris; T.m = Trichophyton mucoides

4.4 Discussion

The use of *B. disticha* for the treatment of wounds and various ailments has been documented throughout history (Watt and Breyer-Brandwijk, 1962). *Boophone disticha* is still one of the most common commercial bulbous plants in the traditional trade industry (Nair and van Staden, 2013) due to the demand of people and traditional healers. The findings from this research showed that the methanol extracts exhibited essential antibacterial properties against the bacteria tested. These results are consistent with the findings of Rabe and van Staden (1997) on aqueous and methanol extracts from 21 plant species in South Africa. The findings revealed that most antibacterial activity was identified in methanol extracts and concluded that organic extracts contribute to the complete identification of the organic compounds present in the plant.

Several aqueous extracts from this study showed very good activity against the bacteria tested, confirming or validating the conventional use of the plant. However, most of the aqueous extracts showed very poor activity. This indicates that water is not the most efficient solvent for plant extraction for the availability of active compounds. Shale et al. (1999), together with Buwa and Afolayan (2009), suggested that water could still be regarded as a suitable solvent for traditional remedies because traditional healers usually use high doses of their mixtures. Contrarily to this, Newton et al. (2002) indicated that the negative findings do not indicate a complete lack of bioactive constituents or that the plant has no activity, but rather suggested that the extracts may function in certain ways by producing antibodies to the patient or by creating internal conditions that are unfavourable for the replication of the microorganism.

Gram-positive bacteria are the most sensitive to crude extracts as opposed to Gram-negative bacteria. The reported resistance difference between the Gram-negative bacteria was not uncommon, as it was reported to be more immune than Gram-positive to antibiotics (Palomboa and Semple, 2001). Gram-negative bacteria are a major health barrier because they have an outer membrane that poses a threat to several environmental substances and protects them from many antibiotic cell membranes (Gill et al., 2015). Previous studies have shown Gram-negative bacteria's resistance to plant extracts, where extracts have been reported to be quite potent in Gram-positive bacteria particularly in comparison to Gram-negative bacteria (Koohsari et al., 2015). The authors have clarified that because of the intrinsic resistance of Gram-negatives and the nature and structure of herbs, as well as the cell walls of Gram-positive bacteria compared to Gram-negative bacteria, they are more sensitive to many antibiotics, antimicrobial chemical compounds and herbal medicines (Sharifa, et al., 2008). Lipopolysaccharides layer and periplasmic space of Gram-negative bacteria are the reasons for their relative resistance (Schwechheimer and Kuehn, 2015). However, *K. pneumoniae*, *S. flexneri*, and *P. vulgari* are

Gram-negative bacteria and solvent extracts displayed extremely good inhibitory activity against them (MIC values of 0.078 mg/ml).

To prove and validate the traditional use of *B. disticha* on the treatment of wounds, Dilika et al. (1996) investigated the antimicrobial properties of plants used on Xhosa initiates after circumcision. They reported that the wound was bandaged with mashed leaves of *Helichrysum pedunculatum, Helichrysum appendiculatum* or *Helichrysum longifolium*. The wound was then dressed with the dry outer scales of the bulb of *B. disticha.* Dilika et al. (1996) showed that *H. pedunculatum* and *H. longifolium* had positive antimicrobial activity against all four bacterial strains used in the disc diffusion assay. Dilika et al. (1996) did not test the antimicrobial activity of *B. disticha*.

However, Cheeseman (2013) conducted an investigation on the antibacterial activity of the outer scales of *B. disticha* which showed poor activity against all the bacterial strains tested. The best MIC value for the outer scales was 1.56 mg/ml. Therefore, it is apparently the positive antimicrobial activity of the *Helichrysum* plants used to treat circumcision wounds, and not the outer scales of *B. disticha,* which could explain the fact that most traditionally circumcised patients, remain free from infection.

The results obtained in this study disagree with those of Cheeseman (2013) because the aqueous bulb outer scales extract of *B. disticha* displayed very high activity against *S. aureus* (MIC values of 0.39 mg/ml). The *B. disticha* plant parts proved to possess antibacterial properties, with roots proving to be the most active plant part. To support the ethnobotanical uses of *B. disticha* described by the indigenous people of the Southern African region, the plant displayed good inhibitory properties against bacteria known to be disease-causing. The plant is indeed a good cure for personal injury and gastrointestinal conditions because of the good inhibitory properties observed against bacterium like *S. flexneri, E. faecalis, K. pneumoniae*

and *P. aeruginosa*. The MBC values established from this study were shown to be potentially fatal to most of the pathogens even though some had MBC values above 12.25 mg/ml.

Miller (1996) argued that the active plant parts had a variety of phytochemicals in the extracts that are known to act in some way to exert antibacterial action. Tsuchiya et al. (1996), found that tannins in medicinal plants showed a better antibacterial activity. The claims were premised on the reality that tannins' potency was linked to their ability to inactivate a variety of enzymes, as well as microbial conductivity and cellular membrane transport proteins. (Tsuchiya et al., 1996). It has been documented that flavonoids and saponins have antibacterial activity, which could be due to their ability to complex with extracellular proteins, soluble proteins and bacterial cell walls (Divakar et al., 2001). These are the phytochemicals that have been reported to be significantly present in this plant in Chapter 3.

The MIC values of *B. disticha* extracts from all plant parts tested against the test bacteria were higher than those for fungi, indicating that fungi were more resistant to *B. disticha* crude extracts than bacteria. This study is in agreement with findings by Buwa and van Staden (2006) that pathogenic fungi are generally more resistant to plant extracts than plant pathogenic bacteri. Fungi have extremely thick cell walls that are characterized by complex polysaccharides called chitins and glucans that help adjust structural stability. In general, the cell wall protects the cell from heat and predators (Gow et al., 2017). Wang et al. (2019) argued that fungal infections are notoriously difficult to treat in humans. Fungi, unlike bacteria, do not respond to standard antibiotic therapy because they are eukaryotes and that fungal infections can be fatal in people who have weakened immune systems (Huffnagle and Noverr, 2013). The findings in this study are similar to those of Cheeseman, (2013) who reasoned on the overall activity of *B. disticha* against *C. albicans* as poor, and further on commented that there were no real differences in activity between the different plant parts.

4.5. Conclusion

Boophone disticha examined in this study has historically been used for the treatment of skin and wound infections. The optimistic findings of this analysis provide a theoretical basis for conventional plant use. Although *B. disticha* extracts had poor activity against the fungal strains tested, they showed very promising antibacterial activity against the bacteria tested. Finally, the findings of this study clearly explain the antibacterial activity of this plant and provide proof to substantiate its use in folk medicine and may serve as an affordable source of bioactive compounds for the diagnosis and treatment of bacterial wounds and diseases.

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CHAPTER FIVE

EVALUATION OF THE ANTI-MYCOBACTERIAL ACTIVITY OF *B. DISTICHA*

5.1. Background of study

Mycobacterium tuberculosis is an aerobic, acid and alcohol fast bacilli that belongs to the *M. tuberculosis* complex (Banuls et al., 2015). The bacterium is characterised by an unusual waxy coating on its cell surface, mainly due to the presence of mycolic acid. The unique lipid rich cell wall (mycolic acid) is possibly responsible for its resistance and is a key factor in virulence, as a result *M. tuberculosis* may occur as gram positive or gram negative (Glickman and Jacobs, 2001). *Mycobacterium tuberculosis* is the common cause of tuberculosis in human beings (Banuls et al., 2015). Approximately 80% of TB affects the lungs and spreads to the public through careless acts of sneezing, coughing and spitting (Chakraborty, 2019). The bacteria may spread through the bloodstream to deposit in any part of the body and manifest themselves at those sites, with TB affecting even the bones, commonly those of the spine and known as Bone-TB. (Roberts and Buikstra, 2019). Goletti et al. (2019) reported that about one third of the world's population is septic to *M. tuberculosis* and TB disease is likely to develop.

The World Health Organization's facts sheet on tuberculosis (TB) listed it as one of the top ten leading causes of death worldwide (WHO, 2019). Furthermore, WHO found that an average of 10.0 million people worldwide (range 9.0–11.1 million) were infected with tuberculosis in 2018, a figure that has remained fairly stable over time. The disease's prevalence varies greatly between countries, ranging from less than five to more than 500 new cases per 100 000 populations per year, with a global average of nearly 130 (WHO 2010). In 2018, an estimated 1.2 million HIV-negative people died of tuberculosis, down from 1.7 million in 2000, and an additional 251 000 HIV-positive people died of tuberculosis (range 223 000–281 000), a 60%

decrease from 620 000 in 2000. (WHO, 2019). In 2018, both genders were affected by TB in 57 percent of all cases; in comparison, women accounted for 32 percent of all TB cases, and children (aged genders in all age groups, but the highest burden is on men (aged 15 years) (Floyd et al., 2018). Almost three decades ago, Rifampicin, Isoniazid, Streptomycin, and Ethambutol were introduced into the TB control program for the treatment of tuberculosis (Gandhi et al., 2010). Non-selective use: inadequate, erroneous prescribing by healthcare providers, low quality medications, and patients discontinuing treatment rashly have all contributed to an unprecedented rise in the production of multidrug resistance (MDR-TB), more severely resistant (XDR-TD), and actually fully drug resistant (TDR-TB) TB strains.

It is usually a curable disease, given that the correct medication is prescribed and completed within a standard six-month period (WHO 2019). Raviglione (2006), reported that only 4% of TB deaths in South Africa were caused by drug-resistant forms of bacteria because MDR tuberculosis was caused by *M. tuberculosis* resistant to isoniazid and rifampicin and XDR tuberculosis was caused by mycobacteria resistant to rifampicin and isoniazid, any fluoroquinolone, and one of the three injectable drugs, capreomycin, kanamycin, and amikacin.

Drug resistance poses a serious threat to tuberculosis control as it raises the possibility of a return to an era in which drugs are no longer effective (Raviglione, 2006). No new anti-TB drugs have been released for decades and MDR-TB, XDR-TB and TDR-TB are on the increase, therefore there is an urgent need for new leadership in the development of novel, effective, safe and affordable drugs against all forms of resistant TB strains. Plant-based products have been frequently used among the numerous natural products used in ethno-pharmacology, owing to its large pool of phytochemicals investigated for a wide range of infections and anti-mycobacterium compounds (Nguta et al., 2015).

Only *B. disticha* is renowned in traditional medicine for the treatment of tuberculosis among the South African Amaryllidaceae (Watt and BreyerBrandwijk, 1962), although no pharmacological studies have been conducted to confirm this property (Nair and van Staden 2014). This chapter was aimed at investing the possible anti-*Mycobacterium tuberculosis* potential of *B. disticha* in attempts to validate the traditional use of this plant in the treatment of TB.

5.2. Materials and method

5.2.1. Plant collection and extraction of the sample

The collection and extraction of *B. disticha* were carried out as previously described in chapter three.

5.2.2 Antimycobacterial activity

Mycobacterium tuberculosis strain (ATCC 25177) was obtained from the University of the Free State and used for this assay. The bacterial strain was preserved in Middlebrook 7H9 broth containing 10% OADC (oleic acid + albumin + dextrose + catalase). The stock of mycobacterial culture was transferred to the supplemented 7H9 broth for inoculum preparation and cultivated for 72 hours on a shaker. Two test tubes, each containing 5 ml of complemented 7H9 broths with mycobacterial culture, were inoculated and grown for 72 hours. Twenty percent of sterile glycerol was added to each culture, and 500 µl aliquots were rendered into sterile Eppendorf tubes. These stocks were referred to as G1 stocks, and held at -30°C. A single stock of G1 was used to inoculate supplemental plates of Middlebrook 7H10 agar (7H 10 + 10% OADC) and incubated at 37°C for 4 days or until growth was observed. From this culture,

a single colony was used to inoculate 5 ml of complemented 7H9 broth. It was grown in a shaker at room temperature for 72 hours and used for the experiment.

Swenson et al. (1982) described the broth microplate dilution method used to assess MIC values for plant extracts against *M. tuberculosis*. Residues of aqueous extract were dissolved in water while residues of the methanol extract were dissolved in DMSO. All extracts were dissolved at a concentration of 100 mg/ml. One hundred microliter of the added 7H9 broth was added to all the wells of micro-titre plates. All extracts were tested at a concentration of 25 mg/ml in well A and serially diluted to 0.195 mg/ml. The optical density was determined and adjusted at 550 nm for the 72-hour broth culture. One hundred microliter of the diluted culture was added to each micro-litre plate well. Controls included good regulation of the solvent used to dissolve plant extracts, of the Middlebrook 7H9 broth alone and of the antibiotic streptomycin (1.56 mg/ml).

The plates were sealed for 72 h at 37°C, and incubated. 40 µl of 0.4 mg / ml of INT solution was added to each plate well after incubation. The plates were coated and incubated at 37°C for 24 h. All extracts were examined in triplicates. The micro-titre plates were further incubated overnight at 37°C for determination of minimum bactericidal concentration (MBC) as described by Balouiri et al. (2016). MBC is the lowest concentration of the plant extract needed to kill 99.9% of the bacteria, where the well remained clear, the bacterial growth was inhibited.

5.3. Results

The results obtained for antimycobacterial activity are shown in Table 5.1. The extracts were considered to be highly active when their MIC values ranged from 0.098 to 0.78 mg/ml, moderately active when their MIC values were 1.56 mg/ml and poor activity with values above 3.125 mg/ml. The high inhibitory activity against *M. tuberculosis* from this study was shown

by the root methanol extract with a MIC value of 0.78 mg/ml. The methanol extract of leaves showed moderate mycobacterial inhibition at MIC value of 1.56 mg/ml and the methanol inner bulb scale extract displayed poor activity at 3.125 mg/ml. The methanol extract of outer scales displayed a poor activity with MIC values of 12.5 mg/ml. The aqueous extracts of *B. disticha* displayed poor activity on all the tested plant parts, with roots exhibiting inhibitory activity at MIC 6.25mg/ml, the bulb inner and outer scales extracts with MIC values above 12.5 mg/ml and the leaves with the MIC values of 6.25 mg/ml. All the extracts for both solvents had MBC values above 12.5mg/ml, this meant that the extracts were not able to kill the bacteria further after incubating for a further 24 hrs.

Table 5.1: Anti-mycobacterial activity of *B. disticha* (MIC/ MBC values in mg/ml)

Solvent	**Plant part**	***M. tuberculosis* mg/ml**
Methanol	Root	0.78/ **12.5**
	Bulb inner scales	3.125/> **25**
	Leaves	1.56/**25**
	Bulb outer scales	12.5/>**25**
Aqueous	Root	6.25.5/> **25**
	Bulb inner scales	>25/ **NA**
	Leaves	12.25/>25
	Bulb outer scales	>25/**NA**
Streptomycin (µg/ml)		**0.098**

5.4 Discussion

Ethnobotanical studies on the use of *B. distcha* reported their pharmacological application on a variety of ailments and traditional medicine for the treatment of tuberculosis. To the best of my knowledge, this study is the first assessment of the anti-tuberculosis potential of *B. disticha,* from the Eastern Cape Province. It was last reported by Watt and BreyerBrandwijk (1962), with Nair and van Staden (2014) later stating that no pharmacological studies have been conducted to confirm this property. The findings of this study are consistent with the report by Watt and BreyerBrandwijk (1962) on the use of *B. disticha* in the treatment of tuberculosis.

Aqueous extracts showed poor activity against the bacterium used in this study. Buwa and Afolayan (2009) in their findings, suggested that water was not a suitable solvent for extracting active ingredients from plants. Although the recommended dosage of traditional healers is often very high, e.g., three to four full cups per day for adults, water can still be considered an appropriate extraction solvent for traditional remedies. Tiwari et al. (2011) suggested that while traditional healers most commonly use water to extract bioactive compounds from plants, it was found that organic solvent extracts have high antimicrobial activity compared to water extracts. The results obtained as shown in Table 5.1 exhibited roots as the most active part of the plant and that methanol was the best extracting solvent. This is because, according to Tiwani, (2017), underground plant parts are widely considered to contain higher levels of bioactive compounds, while leaves and fruits are less commonly used. In addition, underground sections of plants have been reported to be commonly used in traditional medicines, with herbalists claiming the highest concentration of healing agents (Appidi et al., 2008). The presence of phytochemicals such as alkaloids, flavanoids and polyphenols, terpenoids, quinones and phytosteroids etc. justify *B. distcha's* antimycobaterial activity. These compounds are found in all parts of the plants and are widely used for medicinal purposes, including tuberculosis (Hutchings et al., 1996).

5.5. Conclusion

This study highlights the results of *B. disticha* against the virulent strain of *M. tuberculosis*. This offers a tentative scientific proof of this species' use against certain symptoms associated with tuberculosis such as cough, respiratory dysfunctions, fever and headaches. Further screening of *B. disticha* compound isolation possible use in the development of anti-mycobacterial drugs is needed in the fight against TB.

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CHAPTER SIX

EVALUATION OF ANTI-INFLAMMATORY PROPERTIES OF *B. DISTICHA*

6.1. Background of study

Inflammation is the immunological reaction of biological cells to injuries resulting in an overproduction of plasma fluids and blood cells (Sosa et al., 2002). It usually occurs when infections invade the body, reside in a particular tissue or circulate in the blood (Azab et al., 2016). Even though it is a coping strategy, complicated activities and transcription factors involving inflammatory reactions may cause, retain or exacerbate multiple diseases (Saha and Ahmed, 2009). There are two types of inflammation – acute and chronic inflammation. Acute inflammation occurs in response to a temporary infection. The body responds to harmful substances during this cycle, restores cell damage and takes dead cells away (Sosa et al., 2002). This has signs such as redness, swelling, heat and discomfort. Examples of conditions causing acute inflammation include acute bronchitis, a cold or flu-like sore throat or an infected ingrown toenail (Trouillas et al., 2003). Chronic inflammation is influenced by a number of factors, including bacterial infections, viruses and parasites, chemical irritants and indigestible particles (Antonelli and Kushner, 2017). Shacter and Weitzman (2002) stated that the greater the inflammation, the greater the risk of cancer development.

Medicinal plants have long been recognised as important sources of active compounds (Keeble and Moore, 2002). Validated scientific evidence identifies the medicinal and biochemical benefits of plants, thereby growing interest in the discovery and classification of these bioactive metabolites from natural resources (Pan et al., 2013). One of the oldest known forms of relieving inflammation and pain was for Celsius to apply willow leaf extracts in 30 AD (Pan et

al., 2013). A major anti-inflammatory drug (acetyl salicylic acid) commonly used in clinical practice was identified in common use alongside several other non-steroidal anti-inflammatory drugs (NSAIDs) (Antonelli and Kushner, 2017). In South Africa, plants are widely used to treat a range of conditions (Dyubeni and Buwa, 2012). These biologically active plant species are rich in natural compounds such as phenolic compounds (curcumins, flavonoids and tannins), saponins, terpenoids and alkaloids (Kennedy and Wightman, 2011). These plant metabolites are credited with safe and healing properties such as antioxidant, anti-inflammatory, antimicrobial and anticancer activity (Adebayo et al., 2015). It is hypothesized that the mode of action of such phenolic compounds is through their free radical scavenging capacity or through the prevention of pro-inflammatory enzymes such as cyclo-oxygenase (COX) and lipoxygenase (LOX) (Rathinavel et al., 2017).

Nonsteroidal anti-inflammatory medications are commonly used to treat discomfort and inflammatory disorders such as autoimmune diseases like rheumatoid arthritis, osteoporosis, and dementia (Keeble and Moore, 2002). Concerned about the health risks associated with the use of NSAIDs, the focus has shifted to innovative drug therapy (Adebayo et al., 2015).

Arachidonic acid is metabolized during inflammation via the COX pathway for the production of prostaglandins and thromboxan A2 or via the LOX pathway for the production of hydroperoxy-eicosatetraenoic acids and leukotrienes (Malmsten, 1986). In leukocytes, the LOX pathway includes several immunocompetent cells, including mast cells, neutrophils, eosinophils, monocytes, and basophils. After cell activation, arachidonic acid is excreted by phospholipase A2 from the cell membrane and donated to LOX by LOX activating protein, which then metabolizes arachidonic acids in the leukotriene reaction series, a pro-inflammatory cytokine group (Adebayo et al., 2015). As chemo-attractant phagocytes, leukotrienes function to draw cells from the adaptive immunity to inflammatory sites. For example, in an asthmatic attack, the production of leukotrienes by LOX causes bronchioles to be limited, resulting in

bronchospasm (Rathinavel et al., 2017). The selective suppression of LOX is thus a significant therapeutic technique for asthma (Yedgar et al., 2007). Potential interventions to control certain allergic and inflammatory reactions may be given by LOX activity inhibitors. Medicinal plants can also be possible sources of inhibitors of COX-2/LOX that have less side effects than NSAIDs (Rathinavel et al., 2017).

Boophone disticha is most commonly used in traditional medicine in South Africa, for three top groups under which inflammation-related disorders, ' wounds and illnesses ' and ' Central nervous system-related diseases' are listed (Nair and van Staden, 2014). This plant is often used to prepare infusions, ointments, baths or dressings used in local folk medicines for wound healing properties. However, in a number of skin conditions characterized by inflammatory conditions such as sores, rashes and burns, these preparations are also used to provide relief (Watt and Breyer-Brandwijk, 1962; van Wyk et al., 1997; Botha et al., 2005). The aim of this chapter was to evaluate the anti-inflammatory properties of *B. disticha* extracts.

6.2. Materials and method

6.2.1 Plant collection and extract preparation

The collection and extraction of *B. disticha* were carried out as previously described in chapter three.

6.2.2 Anti-inflammatory activity

Anti-inflammatory activity was conducted using the 5-lypoxygenase (5-LOX) assay as described by Baylac and Racine (2003) and Trouillas et al. (2003), with slight modifications. Linoleic acid was used as a base for the 5-LOX enzyme. An antibody that is produced by

rabbits known as anti-arachidonate (Stigma-Aidrich, Germany) was used. Standardization was initially performed using a reference sample consisting of 10 µl of DMSO in a Tween 20 mixture (1: 29 w/w), 2.95 ml of phosphate buffer (pH 6.3), pre-warmed in a 25°C water bath, and 50 µl of linoleate solution (100 µM final concentration).

The absorbance was read at 234 nm for 10 min. Approximately 12 µl of the ice-cold buffer (potassium phosphate) was then diluted with 12 µl (100 U) of the previously frozen enzyme at which each test sample was administered at five separate doses (0.02, 0.05, 0.01.0.2 and 0.4 mg/ml) of separate plant extracts. The mixture was subjected to a cuvette, and the liquids in the cuvette were mixed thoroughly and read using a spectrophotometer. Two variables were prepared and combined with DMSO and Tween 20 mixture (no enzyme inhibition) to serve as negative controls. The development of conjugated dienes was calculated at 234 nm over a period of 10 min. Nordihydroguaiaretic acid (NDGA) was used as a positive control (enzyme inhibition). The IC_{50} value (concentration at which 50% of the enzyme was inhibited) of each sample test was determined using GraphPad. The percentage inhibition of the enzyme activity was calculated by comparing with the controls (Tween® 20/DMSO mixture). The analysis consisted of three replicates.

6.3. Results

Table 6.1 below presents the inhibitory characteristics of plant extracts for anti-inflammatory activity, and Figure 6 shows the percentage inhibition of methanol extracts and aqueous extracts of various plant parts at different doses compared to NDGA. According to the findings of this study, the IC_{50} values of all of the tested methanol extracts had no activity. The roots had no activity in the aqueous extracts. The IC_{50} values of the aqueous leaves, inner and outer scales exceeded the concentration used.

The overall results for the *B. disticha* extracts were poor; at low concentration the plant had negative results. The leaves aqueous and outer-scales aqueous extracts did show little activity at 0.4 mg/ml.

Table 6.1: IC_{50} scavenging activity of *B. disticha* plant extracts

PLANT PART	METHANOL		AQUEOUS	
	IC_{50}	R^2	IC_{50}	R^2
Roots	Na	na	na	na
Leaves	Na	na	>>	0.8668
Bulb inner scales	>>	0.8937	>>	0.4684
Bulb outer scales	Na	na	>>	0.9194

IC_{50} is defined as a concentration (mg / ml) sufficient to achieve 50 per cent of the maximum scavenging capacity >> values greater than the concentration, <<value lower than the concentration used. R^2 ratio of coefficient determination. The IC_{50} of the extract is inversely related to its anti-inflammatory compound's richness (Lower IC_{50} values indicate a higher anti-inflammatory activity). (n a= not active)

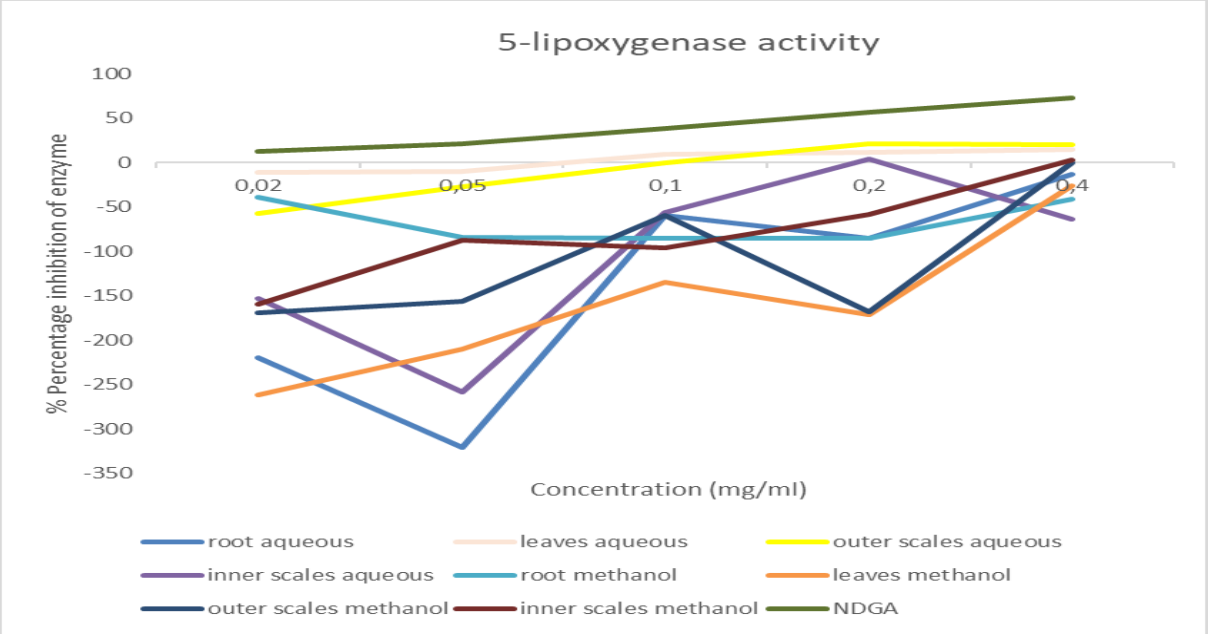

Figure 6.1: The percentage inhibition of 5-lipoxygenase by the methanol and aqueous plant extracts of *B. disticha* in comparison to NDGA.

6.4. Discussion and conclusion

Based on the results obtained from this study poor activity in 5-LOX assay was observed with plant extracts showing very poor to no inhibitory activity. Amoo et al. (2009) observed that a higher activity was correlated with lower or decreased IC_{50} value. This could not be verified in the present study because IC_{50} values were all above the maximum concentration used. NDGA was used as a positive guide for 5-lipoxygenase inhibition studies due to its well-documented strong inhibitory activity on this enzyme (Safayhi et al., 1992). The NDGA showed good anti-inflammatory activity as anticipated since it was used as a positive control. For the positive results obtained, the anti- inflammatory activity was between concentrations of 0.1 mg/ml – 0.4 mg/ml with percentage inhibition of 21.43% being the highest observed from the aqueous bulb outer scales extract. The activity in the leaves and bulb outer scales of the aqueous extract

validates the findings by Vildomat et al. (1997) that dried leaves of this plant moistened with milk or oil were used to treat skin diseases, varicose ulcers, and phlebitis.

Jagger et al. (1996) conducted a study to verify the anti-inflammatory properties of the plant from cyclo-oxygenase, where they discovered that the ethanol bulbs exhibited 55% inhibition against COX-1. In the case of traditional medicine, *B. disticha* is best known for the management of wounds, infections and various types of illnesses (Philander, 2011). Botha et al. (2005) reported that freshly harvested bulbs were softened and the milk produced was used to treat various inflammatory conditions.

In conclusion, the results obtained from this study were mostly negative, but little activity indicated the existence of anti-inflammatory principles in the extracts tested, which provided pragmatic support for their use in traditional medicines. The use of higher concentrations of different solvents would help to produce better results and, since it has been confirmed in the literature that the plant possesses a compound with anti-inflammatory properties, further investigation is needed for plant species growing in the Eastern Cape region.

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CHAPTER SEVEN

GENERAL CONCLUSION AND RECOMMENDATIONS

The modern evidence advocates that extracts from the *B. disticha* might be a cheap potential source of natural antioxidants that could be of great importance for the treatment of infectious diseases and wounds. This study validated the traditional medicinal use of *B. disticha* in the Eastern Cape of South Africa.

The preliminary phytochemical analysis is presented in Chapter 3, with tannins, flavonoids, phenols and alkaloids detected in all of the plant parts tested for both extraction solvents. The detected phytochemicals were then quantified. The methanolic extracts displayed higher screened phytochemical contents compared to water extracts. The methanol extracts prepared from roots displayed highest phenolic, tannin, flavonoid and alkaloid contents, followed by leaves methanolic extracts. Concerning aqueous extracts, high phytochemical contents were observed in roots. The high concentration of phytochemicals in this plant, in general, explains why it is such an important medicinal plant.

In the screening for antimicrobial activity of *B. disticha*, presented in Chapter 4, Gram-positive bacteria were more susceptible to *B. disticha* extracts than Gram-negative bacteria, though *S. flexineri* performed poorly against *B. distcha* extracts. Both extraction solvents demonstrated good activity, with methanol extracts exhibiting the highest activity. This plant demonstrated good antibacterial activity, displaying its potential as a source of antibacterial drugs. Concerning antifungal activity, *C. albicans*, *C. vulgaris* and *T. mucoides* displayed some

resistance towards *B. disticha* extracts. The aqueous extract prepared from the leaves displayed fungal inhibition at 3.125 mg/ml (the lowest concentration) against *C. albicans*.

This plant's antimycobacterial potential is presented in Chapter 5 and the activity against *M. tuberculosis* was most evident in the plant's roots. The positive results confirmed the plant's long history of use and provided documented evidence for the first time that *B. disticha* can be used in the treatment or management of tuberculosis.

The anti-inflammatory properties of *B. disticha* were analysed and are reported in Chapter 6. *Boophone disticha*'s anti-inflammatory activity was generally very low, but the small amount of activity observed in this study was a good indication that the traditional use of the plant species was correct.

Recommendations

Regardless of its toxic status, which has been widely reported in the literature, it is clear that *B. disticha* has a wide range of medicinal applications.

- This study discovered that the roots had the highest activity in almost all of the performed bioactivity assays. In order to preserve the status of *B. disticha*, harvesting only the roots and returning the rest of the bulb to the soil would help prevent it from becoming endangered or extinct.
- *Boophone disticha* extracts could be a low-cost source of natural antioxidants that could be very beneficial in the treatment of complementary progressive diseases.

- For future research purposes, a further investigation on the anti-mycobacterial activity of the plant and compound isolation is recommended.
- A detailed analysis with other enzymes such COX-1, COX-2 or 15-LOX together with adjusted higher concentrations is also required to confirm further the anti-inflammatory activity of the plant.
- Furthermore, research on the effects of collection time on the pharmacological activity of *B. disticha* is necessary. This might provide answers to the poor antifungal and anti-inflammatory activity of the plant. This would help in the development of new drugs.