

Conservation Strategies of the Red Listed *Brackenridgea zanguebarica* Oliv. in Vhembe District Municipality, Limpopo Province, South Africa

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Declaration

I, Tiawoun Makuété André Patrick, declare that the thesis which I hereby submit for the degree of Doctor of Philosophy at the University of Venda is my original work and has not been previously submitted for any degree at any other university or institution. The thesis does not contain other persons' writing unless specifically acknowledged and referenced accordingly.

Signature:

Date:

Dedication

To the memory of my parents, Tiawoun André Raymond and Makuété Jeanne.

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Abstract

Brackenridgea zanguebarica Oliv. (Ochnaceae) commonly known as “Yellow Peeling Plane”, is an important medicinal plant species, and one of the most threatened tree species that is endemic to Thengwe village in Vhembe District, South Africa. It is widely harvested for traditional purposes and difficult to propagate sexually and asexually. Nowadays, it has become threatened and this unique plant population is believed to be declining in the Brackenridgea Nature Reserve (BNR). According to the Red List of South African plants, *B. zanguebarica* is considered a critically endangered medicinal plant. All these facts combined with a lack of knowledge on the conservation of this species prompted this study on its conservation. To ascertain the effective conservation and proper management of this multipurpose tree, this study was conducted with the aim to improve its conservation strategy by investigating current threats to the existence of *B. zanguebarica*, the chemical constituents from the different plant parts, the propagation methods of the species, and the factors delaying the germination of its seeds.

In this study, an assessment of the current population threats was important as a first step in conservation; this would update the status of the species in its natural habitat. The current status of *Brackenridgea zanguebarica* was conducted through an extensive field survey in the Brackenridgea Nature Reserve (BNR), where a belt transect method was deployed to record quantitative information, while the harvesting damage was estimated using a sliding scale from 0 to 5. The structure of the populations in terms of the stem diameter size classes showed a bell shape

pattern. About 60% of individuals showed some signs of plant parts extraction as the stem bark was reported to be the main plant part harvested. The population structure, however, was dominated by juvenile plants due to the selective harvesting of mature individuals; this hinders fruit production leading to poor or slow seed production.

The present study aimed to compare the chemical constituents and the antimicrobial activities of some parts of this plant species. The extracts were screened for phytochemicals using standard methods. Thin-layer chromatography (TLC) was carried out to compare the chemical constituents using various solvent systems of varying polarity. Agar well diffusion and broth microdilution methods were used to evaluate the antimicrobial activity and the minimum inhibitory concentrations (MIC) of plant extracts respectively, against three bacteria and four fungi. The phytochemical analysis revealed a close similarity of various phytoconstituents of the three plant parts (stem bark, twigs and leaves). TLC analysis showed a slight difference in the acetone extract of different plant parts with more bands on the leaves than the stem bark and twigs. Acetone extracts of the three plant parts exhibited varying degrees of antimicrobial activity against *Staphylococcus aureus* and *Candida glabrata*, while none of the extracts showed any activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans*, *C. krusei*, and *C. parapsilosis*. However, analysis of the antibacterial activity of various extracts revealed that the best inhibitory activity was produced by the stem bark extract compared to the leaf and twig extract.

The sustainable way to meet the growing demand of *Brackenridgea zanguebarica* is to increase its availability through propagation practices. Unfortunately, the conservation of *B. zanguebarica* is hampered by lack of information on how it can be propagated. Sexual and asexual propagations were conducted to determine an efficient method to increase the number of individuals of this plant species. Seed propagation was carried out to study the effect of various pre-treatments on germination, in order to understand the germination requirements; vegetative propagation was conducted to assess if *B. zanguebarica* could be successfully propagated via stem cuttings if the appropriate growth hormones and growing media were applied. *Brackenridgea zanguebarica* seed did not germinate at all under any of the conditions tested. However, the results, showed the potential of propagating this species from stem cutting despite the poor results obtained. Thus *B. zanguebarica* was found to be difficult to propagate sexually and asexually.

Seeds serve as a means of reproduction and a vital element to ensure the survival of plant species. Seed germination is controlled by a number of factors. The propagation of *B. zanguebarica* via seed is very difficult and research has not been undertaken to understand possible factors that may delay its germination. This study was aimed to investigate and describe both the morphological and anatomical features of *B. zanguebarica* seed, in order to identify structural features implicated in its poor germination. To achieve this goal, a morphological and anatomical study was conducted based on the observation of seeds under a scanning electron microscope (SEM) and light microscopy (LM). Water uptake potential was assessed by the increase in seed mass. The morpho-anatomical analysis indicated that some structures of the seed, such as seed coat, and the presence of endosperm

surrounding the embryo that consisted of two prominent cotyledons, seem to greatly hinder the germination of this species. The research findings provided information regarding the seed structure which is one important step in identifying useful techniques to improve seed germination for conservation purposes.

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List of abbreviations

ANOVA	Analysis of variance
BNR	Brackenridgea Nature Reserve
DBH	Diameter at breast height
DNPH 2, 4	Dinitrophenylhydrazine
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
FAA	Formalin acetic acid
GMA	Glycol methacrylate
HSV-1	Herpes simplex virus type 1
INT	Iodo-nitrotetrazolium
IUCN	International Union for the Conservation of Nature
LEDET	Provincial Limpopo Department of Economic Development, Environment and Tourism
LM	Light microscopy
LSD	Least Significant Difference
MBC	Minimum bactericidal concentrations
MFC	Minimum fungicidal concentrations
MIC	Minimum inhibitory concentration
MHA	Mueller- Hinton agar medium
MHB	Mueller- Hinton broth
NAA	1-naphthaleneacetic acid
R _f	Retention factor
SDA	Sabouraud dextrose agar

SANBI	South Africa National Biodiversity Institute
SEM	Scanning electron microscopy
THP	Traditional Health Practitioners
TLC	Thin-layer chromatography
TTC	2, 3, 5-triphenyl-tetrazolium chloride
UV	Ultraviolet
WHO	World Health Organization
WWF	World Wild Fund

Conference contributions

1. Tiawoun, M.A.P., Tshisikhawe, M.P. and Gwata, E.T. 2019. Population status of the red listed *Brackenridgea zanguebarica* Oliv. of Thengwe area in Vhembe District Municipality, Limpopo Province, South Africa. 45th Annual SAAB, AMA and SASSB Joint Congress held at the University of Johannesburg, 8-11 January 2019. Johannesburg, South Africa. Oral Presentation.
2. Tiawoun, M.A.P., Tshisikhawe, M.P. and Gwata, E.T. 2016. Potential for conserving the “yellow peeling plane” (*Brackenridgea zanguebarica* Oliv.) plant in Limpopo Province, South Africa. University of Venda/Walter Sisulu International Research Conference, 5-7 October 2016. Polokwane, South Africa. Poster Presentation.

Publications

1. Tiawoun, M.A.P., Tshisikhawe, M.P. and Gwata, E.T. 2018. A review on yellow peeling plane (*Brackenridgea zanguebarica* Oliv.): a critically endangered endemic plant species. *Annual Research and Review in Biology* 29(5): 1-13.
2. Tiawoun M.A.P., Tshisikhawe M.P. and Gwata E.T. 2019. Investigation of current threats to the existence of *Brackenridgea zanguebarica* in a small geographic area in Vhembe, Limpopo Province, South Africa. *Biodiversitas* 20(5): 1487-1495.

Manuscripts

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Chapter 1

GENERAL INTRODUCTION

1.1 Background of the study

Biodiversity is, worldwide, under significant pressure caused both by anthropogenic overexploitation and natural calamities. There is hardly a region of the earth that is not facing this pressure due to the exponential increase in human population. The accelerating and widespread loss of species is unprecedented (Thomas *et al.*, 2004). The task of conserving biodiversity is one of the most important global environmental challenges today.

Retaining the integrity of the world's biodiversity is of great importance (Pimm *et al.*, 1995), hence, from the Earth Summit in Rio de Janeiro in 1992 to the World Summit on Sustainable Development in Johannesburg in 2002, signatory countries were committed to conserving biological diversity in their territories. Monitoring biodiversity of a single species to avoid its extinction is recognized as urgent, crucial. Thus species' maintenance is a major concern worldwide.

South Africa is one of the richest floristic countries in the world, third only after Brazil and Indonesia. It has an exceptionally rich diverse source of plant products with many used for medicinal purposes. It was estimated in 2009 that over half a million of people are directly involved in the medicinal plant trade in South Africa (Mulaudzi, 2009). The high demand for medicinal plant material has led to indiscriminate harvesting and

overexploitation of the natural flora within all ecosystems. In Vhembe District Municipality, medicinal plants play an important role not only as primary health care needs, but also as a source of incomes (Semenya and Potgieter, 2014). Hence, the growing market demand is putting undue pressure on existing resources causing a number of species to be threatened, endangered or rare. The increasing interest of using medicinal plants is endangering many important medicinal plant species, therefore, creating an urgent need for improving methods of conservation and sustainable use of plant resources. There is, thus, considerable interest to conserve the remaining endemic population of *Brackenridgea zanguebarica*.

Brackenridgea zanguebarica commonly known as “Yellow Peeling Plane” and locally known as “Mutavhatsindi” is listed as Critical Endangered plants in the Red List of South African plants (Williams *et al.*, 2013). This is principally the results of plants being illegally overexploited in their natural habitat to fulfil the growing market demand, as well as by its poor regeneration and its relatively small population. The population of this rare medicinal tree species in South Africa is restricted to a small area around the Thengwe village in the Vhembe District of Limpopo Province. *Brackenridgea zanguebarica* is in demand because of its medicinal and its magical value. For example, it is used traditionally in the treatment of various ailments (Neuwinger, 2000; Möller *et al.*, 2006; Bruschi, 2011; Steenkamp, 2003; Sheila *et al.*, 2016), such as conjunctivitis, wounds, anemia, amenorrhea, venereal diseases and jaundice. The presence of several potential chemical constituents such as phenols, flavonoids, tannins and its antimicrobial activities (Möller *et al.*, 2006) have made *B. zanguebarica* valuable as medicine plant. It is also used for magical purposes to protect people against evil spirits and to bring luck (Tshisikhawe and Van Rooyen, 2012).

Stem bark is the plant part that is being overharvested; this may seriously damage the tree's development and lead to its mortality. The plant parts used, and the irrational collection methods ultimately affect the population structure and their availability (Tsigemelak and Dharani, 2016). The increasing harvesting coupled with the poor germination rates make *Brackenridgea zanguebarica* a highly vulnerable species. Despite all these facts, there is a lack of published information concerning the conservation strategy of this species. In order to reduce the harvesting pressure and determine a sustainable harvesting method, the present work sought to investigate the current population status, and evaluate the possibilities of substituting the use of some other plant parts of *B. zanguebarica*. In order to increase the availability of this species, the present study sought, at the same time, to develop a protocol for seed and vegetative propagation and identify, through the morphological and anatomical features of seed, the potential factors that may limit its seed germination. This study is critical to initiating appropriate conservation measures and management plans for this critically endangered and endemic plant species.

1.2 Problem statement

Brackenredgia zanguebarica is a rare endemic medicinal tree species with a small population and limited distribution that contributes to the richness of biodiversity in the Vhembe District Municipality, South Africa. Extensive and destructive harvesting of bark and root for medicinal purposes, along with poor, erratic and slow regeneration have decreased the population. The species is threatened with extinction because of excessive extraction and commercial trade. Botha *et al.* (2007) reported the high

demand for this product on the markets. The high current level of demand for its bark is predicted to escalate soon due to the growing confidence in consumers in this product; Mutshinyalo (2011) reported that, it is one of the most wanted medicinal plants in the Limpopo Province.

Despite the collection of *B. zanguebarica* without its replacement, it is unclear if the germination of the seed of this species can be improved through pre-treatment techniques. In addition, the potential of vegetative propagation of the species has not been adequately determined. There is little attention from scientists to propagate this plant species, thus, our interest is now directed towards questions of conservation, and whether overexploitation and low recruitment contribute to the small population and limited distribution of *B. zanguebarica*. Propagation is one of the best interventions that can ensure that the population continues to grow and evolve in their natural habitat. Information regarding the propagation and the conservation status of this plant species is very scarce. Thus, the challenge for this study is to increase the number of individuals of this species and also to find a way to substitute the use of different parts of *B. zanguebarica* in order to establish a balanced cycle of harvest and renewal. These trees need to be conserved, not only for their value and ecological significance, but also to maintain the natural heritage of the region.

1.3 Broad objective

Develop different methods for improving the conservation and management of *Brackenridgea zanguebarica*.

This broad objective was addressed by investigating the specific objectives below:

- (i) To investigate the current threats to the existence of *B. zanguebarica*.
- (ii) To analyse the qualitative phytochemical and the antimicrobial activities of *B. zanguebarica*.
- (iii) To develop a protocol for seed and vegetative propagation of *B. zanguebarica*.
- (iv) To investigate and describe the morphological and anatomical structure of *B. zanguebarica* seed.

1.4 Hypotheses

- (i) There are currently some threats to the existence of *B. zanguebarica* in the Brackenridge Nature Reserve.
- (ii) There are similar chemical constituents and antimicrobial activities in different plant parts of *B. zanguebarica*.
- (iii) A protocol for seed and vegetative propagation of *B. zanguebarica* can be developed if appropriate treatments are provided.
- (iv) The morphological and anatomical structure of *B. zanguebarica* seed reveal factors limiting its germination.

1.5 Rationale of the Study

Plants provide valuable contributions to the ecosystem; therefore, it is important to understand the consequences of their disappearance (Lyons and Schwartz 2001). Plant contributes significantly to the quality of life as the primary producer in an ecosystem. Medicinal plants have always been part of people's life on earth, thus, there is a close relationship between plants and human beings (Mulaudzi, 2009). Human beings depend on plants for their existence and this relationship must be sustainable. For this reason, their overuse concerns everyone. In this respect, knowledge about the existing population size and structure of medicinal plants, in particular *B. zanguebarica* is very important to improve indigenous knowledge and contribute in motivating local people to conserve the species.

Brackenridgea zanguebarica, like many other medicinal trees that are under threat due mainly to the extensive use by traditional healers may soon become extinct. With the goal to conserve this species, one approach to fulfil the requirements of sustainable harvesting and at the same time satisfy the growing market demand would be, the substitution of stem bark with other parts, namely, twigs or leaves. Cunningham (1988) and Shai *et al.* (2009) reported that leaves, twigs and fruits harvesting do not damage plants in the same way as debarking and uprooting. Research into plant parts substitution may provide new information of value to Traditional Health Practitioners (THP) and scientists, and may contribute to the protection of this endemic plant from extinction.

The species has a limited distribution and is found only around Thengwe village of South Africa, and despite the number of seeds produced every year, their germination

is very poor and irregular. A species with a small and a single population that lacks recruitment remains at risk of extinction (Pimm *et al.*, 1988). The small population of this species increases its risk of extinction from relatively any small disturbances. In this case, artificial propagation techniques through seed and/or vegetative organs will be useful for increasing the plant population in order to expand its distribution into new areas of the region. Understanding the structural features of this seed is important to identify useful techniques to improve seed propagation for conservation purposes and in doing so, it may be possible to alleviate the risk of extinction.

This research is crucial to understanding the rarity and the regeneration complexities of *Brackenridgea zanguebarica*. Knowledge gained from the study can also be applied to similar endangered plant species in the future, thus benefiting the ecosystem and users.

1.6 Organization of the thesis

The thesis is composed of seven chapters. Following the first introductory chapter which outlines the structure of the thesis, the second chapter, which represents the literature review provides the background information on various aspects necessary in strengthening the conservation strategies of *Brackenridgea zanguebarica*. Four of these chapters (Chapters 3-6) address the objectives outlined above and are written in the format of scientific papers for publication. These chapters are presented in the form of self-contained papers with some inevitable repetition between chapters. Chapter Three updates the current status of this species in the Brackenridgea Nature Reserve (BNR), to provide a background as to the necessity of improving its

conservation measures. Chapter Four investigates the chemical constituents and the antimicrobial activities of some plant parts in order to assess the possibility of plant parts substitution in the treatment of various ailments. Chapter Five examines appropriate techniques through sexual and asexual propagation to ensure the availability and the perpetuation of this endemic plant species. Chapter Six describes the morphological and anatomical structure of *B. zanguebarica* seed to identify probable factors that influences its germination. Chapter Seven presents a summary of the preceding four data analysis chapters and the overall conclusion and recommendations of this study which also sets research goals for further studies.

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Chapter 2

A REVIEW ON YELLOW PEELING PLANE (*BRACKENRIDGEA ZANGUEBARICA* Oliv.): A CRITICALLY ENDANGERED ENDEMIC PLANT SPECIES

Abstract

Brackenridgea zanguebarica Oliv. is among the most popular and frequently used tree species in the Vhembe District, Limpopo Province of South Africa. However, increasing illegal overexploitation coupled with the reproductive inefficiency have caused a severe depletion of this plants population, bringing the species to the verge of extinction. *Brackenridgea zanguebarica*, commonly known as “yellow peeling plane” and locally known as “Mutavhatsindi”, is an important tree used for medicinal and magico-religious purposes in the region. This most sought-after species is a critically endangered species with its distribution in South Africa restricted to Thengwe village in the Vhembe District Municipality. It is therefore crucial to develop various propagation protocols in order to increase the availability of this species and expand its distribution area in the region. Despite the risk of extinction there is a lack of scientific knowledge regarding many aspects of this plant species. This study revealed that mostly bark is being collected for medicinal use. The aim of the current study was to compile up-to-date scientific information about this critically endangered plant species to promote its conservation. In view of the importance of conservation of such a Red-Listed plant species, the present review focused on various aspects necessary in strengthening its conservation strategies.

Keywords: *Brackenridgea zanguebarica*, conservation, critically endangered plant, endemic plant, Vhembe District.

2.1 Introduction

Plants with medicinal properties have been used for thousands of years in many countries for the treatment of various ailments (Petrovska, 2015). Medicinal plants have now gained significant importance because of their efficacy, cultural acceptability, affordability and lesser side effects (Thillaivanan and Samraj, 2014). Similar to many Africans, the majority of South Africans relies on medicinal plants for their primary health care and as a source of income (Street and Prinsloo, 2013).

Brackenridgea zanguebarica Oliv., which belongs to Ochnaceae family, is one of the most sought-after plants by traditional healers in the Limpopo Province (South Africa). The plant is locally known as “Mutavhatsindi” and is endemic to Thengwe area in the Vhembe District of Limpopo Province (Figure 2.1). The plant is overexploited for magico-religious purposes by the Vhavenda, the local tribe of the area (Tshisikhawe and Van Rooyen, 2012). There is an urgent need to standardize sustainable harvesting methods of this species so that its natural populations remain stable.

Being of medicinal and magico-religious value, *B. zanguebarica* is traded in many traditional medicine shops or “muthi shops” around the Limpopo Province. It is valued for its bark and root. These plant parts are used in the treatment of several ailments (Neuwinger, 2000; Möller *et al.*, 2006; Bruschi *et al.*, 2011) such as amenorrhea, conjunctivitis, jaundice, anaemia, wounds, diarrhoea, venereal diseases. The bark has a strong antibacterial activity against Gram-positive strains, and various phytochemical constituents like calodenin B have been isolated from the species (Möller *et al.*, 2006). The increasing harvesting of *B. zanguebarica* for medicinal and magical purposes

coupled with its poor natural regeneration ability and its poor conservation strategies in the Brackenridge Nature Reserve (BNR); pose a serious threat to the future availability of this endemic plant in the region. Due to its overexploitation, natural populations are being depleted at an alarming rate, with a high risk of extinction (Theilade *et al.*, 2007). *Brackenridgea zanguebarica* has been listed as Critically Endangered by the International Union for the Conservation of Nature (Williams *et al.*, 2013).

Literature review showed that, there are numerous threatened medicinal plant species in South Africa and *B. zanguebarica* is one of them, and thus listed in the South African Red List of Plants Species (Williams *et al.*, 2013). However, there is little published about this multi-purpose plant. Scientific information related to its taxonomy, description, distribution, traditional uses, phytochemistry, pharmacology, population status, propagation and conservation are scanty. This study is crucial to fulfil this gap as well as to highlight the importance of this plant by making people aware about its conservation, because the removal of a single species can affect the functioning of global ecosystems (Myers *et al.*, 2000).

2.2 Taxonomic distribution and description of *Brackenridgea zanguebarica*

2.2.1 Distribution and Habitat

Brackenridgea zanguebarica is distributed in eastern Africa, including Kenya and Tanzania. In southern Africa, it occurs in the northern area of Mozambique, eastern area of Zimbabwe, northern area of South Africa (Mutshinyalo, 2011) and in some areas

of Zambia. It can also be found in Madagascar. In South Africa, the species has a narrow distribution and occurs only in the Thengwe area of the Vhembe District (Tshisikhawe and Van Rooyen, 2012). This indigenous species is endemic to Vhembe District (Figure 2.1). The mean annual rainfall is 688 mm and annual mean temperature range between 16 and 26.5°C. This plant grows in stony, light grey and sandy loam soil at low altitude in open woodlands, deciduous woodlands and coastal bush (Williams and Raimondo, 2008).

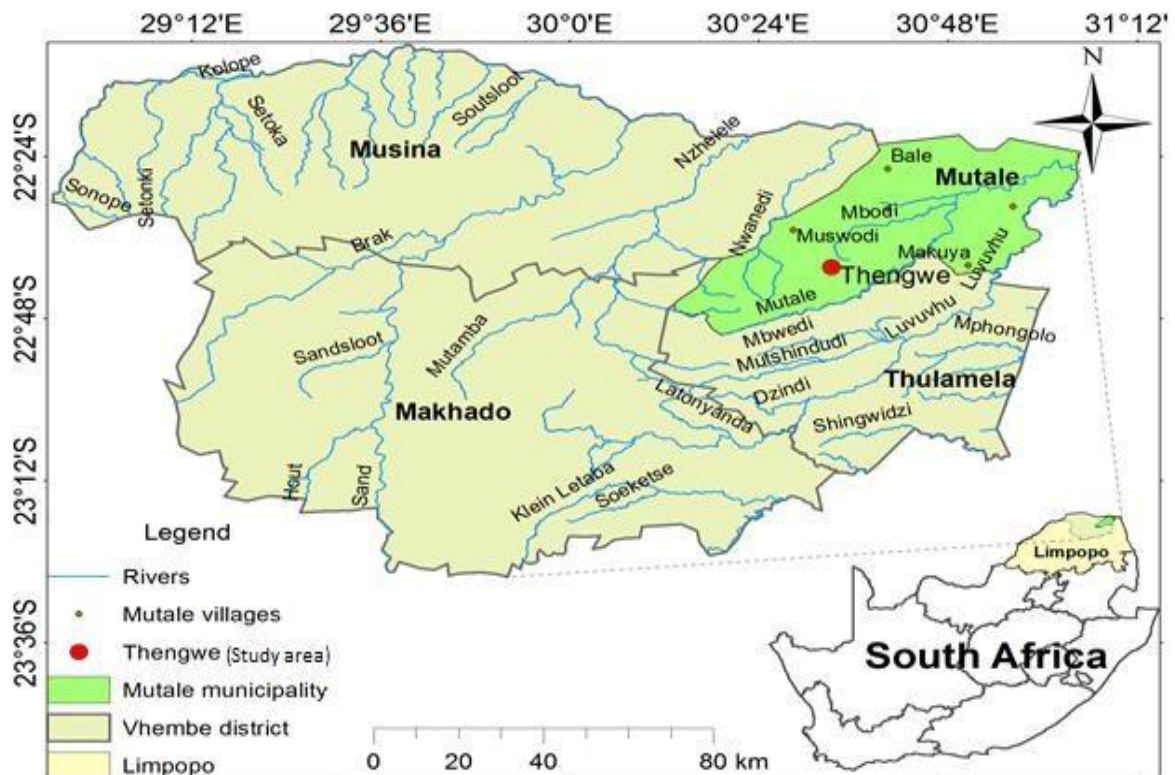


Figure 2.1: Map of Vhembe District showing the study area (Thengwe) in the Vhembe District Municipality, Limpopo Province, South Africa.

2.2.2 Description of the species

Brackenridgea zanguebarica is a plant species belonging to the Ochnaceae family. This is a family of about 500 species and 27 genera worldwide (Amaral and Bittrich 2014). The genus *Brackenridgea* to which *B. zanguebarica* belongs comprises of 12 species distributed in Africa, Madagascar, and Malaysia to Australia (Issa *et al.*, 2013).

Brackenridgea zanguebarica is a shrub or small deciduous tree growing up to 10 m height at maturity (Figure 2.2). It has very rough and corky dark grey to black bark. Scratching of the bark shows the yellow pigment beneath it. Leaves are variable in shape and size, elliptic to obovate, about 4-5 cm long, glossy dark green and hairless, lateral veins fine and numerous; margin with very fine, gland-tipped teeth (Mutshinyalo, 2011). The attractive flowers are whitish or pinkish and very ephemeral. The fruits of this species are ovoid drupes, indehiscent and greenish when unripe then turn in black when mature. The fleshy outer layer attracts the attention of animals, especially birds, as a food. Fruits contain a single seed that is very hard to separate from the pulp when dry. Seeds are small and pale brown in colour. *Brackenridgea zanguebarica* regenerates by coppice, which, according to Upadhaya *et al.* (2009) is a significant regeneration strategy to conserve viable populations of several threatened plant species.



Figure 2.2: A *Brackenridgea zanguebarica* tree in the Brackenridge Nature Reserve.

2.3 Traditional uses

Brackenridgea zanguebarica is medicinally used in many countries to treat various diseases. Neuwinger (2000) documented that the bark of *B. zanguebarica* could be used in the treatment of wounds, conjunctivitis and jaundice. In East Africa, it is used to treat anemia, swollen ankles, amenorrhea, worms and mental illness (Möller *et al.*, 2006). In Mozambique, root and bark macerated in water are used to speed the delivery process, treatment of miscarriage, diarrhoea, venereal diseases and wounds (Bruschi *et al.*, 2011). Bruschi *et al.* (2011) also reported that the root or bark is used to protect against adversities. In Tanzania, the vapours of boiled stem bark are used in the treatment of conjunctivitis (Sheila *et al.*, 2016).

In Limpopo Province of South Africa, this multipurpose tree is one of the most used medicinal tree species (Mutshinyalo, 2011). Steenkamp (2003), revealed that some Traditional Health Practitioners in South Africa used *B. zanguebarica* to treat gynecological disorders while the root is useful in the treatment of amenorrhea. For many years, the Vhavenda have been using the plant (Ponnapali *et al.*, 2012; Tshisikhawe and Van Rooyen, 2012) mainly for magico-religious purposes as protection against witchcraft and all sorts of evil. It is also reported to bring a person luck in a relationship or business. Based on this review it has been noted that in many countries, the most frequently collected parts of *B. zanguebarica* are the root and bark used in the treatment of various ailments. The species seems to be more efficient in the treatment of wounds, amenorrhea, and conjunctivitis. It is reported to protect people and properties (Bruschi *et al.*, 2011) in Mozambique.

2.4 Phytochemical studies

Phytochemicals are non-nutritive plant chemicals that occur naturally and have protective or disease preventive properties (Murugan *et al.*, 2013). Some phytochemicals isolated from *B. zanguebarica* contain various chemical compounds that are responsible for its biological activity. For example, Möller *et al.* (2006) reported several phytochemicals from the bark such as tannins, brackenin, calodenin B, flavonoids, isochamaejasmin and a derivative of isoswertisin. The chemical structures of the major compounds are shown in Figure 2.3. Intensive phytochemical studies have revealed the Ochnaceae family as an important source of biflavonoids which is well represented in some genera (Pegnyemb *et al.*, 2001; Fidelis *et al.*, 2014) such as *Lophira*, *Luxemburgia*, *Ochna* and *Ouratea*.

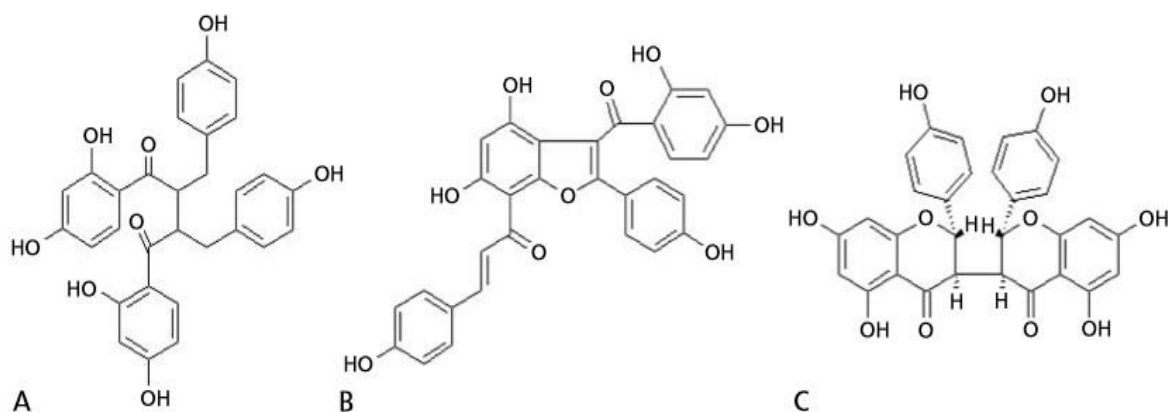


Figure 2.3: Chemical Structures of major compounds occurring in *Brackenridgea zanguebarica* (A: brackenin, B: calodenin B, C: isochamaejasmin).

Brackenin (Figure 2.3A) is an uncommon biflavonoid consisting of two α , and α' -coupled dihydrochalcones which is naturally the only existing compound in this class (Ponnappali *et al.*, 2012). It is a new dimeric dihydrochalcone isolated from the stem bark and root of *B. zanguebarica* that consist of two dihydroisoliquiritigen (Drewes, 1983).

Calodenin B (Figure 2.3B) is an isometric biflavonoid isolated from the stem bark of *B. zanguebarica* (Möller *et al.*, 2006). Ponnappali *et al.* (2012) extracted the calodenin B from the powdered stem bark of *Ochna afzelii* R.Br. ex Oliv. which is one of the members of Ochnaceae family.

Isochamaejasmin (Figure 2.3C) is a biflavonoid that consists of two units of 5, 7, 4'-trihydroxyflavanone joined together at position 3 and 3". The stem bark of *B. zanguebarica* has been found to contain the biflavonoid isochamaejasmin (Möller *et al.*, 2006). Reddy *et al.* (2008) extracted this compound from the stem bark of *Ochna lanceolata* Spreng.

These major compounds extracted from the stem bark and root of *B. zanguebarica* confirmed the presence of this molecule in members of Ochnaceae family. Therefore, there is a significant need to investigate the presence of biflavonoids in all parts of this plant. Although, the two main compounds of *B. zanguebarica* such as Calodenin B and Isochamaejasmin are also found in other genera of the Ochnaceae family, it has been noticed through this review that Brackenin only occur in *Brackenridgea*.

2.5 Pharmacological studies

Pharmacological studies on *Brackenridgea zanguebarica* have shown a range of biological activities. Some research has confirmed the potential of *B. zanguebarica* used by Traditional Health Practitioners for curing and relieving many diseases and diseases. After investigation, this plant has revealed some biological activities such as antibacterial, antiviral, antiproliferative, antifungal and antitumor activities (Möller *et al.*, 2006). However, there is a limited information regarding their specific mode of administration and action.

2.5.1 Antibacterial activity

Möller *et al.* (2006) showed a significant antibacterial activity of the ethanolic bark extracts of *Brackenridgea zanguebarica* that were tested by using a minimum inhibitory concentration (MIC) method against seven Gram-positive pathogenic bacterial strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pyogenes*, methicillin resistant *S. aureus*, whereas all the four Gram

negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Proteus mirabilis*) were resistant. Steenkamp *et al.* (2007) studied the antibacterial activity of *B. zanguebarica* on selected bacterial strains (*S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli*) and the extracts showed activity against the Gram-positive. These results support the use of *B. zanguebarica* in treating bacterial infections. Crude methanol and water extract of the root have been evaluated and the research claims that none of the extracts showed activity against the Gram-negative organisms, whereas, the tested root revealed a high antibacterial effect against Gram-positive organisms (Steenkamp *et al.* 2007). The pharmacological effects of *B. zanguebarica* have been lightly investigated. Bark extracted with ethanol and root extracted with water and methanol showed antibacterial activity against Gram-positive bacterial strains. The acetone leaf extracts of three *Ochna* species investigated such as *Ochna pretoriensis* E.Phillips., *Ochna gamostigmata* Du Toit. and *Ochna pulchra* Hook.f. showed significant antibacterial activity based on their MIC against *E. coli* and *E. faecalis* (Makhafola and Eloff, 2012). All these results suggest that further studies are needed using all the plant parts of *B. zanguebarica* with different solvents in various concentrations.

2.5.2 Antiviral activity

Considering the high prevalence of viral infections without specific treatment and the constant appearance of resistant viral strains, the development of new antiviral agents is necessary (Visintini *et al.*, 2013). The yellow bark used for the preparation of an ethanolic extract of *Brackenridgea zanguebarica* was tested for antiviral activity against Herpes simplex virus type 1 (HSV-1) and it was found that the ethanolic extract

from the bark had effectively a strong antiviral activity against free HSV-1 [5]. Some members of the Ochnaceae family, such as *Ouratea castaneifolia* (DC.) Engl., *Ouratea spectabilis* (Mart. ex Engl.) Engl. and *Ouratea semiserrata* (Mart. & Nees) Engl. have shown activity against selected viruses assayed namely HSV-1, vaccinia virus and murine encephalomyocarditis virus and *O. spectabilis* and *O. semiserrata* were the most active against HSV-1 (Brandão *et al.*, 2011). Thus, the presence of antiviral constituents from some genera of the Ochnaceae family against various viruses should be an opportunity for further studies on *B. zanguebarica* in order to test the potential of its antiviral activity against various viruses.

2.5.3 Antifungal activity

Steenkamp *et al.* (2007) evaluated the antifungal activity of *B. zanguebarica* against *Candida albicans*. The aqueous extract of this medicinal tree species inhibited *C. albicans* growth, while the methanol extracts were ineffective. However, the authors did not reveal the plant part used for the antifungal activity. Few studies have investigated the antifungal properties of this plant species. However, Araújo *et al.* (2013) evaluated the antifungal activity of extracts from *Ouratea hexasperma* and *O. parviflora* on *C. albicans* and the results obtained from *O. hexasperma* with the ethyl acetate extract of the roots and n-butanol extract of the leaves exhibited a higher antifungal activity. These results could be helpful to investigate different plant parts of *B. zanguebarica* with various solvent for antifungal activities.

The pharmacological review of this plant species presented an interesting potential of biological activities against bacteria, viruses and fungi. However, very few studies

(Möller *et al.*, 2006; Steenkamp *et al.*, 2007) have been carried out to explore deeply the biological activities of this species. Therefore, more studies are necessary to investigate the biological activity of other compounds occurring in *B. zanguebarica*.

2.6 Population status

The current population size of *Brackenridgea zanguebarica* is very small, although this species is known to have been more abundant until recently. Todd *et al.* (2004) reported that uncontrolled harvesting of this species has been high, leading to an 86% decline in density from 140 trees/ha in 1990, to 25 trees/ha in 1997. Plant have been intensively collected from the wild due to its highly medicinal and magico religious properties. The bark of this species is often removed from almost the entire stem in different diameter size classes (Figure 2.4) and sometimes the tree is felled, leading to its mortality. Todd *et al.* (2004) stated that 100% of trees sampled outside the Brackenridgea Nature Reserve (BNR) had been debarked and some their roots removed, while inside the BRN, 74% of trees showed evidence of harvesting, and of this 35% were dead. However, Tshisikhawe and Van Rooyen (2012) assessed the harvesting impact of this species in the BNR and noticed stem bark regeneration. Although the extent to which the bark is collected is well-known, the same cannot be said for the duration the bark of this plant takes to regenerate, because no studies have been done on this matter. In addition, *B. zanguebarica* has a poor seed germination and has become increasingly threatened throughout its range.

Harvesting has, to a lesser extent altered the population structure outside and within the BRN (Williams and Raimondo 2008). The number of individuals fluctuated over

time, and the remaining population within the reserve is not a guarantee for the survival of this species. Therefore, more population studies are needed to understand trends of this plant in order to formulate a management plan and conservation measures to maintain this dwindling endemic species. Due to its overexploitation and its extreme rarity, *B. zanguebarica* is Red-Listed as Critically Endangered plant species in South Africa (Williams *et al.*, 2013).



Figure 2.4: *Brackenridgea zanguebarica* tree (sapling) stripped for bark from almost the entire stem.

2.7 Propagation

Both sexual and asexual propagation is poorly known in *Brackenridgea zanguebarica*. Although to date, the artificial propagation of this tree seems to be unsuccessful, the natural regeneration of this plant under natural condition is also very challenging. The germination of seeds under natural condition is unpredictable and very slow. Seeds are very difficult to germinate in the wild which appears to be due to the presence of

parasites affecting them. However, seeds of other species in the Ochnaceae family germinate very readily (Mutshinyalo, 2011). As fallen seeds are often parasitized leading to low viability, freshly mature seed should be collected from the standing trees, and several methods of pre-treatment must be used to overcome the dormancy of *B. zanguebarica* seeds. To date, studies, have not indicated any research effort concerning the vegetative propagation of this medicinal tree. Since propagating of *Brackenridgea zanguebarica* has been very difficult, a review of several successful methods of propagating many medicinal plants could potentially be helpful to gain insight on how to propagate it.

Successful seed propagation techniques of many plants producing drupe fruits have been documented. Grunyuza *et al.* (2008) as well as Mbambezeli and Notten (2002), respectively found that to achieve best results, *Ochna pretoriensis* E. Phillips and *Ochna serrulata* Walp. fruit should be cleaned to remove of the pulp and be sown as quickly as possible. A light scarification with hot water or acid treatment would aid rapid germination of *Ochna natalitia* Walp. (McQuillan, 2015). Lilabati and Sahoo (2015) reported that pre-treatments such as scarification, stratification, gibberellic acid, hot water on seed extracted from the drupe fruit of *Embllica officinalis* Gaertn (Euphorbiaceae) were used to overcome seed dormancy and improve seedling vigour.

As an alternative to overcome seed viability problems and slow seedling growth, various vegetative propagation methods can be used. But, with restricted distribution, and a small population of this plant species, micropropagation methods can potentially be an excellent alternative for conservation of *Brackenridgea zanguebarica*. This is because small pieces of plant material can be used for mass propagation without

damage to the donor plant. Various endangered plants have been successfully propagated using *in vitro* mass propagation methods. The application of tissue culture methods for improving large scale propagation of many medicinal trees has been demonstrated by several authors (Kowalski and van Staden 2001; Usha *et al.*, 2007). The review by Afolayan and Adebola (2004) reported micropropagation protocols for some medicinal plants of South Africa and highlighted the need of using this method for the mass propagation of over-used medicinal plants. During our field work, we observed that *B. zanguebarica* has the potential to regenerate from axillary buds (coppice shoot) which, according to Upadhaya *et al.* (2009) is a significant regeneration strategy to conserve a viable population.

2.8 Conservation and sustainable use

According to the findings of many researchers, various sets of recommendations relating to the conservation of medicinal plants have been developed, such as *in-situ* and *ex-situ* conservation (Chen *et al.*, 2016). However, as stated by Sutherland (2001), the number of wild plant species necessitating specific conservation methods is so large to include all of them in conservation plans. Therefore, the conservation of plant species need the contribution of everyone. According to Hamilton (2004), several actions must be considered in favour of the conservation and sustainable use of medicinal plants in order to continuously benefit from it as a natural resource. Therefore, the conservation and sustainable use of this species could be effective through a combination of indigenous and modern knowledge systems. Both approaches follow the same purpose which is to enable this plant species to maintain itself within the context of the ecosystem in which it naturally occurs.

Mathibela (2013) revealed the importance of indigenous knowledge as a conservation strategy by emphasizing on the traditional management practices or rituals that promote sustainable resource use. According to Kambizi and Afolayan (2006), the management of plants with medicinal properties in the Guruve district of Zimbabwe has been facilitated by several inadvertent or indirect controls. Taboos, seasonal and social restrictions have all helped reduce the harvesting of medicinal plants in the area (Kambizi and Afolayan, 2006).

Mutshinyalo (2011) reported many myths related to the use of *B. zanguibarica*. After the collection of plant materials for medicinal or magical purposes, it is prohibited to take them to the house or be touched by women because they may hamper menstruation. Either a young virgin or an older member of the 'Vhatavhatsindi' clan who is no longer sexually active collect the plant material for medicinal or magical purposes (Tshisikhawe and Van Rooyen, 2012). This is because the material can cause any fertile collector to become sterile. All these traditional strategies served to limit medicinal plants harvesting and improve or promote their conservation.

Despite these indigenous knowledge practices related to conservation of medicinal plant species, there are only few studies (Kisangau, *et al.*, 2011) investigating traditionally concerning endangered, rare and threatened medicinal plants and how they could be integrated into conservation strategies. However, numerous published papers (Norfolk and Cosijn, 2011 Street, 2016) showed several policies, plans, Legislative Acts and laws that have been put together to fill some gaps in traditional knowledge and to control overharvesting of natural resources.

South Africa has done intensive work to conserve plants, including protected areas, nature reserves, parks and Botanical gardens. However, despite this intensive work and regulation towards conservation, it is still difficult to prevent local people from overexploiting plants. The Brackenridgea Nature Reserve (BNR) was established in 1987 as a strategy to protect the population of *Brackenridgea zanguebarica*. It is now classified as a critically endangered medicinal tree in the Red List of South Africa by the IUCN (Williams *et al.*, 2013). But this enforcement is still inadequate as demonstrated by continuing human activities that greatly threaten its population outside and within the reserve. It is now necessary to use various customary conservation practices such as taboos, religious values to protect some threatened plant species. These approaches of conserving biodiversity depending on cultural and religious beliefs seem to be much more reliable than those relying only on statutory regulations. However, a combination of all these approaches is needed to conserve this endemic medicinal tree species. If endemic plants are not protected, they may become extinct. The conservation status of some protected medicinal plant species in the Limpopo Province is reviewed (SANBI, 2018) and summarized in Figure 2.5 and then, presented in Table 2.1 in terms of the number of plant species in different conservation categories in the province.

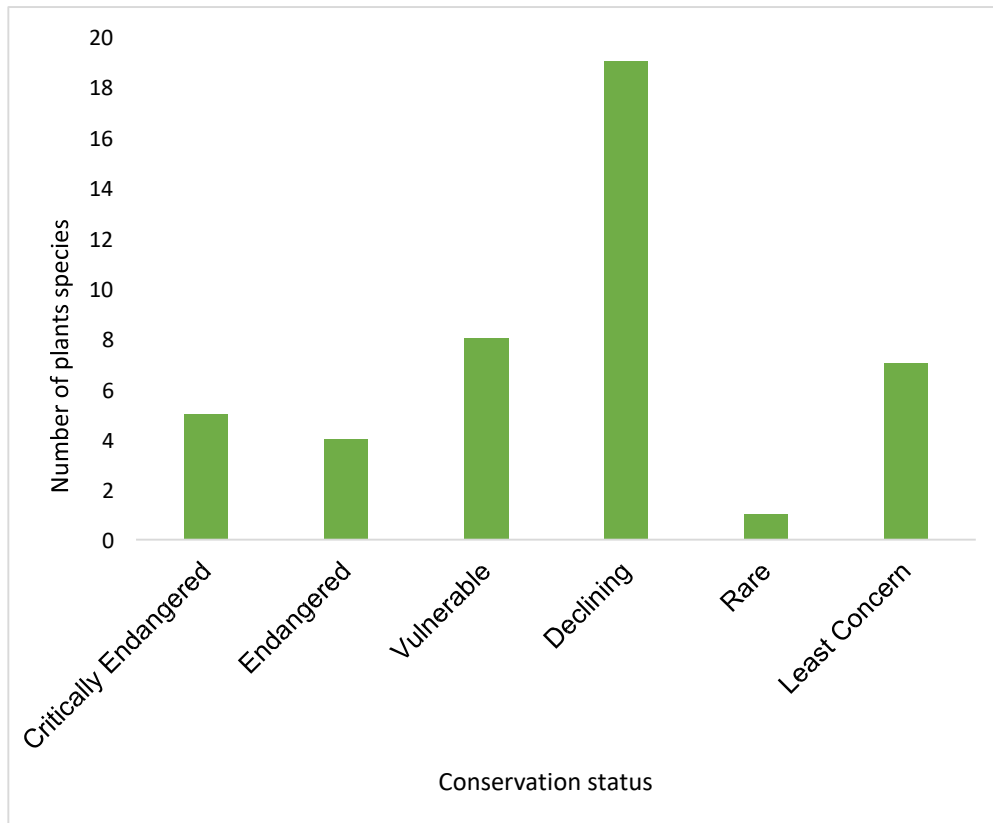


Figure 2.5: Number of plant species in different conservation categories in Limpopo Province.

Table 2.1: Conservation status of some red listed medicinal plants species in Limpopo Province (SANBI, 2018).

Conservation category	Plants species	English common name	Habit	Part harvested	Family
Critically Endangered	<i>Brackenridgea zanguebarica</i>	Yellow peeling plane	Tree	Roots, Bark	Ochnaceae
	<i>Encephalartos dolomiticus</i>	Wolkberg Cycad	Bread Tree	Roots, Corms	Zamiaceae
	<i>Encephalartos hirsutus</i>	Venda Cycad	Bread Tree	Roots, Corms	Zamiaceae
	<i>Encephalartos laevifolius</i>	Kaapsehoop cycad	Bread Tree	Roots, Corms	Zamiaceae
	<i>Siphonochilus aethiopicus</i>	Natal ginger, Wild ginger	Herb	Roots, Rhizomes	Zingiberaceae
Endangered	<i>Encephalartos eugene-marais</i>	Waterberg cycad	Bread Tree	Roots, Corms	Zamiaceae
	<i>Mondia whitei</i>	White's ginger, tonic root	Herb	Roots, leaves	Apocynaceae
	<i>Ocotea bullata</i>	Stinkwood, Cape laurel	Tree	Bark	Lauraceae
	<i>Warburgia salutaris</i>	Pepper-bark tree	Tree	Root-bark, stems	Canellaceae
Vulnerable	<i>Alepidea amatymbica</i>	Giant alepidea, larger tinsel flower	Herb	Rhizomes	Apiaceae
	<i>Bowiea volubilis</i>	Climbing Green Lily, Climbing Onion	Herb	Bulbs	Hyacinthaceae
	<i>Ceropegia cimiciodora</i>		Herb		Apocynaceae
	<i>Cucumis humifructus</i>		Herb		Cucurbitaceae
	<i>Dioscorea sylvatica</i>	Forest Elephant's Foot	Herb		Dioscoreaceae
	<i>Ocotea kenyensis</i>	Northern stinkwood	Tree	Bark	Lauraceae
	<i>Prunus africana</i>	Red stinkwood	Tree	Bark	Rosaceae
	<i>Rhynchosia vendae</i>		Herb		Fabaceae

Near Threatened	<i>Adenia fruticosa</i>	Green-stem	Herb		Passifloraceae
	<i>Clivia caulescens</i>		Herb		Amaryllidaceae
	<i>Curtisia dentata</i>	Assegai	Tree	Bark	Curtisiaceae
	<i>Drimia sanguinea</i>		Herb	Bulbs	Hyacinthaceae
	<i>Elaeodendron transvaalense</i>	bushveld saffron	Tree	Bark	Celastraceae
	<i>Gasteria batesiana</i>	knoppies-beestong	Herb	leaves	Asphodelaceae
	<i>Lithops lesliei</i>	Living stones , Stone plants	Herb		Aizoaceae
Declining	<i>Adenia gummifera</i>	Monkey Rope, Snake-climber	Herb	Roots	Passifloraceae
	<i>Aloe cooperi</i>		Herb	leaves	Asphodelaceae
	<i>Ansellia africana</i>	leopard orchid	Herb	Bulbs	Orchidaceae
	<i>Balanites maughamii</i>	Green Thorn, Torch Wood	Tree	Roots and bark	Zygophyllaceae
	<i>Boophone disticha</i>	Poison bulb, sore-eye flower	Herb	Fresh leaves	Amaryllidaceae
	<i>Callilepis leptophylla</i>	Wild Daisy	Herb		Asteraceae
	<i>Cassipourea malosana</i>	Onionwood	Tree	Bark	Rhizophoraceae
	<i>Crinum macowanii</i>	river crinum, river lily	Herb	Leaves, bulbs	Amaryllidaceae
	<i>Crinum stuhlmannii</i>	Candy-striped crinum	Herb	Bulbs	Amaryllidaceae
	<i>Cryptocarya transvaalensis</i>	Mountain Wild-Quince	Tree	Bark	Lauraceae
	<i>Drimia altissima</i>	Tall White Squill	Herb	Bulbs	Hyacinthaceae
	<i>Elaeodendron croceum</i>	saffron; saffron wood, forest saffron	Tree		Celastraceae
	<i>Eucomis autumnalis</i>	pineapple flower, pineapple lily	Herb	Bulbs	Hyacinthaceae
	<i>Eulophia speciosa</i>	Large Yellow Eulophia	Herb		Orchidaceae
	<i>Gunnera perpensa</i>	Pumpkin, Wild Rhubarb	Herb	Roots	Gunneraceae
<i>Hypoxis hemerocallidea</i>	Star-flower, Yellow Star	Herb	Roots	Hypoxidaceae	

	<i>Ilex mitis</i>	African holly, Cape holly, watertree	Tree		Aquifoliaceae
	<i>Pterocelastrus rostratus</i>	Red Candlewood, Red Cherrywood	Tree	Barks	Celastraceae
	<i>Rapanea melanophloeos</i>	Cape Beech	Tree	Roots, barks	Myrsinaceae
Rare	<i>Euphorbia sekukuniensis</i>	Sekhukhune Candelabra Tree, Sekhukhuni Euphorbia	Tree		Euphorbiaceae
	<i>Alsophila capensis</i>	Cape Tree Fern, Forest Tree Fern	Tree		Cyatheaceae
	<i>Boscia albitrunca</i>	Shepherd's tree	Tree	Roots, leaves, fruits	Capparaceae
	<i>Capparis sepiaria</i>	Wild caper bush	Shrub	Roots, bark	Capparaceae
	<i>Catha edulis</i>	Bushman's tea	Tree	Leaves, bark and roots	
Least Concern	<i>Encephalartos transvenosus</i>	Modjadji cycad	Tree	Roots, bark and leaves	Zamiaceae
	<i>Pittosporum viridiflorum</i>	Cheesewood	Tree	Roots, stem-bark	Pittosporaceae
	<i>Securidaca longepedunculata</i>	Violet tree	Tree	root-bark	Polygalaceae

2.9 Conclusion

Brackenridgea zanguebarica, an indigenous and endemic plant to Thengwe village in the Vhembe District Municipality, popularly known and well reputed for its medicinal and magical properties, is critically endangered according to the listing of the IUCN. This review of literature revealed the frequent traditional uses and a variety of biological activities found in this plant have built a heavy pressure on the remaining population of this plant in the reserve. Considering the restricted distribution of *B. zanguebarica* in South Africa as well as throughout Africa, the species has received only limited attention from researchers. However, most of the studies have been directed to the medicinal aspects of this plant. But there are still many important challenges that need to be overcome in order to discover new potent compounds. This review summarized for the first time some aspects of *B. zanguebarica* so that this information gathered together in one place, can be used as a guide to understand the present situation of this species. Therefore, there is significant necessity to continue doing research in various aspects of this species. Knowledge on the reproduction biology of this endemic plant species is strongly needed, because the availability of such information is crucial to develop a meaningful conservation strategy.

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Chapter 3

INVESTIGATION OF CURRENT THREATS TO THE EXISTENCE OF *BRACKENRIDGEA ZANGUEBARICA* Oliv. IN A SMALL GEOGRAPHIC AREA IN VHEMBE DISTRICT MUNICIPALITY, LIMPOPO PROVINCE, SOUTH AFRICA

Abstract

Brackenridgea zanguebarica is one of the most threatened plant species in South Africa, found only in Thengwe village, Vhembe District Municipality, Limpopo Province, South Africa. Due to the high traditional use of its stem bark and root, the species is now facing the threat of extinction. It has been assessed in accordance with the IUCN Red List of South Africa as a critically endangered species. The aim of this study was to investigate the impact of current threats to the population in the Brackenridgea Nature Reserve, in order to improve its conservation measures. The study was carried out in the reserve, where 10 belt transects of 50 m long and 20 m wide were laid at regular intervals of 5 m to investigate the population of *B. zanguebarica*. Individuals were counted and the structural parameters, e.g. tree height and stem diameter size of each were measured, while the impact of plant harvesting was estimated using a sliding scale of 0 to 5. The structure of the populations in terms of the stem diameter size classes was dominated by juvenile plants that showed the bell shape pattern. In addition, 59.9% out of the total plants recorded presented signs of plant parts extraction, with the stem bark the main part extracted. The population status of *B. zanguebarica* was under severe threat due to the destructive harvesting

of the mature tree parts, leading to poor regeneration of individuals. It is thus recommended that in order to improve its conservation measures.

Keywords: *Brackenridgea zanguebarica*, conservation, endemic plant, harvesting, population status

3.1 Introduction

The extinction and declining populations of many rare plant species worldwide are increasing at an alarming rate. This escalating biodiversity crisis is an indication that the current diversity of nature is not capable of supporting the pressure that the growing humanity is placing on the planet. Overexploitation of the plants' roots and bark has endangered the survival of the mature trees as these are unable to fully recover, hence, eventually hindering fruit production but rather causing poor or slow seedling recruitment (Dhillon and Gustad 2004). The continued decline, coupled with the plant's endemism status and the small geographic range where this tree species occurs, make it particularly vulnerable to natural catastrophes. Rare species restricted to one or to a few populations have a greater chance of becoming extinct (Van Dyke 2003). In this scenario, *B. zanguebarica* has been classified as a protected tree in the Red List of South Africa. In terms of the IUCN criteria, this plant has been listed under the category of critically endangered plant in South Africa (Williams and Raimondo 2008).

Brackenridgea zanguebarica, a small deciduous tree, belongs to the family, Ochnaceae. It has a restricted geographic distribution and its natural distribution zone is known from only one population confined to a small area within Thengwe, in the Vhembe District of Limpopo Province, therefore, endemic to the Vhembe District. The species is habitat-specific as many other rare and endemic plant species have only one or few ranges of distribution. It grows naturally in stony, light gray and shallow sandy loam soil, at low altitude, in open areas with low grass cover (Mutshinyalo 2011). In South Africa, *B. zanguebarica* is considered a critically endangered plant

species according to the South African National Biodiversity Institute (SANBI) Red List categories (Raimondo *et al.*, 2009), mainly due to severe harvesting from traditional herbalists, and commercial harvesting of its bark and roots to meet growing market demand. It is an important indigenous medicinal plant used by the Venda people, mostly for magical purposes (Tshisikhawe and Van Rooyen 2012).

Due to the high anthropogenic pressure, harvesting has depleted the population outside the Brackenridgea Nature Reserve as well as within the reserve, although to a lesser extent (Williams and Raimondo 2008); not a single plant seems to be available outside the reserve. Nowadays, this species only has a small population and is very threatened across its extremely narrow range of distribution. The number of individuals have fluctuated over time, however, the remaining population within the reserve it is not a guarantee for the survival of this species due to the destructive harvesting that is taking place within the reserve.

This species has been listed as protected since the establishment, in 1987, of the Brackenridgea Nature Reserve by the Provincial Limpopo Department of Economic Development, Environment and Tourism (LEDET). Despite its protection, this tree species still faces a high risk of extinction over its small geographical range due to unsustainable harvesting of its stem bark and root. The population density of *B. zanguebarica* inside and outside the reserve was investigated in 1990 and 1997. In this regard, Todd *et al.* (2004) reported that uncontrolled harvesting has resulted in an 86% decline in density from 140 trees/ha in 1990, to 25 trees/ha in 1997. The reserve, obviously, is not performing its role of *in situ* conservation of biological diversity confirming the prediction which states that, if the intensity of collecting this plant

continues at this rate, this species will become extinct in South Africa (Mutshinyalo 2011). Action, therefore, must be taken for the sustainable conservation of this plant species by setting up an effective management scheme.

The conservation of this endemic tree species is hampered by the dearth of information on its population biology and ecology. Because of this, the present study was undertaken to understand how the current threats affect the population biology and ecology of this endemic tree species in the Brackenridgea Nature Reserve. The specific objectives of the study were, to (i) Assess the population density and structure of *B. zanguebarica*; (ii) examine the harvesting impact on the population structure and the natural regeneration of *B. zanguebarica*. The findings of this study might contribute to useful information for the planning of efficient conservation strategies for this critically endangered plant species in the Brackenridgea Nature Reserve by LEDET.

3.2 Materials and Methods

3.2.1 Study site

The study was conducted in The Brackenridgea Nature Reserve (BNR) also known as the Mutavhatsindi Nature Reserve (MNR). The reserve is currently 110 ha in size and the conservation state of this species in this site is not satisfactory due to limited reserve size (Tshisikhawe *et al.*, 2013). The soil is mainly stony and thin with sandy loam (Mutshinyalo 2011). The reserve is within the savanna biome and the vegetation in and around the reserve is classified as Vhavenda Miombo (Mucina and Rutherford

2006), and it is situated in the Vhembe District Municipality of the Limpopo Province (Figure 3.1).

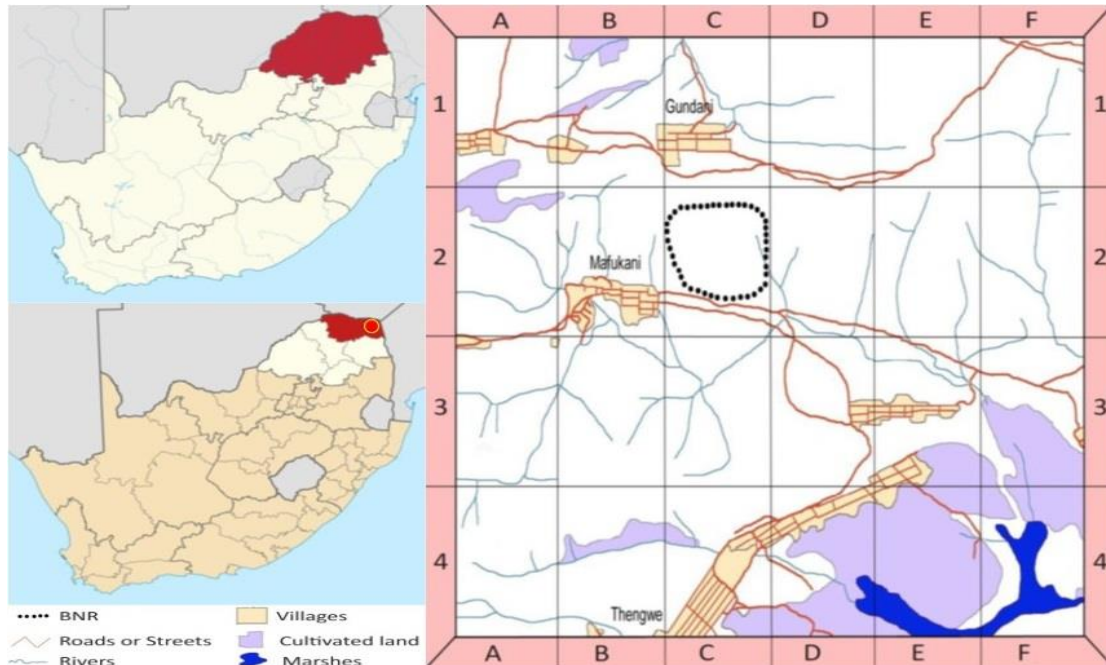


Figure 3.1: Map of the Thengwe village showing the Brackenridge Nature Reserve (BNR), in Vhembe District Municipality, Limpopo Province, South Africa (Tshisikhawe *et al.*, 2013; Wikipedia).

Its coordinates are 22° 24' 0.0"-23° 36' 0.0" S and 29° 12' 0.0" and 31° 12' 0.0" E. The reserve is at an average elevation of 600 meters above sea level. The mean annual rainfall is about 350 mm, but it occasionally varies with the annual mean maximum and minimum temperature being 26.5°C and 16°C, respectively (Mzezewa *et al.*, 2010). The temperature, however, does not vary much over the seasons. Rainfall occurs mainly during summers, from October to March, and mild winters occur from April to September (Mucina and Rutherford 2006). The vegetation of the region is dominated mostly by *Colophospermum mopane*, *Terminalia sericea*, *Grewia flava* and *Combretum apiculatum* (Venter and Witkowski 2010). The dominant ethnic

population group in the surrounding area of the BNR is the Vhavenda who survive mainly on farming.

3.2.2 Field data collection

A field survey was undertaken and samplings collected within the BNR in Thengwe village from November to December 2016. The population sampling was conducted through the vertical belt transect method of Michael (1990). This method is appropriate for recording the presence of as many taxa as possible, in the study area to produce detailed information on the population status of this plant species. In order to examine the population status of *B. zanguebarica*, it is necessary to record the presence of as many trees as possible that will be representative of the population. Ten belt transects of 50 m long and 20 m wide were laid at regular intervals of 5 m in the study area. Each belt transect was divided into two sub-belts of 50 m x 10 m for ease of sampling and five quadrats of 10 x 10 m were laid in each sub-belt as replicates. Ten quadrats were derived from each belt transect. One hundred quadrats of size 10 x 10 m, therefore, were sampled in the study site covering an area of 1 ha. The coordinates of all quadrats were recorded using a Global Positioning System (GPS).

All the *B. zanguebarica* trees, from seedlings to adult plants that were encountered within each quadrat were counted and different parameters, such as the height and stem diameter were measured. In addition, the evidence of anthropogenic bark and root harvested were estimated and scored.

3.2.3 Population density and structure

In each quadrat, the diameter of all samples of *B. zanguebarica* was measured and, the number of individuals in different diameter size classes was enumerated to assess the population structure. The diameter of seedlings and saplings were measured using tree caliper at the base of the plant, while larger individuals with DBH ≥ 5 cm were obtained by measuring their circumference at 1.3 m from the ground level with a tape measure. In order to study the stem size class distribution, seedlings were regarded as samples with a stem diameter ≤ 0.5 cm and height < 50 cm, whereas saplings were samples with a stem diameter between 0.5 and 1.99 cm and with height >50 cm. The DBH of larger individuals were recorded and grouped into eight DBH classes assigned at 4 cm DBH increments: 2.0-5.9 cm, 6.0-9.9 cm, 10.0-13.9 cm, 14.0-17.9 cm, 18.0-21.9 cm, 22.0-25.9 cm, 26.0-29.9 cm and 30.0-33.9 cm (Vesa et al. 2015). These DBH classes were then, separated into three arbitrary categories based on the age stage for easy analysis: juveniles were those with $2.0 \text{ cm} \leq \text{DBH} < 5.9 \text{ cm}$ and $6.0 \text{ cm} \leq \text{DBH} < 9.9 \text{ cm}$; sub-adults: $10.0 \text{ cm} \leq \text{DBH} < 13.9 \text{ cm}$ and $14.0 \text{ cm} \leq \text{DBH} < 17.9 \text{ cm}$ and adults: $\text{DBH} \geq 18.0 \text{ cm}$.

3.2.4 Plant parts harvested

Because of only some plant parts of this species were being over-collected, two major anthropogenic harvesting practices (root and bark) were investigated. The occurrence of any evidence of bark removal and root excavated was recorded for each sample; also chopped stems were noted as well. In order to investigate the impact of harvesting practices on the population structure of this species, all samples were

divided into different size classes based on the stem diameter and harvesting practices were measured by classifying the proportion of extracted bark or root.

The intensity of bark harvesting was estimated and recorded in a sliding scale following the visual classification described (Cunningham 1993). Cunningham (1993), used the visual method to classify the levels of damage intensity into seven categories based on the proportion of bark collected from each sample. This method was applied in this study and interpreted by using the terms of Tshisikhawe *et al.* (2012) with slight modification as follows: 0: No damaged (Null); I: Individual with $\leq 10\%$ damaged (Trace); II: Individual with 11-25% damaged (Light); III: individual with 26-50% damaged (Moderate); IV: Individual with 51-75% damaged (Severe); V: Individual with 76-100% damaged (Very severe) (Table 1 and 2).

Root excavated was assessed according to the level of disturbed surface area at the base of the stem as applied in the study of *Catha edulis* (Botha 2004). Roots excavated were recorded and scored in five classes as follows: 0: no harvesting; I: $<10\%$; II: 11-25%; III: 26-50%; IV: 51-75%; V: $>76\%$, and then, interpreted by using the above terms of Tshisikhawe *et al.*, (2012).

3.2.5 Natural regeneration

In each quadrat, the number of seedlings and saplings were counted and at the same time, all cut stumps with sprouting ability were recorded.

3.2.6 Data analysis

The numbers of the individual samples in different diameter size classes and distribution were used to create Bar graphs of the population structure. Data were entered into Microsoft Excel Spreadsheet 2010 and analyzed using SPSS version 24. Kruskal-Wallis Test (Nonparametric ANOVA) was performed to check for significant differences in the number of individual samples with signs of collection in different diameter size classes and to test if the proportion of trees harvested is diameter-dependent.

3.3 Results

3.3.1 Population density and structure

Two hundred and forty-seven *B. zanguebarica* individuals, in different diameter size classes, were recorded in 1 ha of the study area; this corresponded to a density of 247 individuals/ha. Based on the assessment of diameter size class, the population structure of *B. zanguebarica* showed that individuals are represented in all diameter classes that were arbitrarily selected. The highest number of individuals was observed in DBH (2-5.9 cm) (Figure 3.2A). All individuals in different DBH were arbitrarily grouped into five tree age stages (seedlings, sapling, juveniles, sub-adults, and adults). Out of the total samples of this plant species, 32 were found in the seedlings category, 21 were grouped as saplings, 120 represented the juvenile categories; sub-adults and adults were 57 and 17, respectively (Figure 2.B). About half (48.6%) of individuals per hectare were concentrated in the juveniles classes (2.0-5.9 cm and 6.0-9.9 cm) followed by the sub-adults classes (10.0 cm \geq DBH \leq 25.9 cm) with 23%

of the total tree species. Seedlings contributed to 13% and saplings represented 8.5% of the total tree; however, the adult category was under-represented at 6.9%.

Few individuals were recorded for both the lower and the higher stem diameter, whereas a high number of individuals were noted in the medium class. The population structure of *B. zanguebarica* according to the number of individuals per hectare showed a bell-shaped trend (Figure 3.2B). The number of individuals increases with increasing of stem diameter up to a certain point, then decreases with increase in stem diameter.

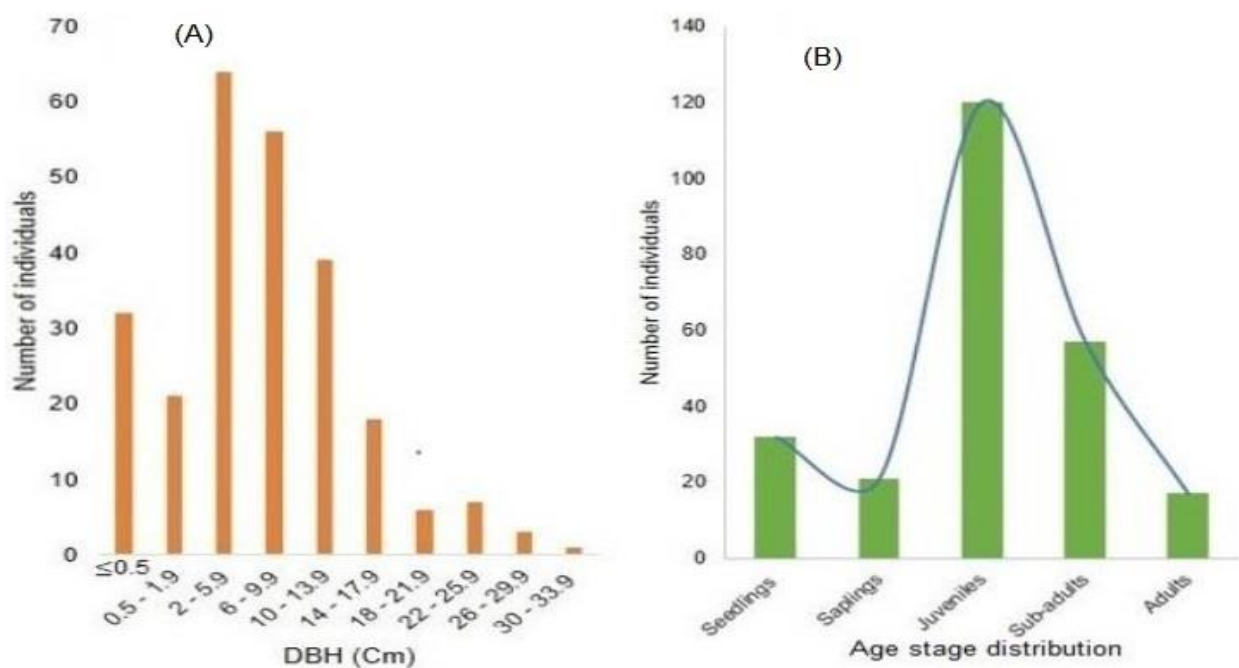


Figure 3.2: Illustration of population of *Brackenridgea zanguebarica* in the Brackenridge Nature Reserve. (A). Distribution of *B. zanguebarica* in various diameter size classes; (B). Patterns of age stage distribution of *B. zanguebarica*.

3.3.2 Proportion of plant parts harvested

A total of two hundred forty-seven individuals of the species *B. zanguebarica* was recorded in the study area of which hundred (40.5%) presented some signs of bark collection, twenty-nine (11.7%) showed evidence of roots excavated while nineteen (7.7%) had cut stumps which were clearly visible. Overall, one hundred and fifty-three plants (60%) out of the total distributed in a different size class and recorded in this study, presented some signs of plant parts extraction. The number of plants showing a different proportion of bark and root extracted is presented in Tables 1 and 2, respectively.

3.3.2.1 Bark harvested

Based on the bark collection, the number of plants and the proportion of bark removal varied for different diameter size classes. One hundred forty-seven individuals (59.5%) out of the 247 found in this population had no evidence of bark extraction, whereas 100 plants (40.5%) showed some signs of bark extraction. The extent of bark removal ranged from simple scaling (trace) to the entire removal of the bark from the tree. All size classes presented signs of bark harvested except the lowest diameter class (seedlings ≤ 2).

Table 3.1: Number of individuals with the sign of bark extraction and proportion of bark harvested in different diameter size classes of *Brackenridgea zangueberica*

DBH class (cm)	Total number of individuals sampled	Proportion of bark removal					Total number of individuals with bark harvested	
		0 (Null) 0%	1 (Trace) 1-10%	2 (Light) 11-25%	3 (Moderate) 26-50%	4 (Severe) 51-75%		5 (Very severe) 76-100%
Seedlings ≤ 0.5	32	32	0	0	0	0	0	0
Saplings 0.5-1.9	21	20	0	1	0	0	0	1
2-5.9	64	53	4	1	3	1	2	11
6-9.9	56	31	3	7	9	3	3	25
10-13.9	39	8	4	5	10	7	5	31
14-17.9	18	3	0	2	4	5	4	15
18-21.9	6	0	1	0	1	4	0	6
22-25.9	7	0	1	0	2	3	1	7
26-29.9	3	0	0	0	1	1	1	3
30-33.9	1	0	0	0	0	0	1	1
Total	247	147	13	16	30	24	17	100

Of those with signs of bark harvested, the highest number (30 plants) was moderately harvested (26-50%), 24 showed signs of severe bark removal (51-75%) while, 17 presented very severe signs of bark damaged (76-100%). Sixteen plants showed evidence of bark lightly exploited (11-25%) and the lowest number (13) were found in the trace category (1-10%). The greater the DBH of the tree, the more it was harvested and the higher the proportion of bark removal (Table 3.1). Kruskal-Wallis analyses revealed a significant relationship between the number harvested and the proportion of bark harvested in the diameter size class ($p < 0.05$).

Concerning the proportion of bark removal for each diameter class, none of the seedlings showed signs of bark removal. The sapling class had 1 plant where 11-25% of the bark had been collected. In the juvenile category (2-5.9 cm and 6-9.9 cm), 36 plants (30%) out of the 120 in this category showed signs of bark collection with different percentages. Among these 36, 5 had 76-100% of bark damaged, 4 and 12 plants were found with 51-75% and 26-50% of bark extraction, respectively. Eight plants showed 11-25% and 7 had 1-10% of their bark extracted. In the sub-adult category (10-13.9 cm and 14-17.9 cm), 46 (80.7%) out of 57 plants presented bark extraction. Four plants presented traces of bark damaged, and seven were lightly harvested. Fourteen individuals had moderately extracted bark while 12 were found with severe levels of bark removal. Nine plants were recorded in the highest proportion (76-100%) of bark extracted. In the Adult category (18-21.9 cm to 30-33.9 cm), 17 plants (100%) showed signs of bark damage (Table 3.1).

Figure 3.3 shows the percentage of plants with bark removal in different age stage distribution. In the adult categories, all the plants showed signs of bark extraction from

trace to very severe, whereas, in sub-adults' categories, 80.7% of the plants presented signs of bark removal. 30% and 4% of plants were found with signs of bark harvested in the juvenile and seedlings categories. Almost all individuals of the five largest diameter classes that were found within this population, clearly, showed signs of bark removal (Table 3.1). The majority of the larger stems had been intensely harvested in the past; hence, the larger the stem, the higher the percentage of bark removal (Figure 3.3). According to the Kruskal-Wallis Test, the number of individuals with the sign of bark harvested is considered insignificant between diameter size classes; P value of 0.5382 is considered insignificant ($p < 0.05$).

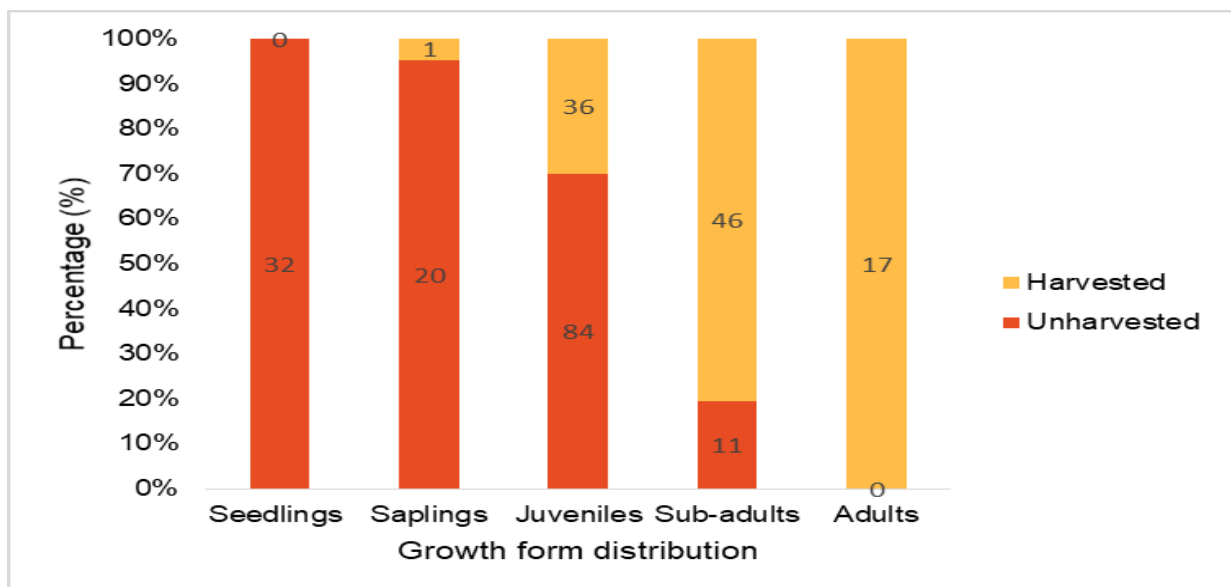


Figure 3.3: Percentage of individuals with bark removal in different growth form distribution of *Brackenridgea zanguebarica*.

3.3.2.2 Root harvested

Among the total recorded plants (247) in all the diameter size classes, 29 plants (11.7%) showed some signs of root excavated as opposed to 218 (88.3%), which had

no sign of root excavated. Of the 29 plants presenting signs of root damage, 23 (79.3%) had 1-10% (trace) and 11-25% (light) of their root extracted. Two plants presented moderate root extraction, 2 others had been severely harvested, while two showed more than 76% of root collection (Table 3.2).

Table 3.2 shows the number of plants with signs of a root collection and the proportion of root harvested in different diameter size classes. The two first classes (seedlings and saplings) showed no sign of root damage. In the juvenile category (2-5.9 cm and 6-9.9 cm) 4 samples had 1-10% of root extracted, 2 indicated light root removal and 2 showed 51-75% of root damage. Fifteen samples were found with evidence of root extracted in the sub-adult category (10-13.9 cm to 14-17.9 cm); of these, 8 presented trace of root damage, 4 showed slight proportion and 2 plants' roots had been moderately collected. In the adult category, 6 plants showed signs of root damaged of which 2, 3 and 1 had 1-10%, 11-25% and 76-100% of their roots removed, respectively (Table 3.2). The number of individuals with signs of root collection, in different diameter size classes, according to Kruskal-Wallis is significantly different ($p < 0.05$) between diameter size classes. Figure 3.4 shows that the larger the DBH class, the higher the percentage of plants with roots excavated. 35.3% of the samples in the adult categories showed signs of roots collection followed by 26.3 % in sub-adult categories and 6% in juveniles.

Table 3.2: Number of individuals with the sign of root collection and proportion of root harvested in different diameter size classes of *Brackenridgea zanguebarica*

DBH class (cm)	Total number of individuals sampled	Proportion of roots harvested					Total number of individuals with roots harvested	
		0 (Null) 0%	1 (Trace) 1-10%	2 (Light) 11-25%	3 (Moderate) 26-50%	4 (Severe) 51-75%		5 (Very severe) 76-100%
Seedlings ≤ 0.5	32	32	0	0	0	0	0	0
Saplings 0.5-1.9	21	21	0	0	0	0	0	0
2-5.9	64	61	0	2	0	1	0	3
6-9.9	56	51	4	0	0	1	0	5
10-13.9	39	31	4	3	0	0	1	8
14-17.9	18	11	4	1	2	0	0	7
18-21.9	6	4	0	2	0	0	0	2
22-25.9	7	5	1	1	0	0	0	2
26-29.9	3	2	1	0	0	0	0	1
30-33.9	1	0	0	0	0	0	1	1
Total	247	218	14	9	2	2	2	29

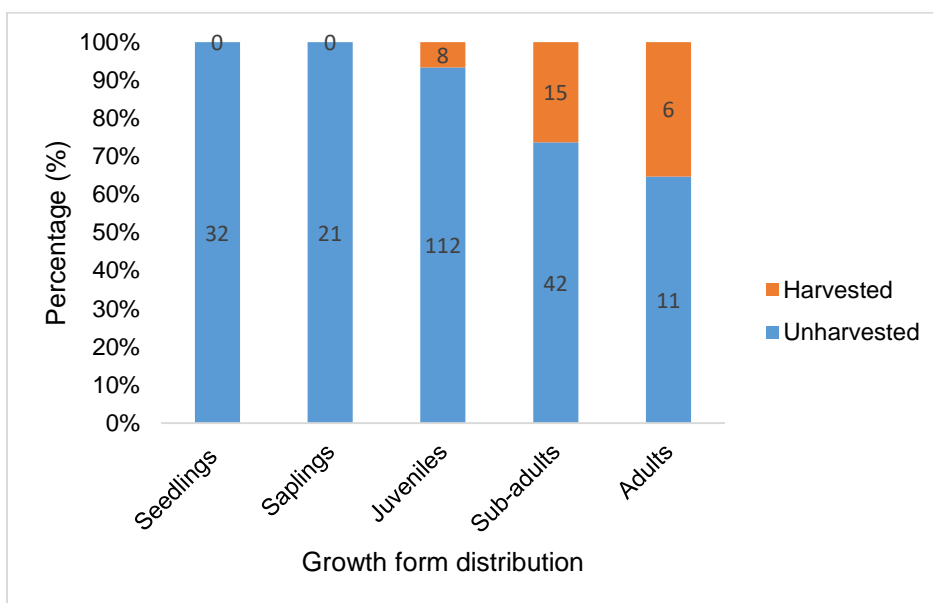


Figure 3.4: Percentage of individuals with root excavated in each growth form distribution of *Brackenridgea zanguebarica*.

3.3.2.3 Cut Stump

In this study, stem harvested were recorded only in five DBH size classes; from size class 2-5.9 cm up to size class 18-21.9 cm. In the study area (1 ha), the total number of individuals with their stem cut off was nineteen out of two hundred and forty-seven (7.7%) of which 8 (42.1%) occurred in the diameter size class (10-13.9 cm). Four plants were observed with their stems chopped off in each of the diameter size classes 6-9.9 cm and 14-17.9 cm. In the juvenile category, 5% of plants presented chopped stems, whereas only 1 (5.8%) was noted with stems cut off in the adult category. Overall, the majority of the samples presenting chopped stems were concentrated in the sub-adult category (21%). In smaller diameter classes (seedlings and saplings) stems chopped off were not observed (Figure 3.5).

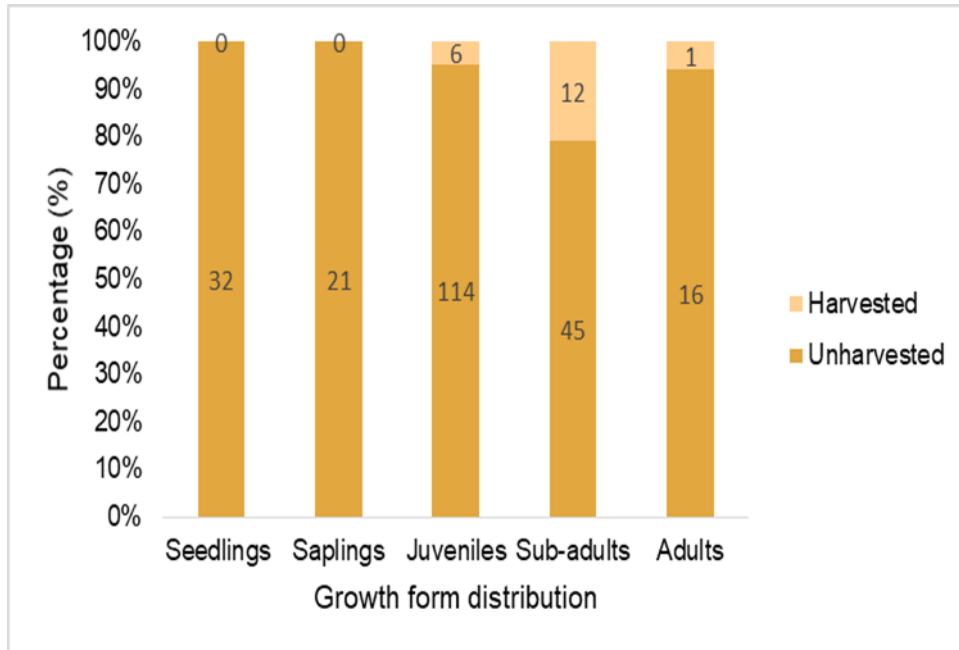


Figure 3.5: Percentage of individuals with cut stump in each growth form distribution of *Brackenridgea zanguebarica*.

3.3.3 Natural regeneration

The low number of seedlings (32) and saplings (21) (Figure 3.2B) encountered in this study showed low rates of regeneration. Figure 3.2B indicated a few individuals from the lower (32 individuals) and higher (16 individuals) stem diameter. Sprouting from few cut stumps (19) was recorded only in the Juvenile, Sub-adult and Adult categories (Figure 3.5). Only 3 out of 19 (15.8 %) cut stumps showed coppice ability (Figure 3.6).



Figure 3.6: Regeneration from axillary bud (coppice shoot) of *Brackenridgea zanguebarica* in its natural habitat.

3.3.4 The main threat to *Brackenridgea zanguebarica*

The results of this study revealed that the stem bark was the most collected plant part with about 40.5% followed by root (11.7%) and cut stump (7.7%). Overall, 60% of individuals showed signs of plant part extraction and 40% of this species showed no damage.

3.4 Discussion

3.4.1 Population density and structure

The results in terms of DBH size distributions of *B. zanguebarica* population revealed that the majority (48.6 %) of the plants were in the juvenile categories, although, few plants in both lower and higher diameter size classes were also observed. This result indicated that the low number (6.9 %) of adult trees is probably due to the destructive harvesting of bark and root. The higher number of individuals in the juvenile stage could be due to the harvesting status of these plants; they are considered not mature enough to be intensely extracted by the harvesters. The higher number of individuals in the juvenile stage could further be due to irregular recruitment episodes when conditions were favorable (Tsheboeng and Murray-Hudson 2013).

The gradual decrease in density from the sub-adult to adult stages is due to past anthropogenic activities that targeted mature plants for bark and root collection, which might have led to reduced reproduction. Similar results have also been reported that populations of species of anthropogenic importance are often characterized by a decrease in occurrences in larger size classes (Botha *et al.*, 2004). Williams *et al.* (2007) revealed that harvesters tend to select plants in the larger size classes. The structural population pattern of *B. zanguebarica* in this study contradicts that of Tshisikhawe and Van Rooyen (2012) from the same study area, who reported that the population structure of *B. zanguebarica* exhibited the inverse J-shaped curve, indicating a healthy population structure. This contradiction might be associated with the difference interval of the diameter size classes utilized in each study.

3.4.2 Plant parts harvested

3.4.2.1 Bark harvesting

A large number (40.5%) of plants showed evidence of bark extraction. In almost all diameter size classes, signs of stem bark collection were noted, which highlights the growing market demand for this multipurpose tree in the area. Despite, the collection of stem bark in almost all the diameter size classes, there was a tendency for more harvesting from trees belonging to the larger diameter classes. This is comprehensible because trees with a large stem diameter have more available bark to harvest than the small ones. These results correspond with other studies, such of *Garcinia lucida* Vesque (Guedje *et al.*, 2007), *Anadenathera colubrina* (Soldati *et al.*, 2011) and *Myracrodruo nurundeuva* (Lins Neto *et al.*, 2008). Bark extraction from large plants could affect the survival of reproductive trees as they will not be able to fully recover and will eventually hinder fruit production (Dhillion and Gustad 2004); this will lead to poor seed production. The poor reproduction of this tree species probably increases with the intensity of bark extracted.

The intensive bark collection of *B. zanguebarica* has caused serious damage to its population because harvesters seem to have no preference about size class. This result supports the findings of the study that revealed the non-size class effect on the extent of bark and foliage harvest of *Azelia africana* and *Pterocarpus erinaceus* in Eastern Burkina Faso (Nacoulma *et al.*, 2011). If the bark is collected from the small-size class trees, it affects the growth and development of this species. Bark harvesting from small plants was reported in some studies such as the study of people's

knowledge and extractivism of *Stryphnodendron rotundifolium* Mart. in Northeastern of Brazil (Feitosa *et al.*, 2014).

The extraction of bark from this multipurpose tree, either from small size classes or from bigger reproductive trees, have had a detrimental impact on the population structure. This species, therefore faces a serious threat from the random collection of bark, which has eventually led to a critically endangered plant. If this trend continues to occur, it is predicted that the population will be extinct in the next years to come.

3.4.2.2 Root harvesting

In this study, few trees showed evidence of root extraction. A similar situation of root extraction was revealed by Todd (1999), with the number of the tree with root harvested concentrated in the higher diameter classes. Harvesters seem to focus their activities on higher-diameter size classes. The low proportion of root harvested of this tree species may reveal that, this plant part seems not to be the most preferred by harvesters. However, the harvesting impact of root might be responsible for the death of some individuals of this tree species.

3.4.2.3 Cut stump

The variation in the number of cut stumps in the different diameter classes reveals the dominance of plants in the intermediate size class. The high number of cut stumps in the medium diameter class may be due to high density of medium diameter resulting in a large probability to be found and cut. Few cut stump in this category showed the sprouting ability, which is an important reproduction mechanism to maintain a

population viable. Even though many factors influence the ability of a stump to sprout, some studies reveal that one of the most important factors in terms of coppice regeneration is the age of the tree (Johansson and Hjelm 2012).

3.4.3 Natural regeneration

The majority of individuals found in the medium diameter class of this tree may be due to irregular recruitment and mortality of trees of the larger diameter size classes. The *B. zanguebarica* status showed a bell-shaped pattern with fewer individuals in the seedling, sapling, sub-adult and adult stages compared to the juvenile stage (Figure 3.2B). Species with such regeneration pattern is an indication of an unstable population (Helm and Witkowski 2012), and which are under threat due to lower recruitment levels (Colling *et al.*, 2002).

Few numbers of cut stumps observed only in juvenile and sub-adult categories showed the sprouting ability. This may indicate that natural regeneration by coppicing of this species is influenced by the stem diameter size. These findings are also in accord with those of Sangeda and Maleko (2018) who stated that *Brachystegia boehmii* and *B. spiciformis* of large size diameter classes did not show much vegetative regeneration. The number of shoots per stump and how long they can start to sprout were not investigated in this study. However, this study showed that *B. zanguebarica* could regenerate vegetatively and this could assist in increasing the regrowth rates of the species.

3.5 Conclusion and recommendations

The overall findings of this study show that the population of *B. zanguebarica* that occurs in the BNR is facing many stresses from human activities which need the reinforcement of certain conservation measures. The harvesting impact on population structure and regeneration showed that *B. zanguebarica* is severely overexploited in the reserve. The destructive harvesting of large individuals influenced the natural regeneration of this plant. The poor natural regeneration is characterized by few seedlings and saplings. Bark harvesting for medicinal and magical purposes is the main threat to the decline of *B. zanguebarica*. The bell-shaped size class distribution pattern characterizes the population structure of *B. zanguebarica* due to the high number of medium-sized trees with a poor number of individuals in both lower and higher DBH size classes. This indicates that the population of *B. zanguebarica* in the reserve is under threat. The ongoing anthropogenic activities are influencing the population structure and the regeneration status of this tree species. The species has become a critically endangered plant due to the intensive and improper collection of plant parts to supply the growing market demand. This trend may reduce the capacity of this tree species to maintain its population in the coming years. Hence, proper conservation measures have to be formulated to protect the remaining population of *B. zanguebarica*, taking into account not only its multiple values but also for future research. This study is an important step towards the conservation of this threatened plant species. Hence, the following points are made as recommendations: (i) Protection and continuous inventory of the existing populations should be conducted, and it needs the participation of local people. (ii) Conservation through sexual and asexual propagation is urgently needed for overcoming the challenges of natural

regeneration. (iii) Regular overnight patrols to reduce illegal harvesting should be implemented by the manager of the reserve. (iv) *Ex situ* conservation and plantation of *B. zanguebarica* are necessary to expand the new production area in the region.

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Chapter 4

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITIES OF DIFFERENT PLANT PARTS OF *BRACKENRIDGEA ZANGUEBARICA* Oliv. AS AN APPROACH FOR ITS CONSERVATION

Abstract

Brackenridgea zanguebarica of Ochnaceae family, is a multipurpose tree species prioritized for conservation in South Africa. The stem bark is mainly and widely harvested for its important therapeutic properties; this has increased the risk of extinction of this plant species. With the aim of conserving this threatened plant species, the present study was undertaken to analyse the phytochemical and the antimicrobial activities of some plant parts to evaluate the possible substitution of other plant parts which may assist in a rational for sustainable collection. To achieve this, different plant parts, namely: leaves, stem bark and twigs were collected, shade dried, powdered and separately extracted with acetone. The plant extracts were screened for phytochemicals using standard methods, Thin-layer chromatography (TLC) was carried out using various solvent systems of varying polarity. Agar well diffusion and broth microdilution methods were used respectively to evaluate the antimicrobial activity and the minimum inhibitory concentrations (MIC) of plant extracts against three bacteria and four yeasts. The phytochemical analysis revealed a close similarity of various phytoconstituents of the three plant parts. TLC analysis showed a slight difference in the acetone extract of different plant parts with more bands on the leaves than the stem barks and twigs. Acetone extracts of the three plant parts exhibited

varying degrees of antimicrobial activity against *Staphylococcus aureus* and *Candida glabrata*, while none of the plant extracts showed any activity against *Escherichia coli*, *Klebsiella pneumonia*, *Candida albicans*, *C. krusei*, and *C. parapsilosis*. Overall, the stem bark extract had the highest antimicrobial activity with a zone of inhibition of 15.8 mm for the bacteria (*S. aureus*) and 19.7 mm for the yeast (*C. glabrata*), followed closely by twig and leaves extracts. Stem bark extract had the lowest MIC value ranging from 0.5 to 2.5 mg/ml against all microorganisms tested when compared to leaves' and twigs' extracts. The current study conclude that the three plant parts tested had similar chemical constituents and antimicrobial activity, which indicate the possible substitution of these plant parts in the treatment of various ailments.

Keywords: Phytochemical, Antimicrobial, TLC, *Brackenridgea zanguebarica*, Microorganisms.

4.1 Introduction

For decades, many plant species have been heavily exploited for their highly desirable therapeutic properties. Demand for traditional medicine has now exceeded the supply in the growing market, leading to the depletion of plant species in their natural habitat. Approximately 80% of the world's populations rely on traditional medicine for their primary health care (WHO, 2004). Countries like South Africa have for many years been using plants as medicine to treat of various ailments (Amoo *et al.*, 2009). The unsustainable collection of medicinal plant materials has been based on the assumption that plants will be available on a continuing basis (WHO/ IUCN/ WWF, 1993). Today, many medicinal plant species like *Brackenridgea zanguebarica* face extinction. In the future, this plant may be totally unavailable due to illegal and indiscriminate collection. It has been classified as a critically endangered plant in the Red List of South African plants (William *et al.*, 2013).

Brackenridgea zanguebarica is one of the threatened medicinal plants prioritized for conservation in South Africa. It is an ethno-medicinally important tree species, found only in Thengwe village of South Africa (Tshisikhawe and Van Rooyen, 2012). Commonly known as 'yellow peeling plane' and locally known as 'Mutavhatsindi', it belongs to the Ochnaceae family. *Brackenridgea zanguebarica* has been used for many years by the Vhavenda people for its medicinal and magical properties (Tshisikhawe and Van Rooyen, 2012). Its stem bark and roots have been reported to being used in the treatment of various ailments (Neuwinger, 2000; Steenkamp, 2003; Möller *et al.*, 2006; Bruschi, 2011; Sheila *et al.*, 2016), such as conjunctivitis, wounds, anemia, amenorrhea, venereal diseases and jaundice. The stem bark has been

proved to be widely harvested for the presence of chemical compounds such as phenols, flavonoids, tannins with a range of valuable biological activities such as antibacterial, antiviral, antiproliferative, antifungal and antitumor properties (Möller *et al.*, 2006). The stem bark of *B. zanguebarica* is the most commonly and extensively used plant part in traditional medicine (Tiawoun *et al.*, 2019).

The intensive and unsustainable harvesting of plant species for medicinal purposes pose a threat (Mander 1998). *Brackenridgea zanguebarica* is under threat due mainly to the destructive method of bark extraction, which exposes the uncovered stem to insects, pests and fungal infection; this practice may eventually hinder fruit production and affect the plant's population dynamics in the wild. This has also resulted in this endemic species becoming rare and threatened with extinction in the Brackenridgea Nature Reserve (BNR). In order to prevent further depletion due to unsustainable overexploitation and to conserve this valuable multipurpose tree species, it was crucial to investigate if different parts of this plant species have either similar or different compounds with beneficial effects on the treatment of various ailments. The need for sustainable harvesting and to provide at the same time some primary health care could be fulfilled by the substitution of different parts of the same plant (Zschocke *et al.*, 2000). Plant parts substitution may provide reliable and viable possibilities to overcome the overexploitation problem of *B. zanguebarica*. Although the stem bark of this species is predominantly used for its medicinal properties, there is a lack of information regarding the medicinal properties of other parts, like leaves and twigs that could serve as a beneficial alternative to bark harvesting.

Due to the illegal, indiscriminate and intense stem bark removal of *B. zanguebarica* in the Brackenridge Nature Reserve and a lack of proper propagation practices, this plant species is severely threatened. Rational and sustainable harvesting by substitution of various parts of the plant may provide an alternative approach for the conservation of this critically endangered plant species. This study aimed to evaluate the phytochemical and the antimicrobial activity of various aerial parts of *B. zanguebarica*.

4.2 Materials and Methods

4.2.1 Collection, identification and preparation of plant material

Stem bark, twigs and leaves of *Brackenridgea zanguebarica* were collected during June 2018 from mature trees growing in the Brackenridge Nature Reserve, situated at Thengwe village. The village is at 22° 24' 0.0" - 23° 36' 0.0" S and 29° 12' 0.0" - 31° 12' 0.0" E. The botanical identification of the plant material was confirmed at the Herbarium of the Department of Botany, University of Venda with voucher number: TEU037. The collected samples were shade-dried on the laboratory bench at room temperature for 14 days. The dried samples were crushed into small pieces, ground to fine powder using an electric blender, and then stored separately in airtight containers at ambient temperature until needed for extract preparation.

4.2.2 Preparation of extracts

Acetone was used as extraction solvents because it extracts both polar and non-polar compounds (Shai *et al.*, 2009) and because it has been reported to be harmless to

fungi and bacteria (Masoko *et al.*, 2007). For preparation of extracts, 50 g each of the stem bark, twigs and leaf powder obtained was macerated separately with 500 ml of acetone at laboratory temperature for 72 hours. After this period, the mixture was filtered using sterile Whatman No. 1 filter paper to obtain an extract. This procedure was repeated twice to exhaustively extract the plant material with 250 ml and 125 ml of acetone, respectively. The plant extracts from the three procedures were combined and concentrated to dryness in rotary vacuum evaporator and thereafter, it was placed under a fan to evaporate the solvent.

4.2.3 Preliminary phytochemical screening

Ten grams of the dried acetone extracts of *B. zanguebarica* stem bark, twigs, leaves were re-dissolved in 100 ml of acetone solvents to obtain the stock solutions for the preliminary phytochemical screening test. Standard procedures as described by Sofowara (1993) as well as Trease and Evans (2002) were used to identify various phytochemicals, such as alkaloids, cardiac glycoside, coumarins, flavonoids, phenols, saponins, steroids, tannins and terpenoids. The interpretation of the results was done by direct eye observation of the colouration profile of the reactions and/or the formation of precipitates (Nemudzivhadi and Masoko, 2015; Geetha *et al.*, 2016).

Test for alkaloids: Take 1 ml of plant extract, add 6 drops of Mayer's reagent. The formation of yellowish creamish precipitate indicate the presence of alkaloids (Harbone, 1998; Edeoga *et al.*, 2005).

Test for cardiac glycoside: To 2 ml of plant extract, add 2.5 ml of glacial acetic, 1 ml of ferric chloride and then add 1 ml of concentrated sulphuric acid. Appearance of

upper red layer with green fluorescence indicates the presence of cardiac glycoside (Harbone, 1998).

Test for coumarins: To 2 ml of plant extract, add 3 ml of 10% sodium hydroxide. Appearance of yellow color indicates the presence of coumarins (Harbone, 1998).

Test for flavonoids: Take 1 ml of plant extract and add 1 ml of sulphuric acid. Formation of orange color confirmed the presence of flavonoids (Kokate, 2000).

Test for phenols: To 1 ml of plant extract was added 4 drops of 3% ferric chloride. The appearance of deep blue color shows the presence of phenol (Kokate, 2000).

Test for saponins: To 1 ml of plant extract add 1 ml of sodium hydroxide and shake vigorously. The stable persistent froth/foam indicates the presence of saponins (Sofowora, 1993).

Test for steroids: To 2 ml of plant extract, add 2 ml of chloroform and 2 ml of concentrated sulphuric acid. Appearance of upper reddish brown ring showed the presence of steroids (Harbone, 1998).

Test for tannins: To 1ml of plant extract was added 2 ml of water and 2 drops of 5% ferric chloride solution. Formation of dirty green precipitate indicates the presence of tannins (Harbone, 1998; Edeoga *et al.*, 2005).

Test for terpenoids: To 2 ml of plant extract, 2 ml of acetic acid was added and then 1 ml of concentrated sulphuric acid. Formation of deep red color indicates the presence of terpenoids (Harbone, 1998).

The interpretation of the results was done by direct eye observation of the coloration profile of the reactions and/or the formation of precipitates (Nemudzivhadi and Masoko, 2015; Geetha *et al.*, 2016).

4.2.4 Thin layer chromatography

To analyse the presence of various phytochemical compounds, the acetone extract of stem bark, twigs and leaves of *B. zanguebarica* was performed by separation on TLC using aluminium-backed TLC plates coated with Merck, Silica gel 60 GF 254. The extracts were taken in a capillary tube and spotted at about 5 cm from the bottom of the TLC plate and placed in the TLC chambers, previously saturated with different solvent systems of varying polarity. The three solvent systems used to separate the compounds were: benzene/ethanol/ammonia hydroxide (BEA) (18:2:0.2), chloroform/ethyl acetate/formic acid (CEF) (10:8:2), and ethyl acetate/methanol/water (EMW) (10:5.4:4) (Kotze and Eloff, 2002). The separated classes of compounds on the TLC plates were visualized under ultraviolet (UV) light (365 nm). For classes of compounds that were not visible under UV light, the TLC plates were sprayed with vanillin-sulphuric acid reagent (0.2 g vanillin (Sigma ®): 56 ml methanol: 2 ml concentrated sulphuric acid) and heated at 110°C for optimal colour development and viewed in daylight (Masoko and Makgapeetja, 2015). The colour of the spots was viewed and the retention factor (R_f) values of all the spots were determined by the following formula:

$R_f = \text{Distance travelled by the plant extract (solute)}/\text{distance travelled by the solvent system.}$

4.2.5 Antimicrobial Activity Assays

4.2.5.1 Microorganisms and growth conditions

The microorganisms used for the determination of antimicrobial activities of the different extracts were obtained from the Department of Microbiology, University of Venda. Acetone extracts of leaves, stem bark and twigs of *B. zanguebarica* were evaluated against the following microbial strains: one Gram positive bacteria: *Staphylococcus aureus* (ATCC 33594); two Gram negative bacteria: *Escherichia coli* (ATCC 35218), *Klebsiella pneumonia* (ATCC 700603) and four yeasts (clinical isolate): *Candida albicans*, *C. glabrata*, *C. krusei* and *C. parapsilosis*. Each microorganism, prior to the antimicrobial testing was cultured on selective media. Mannitol salt for *S. aureus*; MacConkey for *E. coli* and *K. pneumonia* and for fungi, Sabouraud Dextrose Agar (SDA) were used for developing surface colony growth.

4.2.5.2 Agar Well Diffusion Method

The agar's well-diffusion assay was used to evaluate the anti-bacterial and anti-yeast activities of the acetone extracts of different plant parts of *B. zanguebarica*. The experiments were done in triplicates. Small holes of 6 mm diameter were made in Mueller- Hinton agar medium (MHA) plate of 90 mm diameter, using a sterile cork borer. The sterile swab stick was dipped into an overnight culture of bacterial or yeast suspensions strain (adjusted to turbidity of 0.5 McFarland Standard) and the swab stick moistened with bacterial or yeast was spread over the solid media (MHA). About 20 μ L of each plant part extract (30 mg/ml) was dispensed into each hole and the

plates were incubated at 37°C for 24 h. At the end of the incubation period, the diameters of growth inhibition zones around the holes, if any, were measured in millimetre. The inhibitory activity of the plant was indicated by a clear zone of no microbial growth around each well.

4.2.5.3 Determination of Minimum inhibitory concentration (MIC) values

The determination of minimum inhibitory concentrations (MIC) of different plant part extracts was done using broth microdilution method and performed in 96 well Microtiter plate. 180 µl of Mueller- Hinton broth (MHB) (Rochelle Chemicals SA) was distributed into each well, in the first row (A) of the Microtiter plate and 100 µl from the second row (B) to the last row (H). The acetone extract of different plant parts to be tested was 30 mg/ml. Twenty microliters extracts from each plant part was added from the third to the twelfth well on the first row, while 20 µl of distilled water and gentamicin (for bacteria)/ miconazole (for yeast) was added to the first and the second well on the first row as negative and positive controls, respectively. A two-fold serial dilution was done by mixing the contents in each well of the first row and transferring 100 µl to the next well for the same column, and the same procedure was done up to the last well of the same column, and the last 100 µl from the last well was discarded (Samie *et al.*, 2017). Then, 100 µl of different microorganism suspensions was added to each well and the Microtiter plate was incubated for 24 h at 37°C. The following day, 40 µl of Iodo-nitrotetrazolium (INT) dissolved in distilled water (0.2 mg/ml) was added in each well and the plate was incubated at 37°C for about 15 min (Buwa and van Staden, 2006). The results were read and the clear wells indicated the presence of compound in the extracts that inhibited the growth of the microorganisms tested. The

lowest concentration of each extract showing no growth was taken as its minimum inhibitory concentration (MIC).

4.2.5.4 Determination of Minimum bactericidal/fungicidal concentrations (MBC/MFC) values

The MBC and MFC were determined for each of the extracts by sub-culturing the media from each well showing no visible growth during MIC determination onto MHA for bacteria and SDA plates for yeast. The plates were incubated at 37°C until growth was seen in the control plates. The MBC/MFC was defined as the consequent concentrations required for killing 99.9% of the cells (Scorzoni *et al.*, 2007).

4.3 Results

4.3.1 Qualitative phytochemical analysis of acetone extract of stem barks, leaves and twigs of *Brackenridgea zanguebarica*

With the standard procedures described by Trease and Evans (2002) as well as Sofowara (2008), the qualitative phytochemical analysis of acetone extract of stem bark, leaves and twigs of *Brackenridgea zanguebarica* showed the presence of various kinds of chemical constituents such as alkaloids, cardiac glycosides, coumarins, flavonoids, phenols, quinones, saponins, steroids, tannins and terpenoids. Among the 10 phytoconstituents tested, nine were positive. However, results revealed the presence of almost similar phytochemical constituents in different plant parts

studied with the absence of saponins in stem bark extracts and the absence of steroids in the leaf and twig extracts. (Table 4.1).

Table 4.1: Preliminary qualitative phytochemical analysis of acetone extract of stem bark, leaves and twigs of *Brackenridgea zanguebarica*.

Plant constituents	Plant extracts		
	Leaves	Stem bark	Twigs
Alkaloids	+	+	+
Cardiac glycosides	+	+	+
Coumarins	+	+	+
Flavonoids	+	+	+
Phenols	+	+	+
Quinones	+	+	+
Saponins	+	–	+
Steroids	–	+	–
Tannins	+	+	+
Terpenoids	+	+	+

Keys: + = Presence, – = absence

4.3.2 Thin layer chromatography of acetone extracts of stem barks, leaves and twigs of *Brackenridgea zanguebarica*

For the acetone extracts of leaves, stem bark and twigs, the TLC chromatogram developed in the BEA revealed a total of 19 bands when visualized under UV light; leaves, stem bark and twigs showed 8, 5 and 6 bands respectively (Figure 4.1A). Out of the 19 bands found under UV, 4 were common to stem bark, leaves and twigs with Rf values 0.15, 0.46, 0.53, 0.69 (Table 4.2). When visualized in daylight, the acetone extract of the three plant parts revealed 6 bands, viewed only on the leaf extract with Rf values between 0.13 to 0.91 (Figure 4.1B and Table 4.2). The acetone extracts of stem bark and twigs failed to exhibit any separation when viewed in daylight.

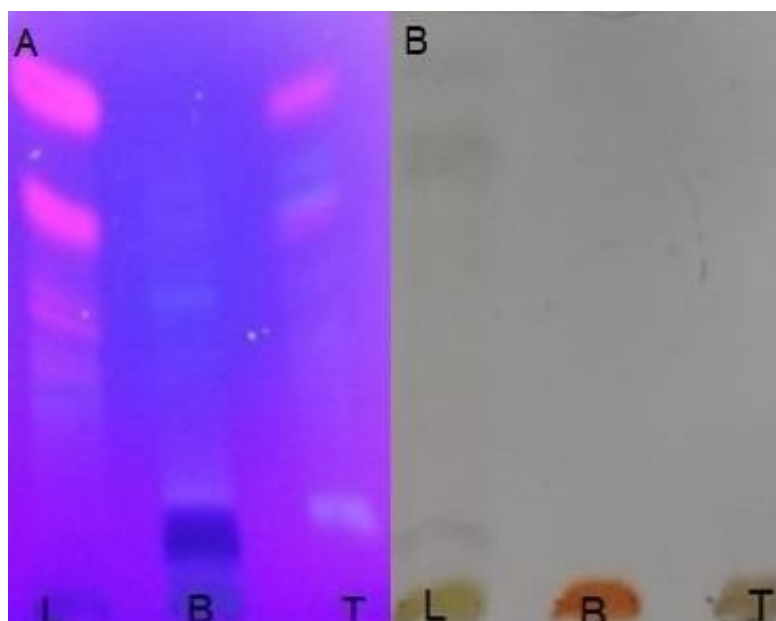


Figure 4.1: Chromatograms of acetone extracts of leaves (L), stem barks (B) and twigs (T) of *Brackenridgea zanguebarica* developed with benzene/ethanol/ammonia hydroxide (18:2:0.2). (A) TLC plates viewed under UV 365 nm, (B) TLC plates viewed under daylight after being sprayed with vanillin reagent.

The chromatogram developed in the CEF of the acetone extracts of the three plant parts showed a total of 19 bands under UV; leaves, stem bark and twigs showed 8, 7 and 4 bands, respectively. Out of the 19 bands found under UV, 3 were common to the three plant parts with Rf values 0.46, 0.92, 0.96 (Figure 4.2A, Table 4.2). In daylight, 19 bands were recorded; leaves, stem bark and twigs showed 8, 6 and 5 bands, respectively, and 2 bands were common to the three plant parts with Rf values of 0.04, 0.26 (Figure 4.2B, Table 4.2).

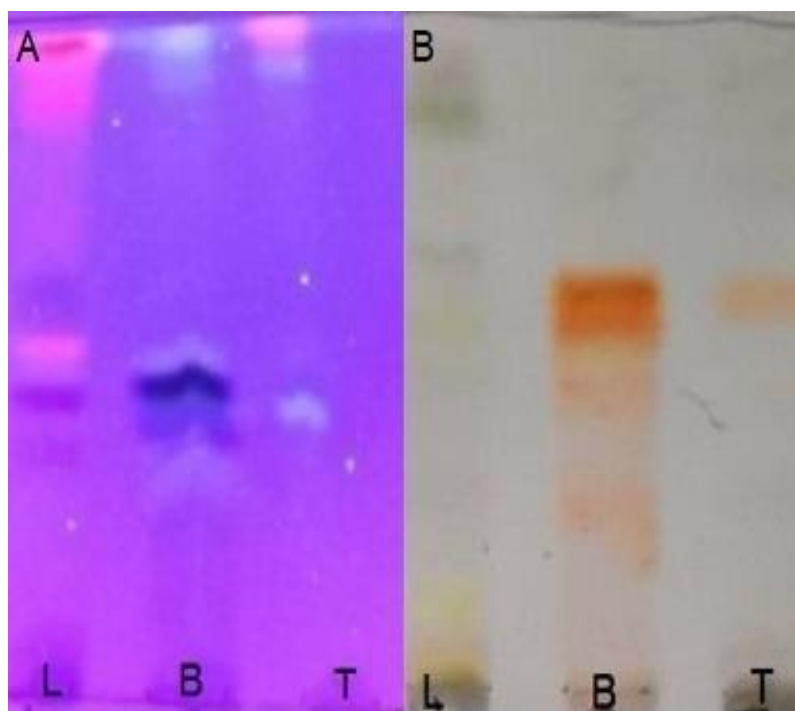


Figure 4.2: Chromatograms of acetone extracts of leaves (L), stem bark (B) and twigs (T) of *Brackenridgea zanguebarica* developed with chloroform/ethyl acetate/formic acid (10:8:2). (A) TLC plates viewed under UV 365 nm, (B) TLC plates viewed under daylight after being sprayed with vanillin reagent.

For chromatogram developed in EMW, the acetone extract of the three plant parts revealed the presence of 17 bands under UV; leaves, stem bark and twigs which showed 10, 3 and 4 bands respectively (Figure 4.3A, Table 4.2). Among the 17 bands recorded, 2 showed close similarity to the three plant parts, with Rf values 0.81, 0.93. In daylight, 20 bands were visualized and leaves, stem bark and twigs showed 9, 5 and 6 bands, respectively (Figure 4.3A, Table 4.2). Three of the Rf values 0.72, 0.63, 0.36 were found to be common to all.

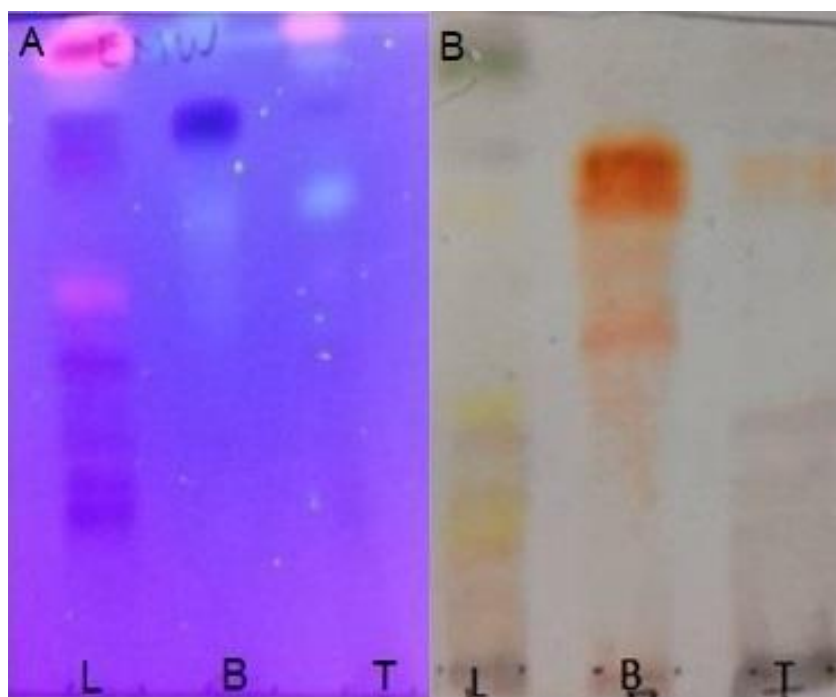


Figure 4.3. Chromatograms of acetone extracts of leaves (L), stem bark (B) and twigs (T) of *Brackenridgea zanguebarica* developed with ethyl acetate/methanol/water (10:5.4:4). (A) TLC plates viewed under UV 365 nm, (B) TLC plates viewed under daylight after being sprayed with vanillin reagent.

Table 4.2: Rf values and colors of each phytoconstituents present in various extracts in different solvent system and visualized under UV 365 nm and in daylight after spraying with vanillin reagent.

Solvent system	Visualization	Plant parts extract	Rf values	Colours of bands
BEA	UV	Leaves	0.15, 0.23, 0.30, 0.38, 0.46, 0.53, 0.69, 0.92	Purple, Purple, Purple, Blue, Pink, Pink, Pink, Pink.
		S. bark	0.15, 0.46, 0.53, 0.69, .073	Dark blue, Blue, Blue, Blue, Blue,
		Twigs	0.15, 0.46, 0.53, 0.69, 0.76, 0.92	Grey, Purple, Purple, Pink, Blue, Pink
	Daylight	Leaves	0.13, 0.16, 0.33, 0.41, 0.57, 0.91	Grey, Grey, Grey, Grey, Green, Grey
		S. bark	0	Not visible
		Twigs	0	Not visible
CEF	UV	Leaves	0.07, 0.38, 0.46, 0.53, 0.61, 0.75, 0.92, 0.96	Purple, Purple, Purple, Pink, Blue, Pink, Pink, Red
		S. bark	0.15, 0.38, 0.46, 0.49, 0.53, 0.92, 0.96	Purple, Grey, Blue, Dark blue, Grey, Grey, Grey
		Twigs	0.46, 0.49, 0.92, 0.96	Grey, Purple, Grey, Pink
	Daylight	Leaves	0.04, 0.07, 0.15, 0.26, 0.55, 0.63, 0.87, 0.92	Yellow, Grey, Yellow, grey, Yellow, Grey, Green, Grey Orange, Orange, Orange, Orange, Orange, Orange
		S. bark	0.04, 0.23, 0.26, 0.39, 0.47, 0.55	
		Twigs	0.04, 0.23, 0.26, 0.46, 0.55	Grey, Grey, Grey, Yellow, Orange
EMW	UV	Leaves	0.24, 0.32, 0.37, 0.40, 0.49, 0.57, 0.77, 0.81, 0.90, 0.93 0.70, 0.81, 0.93	Purple, Purple, Purple, Purple, Purple, Pink, Purple, Blue, Pink, Red Grey, Blue, Grey
		S. bark	0.81, 0.93	
		Twigs	0.70, 0.81, 0.90, 0.93	Grey, Blue, Grey, Pink
	Daylight	Leaves	0.09, 0.18, 0.27, 0.32, 0.36, 0.63, 0.72, 0.81, 0.94	Grey, Grey, Grey, Grey, Yellow, Grey, Yellow, Grey, Green
		S. bark	0.36, 0.45, 0.54, 0.63, 0.72	Orange, Orange, Orange, Orange, Orange
		Twigs	0.09, 0.18, 0.27, 0.36, 0.63, 0.72	Grey, Grey, Grey, Grey, Grey, Orange

BEA, CEF and EMW used as solvent systems of different polarity showed good resolution. The intermediate polarity solvent system CEF gave a more separated profile of classes of compounds in all the three plant parts extracts, followed by EMW and BEA (Table 4.2). Several bands were visible in both UV and daylight with EMW and CEF plates, whereas with BEA plate, more bands were visible with UV. In BEA, CEF and EMW under UV, the classes of compounds in the leaves and twigs were dominated by purple and pink bands, whereas, the stem bark contained more grey and blue bands. After spraying with vanillin reagent and viewed in daylight, the classes of compounds in the leaves and twigs were dominated by grey and yellow bands and the stem bark showed more orange bands. The presence of different colours confirmed the presence of classes of compounds. Overall, the leaf extracts showed more bands, under both UV and day light, compared to stem bark and twigs.

4.3.3 Antimicrobial activity

4.3.3.1 Agar Well Diffusion Assay

The anti-microbial activity of acetone extracts of leaf, stem bark, and twig of *Brackenridgea zanguebarica* was examined against three pathogenic bacteria (Gram positive: *Staphylococcus aureus*; Gram negative: *Escherichia coli* and *Klebsiella pneumonia*), and four yeasts (*Candida albicans*, *C. glabrata*, *C. krusei* and *C. parapsilosis*) using Well Diffusion Method. All extracts exhibited varied levels of anti-microbial activity against these pathogenic microorganisms. The antimicrobial activities were evaluated by measuring the zone of inhibition. Based on the results, all

the different plant part extracts showed anti-microbial activity against *S. aureus* and *C. glabrata*.

The acetone stem-bark extract was the most effective, showing the highest zone of inhibition (15.8 mm) against Gram positive *S. aureus*, followed closely by the twig extract (14.3 mm) and the leaf extract (11.5 mm); whereas, no activity was found with the extract of these different parts of *B. zanguebarica* against Gram negative *E. coli* and *K. pneumonia*. Likewise, the acetone stem bark extract had the highest antifungal activities against *C. glabrata* with a zone of inhibition of 19.7 mm followed by the twig extract (18.2 mm) and the leaf extract (9.6 mm). The plant parts tested, however, did not show any antifungal activity against the other species of yeasts (*C. albicans*, *C. parapsilosis* and *C. krusei*). Overall, this work showed that acetone extracts of three plant parts displayed some anti-microbial activities. The acetone extracts of stem bark exhibited the highest antimicrobial activity compared with those of twigs and leaves (Figure 4.4, Table 4.3).

4.3.3.2 Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was tested for the acetone extract of leaf, stem bark and twig of *Brackenridgea zanguebarica* (Table 4.3). MIC assay of the plant part extract varied between the different tested strains. MIC values of 0.62 - 3.0 mg/ml were recorded from the acetone extracts of the three different plant parts against all the microorganisms tested, however, MIC values of different plant extracts varied between 1.1 – 3 mg/ml in the case of bacteria and 0.62 – 3 mg/ml for yeasts tested. Overall, stem bark extract had the lowest MIC value (0.62 – 2.5 mg/ml), followed by the leaf extracts (0.62 – 3 mg/ml) and twig extracts (1.75 – 3 mg/ml) against all

microorganisms tested (Table 4.3). When different parts of *B. zanguebarica* were compared for their antimicrobial activities, the stem bark gave the best results, followed by twigs and leaf extracts. Despite, the slight differences concerning the zone of inhibition, the acetone extracts of the three plant parts of this species inhibited the growth of *S. aureus* and *C. glabrata* (Figure 4.4A and 4.4B).

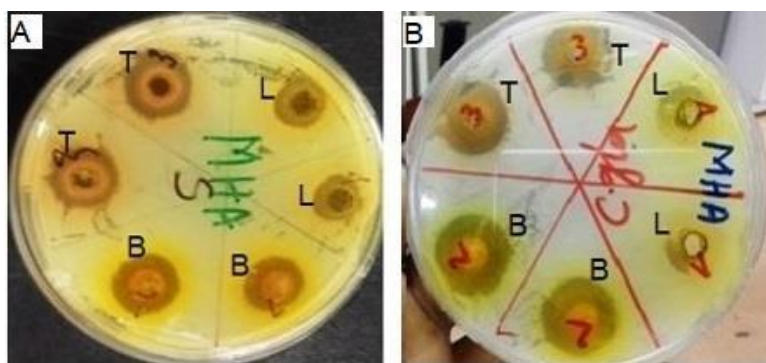


Figure 4.4: Antimicrobial activity of acetonic extracts of leaves (L), stem bark (B) and twigs (T) of *B. zanguebarica* against two microorganisms. (A) *S. aureus*; (B) *C. glabrata*.

Table 4.3: Antimicrobial activity and Minimum inhibitory concentration (MIC) values of acetone extracts of leaves, stem bark and twigs of *Brackenridgea zanguebarica*.

	Tested microorganisms	Extract (mg/ml)					
		Leaves		Stem bark		Twig	
		Zone of inhibitions (mm)	MIC (mg/ml)	Zone of inhibitions (mm)	MIC (mg/ml)	Zone of inhibitions (mm)	MIC (mg/ml)
Bacteria	<i>Staphylococcus aureus</i>	11.5	1.5	15.8	1.5	14.3	2
	<i>Escherichia coli</i>	0	1.5	0	1.1	0	3
	<i>Klebsiella pneumonia</i>	0	3	0	2.5	0	3
Yeasts	<i>Candida albicans</i>	0	>3	0	2	0	2.5
	<i>Candida glabrata</i>	09.6	1	19.7	0.5	18.2	1.75
	<i>Candida krusei</i>	0	0.62	0	0.62	0	3
	<i>Candida parapsilosis</i>	0	1.12	0	0.75	0	2

4.3.3.3 Minimum bactericidal/fungicidal concentration (MBC/MFC)

Acetone extract of different parts (leaf, stem bark, twig) of *Brackenridgea zanguebarica* showed bactericidal activity against *Staphylococcus aureus*, but none of the extracts showed bactericidal effect against *Escherichia coli* and *Klebsiella pneumoniae*. The acetone extract of different plant parts had no fungicidal activity against all the fungi tested.

4.4 Discussion

This study reveals the presence of several important chemical constituents (Alkaloids, Cardiac glycosides, Coumarins, Flavonoids, Phenols, Quinones, Saponins, Steroids, Tannins and Terpenoids) from a qualitative analysis of the three plant parts (leaves, stem bark and twigs) of *Brackenridgea zanguebarica* which is an indication that this plant species has medicinal values. In this study, the results obtained, showed almost similar bioactive compounds in the acetone extract of leaves, stem bark and twigs. Similar secondary metabolites, such as flavonoids, phenols and tannins were also observed in the ethanolic extract of *B. zanguebarica* stem bark (Möller *et al.* 2005). The possibilities of using leaves and twigs instead of stem bark may help the sustainable utilization and promote the conservation of *B. zanguebarica*. As Cunningham (1988) and Shai *et al.* (2009) stated, aerial plant parts, such as leaves, twigs and fruit, harvesting does not damage plants in the same way as debarking and uprooting.

Thin layer chromatography profiling of these acetonic plant parts extract in (BEA) (18:2:0.2), (CEF) (10:8:2) and (EMW) (10:5.4:4) showed different bands representing various classes of compounds. The phytochemical constituents with the same R_f value, found in the three plant parts under UV and in daylight, indicates the presence of similar class of compounds. However, stem bark and twig extracts have very close and identical classes of compounds compared to leaves. Despite the slight difference with the leaf extracts, twigs and leaves may be used in place of stem bark. Due to the results of phytochemical screening and TLC, the presence of various classes of compounds in all the tested plant parts, explains the growing interest in the manufacturing of traditional medicines from this species; hence, leading to its excessive and destructive collection. A literature search showed that TLC of this plant has not been previously studied, thus, with the present study, the R_f values may help in selection of appropriate solvent system for further separation of classes of compounds from plant extracts as stated by authors such as Sharma and Paliwal (2013) as well as Ahamed *et al.* (2017).

Different plant part extracts of *Brackenridgea zanguebarica* were evaluated to compare their anti-microbial activity against some selected pathogenic microorganisms in order to know if these plant parts apart from the stem bark can be substituted for the treatment of bacterial and fungal infections, as the plant species is threatened. The results of this study showed that certain parts of *B. zanguebarica* extracts have anti-microbial activity against bacteria (*Staphylococcus aureus*) and yeast (*Candida glabrata*). When different parts of *B. zanguebarica* were compared for their antimicrobial activities, the stem bark gave the best results, followed by twig and leaf extracts. Raja *et al.* (2013) and Bassey *et al.* (2016) reported that, the difference

in the anti-microbial efficacy could be due to variable distribution of phytochemical compounds in different plant parts. None of the plant part extracts was active in inhibiting the growth of other pathogenic microorganisms tested in this study. This result is an indication of similar antimicrobial activity produced by the three plant parts against the selected microorganisms, however, the stem bark was more active than the twig and leaf. Other studies such as Srivastava *et al.* (2016) have shown the similarity between the different plant parts when evaluating the antimicrobial activity against some pathogenic microorganisms. But, Shai *et al.* (2009), revealed that the extracts of the leaves and twigs of *Curtisia dentata* are more active against some tested microorganisms than the stem bark extract. This work supports the possibility of plant parts substitution as leaves, stem bark and twigs inhibited the growth of *S. aureus* and *C. glabrata*, but they were ineffective against other microorganisms tested in this study.

For all the tested plant part extracts, *S. aureus* was the most sensitive compared to *E. coli* and *K. pneumoniae*. None of the extracts showed any activity against the Gram-negative bacteria. The results of this work agree with other studies (Martin, 1995; Paz *et al.*, 1995) that Gram-positive bacteria are more susceptible as compared to Gram-negative bacteria. Möller *et al.* (2005) reported that the ethanolic bark extract of *B. zanguebarica* had a high anti-bacterial activity against seven Gram-positive bacteria. Furthermore, the methanol and water extracts of *B. zanguebarica* root had been reported as having a high antibacterial effect against Gram-positive organisms, namely, *S. epidermidis* and *S. aureus* (Steenkamp *et al.*, 2007). The difference in sensitivity between Gram-positive and Gram-negative bacteria can be attributed to the

chemical composition and structure of the cell wall of these two types of microorganisms (Goyal *et al.*, 2008).

4.5 Conclusion

The present investigation indicated the presence of important phytochemicals with antimicrobial activities in the different plant parts tested. The three plant parts extracts of *B. zanguebarica* are a rich source of various secondary metabolites as revealed by phytochemical studies and TLC analysis. All three plant parts had almost comparable chemical compounds. Acetone extracts from the tested plant parts were found to be effective in inhibiting the growth of bacteria and fungi, but the stem bark was more potent against *S. aureus* and *C. glabrata*, although similar antimicrobial activities were found in the different plant parts investigated in this work. This study confirms the possibility that the stem bark can be substituted with leaves and twigs extracts for traditional medicine. Very little work has been done so far on the phytochemical and antimicrobial activity of various parts of this plant, therefore, further studies are required which would focus on water (polar molecule) extracts in order to confirm its continual traditional use; it is also recommended for researchers to perform other investigations, such as the cytotoxicity test.

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Chapter 5

PROPAGATION POTENTIAL FOR THE CONSERVATION OF *BRACKENRIDGEA ZANGUEBARICA* Oliv.: A CRITICALLY ENDANGERED PLANT SPECIES, ENDEMIC TO VHEMBE DISTRICT IN LIMPOPO PROVINCE (SOUTH AFRICA)

Abstract

Brackenridgea zanguebarica, is an important multipurpose tree valued for its magical and medicinal uses in the Vhembe District. The indiscriminate and unsustainable harvesting coupled with the poor seed germination in the wild, is threatening its regeneration; which pose a challenge in efforts aimed at its conservation. This study was conducted to identify suitable methods for propagating *B. zanguebarica* using seeds and stem cuttings. To achieve this, seed propagation was carried out to evaluate the effect of various pre-treatments; as well as vegetative propagation to assess if *B. zanguebarica* could be successfully propagated via stem cuttings with, appropriate treatments. Data were recorded on different vegetative growth parameters. The results showed that *B. zanguebarica* seeds did not germinate at all under any of the conditions tested. However, the results, showed the potential of propagating this species from stem cutting despite the poor results obtained, where 51% of cuttings across all treatment produced buds and 17% only developed leaves without any root development. The growing media had insignificant ($p < 0.05$) effect on the vegetative growth parameters, but growth hormones showed a significant effect in some vegetative growth parameters of stem cuttings where IAA performed better than IBA and NNA. The findings showed that *B. zanguebarica* is difficult to propagate

sexually and asexually, hence, further studies are still needed to identify suitable methods for both seed and vegetative propagation of this plant.

Key words: Multipurpose tree; seed propagation; stem cuttings; auxins; growing media.

5.1 Introduction

The on-going disappearance of biological diversity due to various anthropogenic activities has increased drastically during the last few decades, to the point where many species are now threatened with extinction (Hayes and Hayes, 2013). Many plant species are threatened due to indiscriminate overexploitation coupled with insufficient efforts made for the renewal of natural resources (Chen *et al.*, 2016; Kandari *et al.*, 2012). The most affected species are those with small population and narrow geographical range (Wilson *et al.*, 2004). This trend is alarming and immediate conservation measures are required to safeguard many of these species (Sharanappa and Rai, 2011). Many plant species have the potential to be propagated via either sexual or asexual means. These propagation techniques play an important role in the conservation of numerous threatened plant species with poor natural regeneration (Patel, 2015). These propagation techniques offer important strategies which can determine the perpetuation of plant population and a promising way for saving plants from extinction.

Brackenridgea zanguebarica Oliv. is an important woody medicinal plant with only one small population found in Thengwe village within the Vhembe District in Limpopo Province, South Africa. It has been identified as an endemic plant species of that region. It is locally known as “Mutavhatsindi” and commonly known as “Yellow peeling plane”. The plant belongs to the family Ochnaceae and is a small tree that can grow up to 10 m high. Its roots and bark are widely harvested and traditionally used in the treatment of various ailments and is also supposed to have magical purposes (Steenkamp, 2003; Möller *et al.*, 2006; Bruschi *et al.*, 2011). Due to its multipurpose

uses, the indiscriminate extraction and overexploitation of this important plant is depleting its population number at an alarming rate. Presently, the species is under pressure and has become the focus of increasing conservation concern. It has been categorized according to the International Union for Conservation of Nature (IUCN) Red List of South Africa as a Critically Endangered plant species (Williams *et al.*, 2013). Detailed information concerning the propagation is required to increase the availability of this species and to expand its growth to new areas in the region; which can go a long way in remedying the situation. Cultivation is crucial for *in situ* and *ex situ* conservation for this threatened species.

Cultivation with the goal of conserving this multipurpose tree species is hampered by lack of information on how this plant species can be propagated. Therefore, there is a need to take an initiative for mass propagation and re-establishment of this species in nature. The natural regeneration of *B. zanguebarica* from seeds in the wild is unsatisfactory in generating adequate seedlings (Mutshinyalo, 2011). Furthermore, seeds take a long time to germinate. This is probably due to extremely complex seed dormancy that are not well understood. In addition, the vegetative regeneration process has also not been fully investigated. Due to the poor propagation by seed of this tree in the wild, it was considered useful to undertake, for the first time, a study on the propagation of this species. In order to minimize dependence of propagation by seeds, the effect of growth media and hormonal on vegetative propagation can be viewed as an effective alternative and supplement method to seed propagation of this species. The use of growth media and rooting hormone on stem cuttings is very important in plant propagation in stimulating adventitious root as well as to hasten root initiation. Many studies showed that growth media and hormonal concentration for

vegetative propagation have been successfully improved root initiation on stem cuttings (Kassahun and Mekonnen, 2012; Kiuru *et al.*, 2015; Okunlola and Oyedokun, 2016).

To ensure the conservation and the perpetuation of *B. zanguebarica*, the present study was aimed at investigating the possibilities of enhancing its propagation by developing an understanding of sexual and asexual propagation of this species. The specific objectives were therefore to develop a protocol for seed germination using several pre-treatments and assess *B. zanguebarica* stem cuttings response to various auxins and different growing media in order to develop an efficient method for vegetative propagation.

5.2 Materials and Methods

5.2.1 Plant collection and study area

The collection of fruits and stems from *B. zanguebarica* trees was conducted in the Brackenridgea Nature Reserve (BNR) and transported to the University of Venda. This reserve is in Vhembe District between the latitudes of 22° 24' and 23° 36'S, and longitudes of 29° 12' and 31° 12'E in Limpopo Province, South Africa. The climate of the study area is semi-arid (Nel and Nel, 2009) with an average annual rainfall of about 350 mm (Mzezewa *et al.*, 2010); and the mean annual maximum and minimum temperatures are 26.5°C and 16°C respectively. The soil type is mainly stony with shallow sandy loam (Mutshinyalo, 2011).

To assess the propagation capacity of *B. zanguebarica* seeds and stem cuttings, the experiments were carried out in the shade house at the School of Agriculture, University of Venda (South Africa).

5.2.2 Seed propagation

The fresh and mature black fruits (Figure 5.1A) used in the experiments were collected from *B. zanguebarica* trees on the 30th of January 2017. Seeds were manually depulped from the fruits (Figure 5.1B), washed with distilled water and left to dry for 24 hours at room conditions. A total of 360 seeds were used for all the pre-treatments including control.



Figure 5.1: *Brackenridgea zanguebarica* seed. (A) Fresh fruits (B) seeds depulped from the fruits.

After this procedure, the seeds were subjected to nine pre-treatments:

T1: Seeds soaked in Sulphuric acid for 30 minutes,

T2: Seeds soaked in Sulphuric acid for 60 minutes,

T3: Seeds soaked in hot water (100°C) for 5 minutes,

- T4:** Seeds soaked in hot water (100°C) for 10 minutes,
- T5:** Seeds soaked in cold water for 24 hours,
- T6:** Seeds soaked in cold water for 48 hours,
- T7:** Seeds cold stratified at 4°C for 60 days,
- T8:** Seeds cold stratified at 4°C for 90 days,
- T9:** Control (untreated seeds).

Forty seeds were used for each pre-treatment and each pre-treatment had four replicates of 10 seeds. Each seed was sown per perforated black plastic bag containing Hygromix growth medium and placed on a metal bench in the shade house at the School of Agriculture on the 1st of February 2017. Watering was done every two to three days to maintain the moisture content for better seed germination conditions throughout the duration of the experiments of twelve months.

5.2.3 Stem cuttings propagation

5.2.3.1 Source of plant material and preparation of stem cuttings

Healthy semi-hardwoods stem were collected in February 2018 from freshly branches of *B. zanguebarica* trees growing in the BNR. Two hundred stem cuttings were uniformly trimmed into 10 cm long pieces, with each cutting having 4 to 5 nodes; these were used for all the treatments. After taking all the leaves off, the cuttings were treated with three types of auxins, namely, Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) at different concentrations and the controls were treated with distilled water. All the cuttings were then, planted in two types of growing media: site soils (topsoil collected in the reserve) and Hygromix.

5.2.3.2 Preparation of plant hormones (auxins) and growing medium

Each plant hormone (hormone powder) was prepared by dissolving a calculated amount of IAA, IBA and NAA in distilled water to obtain different concentration of solution. The same concentrations (50 mg/l, 100 mg/l, and 150 mg/l) of growth regulators were prepared for each auxin and the control solution was prepared with distilled water. The basal ends of about 2 cm of the cuttings were dipped for 30 seconds in the prepared auxin treatment. Each treatment was replicated four times and each replication consisted of 5 cuttings. A total of 200 stem cuttings were used for this experiment and the treated cuttings were individually planted into a perforated black plastic bags filled with growing media (site soil and Hygromix). Each growing medium had 100 cuttings that were maintained in the shade house and after every two to three days, until the termination of the experiment (90 days).

5.2.4 Data recording

The following parameters were recorded in order to analyze the data: (1) Days to first bud appearance, (2) Percentage of cuttings that produced buds, (3) Average numbers of buds per cuttings after 45 days, (4) Days to first leaf development, (5) Percentage of cuttings that produced leaves, (6) Average number of leaves per cuttings after 90 days, and (7) Percentage of cuttings that produced roots after 90 days.

Days to first bud/leaf appearance: It was recorded from the day of planting to the minimum days taken to first bud/leaf appearance from one cutting of each treatment and then averaged to get the mean value.

Percentage of cuttings that produced buds (or leaves) = $\frac{\text{Number of cuttings with buds (or leaves)}}{\text{Total number of cuttings planted}} \times 100$.

Average number of buds per cuttings: The total number of buds per cuttings was counted in each treatment after 45 days and then averaged to get mean the value.

Average number of leaves per cuttings: The total number of leaves per cuttings was counted in each treatment after 90 days and then averaged to get the mean value.

Percentage of cuttings that produced roots = $\frac{\text{number of cuttings with root}}{\text{Total number of cuttings planted}} \times 100$.

5.2.5 Data analysis

Data collected were subjected to analysis of variance (ANOVA) and where significant difference occurred, the means were compared with the Least Significant Difference (LSD) test and the significance level was set at $P < 0.05$.

5.3 Results

5.3.1 Seed germination

In this study, *Brackenridgea zanguebarica* seeds subjected to numerous pre-treatments did not germinate at all after 12 months of experiment. None of the treatments was able to overcome the seed dormancy, hence, these treatments had insignificant effect on the germination of seeds of this plant species.

5.3.2 Stem cuttings propagation

5.3.2.1 Effect of different types and concentration of auxin (IAA, IBA, NAA) on some vegetative growth parameters of *Brackenridgea zanguebarica* stem cuttings

Stem cuttings treated with different types of auxins in various concentrations, including the untreated cuttings, produced their first bud in the range of 9.75 to 12.75 days after planting (Table 5.1). However, stem cuttings treated with 100 mg/l of IAA showed the first bud in day 9.75 after planting and the maximum days taken to first bud opening were recorded on the cuttings treated with 150 mg/l of IBA. The mean number of days to first bud appearance in all the treatments and control was 11.1 days. The untreated cuttings produced their first bud on day 11. Cuttings treated with IAA produced earlier buds (10.41 days) compared to IBA and NAA (Table 5.1). The number of days to first bud developed with the different types and concentration of auxins was significantly different at $P < 0.05$ (Table 5.1).

Visible buds were recorded in almost all the stem cuttings used as a control (80%), followed by 60% of cuttings treated with 50 mg/l, 100 mg/l of IAA and 50 mg/l of IBA. Whereas, the lowest percentage (30%) was found with cuttings treated with 150 mg/l of IAA and 100 mg/l of NAA (Table 5.1). Concentration of auxins influenced the percentage of stem cuttings that produced buds. The average of cuttings treated with IAA and IBA produced 50% and 48.3% of buds respectively and the lowest budding percentage 43.3% was found with cuttings treated with NAA. However, the percentage

of stem cuttings that produced buds was not significantly influenced by the type of auxins at $P < 0.05$ (Table 5.1).

The number of buds produced per stem cutting varied from 1 – 10, although, the average number of buds per stem cutting in all the treatments ranged between 0.7 and 3.2 with the highest number recorded in cuttings treated with 100 mg/l of IAA and the lowest number was noted in 150 mg/l of IAA (Table 5.1). Per cutting, the number of buds varied significantly due to auxins concentrations. This number per cutting was significantly affected by the type and concentration of auxins (Table 5.1). Most of the buds wilted and started dying during their development. By day 45 no further bud growth was observed in any of the treatments and control cuttings. Only few buds survived and produced leaves.

Across all the treatments, the stem cuttings treated with 100 mg/l of IAA developed the first leaves on day 18.5, while on day 22.5 cuttings treated with 100 mg/l of IBA produced their first leaves. All the treated and untreated cuttings developed their first leaves between days 18.5 and 22.5 (Table 5.1). The mean number of days taken for first leaves growth in cuttings treated with IAA was 20.16 days, while the cuttings treated with NAA took 21 days to show their first leaves development followed by cuttings treated with IBA on 21.25 days later (Table 5.1). The first days of leaves growth were significantly influenced by different auxins concentrations and type at $p < 0.05$ (Table 5.1).

The percentage of cuttings that produced leaves from different treatments including controls varied significantly due to type and concentration of auxin. Cuttings treated with IAA had the highest percentage (26.67%) followed by cuttings treated with NAA

(15%), while cuttings treated with IBA had 11.67% (Table 5.1). Cuttings treated with NAA: 15% and NAA: 11.67% did not show any significant difference (Table 1). Among the auxins concentration, the highest percentage of cuttings (40%) that produced leaves was recorded in 100mg/l of IAA, while cuttings treated with 150 mg/l of IBA and 100 mg/l of NAA produced the least number of leaves (Table 5.1).

From the beginning of leaf development to the end of the experiment (90 days), leaf growth was very slow and many of them wilted and died. The different results on leaves opening can be attributed to the different types and concentration of auxins. The results of the control varied significantly as well as among the different treatments. The low rate of leaf development was attributed to the mortality of many buds that did not survive for long. The numbers of leaves per cuttings varied between 1 and 8. The higher numbers of leaves per stem cutting were obtained in 100 mg/l of IAA (1.8 leaves) followed by 100 mg/l of IBA (1.5 leaves), and 150 mg/l of NAA (1.4 leaves) (Table 5.1). The cuttings treated with 150 mg/l of IAA and 50 mg/l of IBA had a low value of 0.2 leaves, while cuttings treated with 150 mg/l of IBA produced the least number of leaves (Table 5.1). Cuttings treated with IAA produced the highest number of leaves per cutting, followed by NAA and IBA (Table 5.1). The application of different auxin concentrations had a significant effect on the mean number of leaves per stem cutting, although auxin type did not show any significant difference (Table 5.1). Auxin types and concentration did not have any effect on root development of *B. zanguebarica*.

Overall, cuttings treated with IAA had significant effect on the vegetative growth parameters of this species than IBA or NAA.

Table 5.1: Effect of different types and concentration of auxin (IAA, IBA, NAA) on some vegetative growth parameters of *Brackenridgea zanguebarica* stem cuttings.

Auxins	Concentration (mg/l)	Growth Parameters						
		Buds			Leaves		Roots	
		Days to first buds appearance	Percentage of cuttings that produced buds	Average number of buds per cuttings after 45 days	Day to first leaves development	Percentage of cuttings that produced leaves	Average number of leaves per cutting after 90 days	Percentage of cuttings that produced roots
IAA	50	10.50abc	60bc	2.9cd	20.75b	30c	0.6a	0
	100	9.75a	60bc	3.2d	18.50a	40d	1.8b	0
	150	11.00bcd	30a	0.7a	21.25b	10ab	0.2a	0
IBA	50	11.50cd	60bc	2.5cd	20.75b	10ab	0.2a	0
	100	11.00bcd	50ab	3.1cd	22.50c	20bc	1.5b	0
	150	12.75e	40ab	1a	20.50b	5a	0.1a	0
NAA	50	11.50cd	50ab	1.4ab	21.25bc	10ab	0.4a	0
	100	10.25ab	30a	1.2a	20.25b	5a	0.4a	0
	150	11.75e	50ab	2.3bc	21.50bc	30c	1.4b	0
Control	00	11.00bcd	80c	2.3c	21.25bc	10ab	0.3a	0
	Mean	11.11	51	2.08	20.90	17	0.69	0
	LSD at P < 0.05	1.19	20.42	0.91	1.35	10.04	0.58	0
Average of each treatment								
	IAA	10.41a	50a	6.8b	20.16a	26.67b	2.6a	0
	IBA	11.75c	48.3a	6.6ab	21.25b	11.67a	1.8a	0
	NAA	11.16b	43.3a	4.9a	21ab	15a	2.2a	0
	Mean	11.10	47.2	6.1	20.80	17.78	0.73	0
	LSD at P < 0.05	0.57	9.23	1.89	0.99	5.75	1.1	0

Means followed by the same letter in a column are not significantly different at P < 0.05, according to Least Significant Difference (LSD) test.

5.3.2.2 Effect of growing media on some vegetative growth parameters of *Brackenridgea zanguebarica* stem cuttings

The results indicated that the type of growing media did not have any significant effect on the days taken for the first bud appearance. However, among the growing media, the earlier bud emergence (9.75 days) was recorded on cuttings set in site soil; the first bud appearance was observed on Hygromix medium on day 10.5 (Table 5.2). From the day of planting to day 45, buds were developing, however, many wilted. After day 45 no further buds appeared and the results showed that cuttings planted on site soil produced more buds than cuttings set in Hygromix. Fifty six percent of cuttings produced buds when planted in site soil and 46% were recorded when cuttings were planted in Hygromix. The mean percentage from the two media was 51% of cuttings. The growth media, therefore, had no significant effect on the percentage of cuttings that produced buds (Table 5.2).

Stem cuttings propagated in the site soil produced higher number of buds per cutting than those from Hygromix. The average number of buds developed was higher on stem cuttings propagated in site soil with 2.17 buds/cuttings while stem cuttings propagated in Hygromix gave the least number of buds with 1.98 buds/cuttings. The mean number of buds per cutting was 2.08. No significant difference in number of buds per cutting was observed between the two media (Table 5.2).

Leaf development started 18.25 days after planting the cuttings in the site soil and 19.5 days when the cuttings were planted in the Hygromix medium. The mean number of days taken for the first leaf opening in both growth media was 18.88. However,

significant differences were not found between the two growth media regarding the number of day taken for first leaf opening. The percentage of cuttings developing leaves was not significantly affected by the type of growing media ($P < 0.05$). The maximum percentage of cuttings (20%) that developed leaves was recorded in site soil and the minimum of 14% was recorded in Hygromix. The number of leaves per stem cutting was not significantly affected by the growing media. The site soil had the highest number of leaves (0.72 leaf/cutting), which did not significantly differ from the hygromix medium that produced 0.65 leaf/cutting. The mean number of leaves per cuttings was 0.68 (Table 5.2). None of the media stimulated the growth of the roots.

Overall, there was no significant difference between cuttings planted in site soil and Hygromix on the different vegetative growth parameters of *Brackenridgea zanguebarica*.

Table 5.2: Effect of growth media on some vegetative growth parameters of *Brackenridgea zanguebarica* stem cuttings

Growth medium	Growth Parameters						
	Buds			Leaves		Roots	
	Days to first buds appearance	Percentage of cuttings that produced buds	Average number of buds per cuttings after 45 days	Day to first leaves development	Percentage of cuttings that produced leaves	Average number of leaves per cutting after 90 days	Percentage of cuttings that produced roots
Site soil	09.75 ^a	56 ^a	2.17 ^a	18.25 ^a	20 ^a	0.72 ^a	0
Hygromix	10.5 ^a	46 ^a	1.98 ^a	19.50 ^a	14 ^a	0.65 ^a	0
Mean	10.12	51	2.08	18.88	17	0.69	0
LSD at P < 0.05	0.95	10.94	0.35	1.37	10.18	0.49	0

Means followed by the same letter in a column are not significantly different at P < 0.05, according to Least Significant Difference (LSD) test.

5.3.2.3 Effect of auxin concentration and growth media on some vegetative growth parameters of *Brackenridgea zanguebarica* stem cuttings

Stem cuttings of *B. zanguebarica* treated with various concentrations of auxins and planted in two different growth media started with buds appearance (Figure 5.2A) from day 9 up to day 45. For all the treated cuttings including the controls, the first bud appearance was in the range of 9.5 to 13 days. The overall results of the interaction between concentration of auxin and growth media showed that cuttings treated with 100 mg/l of IAA and set in site soil took the minimum number of days (9.5 days) to produce the first bud, while cuttings treated with 150 mg/l of IBA planted in the site soil produced the first bud on day 13 (Table 5.3). The interaction between concentration of auxin and growth media significantly affected the number of days taken for first buds appearance ($P < 0.05$).

At day 45, buds growth were recorded in all the interactions (auxin concentration and growth media) but not in all the stem cuttings. In this interaction, the highest percentage (85%) of cuttings that produced buds was recorded when untreated cuttings were propagated in the site soil. The lowest percentage (30%) was noted when the cuttings were treated with 150 mg/l of IAA, 100 mg/l of NAA and planted in site soil and Hygromix, respectively (Table 5.3). The interaction between auxins concentration and type of growing media significantly affected the percentage of cuttings that produced buds ($P < 0.05$).

The interaction between auxin concentration and growth media showed that the number of buds per cutting varied from 0.4 bud/cutting in site soil when cuttings were treated with 150 mg/l of IAA to 3.7 buds/cutting in the site soil for cuttings treated with

100 mg/l of IAA (Table 5.3). The interaction between the growth media and auxin concentration showed significant effect on the number of buds per cutting (Table 5.3).

Despite many buds wilted and died, some of the survived buds developed into leaves (Figure 5.2B). Stem cuttings treated with 100 mg/l of IAA and set in the site soil began with the first leaves development on day 17.5 after planting. The maximum day taken for the first leaf growth was recorded on day 23 when cuttings had been treated with 150 mg/l of NAA and planted in the site soil. The mean number of days taken to first leaves growth in this interaction was 21 (Table 5.3). Cuttings treated with 100 mg/l of NAA and set in Hygromix medium and those treated with 150 mg/l of IBA and planted in the site soil did not produce leaves until the end of the experiment. The interaction between auxins concentrations and growth media showed a significant difference in time taken for first leaves development.

The interaction of different auxins concentration and growth media had a significant effect on the percentage of cuttings that produced leaves (Table 5.3). Treatment involving 100 mg/l of IAA and propagated in the site soil showed the highest percentage (40%) of cuttings that produced leaves and the lowest percentage (5%) was recorded on cuttings treated with 150 mg/l of IAA, 50 mg/l and 150 mg/l of IBA and propagated in Hygromix medium. No cuttings produced leaves when treated with 150 mg/l of IBA and planted in the site soil and 100 mg/l of NAA in Hygromix.

At the end of the experiments (90 days) some leaves were well expanded (Figure 5.2C); the number of leaves per stem cutting treated with different auxin, including control, and planted in different growth media varied from 0.1 – 2. The maximum number of leaves was recorded when cuttings were treated with 100 mg/l of IAA and

propagated in the site soil (Table 5.3). The average number of leaves per stem cutting was affected by the interaction of various concentrations of auxins and growth media. The interaction between site soil and 100 mg/l of IAA was considered more productive than other treatments, whereas, cuttings treated with 150 mg/l of IBA planted in site soil and the cuttings treated with 100 mg/l of NAA planted on Hygromix did not produce leaves. Overall, it was detected that the interaction with concentration of auxin and growth media did not promote roots development of *B. zanguebarica*.

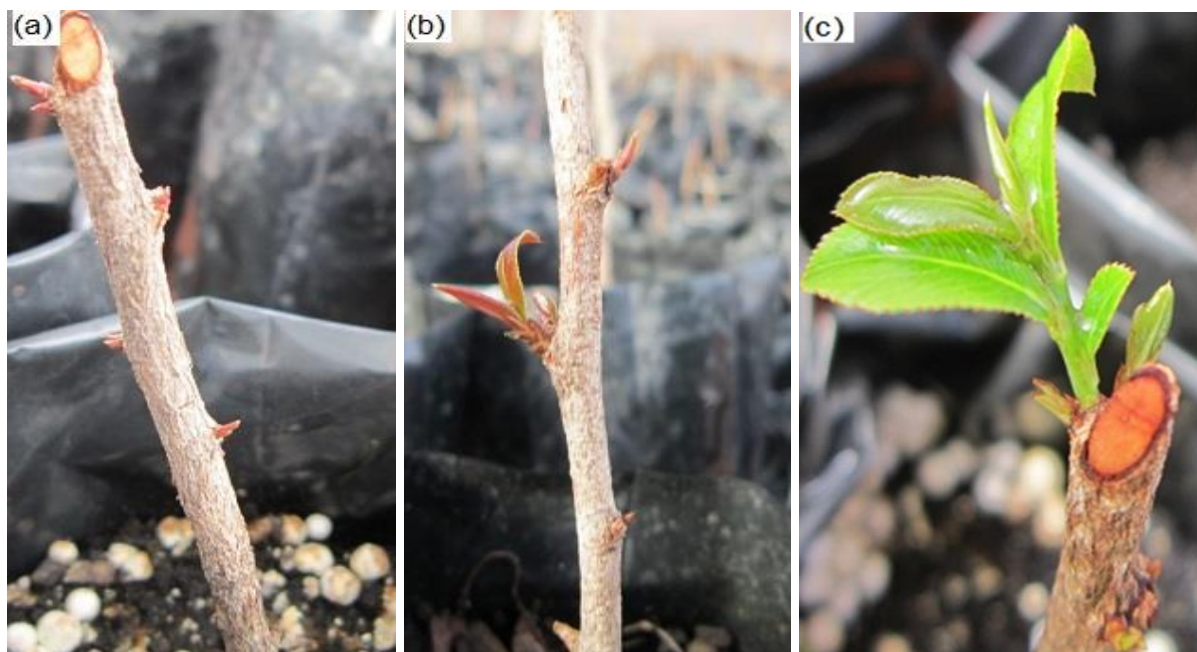


Figure 5.2: Example of different stages of vegetative growth parameters used to score the response of *Brackenridgea zanguebarica* stem cuttings to different treatments in both growth media. (A) Buds emergence; (B) leaves appearance; (C) leaves expansion.

Table 5.3: Effect of concentration of auxin and growth media on some vegetative growth parameters of *Brackenridgea zanguibarica* stem cuttings.

Growing medium		Auxins concentration		Growth Parameters					
				Buds		Leaves		Roots	
				Days to first buds appearance	Percentage of cuttings that produced buds	Average number of buds per cuttings after 45 days	Day to first leaves development	Percentage of cuttings that produced leaves	Average number of leaves per cutting after 90 days
site soil	IAA	50	10.5abc	70efg	3.1fg	21cde	25cde	0.5ab	0
		100	09.5a	60cdef	3.7g	18.5b	40e	2f	0
		150	11.5bcde	30a	0.4a	21cde	15abc	0.4ab	0
	IBA	50	11.5bcde	60cdef	2.9efg	20.5bcd	15abc	0.25ab	0
		100	11abcd	65defg	3.5g	22.5de	20bcd	1.5cdef	0
		150	13e	40abc	1ab	0a	0a	0a	0
	NAA	50	11.5bcde	60cdef	1.6abcd	21.5cde	10abc	0.7abcd	0
		100	10ab	30a	0.9ab	20bc	10abc	0.6abc	0
		150	12cde	55bcdef	2.6cdefg	23e	35de	1bcde	0
	control	00	12cde	85g	2.8defg	20bc	15abc	0.5ab	0
Hygromix	IAA	50	10.5abc	50abcde	2.7cdefg	20.5bcd	35de	0.7abcd	0
		100	10ab	60cdef	2.7cdefg	18.5b	40e	1.6def	0
		150	10.5abc	30a	1ab	21.5cde	5ab	0.1ab	0
	IBA	50	11.5bcde	60cdef	2.1bcdef	21cde	5ab	0.15ab	0
		100	11abcd	35ab	2.7cdefg	22.5de	20bcd	1.5cdef	0
		150	12.5de	40abc	1ab	20.5bcd	5ab	0.2ab	0
	NAA	50	11.5bcde	40abc	1.2ab	21.5cde	10abc	0.1ab	0
		100	10.5abc	30a	1.5abc	0a	0a	0a	0
		150	11.5bcde	45abcd	2bcdef	20bc	25cde	1.8ef	0
	control	00	10ab	75fg	1.8bcde	22.5de	5ab	0.1ab	0
Mean			11.05	51	2.08	20.9	17	0.69	0
LSD at P < 0.05			1.62	24.24	1.25	1.6	15.82	0.92	0

Means followed by the same letter in a column are not significantly different at P < 0.05, according to Least Significant Difference (LSD) test.

5.4 Discussion

Seeds subjected to all the pre-treatments including control in this study did not germinate at all. The inability of all pre-treated seeds of this plant to germinate was probably as a result of the hard seed coat that prevented water imbibition and gas exchange. It is obvious that *B. zanguebarica* seeds exhibit deep physical dormancy leading to poor seed germination. There are a number of reports (El-juhany *et al.*, 2009; Babashpour-Asl, *et al.*, 2011; Zhang *et al.*, 2015) that revealed that seeds of many species showed delayed germination because of hard seed coats. This physical dormancy is clearly one of the problems that make this plant species difficult to propagate from seed, and that could explain the scarcity of this plant species in the wild. Seeds in the field are exposed to a variety of ecological conditions such as temperature, moisture and various degrees of light. However, the poor germination of *B. zanguebarica* seeds in the field benefit from all these conditions. The unsuccessful germination of *B. zanguebarica* seeds in the shade house might have to do with the unfavorable constant environmental conditions, than naturally fluctuating field conditions.

This trial contributes to an understanding of the complexity of seed propagation of *B. zanguebarica*. Hence, further experimentations should include the combination of treatments. For conservation purposes, others pre-treatment methods are required in order to improve the germination of this seed. For example, physical dormancy can be broken by several other pre-treatments such as fluctuating temperatures, fire and passage through the digestive tracts of animals (Baskin and Baskin, 1998). Baskin (2003), revealed that the fluctuating temperatures promoted dormancy break in

impermeable seeds of *Stylosanthes humilis* and *S.hamata* (Fabaceae). Additionally, the application of gibberellic acid (GA3) is also necessary in case this seed may present physiological dormancy. A lack of information about the seed propagation of *Brackenridgea zanguebarica* remains an immense challenge to developing a successful technique for promoting germination and conserving this plant species. Since all the pre-treatments did not stimulate germination in this species, an improvement of propagation method is needed. Preference should be given to vegetative propagation than from seeds, for large scale propagation.

With auxin type and concentration, together with the interaction of auxins and growing media, it was possible to record from *B. zanguebarica* stem cuttings, some of the vegetative growth parameters. However, based on the performance of the untreated cuttings in all the parameters studied, it was clear that the application of all these treatments did not significantly influence the vegetative growth parameters. All treated and untreated cuttings were ineffective to induce root growth on stem cuttings. Though the cuttings were more responsive to auxins treatment than control, the difference was not statistically significant, indicating that *B. zanguebarica* stem cuttings can develop buds and leaves without the requirement of hormones. Similar results were also found in *Lippia javanica* and *Vitex leucoxyton* stem cuttings which showed no significant differences between cuttings with growth hormone application and untreated cuttings on some of the vegetative growth parameters studied (Tiwari *et al.*, 2015).

Different auxins have been successfully used for the rooting of many plant species. According to Stuepp *et al.* (2017), plant growth hormones control all phases of growth and development from embryogenesis to senescence. The same authors reported

that, auxins are critical in the regulation of many aspects of plant growth and development and their endogenous levels are considered important in the process of root induction. Root growth, however, was not recorded in this study after the application of auxins to the stem cuttings. Puri and Swamy (1999) as well as Guo *et al.* (2009) state that, the effects of auxins application on stem cuttings vary between species because of their genetic differences.

A growing medium with an adequate supply of nutrients is important as it promotes the growth and development processes of plants. The mean values for the different parameters showed that the cuttings of *B. zanguebarica* responded positively to both the growing media used in this study (Table 5.2). From this results, it is clear that the growth and development of *B. zanguebarica* stem cuttings may not only depend of soil nutrients. According to Takoutsing *et al.* (2014), a good growing medium must have low water holding capacity and have a considerable level of porosity. Site soil seems to have the characteristics of a better growing medium than Hygromix, which has a high water holding capacity. Hence, all the growth parameters in site soil were better than in Hygromix medium, although the differences were not significant.

Stem cutting is the most frequent technique used for vegetative propagation of many plant species (Hassanein, 2013) and growth regulators and growing media were found to be effective factors for rooting of many plant species (Akinyele, 2010). The performance of untreated cuttings in all the parameters demonstrated that auxin application and growth media may not necessarily be the major factor influencing buds and leaves growth on stem cuttings of this species. Wahab *et al.* (2001); Hartmann and Kester (1990) stated that the development of buds and leaves from stem cuttings

can be attributed to high levels of plant carbohydrate reserve, however, Leakey and Coutts (1989), reported that carbohydrate content can quickly become depleted in cuttings without leaves or with very small leaf area, but increases if leaf area is appropriate; at least until roots start to develop. Stem cuttings without leaves were used in this study and the rapid buds and leaves development was probably due to the use of high carbohydrate reserve and slow reconstitution by stem cuttings, leading to the absence of root development. Similar findings have been obtained by Takoutsing *et al.* (2014) in leafless cuttings of *Garcinia lucida*, therefore, the type and concentration of auxins applied on *B. zanguebarica* stem cuttings may not have any direct effect on bud and leaf development. These results are similar to the findings of Ullah *et al.* (2005) who reported that different growth regulators did not have significant effect on the shoot development of *Psidium guajava*. Even though growth regulators and growing media are found to be effective factors for root development of many plant species, several researchers reported that root development from treated cuttings may also depend on a number of factors, such as type of cuttings, growing media, growth regulators, size of cuttings, season of planting (environmental conditions), cutting source (young seedlings or juvenile) and leaf area, (Tchinda *et al.*, 2013; Naidu and Jones, 2009; Takoutsing *et al.*, 2014).

The inability of root development in *B. zanguebarica* from stem cuttings may be due to some of these factors and also to the application method of the growing media and growth regulators that did not produce the expected effects. For instance, Ahmad (2006) reported that dipterocarp species failed to root when the stem cuttings dropped their leaves one or two days after planting. Similarly, several studies have reported that stem cuttings without leaves presented a high mortality rate in vegetative

propagation (Ofori et al., 1996; Akinyele, 2010). However, many other studies revealed that the presence of leaves on cuttings is necessary to stimulate root initiation (Hartmann *et al.*, 1990; Zem *et al.*, 2016; Belniaki *et al.*, 2018). Therefore, buds and leaves development on stem cutting of this species was an important stage to stimulate root initiation; this has shown the possibility to propagate this species by stem cuttings if appropriate treatments are provided. For this reason, more studies with different interactions of growing media and hormones need to be conducted as these factors may promote root growth and development of *B. zanguebarica* stem cuttings.

5.5 Conclusion

This is the first study to have reported on the propagation by seed and stem cuttings of *B. zanguebarica*, with the aim of improving and promoting its conservation. The results of this study demonstrated that, *B. zanguebarica* seeds exhibited the difficulties in germination under all the conditions tested. However, stem cuttings, showed the potential of propagating this species despite the poor results obtained. Despite the unsatisfactory results from both techniques, this study, nevertheless has improved the scientific understanding of seed and vegetative propagation of *B. zanguebarica*. Considering the threatened status of this multipurpose tree, it is recommended that further research should include experimentation with different treatments such as crushing the seed coat. For vegetative propagation, mixture of auxins, reliable growing media, size of cuttings, environmental conditions and cutting source are required. Tissue culture propagation should be tried as another means for mass propagation.

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CHAPTER 6

MORPHOLOGICAL AND ANATOMICAL FEATURES OF THE SEED OF *BRACKENRIDGEA ZANGUEBARICA* Oliv. AFFECTING ITS GERMINATION

Abstract

Seeds are essential in the regeneration and survival of many plant species; however their germination is controlled by a number of external and internal factors. *Brackenridgea zanguebarica* is propagated mainly through seeds and have an extremely poor germination rates, which delays its regeneration. This study was aimed at investigating and describing, for the first time, both the morphological and anatomical features of *B. zanguebarica* seed in order to identify structural features that may be implicated in its poor germination. To achieve this goal, the morphological study was based on an observation of seeds under a scanning electron microscopy, whereas for an anatomical study, longitudinal and transverse sections of seed were observed under light microscopy; seed imbibition was assessed by an increase in seed mass. The morpho-anatomical analysis indicated that some structures of the seed, such as its hardened endocarp and the presence of endosperm surrounding the embryo, which consisted of two prominent cotyledons, seem to greatly delay the germination of the seed. The seed coat and endosperm layer act as a barrier to water absorption, hence hamper seed germination. The present research has also presented an opportunity to understand more about the seed biology of this species, which is one important step to identifying useful techniques to improve seed propagation for conservation purposes.

Keywords: seed coat, embryo, endosperm, morphology, anatomy.

6.1 Introduction

Brackenridgea zanguebarica belongs to the family Ochnaceae. This is a small, up to 10 m in height, single-stemmed deciduous tree with very rough black bark surface and with yellow inner bark. It is in flower from September to November, while the fruiting takes place in the wet season, from December to February. Flowers are white to cream or pink, showy but very short-lived. The fruits are aggregated, consisted of 2 or 4 indehiscent drupelets; and each drupelet contains one seed.

Brackenridgea zanguebarica has a very restricted geographic distribution in South Africa; this species is known to only one population group and is confined to a small area within Thengwe in the Vhembe District of Limpopo Province (Tshisikhawe and Van Rooyen, 2012). The tree is commonly known as “Yellow Peeling Plane” and is locally known as “Mutavhatsindi” in Tshivenda. The species is protected in the Brackenridgea Nature Reserve forming an important component of its vegetation, but both destructive harvesting and extreme poor-seed germination have created serious difficulties for the protection of its population. It has been classified by the International Union for Conservation of Nature (IUCN) red list of South Africa as a critically endangered plant species (Williams *et al.*, 2013).

Seeds serve as a means of reproduction and a vital element to ensure the survival of the species. The survival of plants, such as *Brackenridgea zanguebarica*, is essentially carried out through sexual propagation in the wild (Mutshinyalo, 2011). Unfortunately, seed germination rate of *B. zanguebarica* is very low and unpredictable, which hamper the regeneration of this species' population. Such delayed germination

is often caused by complex interactions between numerous internal and external factors surrounding the seed (Bewley and Black, 1994), therefore, germination of *B. zanguebarica* seeds is a significant challenge, which needs an understanding of the morphology and anatomy of these seeds to develop better propagation strategies.

Besides the contribution to the identification and classification of plant taxa, a study of seed structure is necessary to clarify the ecological and physiological background of its germination. Seed germination is the emergence of the radicle through the surrounding structures, such as endosperm and testa (Sliwinska *et al.*, 2009). Seeds of some species can germinate soon after being shed, whereas others germinate only after a period of dormancy (Burrows, 1989) due to the complex structure of the seed. Numerous studies (Jorgea and Ray, 2005, Gagliardi and Marcos-Filho, 2011) have been documented to understand the relationship between various characteristics of seeds and germination. Commonly, germination is delayed by one or more factors, such as the seed coat or the endosperm surrounding the embryo being sometimes immature. For example, Chia *et al.* (2016) found that the delay of germination under adequate conditions of *Persoonia longifolia* (Proteaceae) may be related to the nature of the endocarp and embryo. According to Puteh *et al.* (2011), endosperm and testa, surrounding the embryo in seeds of wild banana (*Musa acuminata*), can block water uptake during germination. Seed coat impermeability of five *Vachellia* species (*V. aroma*, *V. atramentaria*, *V. caven*, *V. gilliesii* and *V. praecox*) was mainly attributed to the characteristics of the lignified epidermis (Venier *et al.*, 2012). Impermeable seed coats and other seed layers prevent seeds from water uptake and gaseous exchange, and therefore retard their germination (Li and Foley, 1997). Finch-Savage and Leubner-Metzger (2006), however, showed that long stratification periods are

necessary to break seed dormancy and induce germination. A mechanical or chemical scarification can break physical dormancy caused by water impermeable layers of seed coat (Chaves *et al.*, 2017). Therefore, knowledge gained from the structural features of seeds can contribute to the explanation and prediction of seeds performance under certain conditions, as well as indicating the most suitable pre-treatments methods that could promote seed germination (Salmeri *et al.*, 2015).

Despite the unpredictable and very low seed germination rate, the seed structure of *Brackenridgea zanguebarica* has not been studied. Some traits of embryo shape in other members of *Brackenbergia* have been described by Van Tieghem (1902) and Vyshenskaya (1991), and the gynoecium morphology of this genus has been examined by Guédès and Sastre (1981) and Matthews *et al.* (2012). This study was conducted with the aim of understanding the fruit and seed features of *B. zanguebarica* that may affect its germination. The understanding of factors limiting seed germination of this plant species can serve as a prerequisite for future experiments to promote the germination which is vital for species of conservation concern.

6.2 Materials and Methods

6.2.1 Study area and seeds collection

The botanical material used consisted of fruits and seeds of *Brackenridgea zanguebarica*. Fruits (drupelets) of *B. zanguebarica* are considered mature when completely black. Mature fresh drupelets were hand-collected from randomly selected individual plants, in the Brackenridgea Nature Reserve (BNR) during the fruiting

period, in January 2017. This reserve is within the savanna biome and the vegetation in and around the reserve is classified as Vhavenda Miombo (Mucina and Rutherford 2006). The fruits collected were stored in laboratory conditions until late July 2017, when the morphology and anatomy experiments were performed. The drupelets were soaked in tap water for 48 hours under laboratory conditions, then flesh was scrubbed off manually, and pyrenes (stones) were washed with tap water to clean them off completely. Ten *B. zanguebarica* pyrenes were randomly chosen and used for morphological and anatomical analysis. The morphological and anatomical studies of pyrenes were performed at the Department of Botany, University of Johannesburg, South Africa.

6.2.2 Water absorption

To determine the amount of water imbibed, four sets of 20 randomly selected *Brackenridgea zanguebarica* drupelets and pyrenes (with and without flesh) were weighed on an electronic balance to determine their weight (imbibed and dry). The pyrenes were thereafter, placed in individual Petri dishes lined with two layers of filter paper moistened with 15 ml of tap water and maintained under laboratory conditions for the duration of the experiment. Each set of imbibed pyrenes was periodically removed and weight at every one-hour intervals for the first six hours and then at every twelve hours up to sixty-six hours. After each period of imbibition, the surface of pyrenes was blotted dry with paper towel and reweighed to provide imbibed seed weight (W_i) and immediately returned in the Petri dishes for the next tests. The amount of water imbibed was determined by the pyrene mass increase, and the percentage of increase in pyrene mass which was calculated using the equation:

$$\% \text{ Increase in mass} = (W_i - W_d) / W_d \times 100$$

Where: W_i is the weight of imbibed drupelets/pyrenes and W_d is the weight of dry drupelets/pyrenes (Turner *et al.* 2006).

6.2.3 Morphological and anatomical analysis of *Brackenridgea zanguebarica* fruits/seeds

Ten *Brackenridgea zanguebarica* drupelets were randomly chosen and thoroughly studied for external and internal morphological characteristics. Undamaged drupelets were externally observed for shape, colour, surface texture, and the pyrene size (length and width in mm) were measured using digital callipers. For internal structure, a serial of longitudinal and transverse sections of pyrenes containing seeds were made with a sharp blade, mounted on stubs, coated with gold-palladium and examined with the scanning electron microscope (SEM). For anatomical examination, pyrenes were soaked for two weeks in 4% ethylenediamine to soften the hard seed coat (Carlquist, 1982). After softening, pyrenes were soaked in distilled water, dehydrated in a graded ethanol-butanol series, infiltrated and embedded in glycol methacrylate (GMA) according to a modification of the Feder and O'Brien (1968) method. Transverse and longitudinal sections of about 5 μm thick were cut by using a Porter Blum MT-1 ultramicrotome. Sections were then stained with Schiff - toluidine blue before being mounted in Entellan. The anatomical structure of pyrenes were examined under light microscope Olympus CX41RF with digital camera Olympus XC50.

6.2.4 Data analysis

Data were subjected to Analysis of Variance (ANOVA) with the program, SAS 9.1 and significant differences between each treatment were separated using Least Significant Difference (LSD) test at $p < 0.05$.

6.3 Results

6.3.1 Water absorption

The initial dry weight of twenty *Brackenridgea zanguebarica* drupelets with and without flesh was taken. The mass of twenty drupelets at the beginning of the experimentation was 1.10 g for seeds with flesh and 0.74 g for pyrenes (Table 6.1). Both drupelets and pyrenes showed the same trend with the increase in weight of seed due to water absorption, which showed an increase in time, however, the increase in weight due to water imbibition was at maximum after 42 hours for drupelets and after 6 hours for pyrenes. In different periods of time, drupelets showed a slight high percentage of water absorption compared with the pyrenes (Table 6.1), but statistically water absorption was not significantly different between seeds with flesh (fruits) and seeds without flesh during different periods of time at $p < 0.05$. After 66 hours of imbibition, seed coat remained very hard to the touch.

Table 6.1: Weight and Percentage increase in mass after imbibition of *Brackenridgea zanguebarica* drupelets and pyrenes a specific time interval.

Time (hr)	Drupelets (20)		Pyrenes (20)	
	Weight (g)	Increase in mass (%)	Weight (g)	Increase in mass (%)
0	1.10	0.00	0.74	0.00
1	1.42	29.09	0.80	8.10
2	1.44	30.90	0.80	8.10
3	1.47	33.63	0.81	9.45
4	1.50	34.54	0.82	10.81
5	1.50	35.45	0.82	10.81
6	1.51	35.45	0.83	12.16
18	1.54	37.27	0.83	12.16
30	1.55	40.00	0.83	12.16
42	1.57	41.81	0.83	12.16
54	1.57	41.81	0.83	12.16
66	1.57	41.81	0.83	12.16

6.3.2 Morphology and anatomy of fruits and seeds in *Brackenridgea zanguebarica*

Aggregate fruits of *Brackenridgea zanguebarica* bear 2-4 indehiscent one-seeded drupelets (Figure 6.1A). Each drupelet is circular to ovate in shape, greenish when unripe and black in colour when mature; its average size is 5.5 mm in length and 4.1 mm in width with an abscission zone free of flesh (Figure 6.1B). The mean weight of 20 drupelets was approximately 1.10 g. The drupelet has a fleshy mesocarp (flesh) with a thin exocarp and a very hard endocarp. The mesocarp is impregnated with fatty substances that covers the seed (Figure 6.1C). Pyrenes

(stones) are oval, brownish, about 5 mm in length and 3.8 mm in width. Endocarp showed a wrinkled structure as revealed by SEM (Figure 6.1D).



Figure 6.1: Fruit and seed morphology of *Brackenridgea zanguebarica*. (A) Aggregate fruit bearing 4 indehiscent drupelets, (B) Drupelet with abscission zone, (C) Extraction of seeds from the flesh with oil content, (D) wrinkled surface of endocarp viewed under SEM.

The anatomy of *Brackenridgea zanguebarica* fruit, using LM, showed a diversity of cells in its structure. The pericarp is composed of layers of cells, such as exocarp, mesocarp, endocarp (Figure 6.2A). The exocarp consists of a single layer of cells which is not well differentiated. The mesocarp consists of 9 or 10 layers, very tightly packed with a group of vascular bundles. The endocarp consists of a single layer of sclereids of markedly compressed cells (Figure 6.2B).

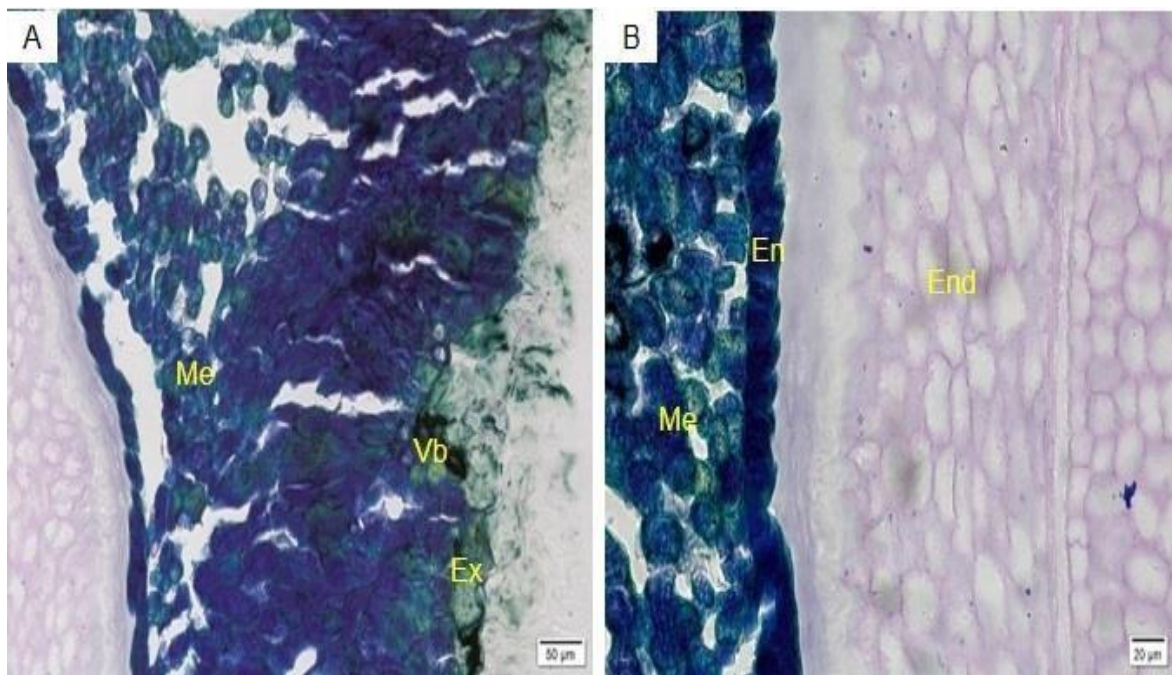


Figure 6.2: Light micrographs of the fruit of *Brackenridgea zanguebarica*. (A) Detail of the pericarp. (B) Pericarp and part of the seed. Ex, Exocarp; Me, Mesocarp; En, Endocarp; Vb, Vascular bundle; End, Endosperm; Co, Cotyledon.

The testa of *Brackenridgea zanguebarica* is hard and thin. The transverse section of seed coat (testa) of *B. zanguebarica* showed that the homogenous endocarp in its structure composed of cross and tube cells (Figure 6.3A). The sections of *B. zanguebarica* seeds viewed under SEM revealed the presence of an embryo enclosed by an endosperm layer, and itself surrounded by the seed coat layers (Figure 6.3B). Figure 6.3C shows the detailed view of a compressed parenchyma layer.

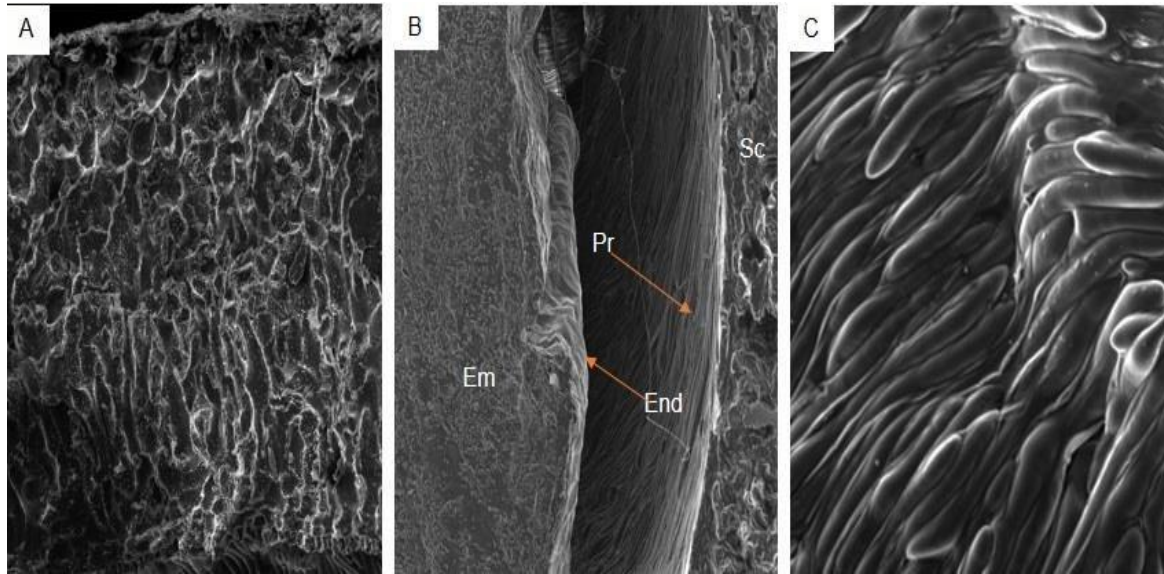


Figure 6.3: Scanning electron micrographs (SEM) of *Brackenridgea zanguebarica* seeds. (A) Homogenous endocarp structure. (B) Cross-section of the seed showing Pr, parenchyma layer; End, Endosperm; Em, Embryo; Sc, Seed coat. (C) Detailed view of parenchyma layer.

The longitudinal section of the seed showed a strongly curved embryo, which occupied the outmost seed cavity (Figure 6.4A). Figure 6.4B showed a curved embryo extracted from the extremely hard seed coat. The transverse section of *B. zanguebarica* seed showed two air-filled spaces, around which the embryo is enclosed by a protective covering by the seed coats (Figure 6.4C).

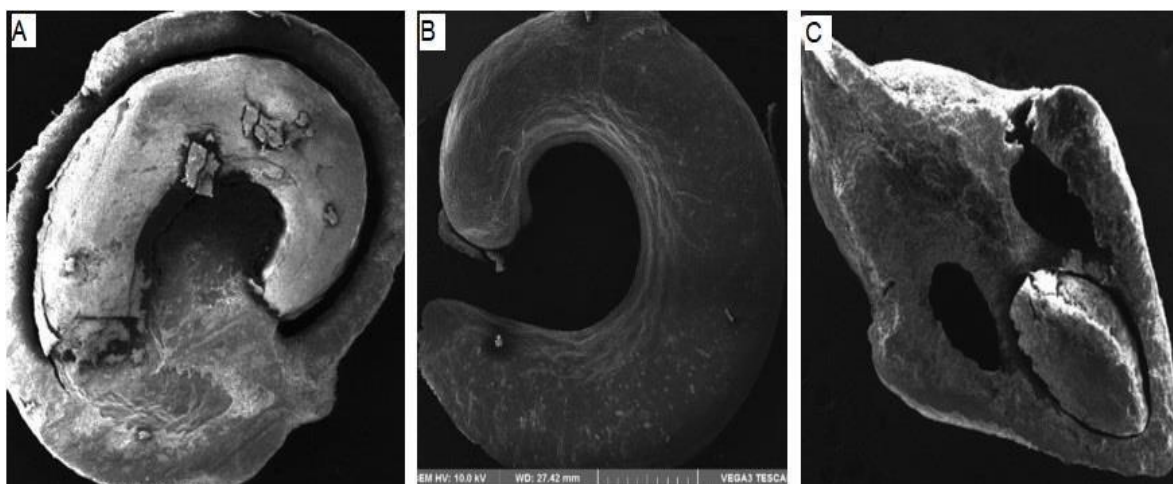


Figure 6.4: Scanning electron micrographs (SEM) of *Brackenridgea zanguebarica* seeds. (A) Longitudinal section showing the curved embryo enclosed in the seed coat; (B) Embryo isolated from seed coat; (C) Cross-section showing two air-filled spaces in the inner seed coat.

Brackenridgea zanguebarica seed was examined under light microscopy (LM) for external and internal characteristics that may be useful in understanding the delay in its germination. Anatomically, the general aspect of this seed is characterized from outside inwards by a seed coat, endosperm and the embryo (Figure 6.5A). The seed coat anatomy of *B. zanguebarica* observed under LM revealed the presence of multi-layered testa. The exotesta and endotesta are composed of a single layer, whereas the mesotesta is composed of 6 or 7 layers (Figures 6.5C and 6.5D). The seed is exalbuminous with 7 or 8 layers of endosperms. The embryo presents two large cotyledons. Figures 6.5B and 6.5D showed two discernible large cotyledons covered by a copious endosperm and itself surrounded by a seed coat. The vascular bundles are found on the seed coat and abundant in the cotyledon (Figures 6.5C and 6.5D).

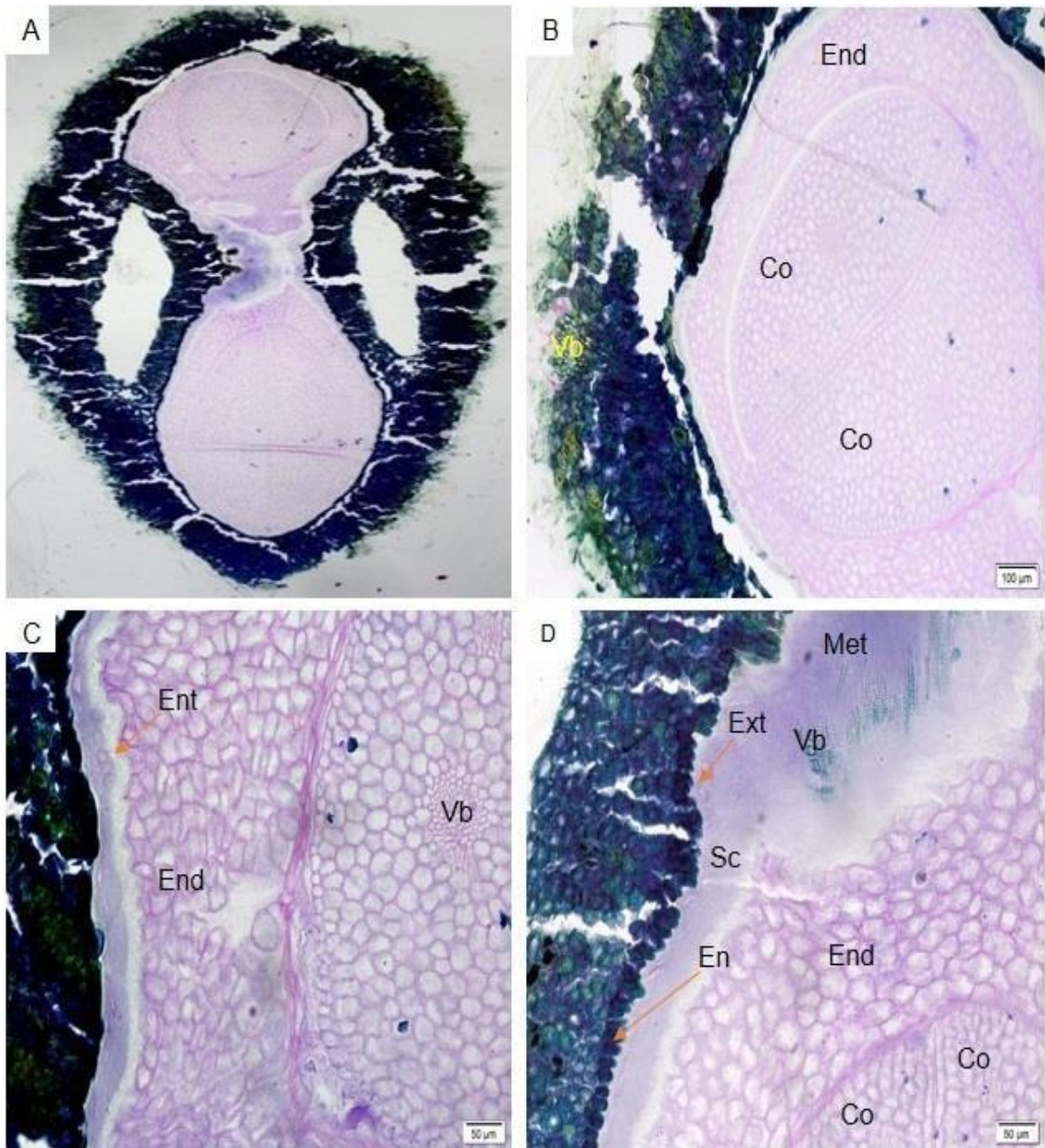


Figure 6.5: Anatomical details of longitudinal section of the seed of *Brackenridgea zanguebarica* observed with light microscope. (A) General aspect of the seed (bar = 50 µm); (B) View of two prominent cotyledons; (C and D) Detail of seed. Co, Cotyledon; End, Endosperm; Ext, exotesta; Met, mesotesta; Ent, endotesta; Sc, Seed coat; Vb, Vascular bundles.

6.4 Discussion

6.4.1 Estimation of water uptake over time

Water constitutes a basic requirement for seed germination. The increased weight of drupelets was higher than that of pyrenes indicating that fleshy mesocarp has a capacity of absorbing water. Drupelets imbibed slightly more water than pyrenes. The increase in mass of drupelets may not necessarily prove, however, that water penetrated through hard endocarp reaching the seed. Similar findings had been reported by Hidayati *et al.* (2001), who revealed that the fibrous layers of *Symphoricarpos orbiculatus* fruits (drupes) are permeable to water, whereas the cuticle is impermeable.

The increased weight of the seeds was low with about 12.16% of water uptake after 66 hours, suggesting that the endocarp can hamper water uptake into the seed. Mensah and Ekeke (2016) reported that the seed coat of *Senna obtusifolia* (Fabaceae) acts as a barrier to germination, with 13.5% of water absorption after 24 hours exposure. Venier *et al.* (2012) reported that water impermeability is due to histological structures of the seed coat. The restriction of water uptake caused by the nature of covering tissues is probably responsible for delaying the germination of *Brackenridgea zanguebarica* pyrenes. Although, this type of hard woody covering structures occurs in many plant species, the amount of water absorption is species-specific. For instance, the seeds of *Senna obtusifolia* (Fabaceae) and *Luffa cylindrica* (Cucurbitaceae) have an impermeable seed coat (Chaodumrikul *et al.*, 2016; Mensah and Ekeke, 2016). In contrast, some species known for their hard-woody seed coat

had seed coat permeability without disruption of the coat, such as *Opuntia tomentosa* (Cactaceae) and *Persoonia longifolia* (Proteaceae) (Orozco-Segovia *et al.*, 2007; Chia *et al.*, 2016).

Due to the complexity of monitoring water absorption, it was difficult to determine whether the slight increase in the mass of the seed, indicates that water has penetrated the seed, however, the low water uptake by the hard endocarp may be one of the factors that delays the germination of *B. zanguebarica*. We cannot exclude, however, that impermeability of oxygen and the retention or the presence of chemical inhibitors are also not responsible for delay of seed germination (Taiz and Zeiger, 2002); therefore, further investigation is needed to elucidate other factors delaying the germination of *B. zanguebarica* seeds.

6.4.2 Morphological and anatomical analysis of *Brackenridgea zanguebarica* fruits/seeds

The present study provided for the first time some useful information on the morphological and anatomical structure of *Brackenridgea zanguebarica* seeds that may be related to its poor germination. In this study, the morphological description of *B. zanguebarica* pyrene was like that which has been described in a few species of the Ochnaceae family (Van Tieghem, 1902; Vyshenskaya, 1991; Amaral and Bittrich, 2014). The results of this study showed that the pericarp of *B. zanguebarica* fruit enclosed the seed with lipid (Figure 6.1C). The presence of lipid was also reported in the fruit of *Ochna serrulata* and *Lophira lanceolata* that also belong to the Ochnaceae family (Gosper *et al.*, 2006; Nonviho *et al.*, 2015). Ning *et al.* (2005) however, reported

that the presence of lipid on the surface of the seed coat may prevent moisture uptake because of their hydrophobic nature. Furthermore, Venier *et al.* (2012) revealed that the impermeability of seed coats to water is related to the presence of chemical substances such as lipids. Moisture is required for seed germination and therefore, the presence of oily substance on the flesh surrounding the pyrene of *B. zanguebarica* could be considered as one of the elements, restricting water uptake, hence delaying seed germination.

The endocarp is composed of cross and tube cells and plays an important role during germination. The testa morphological feature of *Brackenridgea zanguebarica* seed is very hard to crack in normal condition and inhibit germination since it prevents water imbibition and gas exchange. The testa of different species vary in structure and composition; Although there are a number of tissues within the seed coat of many plant species, such as cuticle, palisade layer or macrosclereids, hypodermis, and parenchyma cells associated with the hilum and micropyle areas that play specific roles in seed germination (Atwater, 1980, Smýkal *et al.*, 2014; De Souza and Marcos-Filho, 2001). In order to germinate, most seeds specially need water and oxygen that can reach the embryo in many ways, but due to the structure of this seed, water and gas exchange seem to be very difficult. From this study on water absorption by this seed, the structure of the seed coat of *B. zanguebarica* is revealed to be very thin, but may not be the only structure responsible for water impermeability and gas uptake. With this seed coat structure, the hardened endocarp of this species is seemingly responsible for the delay of seed germination. With the poor seed imbibition observed in this study, the nature of hilum and micropyle cavities seem not to freely let oxygen and water to reach the embryo. Maekawa and Carpenter (1991) stated that, however,

at high moisture levels, water can cover seed hilums, thus limiting oxygen exchange. The size of these cavities that can easily enable water and oxygen to reach the embryo is unclear to our understanding, hence researches are needed.

The structure of the endosperm of mature seeds vary between different species (Yan *et al.*, 2014) and although data on the anatomy of seeds in the sub-family of Ochnoideae are limited, Amaral and Bittrich (2014) reported that the endosperm was absent in the ripe seeds of species of this sub-family. *Brackenridgea zanguebarica* belongs to this subfamily and the finding of the current study revealed the presence of an endosperm layer in this seed. The presence of endosperm in our seeds can indicate that our seeds are still not ripe, as the nutrients from the endosperm have not yet been consumed by the embryo. Muller *et al.* (2006), however, shows that the endosperm in Brassicaceae is dissolved around cotyledons, but it persists near radicle, preventing its growth. Similar results were reported by Salanenka *et al.* (2009), who stated that envelope surrounding the embryo of *Cucumis sativus* seed acts as a barrier to permeability and radicle emergence. Puteh *et al.* (2011) add that endosperm and testa surrounding the embryo in some seeds of *Musa acuminata* block water uptake during germination. From all these previous studies, we can speculate that a similar condition can be found in *B. zanguebarica* seed, hence retarding its germination. According to Yan *et al.* (2014), the endosperm plays a significant role by controlling embryo growth and by acting as a mechanical barrier during seed development and germination. Steinbrecher and Leubner-Metzger (2017) suggest endosperm weakening as a prerequisite for radicle emergence.

6.5 Conclusion

In order to solve the problems related to the delay in germination, the morphological and anatomical description of *Brackenridgea zanguebarica* seeds have helped to identify specific cell layers in their structure. This present work revealed that *B. zanguebarica* seed is composed essentially of an embryo surrounded by an endosperm which is also surrounded by the hard seed coat. The delay in germination is probably attributed to the nature of the endocarp which is very hard and can restrict gas and water exchange during seed germination. Moreover, the delay in germination of this seed may also be attributed to the presence of endosperm that is not totally consumed by the embryo. The rupture of the endocarp and the rupture of the endosperm are two essential consecutive procedures during the germination. The seed must be matured before it can germinate to, probably, allow the consumption of the endosperm by the embryo. These different layers alone or in combination can strongly hamper the germination of *B. zanguebarica* seed by creating a barrier to water and nutrients and by also limiting the emergence of the embryo. The present study, however, has only provided a basic understanding on *B. zanguebarica* seed structure, therefore, further research is now required to identify and elaborate the functions of the relevant seed layers, with respect to their role in germination.

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Chapter 7

GENERAL SUMMARY, CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

This chapter summarizes the research findings, presents the conclusions drawn from the results and the recommendations for further research based on the four preceding data analysis chapters. Due to its high economic and medicinal value, *Brackenridgea zangueberica* has become threatened and endangered. Considering the present situation and future needs, the broad objective of this study was to develop different methods for improving the conservation and management of this plant species. This objective was achieved by significant findings from the different chapters.

7.1 General summary

In South Africa, many plant species are critically endangered (SANBI, 2018) and require strong and immediate intervention to sustain their populations. In chapter three, the present study reveals the ongoing and the destructive harvesting activities of this critically-endangered plant species. Besides its extremely small population sizes, the major threat is the anthropogenic disturbances. The population of *Brackenridgea zangueberica* was extremely disturbed and the stem bark was reported to be the main plant part extracted. Some individuals also showed signs of roots collection and chopped stem. All these threats may lead to death of some of the individuals, which will result in the depletion of this population. The results of our field study revealed that the population of this species was dominated by juvenile plants

that showed the bell shape pattern, hence, the population of *B. zangueberica* is unstable and under threat.

Brackenridgea zangueberica is being used as traditional medicines to cure various diseases leading to the excessive collection of its product for the growing market demand. The aim of chapter four was to compare the chemical constituents and antimicrobial activity of different plant parts in order to evaluate other possible substitutions which may assist in its rational and sustainable collection. The phytochemical screening revealed a close similarity of various phytoconstituents of the three plant parts. The acetone extracts of the three plant parts have confirmed anti-microbial activity against gram positive bacteria (*Staphylococcus aureus*) and yeast (*Candida glabrata*) whereas none of the extracts showed activity against gram negative bacteria; however, stem bark extracts showed greater growth inhibition followed closely by twig and leaf extracts, respectively.

The indiscriminate collection of *Brackenridgea zangueberica* due to its importance to traditional medicine has threatened its survival. Chapter five of this study was conducted to identify suitable methods for the propagating of *B. zangueberica*. The results showed that *B. zangueberica* seed did not germinate at all but showed the possibility of propagating this species from stem cuttings despite the poor results obtained. Seed and vegetative propagation provide a good strategy for plant conservation, however, this species appear to be difficult to propagate sexually and asexually. This work, therefore contributes to an understanding of plant propagation for conservation purpose.

Seed is a crucial element to ensure the survival of plant species. The survival of plants, such as *Brackenridgea zanguebarica* is essentially carried out through sexual propagation, however, the germination of this species, through this process is very poor and unpredictable. In chapter six, therefore, the study aimed at investigating and describing the morphological and anatomical features of *B. zanguebarica* seed in order to reveal some structural features that may be implicated in its poor germination. The outcomes revealed that some structures of the seed, such as the hard endocarp and the presence of endosperm surrounding the embryo seem to strongly delay the germination of this species. The seed coat and endosperm layer act as a barrier to water absorption, hence hamper seed germination of this species.

7.2 Conclusions

Brackenridgea zanguebarica is one of the multipurpose plants, well-known in Vhembe District with a long history of traditional use. The over-exploitation of this plant species for many purposes has historically had a negative impact on natural populations. The destructive harvesting along with the poor regeneration have put this endemic plant species under significant stress and is now red-listed as a critically-endangered plant. It is, therefore, an urgent need to develop an effective conservation strategy to safeguard this valuable medicinal tree that plays also a significant role on the biodiversity of the region. To achieve this goal, the contribution of the present study was to update the population status of *B. zanguebarica*, to assess the possibility of plant parts substitution, to provide an approach for propagation and to understand through the anatomy and morphology features of the seeds, some of the factors influencing its germination.

Brackenridgea zanguebarica, one of South Africa's critically-endangered plant species, occurs only in Vhembe District. The current population status of this plant species undertaken as part of this research, revealed the ongoing collection of plant material, specially the stem bark. Although, the population structure was dominated by juvenile plants, the population status is facing severe regeneration constraints with a low number of seedlings and a destructive harvesting of mature individuals. The population structure of *B. zanguebarica* was characterized by the bell-shaped size class distribution pattern due to the high number of medium-sized trees with a poor number of individuals in both lower and higher DBH size classes. Despite the protection of the population of this unique medicinal plant, it is still under threat of overexploitation in the reserve. This plant species needs a quick and strong conservation strategy to avoid its depletion in the Brackenridgea Nature Reserve (BNR). This study was therefore an important step towards the conservation of this threatened plant species.

The acetonetic leaf, stem bark and twig extracts of *Brackenridgea zanguebarica*, tested positive for the presence of various secondary metabolites such as alkaloids, cardiac glycosides, coumarins, flavonoids, phenols, quinones, saponins, steroids, tannins and terpenoids. These plant parts tested had revealed important antimicrobial activities against *Staphylococcus aureus* and *Candida glabrata*. All three plant parts had almost comparable chemical compound and possess similar antimicrobial activities despite a slight difference observed in their minimum inhibitory concentration (MIC) and diameter of inhibition zones. However, all the three plant parts can be considered as potential antimicrobial against various pathogenic microorganisms.

The conservation of *B. zanguebarica* is hampered by the lack of adequate propagation techniques. This work also aimed at identifying suitable propagation methods to increase the availability of this multipurpose tree, inside and outside the reserve. Seeds subjected to different pre-treatment and the application of various auxin to stem cuttings did not positively affect the seed germination and cuttings' growth and development. None of the methods were successful in improving seed germination and to induce root formation on stem cuttings. In the case of sexual propagation, poor seed germination in *B. zanguebarica* can be attributed to the complex seed dormancy that was not able to be overcome by different pre-treatments applied in this study. Asexual propagation had shown a promising result despite the very low growth parameters recorded but there were no signs of root development on stem cuttings in all the different treatments. For successful propagation, stem cuttings may depend on several factors, such as the type of cuttings, culture media, type and concentration of growth hormone that need to be investigated. The findings of this study clearly indicate that *B. zanguebarica* is difficult to propagate, via seed and stem cuttings.

The contribution of this study was an understanding of some of the factors influencing germination of *B. zanguebarica* seeds. The morphological and anatomical observation of *B. zanguebarica* seeds revealed the presence of a hard seed coat. The hard endocarp and the presence of endosperm surrounding the embryo that consisted of two prominent cotyledons seem to strongly delay the germination of this species. The seed coat and endosperm layer act as a barrier to water absorption, hence hamper seed germination of this species. The delay in seed germination of *B. zanguebarica*

could be found individually or in combination with the endosperm, embryo, or seed coat.

7.3 Limitations of study

Despite the efforts to minimise all limitations in this study, there were certain constraints within which the research was completed. The difficulty to access several sites in the Brackenridgea Nature Reserve presented some inadequacies in Chapter 3; due to the fact that samples collected from few sites could not be considered as an appropriate representation of the population of *Brackenridgea zanguebarica* in the reserve. Another obstacle that was encountered in this research study was the long period experimental seeds germination remained in the shade house, making the monitoring activities imprecise. Furthermore, due to the limitations of equipment at the Department of Botany, University of Venda and limited funding, it was not possible to replicate the experiments demonstrated in Chapter 6 since this work was performed at the Department of Botany, University of Johannesburg, South Africa.

7.4 Recommendations

Important lessons from this research could be applied to other threatened plant species; because the loss of a single species can have adverse effects on the ecosystem (Symstad *et al.*, 1998). Due to intense and frequent harvesting, Tshisikhawe and Van Rooyen (2012) describe this plant species as rare and that it is only found at Thengwe in South Africa. Although classified as critically-endangered in the Red List of South Africa (Williams *et al.*, 2013), the collection is still taking place

in the reserve as observed during field work, therefore, it is necessary to take action before the populations of this plant species decline to critical levels. The current study clearly shows that *Brackenridgea zanguebarica* confirms its conservation status of critically-endangered plant species. To ensure the conservation of this highly valued species, further research as well as the participation of local people is strongly required. The following points are therefore made as recommendations to improve the conservation of this red-listed plant species:

- Regular overnight patrols to minimize the illegal anthropogenic activities should be implemented by the manager of the reserve.
- Continuous population assessment of this plant species should be conducted to monitor its regeneration and detect trends in the existing population.
- Raising awareness of local communities to the ecological consequences of depleting this natural resource.
- Further investigations are necessary to identify more chemical components from different part of *Brackenridgea zanguebarica* using different solvent system.
- Further pharmacological studies should be carried out on various parts of *Brackenridgea zanguebarica* to establish their bioactivity potential for a wide range of pathogenic microorganisms.
- Further studies are required to be focused on water extracts in order to confirm the traditional use of *Brackenridgea zanguebarica*.

- More comparative studies of different plant parts need to be done to open up the possibility of plant parts substitution for various infectious diseases that will reduce the intensity of the removal of stem bark.
- Further studies are needed to identify suitable methods for both seed and vegetative propagation of this plant species that did not positively respond to any of the treatments undertaken in this work.
- Tissue culture propagation should be tried as other means for mass propagation and seedlings should be transplanted inside and outside the Brackenridgea Nature Reserve (BNR) in order to expand the production area to new areas in the region.
- The investigation of the hard seed coat and seed viability is required to achieve fast germination.

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APPENDIX



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A Review on Yellow Peeling Plane (*Brackenridgea zanguebarica* Oliv.): A Critically Endangered Endemic Plant Species

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Authors' contributions

This work was carried out in collaboration between all the authors. Author MAPT designed the study, performed literature review and wrote the first draft of the manuscript. Authors MPT and ETG managed the literature search and finalization of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Brackenridgea zanguebarica Oliv. is among the most popular and frequently used tree species in Vhembe District, Limpopo Province of South Africa. However, the increasing illegal overexploitation coupled with the reproductive inefficiency have caused a severe depletion of this plant population bringing the species on the verge of extinction. *B. zanguebarica*, commonly known as "yellow peeling plane" and locally known as "Mutavhatsindi" is an important tree usually used both for medicinal and magico religious purposes in the region. This most sought after species is a critically endangered species with its distribution in South Africa restricted to Thengwe village in Vhembe District municipality. It is therefore crucial to develop various propagation protocols in order to increase the availability of this species and expand its distribution area in the region. Despite the

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risk of extinction and the multipurpose uses, there is a lack of scientific knowledge about many aspects of this plant species. This study revealed that mostly bark is being collected for medicinal use. The aim of the current study was to compile up-to-date scientific information about this critically endangered plant species to promote its conservation. In view of the importance of conservation of such a red-listed plant species, the present review focused on various aspects necessary in strengthening its conservation strategies.

Keywords: *Brackenridgea zanguebarica*; conservation; critically endangered plant; endemic plant; Vhembe District.

1. INTRODUCTION

Plants with medicinal properties have been used since thousands of years in many countries for the treatment of various ailments [1]. Medicinal plants have now gained large importance because of their efficacy, cultural acceptability, affordability and lesser side effects [2]. As many Africans, the majority of South Africans relies on medicinal plants for their primary health care and as a source of income.

Brackenridgea zanguebarica Oliv., which belongs to Ochnaceae family is one of the most sought-after plant by traditional healers in the Limpopo Province (South Africa). The plant is locally known as “Mutavhatsindi” and is endemic to Thengwe area in Vhembe District of Limpopo Province (Fig. 1). The plant is overexploited for magico religious purposes by Vhavenda [3]. There is immense need to standardize sustainable harvesting methods of this plant so that natural populations of this species remain stable.

Being medicinally as well as magico religious *B. zanguebarica* is traded in many traditional medicine shops or “muthi shops” around the province. It is valued for its bark and root, which are always collected from the wild. These plant parts are used in the treatment of several ailments such as amenorrhea, conjunctivitis, jaundice, anaemia, wounds, diarrhea, venereal diseases and other ailments [4,5,6]. Some findings revealed that the bark has a strong antibacterial activity against Gram-positive strains and various phytochemical constituents like calodenin B have been isolated from the species [5]. The increasing harvesting of *B. zanguebarica* for medicinal and magical purposes coupled with its poor natural regeneration ability and its poor conservation strategies in the reserve; pose a serious threat to the future availability of this endemic plant in the region. Due to its overexploitation, natural populations are getting depleted at alarming rate with high risk of extinction in the wild [7].

B. zanguebarica has been listed as Critically Endangered plant by the International Union for the Conservation of Nature (IUCN) [8].

Literature review showed that, there are numerous threatened medicinal plant species in South Africa and *B. zanguebarica* is one of them which is listed in the South African Red List of Plants Species [8]. However, there is little published information available about this multipurpose plant. Scientific information of this plant in the areas of taxonomy, description, distribution, traditional uses, phytochemistry, pharmacology, population status, propagation and conservation is scanty. To fulfill this gap as well as to highlight the importance of this plant by making people aware about its conservation this attempt has been made. The conservation of this plant is essential in maintaining the biodiversity of the region, because the removal of a single species can affect the functioning of global ecosystems [9].

2. TAXONOMIC DISTRIBUTION AND DESCRIPTION OF BRACKENRIDGEA ZANGUEBERICA

2.1 Distribution and Habitat

B. zanguebarica is distributed in eastern Africa including Kenya and Tanzania. In southern Africa, it occurs in the northern side of Mozambique, eastern part of Zimbabwe, northern part of South Africa [10] and in some parts of Zambia. It can also be found in Madagascar. In South Africa, the species has a narrow distribution and occurs only at Thengwe area in Vhembe District [3]. This indigenous species is reported to be endemic to Vhembe District (Fig. 1). The area is semi-arid with summers from October to March and winters from April to September [11]. The mean annual rainfall is 688 mm and annual mean temperature range between 16 - 26.5°C. Naturally, this plant grows in stony, light gray and sandy loam soil at low altitude in open woodland, deciduous woodland and coastal bush [12].

2.2 Description of the Species

B. zanguebarica is a plant species belonging to the Ochnaceae family. This is a family of 30 genera and 250 species worldwide [13]. The genus *Brackenridgea* to which *B. zanguebarica* belongs comprises of 12 species distributed in Africa, Madagascar, Malaysia to Australia [14].

B. zanguebarica is a shrub or small deciduous tree growing up to 10 m high at maturity (Fig. 2). It has very rough and corky dark gray to black bark. The scratching of the bark shows the yellow pigment beneath it. The leaves are variable in shape and size, elliptic to obovate, about 4-5 cm long, glossy dark green and hairless, lateral veins fine and numerous; margin with very fine, gland-tipped teeth [10]. The attractive flowers are whitish or pinkish and very ephemeral. The fruits of this species are ovoid drupes indehiscent and greenish when unripe and then turn black when mature. The fleshy outer layer attracts the attention of animals, especially birds as a food. The fruits contain a single seed which is very hard to separate from the pulp when dry. The seeds are small and pale brown in color. *B. zanguebarica* regenerates by coppice, which according to [15] is a significant regeneration strategy to conserve viable populations of several threatened plant species.

3. TRADITIONAL USES

B. zanguebarica is medicinally used in many countries for treatment of various diseases. In Africa, the species has been used as a traditional African remedy to treat various ailments. A study by Neuwinger [4] documented that the bark of *B. zanguebarica* could be used in the treatment of wounds, conjunctivitis and jaundice. In East Africa, it is used in the treatment of anaemia, swollen ankles, amenorrhoea, worms and mental illness [5]. In Mozambique, root and bark macerated in water are used to speed the delivery process, treatment of miscarriage, diarrhea, venereal diseases and wounds [6]. Bruschi et al. [6] also reported that the root or bark is used to protect against adversities. In Tanzania, the vapors of boiled stem bark are used in the treatment of conjunctivitis [16]. In Limpopo Province of South Africa, this multipurpose tree is one of the most used medicinal tree species [10]. Steenkamp [17], revealed that some traditional healers in South Africa used *B. zanguebarica* to treat gynecological disorders and the root is useful in the treatment of Amenorrhoea. For many years, the Vhavenda have been using the plant mainly for magico religious purposes as protection against witchcraft, adversities and all sorts of evil [18,3]. It is also reported to bring a person luck in a relationship or business.

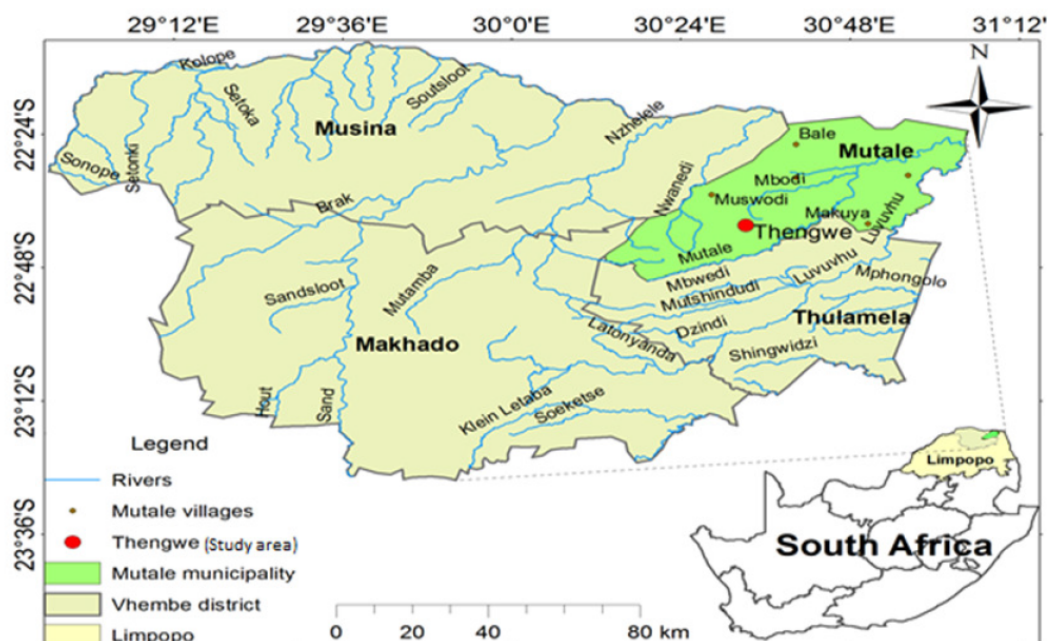


Fig. 1. Map showing the distribution of *B. zanguebarica* Oliv. within Thengwe village in Vhembe District, Limpopo Province, South Africa

Based on this review it has been noted that in many countries the most frequently collected parts of *B. zanguebarica* are the root and bark used in the treatment of various ailments. The species seems to be more efficient in the treatment of wounds, amenorrhoea, and conjunctivitis. It has been reported by most authors that this plant species is not only used for its medicinal importance but also for its highly magical significance. It is reported to protect people and properties against adversities in Mozambique and South Africa [6,3].



Fig. 2. Standing *B. zanguebarica* tree in the reserve

4. PHYTOCHEMICALS STUDIES

Phytochemicals are non-nutritive plant chemicals that occur naturally and have protective or disease preventive properties. Some phytochemicals isolated from *B. zanguebarica* contain various chemical compounds that are responsible for its biological activity. Möller et al. [5] reported several phytochemicals from the bark of *B. zanguebarica* like tannins, brackenin, calodenin B, flavonoids, isochamaejasmin and a derivative of isoswertisin. The same author showed brackenin, calodenin B, isochamaejasmin as the important compounds isolated from the dry bark of *B. zanguebarica*. The chemical structures of the major compounds

are shown in Fig. 3. Intensive phytochemical studies have revealed the Ochnaceae family as an important source of biflavonoids which is well represented in some genera such as *Lophira*, *Luxemburgia*, *Ochna*, *Ouratea* than others [19,20].

Brackenin is an uncommon biflavonoid consisting of two α , and α' -coupled dihydrochalcones which is naturally the only existing compound in this class [18]. It is a new dimeric dihydrochalcone isolated from the stem bark and root of *B. zanguebarica* that has been shown to consist of two dihydroisoliquiritigen [21].

Calodenin B is an isometric biflavonoid isolated from stem bark of *B. zanguebarica*. Ponnappali et al. [18] extracted the calodenin B from the powdered stem bark of *Ochna afzelii* R Br. which is one of the members of Ochnaceae family.

Isochamaejasmin is a biflavonoid that consists of two units of 5,7,4'-trihydroxyflavanone joined together at position 3 and 3". The stem bark of *B. zanguebarica* has been found to contain the biflavonoid isochamaejasmin [5]. Reddy et al. [22] extracted this compound from the stem bark of *Ochna lanceolata* Spreng.

These major compounds extracted from the bark, root and stem of *B. zanguebarica* confirmed the presence of this molecule in the members of Ochnaceae family. Most of these compounds have been isolated from the bark of the species of this family. Therefore there is a significant need to investigate the presence of biflavonoids in all parts of this plant. Although, the two main compounds of *B. zanguebarica* such as Calodenin B and Isochamaejasmin are found in other genera of the Ochnaceae family, it has been noticed through this review that Brackenin is found only in Brackenridgea genus.

5. PHARMACOLOGICAL STUDIES

Pharmacological studies on *B. zanguebarica* have shown a range of biological activities. Some research has confirmed the potential of *B. zanguebarica* used by traditional healers for curing and relieving many diseases. After investigation, this plant has revealed some biological activities such as antibacterial, antiviral, antiproliferative, antifungal and antitumor activities [5]. However, there is lack of information regarding their specific mode of administration and action.

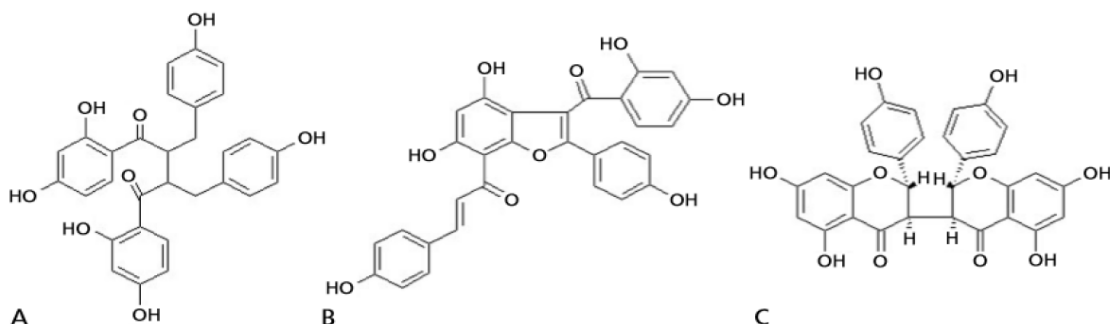


Fig. 3. Chemical Structures of major compounds occurring in *B. zanguebarica* (A: Brackenin, B: Calodenin B, C: Isochamaejasmin)

5.1 Antibacterial Activity

Möller et al. [5] showed a significant antibacterial activity of the ethanolic bark extracts of *B. zanguebarica* that were tested by using a minimum inhibitory concentration (MIC) method against eleven pathogenic bacterial strains of which seven were Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pyogenes*, methicillin resistant *S. aureus*) and four were Gram-negative (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*). Steenkamp et al. [23] studied the antibacterial activity of *B. zanguebarica* on selected strains (*S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*) and the extracts showed activity against the Gram-positive. These results support the use of *B. zanguebarica* in treating bacterial diseases. Crude methanol and water extract of the root have been evaluated and the research claims that none of the extracts showed activity against the Gram-negative organisms whereas, the tested root revealed a high antibacterial effect against Gram-positive organisms [23]. The pharmacological effects of *B. zanguebarica* have been fairly investigated. Bark extracted in ethanol and root extracted in water and methanol showed antibacterial activity against Gram-positive bacterial strains. The acetone leaf extracts of three *Ochna* species investigated such as *O. pretoriensis*, *O. gamostigmata* and *O. pulchra* showed significant antibacterial activity based on their MIC against *E. coli* and *E. faecalis* [24]. All these results suggest further studies using all the plant parts of *B. zanguebarica* with different solvents in different concentrations.

5.2 Antiviral Activity

Considering the high prevalence of viral infections without specific treatment and the constant appearance of resistant viral strains, the development of new antiviral agents is necessary [25]. The yellow bark used for the preparation of an ethanolic extract of *B. zanguebarica* was tested for antiviral activity against Herpes simplex virus type 1 (HSV-1) and it was found that the ethanolic extract from the bark had effectively a strong antiviral activity against free HSV-1 [5]. Some members of the Ochnaceae family such as *Ouratea castaneaefolia*, *O. spectabilis*, *O. castaneaefolia* and *O. semiserrata* have shown activity against selected viruses assayed namely HSV-1, vaccinia virus and murine encephalomyocarditis virus and *O. spectabilis* and *O. semiserrata* were the most active against HSV-1 [26]. Thus, the presence of antiviral constituents from some genera of the Ochnaceae family against various viruses should be an opportunity to further studies on *B. zanguebarica* in order to test the potential of its antiviral activity against various viruses.

5.3 Antifungal Activity

Steenkamp et al. [23] evaluated the antifungal activity of *B. zanguebarica* against *Candida albicans* and the aqueous extract of this medicinal tree species inhibited *C. albicans* growth, while the methanol extracts were ineffective. However, the authors did not reveal the plant part extracts for the antifungal activity. Few studies investigated the antifungal properties of this plant species. However, Araújo et al. [27] evaluated the antifungal activity of extracts from *Ouratea hexasperma* and *O. parviflora* on *C. albicans* and the results obtained

from *O. hexasperma* with the ethyl acetate extract of the roots and n-butanol extract of the leaves exhibited a higher antifungal activity. These results from some members of Ochnaceae family could be helpful to investigate different plant parts of *B. zanguebarica* with various solvent for antifungal activities.

The pharmacological review of this plant species presented an interesting potential of biological activities against bacteria, viruses and fungi. However, very few studies have been carried out to explore deeply the biological activities of this species. Therefore, more studies are necessary to investigate the biological activity of other compounds occurring in the plant part extracts.

6. POPULATION STATUS

The current population size of *B. zanguebarica* is very small, although this species is known to have been more abundant until recently. Todd et al. [28] reported that uncontrolled harvesting of this species has been high, leading to an 86% decline in density from 140 trees/ha in 1990, to 25 trees/ha in 1997. The plant has been intensively collected from the wild due to its highly medicinal and magico religious properties. The bark of this species is often removed from almost the entire stem in different diameter size

classes (Fig. 4A and 4B) and sometimes the tree is cut off (Fig. 4C), leading to its mortality. Todd et al. [28] stated that 100% of trees sampled outside the Brackenridge Nature Reserve (BNR) had been debarked and their roots removed while, inside the reserve, 74% of trees showed evidence of harvesting, and of this 35% were dead. However, Tshisikhawe and Van Rooyen [3] assessed the harvesting impact of this species in BNR and recorded stem bark regeneration. Although the extent to which the bark is collected is well known, the same cannot be said for the duration the bark of this plant takes to regenerate because no studies have been done on this matter. *B. zanguebarica* has a poor seed germination and has become increasingly threatened throughout its range.

Harvesting has to a lesser extent altered the population structure outside the reserve as well as within the reserve [12]. The numbers of individuals have fluctuated over time and the remaining population within the reserve is not a guarantee for the survival of this species. Therefore more population studies are needed to understand trends of this plant in order to formulate a management plan and conservation measures to maintain this dwindling endemic species. Due to its overexploitation and its extreme rarity, *B. zanguebarica* is Red-Listed as Critically Endangered plant species [8].

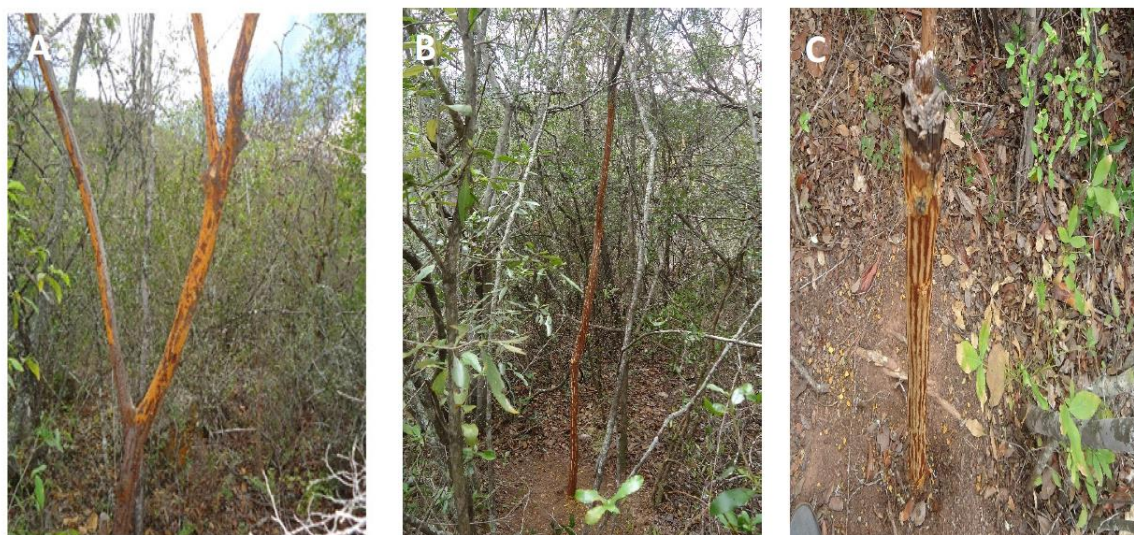


Fig. 4. *Brackenridgea zanguebarica* tree stripped for bark from almost the entire stem in different growth stage: (A) Adult, (B) Juvenile (C) Stem cut off

7. PROPAGATION

Both sexual and asexual propagation is poorly known in *B. zanguebarica*. Although to date, the artificial propagation of this tree seems to be unsuccessful, the regeneration of this plant under natural condition is also very challenging [3].

The germination of seeds under natural condition is unpredictable and very slow. Seeds are very difficult to germinate in the wild and this poor germination appears to be due to the presence of parasites affecting them. However, seeds of other plants species in the Ochnaceae family germinate very readily [10]. As fallen seeds are often parasitized leading to low viability, freshly mature seed should be collected from the standing trees and several methods of pre-treatment must be used to overcome the dormancy of *B. zanguebarica* seeds. Asexual propagation of this species is still unknown in practice. To date, studies have not indicated any research effort concerning the vegetative propagation of this medicinal tree. Since propagating of *B. zanguebarica* has been very difficult, a review of several successful methods of propagating many medicinal plants could potentially be helpful to gain insight on how to propagate *B. zanguebarica*.

Successful seed propagation techniques of many plants producing drupe fruits have been documented. [29,30], respectively found that to achieve best results, *Ochna pretoriensis* E.Phillips and *Ochna serrulata* Walp. fruit should be cleaned to get rid of the pulp and be sown as quickly as possible. A light scarification with hot water or acid treatment would aid rapid germination of *Ochna natalitia* Walp. [31]. Lilabati and Sahoo [32] reported that pre-treatments such as scarification, stratification, gibberellic acid, hot water on seed extracted from the drupe fruit of *Emblica officinalis* Gaertn (Euphorbiaceae) were used to overcome seed dormancy and improve seedling vigour.

As an alternative to overcome seed viability problems and slow seedlings growth, various vegetative propagation methods can be used. But, with restricted distribution and a small population of this plant, micropropagation methods can be an excellent alternative for conservation of *B. zanguebarica* because small pieces of plant material can be used for mass propagation without damage to the donor plant. Various endangered plants have been successfully propagated using *in vitro* mass

propagation methods. The application of tissue culture methods for improving large-scale propagation of many medicinal trees has been demonstrated by several authors. The review by Afolayan and Adebola [33] reported micropropagation protocols for some medicinal plants of South Africa and highlighted the need of using this method for the mass propagation of over-used medicinal plants. During our field work, we observed that *B. zanguebarica* has the potential to regenerate from axillary buds (coppice shoot) which according to Upadhaya et al. [15] is a significant regeneration strategy to conserve a viable population of plant species.

8. CONSERVATION AND SUSTAINABLE USE

According to the findings of many researchers, various sets of recommendations relating to the conservation of medicinal plants have been developed, such as *in-situ* and *ex-situ* conservation [34]. However, as stated by Sutherland [35], the number of wild plant species necessitating specific conservation methods is so large to include all of them in conservation plans. According to Hamilton [36], several actions must be considered in favor of the conservation and sustainable use of medicinal plants in order to benefit continuously from the natural resource. A combination of indigenous and modern knowledge can be a fundamental starting point in any conservation strategy. Medicinal plants with limited distribution, small population, destructive harvesting and poor germination like *B. zanguebarica* generally resulted in species extinction. Therefore, the conservation and sustainable use of this species could be effective through a combination of indigenous and modern knowledge systems. Both approaches follow the same purpose which is to enable this plant species to maintain itself within the context of the ecosystem in which it naturally occurs.

Mathibela [37] revealed the importance of indigenous knowledge as a conservation strategy by emphasizing on the traditional management practices or rituals that promote sustainable resource use. According to Kambizi and Afolayan [38], the management of plants with medicinal properties in the Guruve district of Zimbabwe has been facilitated by several inadvertent or indirect controls. Taboos, seasonal and social restrictions have all helped reduce the harvesting of medicinal plants in the area [38].

Mutshinyalo [10] reported a large number of myths related to the use of *B. zanguebarica*.

After the collection of plant materials for medicinal or magical purposes, it is prohibited to take them to the house or be touched by women because they may hamper menstruation. Either a young virgin or an old member of the Vhatavhatsindi clan who is no longer sexually active collect the plant for medicinal or magical purposes [3]. This is because plant can make any sexually active collector become sterile. All these traditional strategies served to limit medicinal plant harvesting and improve or promote plant conservation.

Despite these indigenous knowledge practices related to conservation of medicinal plant species, there are a few studies investigating traditionally concerning endangered, rare and threatened medicinal plants and how they could be integrated into conservation strategies. However, numerous published papers showed several policies, plans, acts and laws that have been put together to fill some gaps in traditional knowledge and to control overharvesting of natural resources.

South Africa has done extensive work to conserve plants, including protected areas, nature reserves, parks and Botanical gardens. However, despite this intensive work and

regulation towards conservation, it is still difficult to prevent local people from overexploitation of the plants around them. The Brackenridgea Nature Reserve (BNR) was established in 1987 as a strategy to protect the population of *B. zanguebarica*. It is now classified as a critically endangered medicinal tree in the Red List of South Africa by the IUCN [8]. But, this enforcement is still inadequate and has been shown by human activities that greatly threatened the population of this plant outside the reserve as well as within the reserve. It is now necessary to use various customary conservation practices such as taboos, religious values to protect some threatened plants species. These approaches of conserving biodiversity depending on cultural and religious beliefs are usually much more reliable than those relying only on the statutory regulation. A combination of all these approaches is needed to conserve this endemic medicinal tree species. If endemic plants are not protected, they may become extinct. The conservation status of some protected medicinal plant species in the Limpopo Province is reviewed and presented in Table 1 and summarized in Fig. 5 in terms of the number of plant species in different conservation categories in the province.

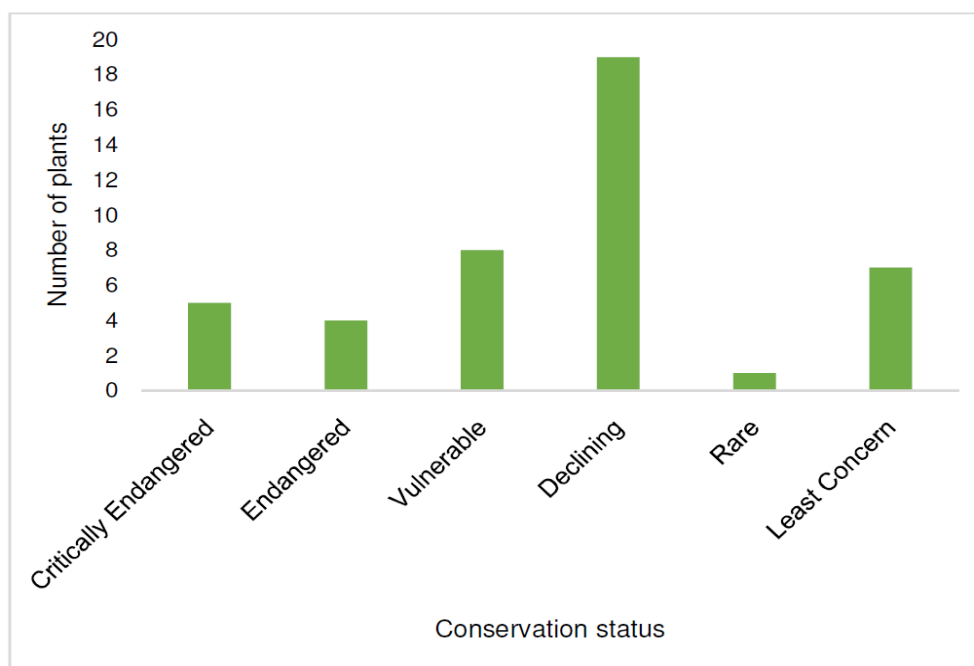


Fig. 5. Number of plant species in different conservation categories in Limpopo Province

Table 1. Conservation status of some red listed medicinal plants species in Limpopo Province [39]

Conservation category	Plants species	English common name	Habit	Part harvested	Family
Critically Endangered	<i>Brackenridgea zanguebarica</i>	Yellow peeling plane	Tree	Roots, Bark	Ochnaceae
	<i>Encephalartos dolomiticus</i>	Wolkberg Cycad	Tree	Roots, Corms	Zamiaceae
	<i>Encephalartos hirsutus</i>	Venda Cycad	Tree	Roots, Corms	Zamiaceae
	<i>Encephalartos laevifolius</i>	Kaapsehoop cycad	Tree	Roots, Corms	Zamiaceae
	<i>Siphonochilus aethiopicus</i>	Natal ginger	Herb	Roots, Rhizomes,	Zingiberaceae
Endangered	<i>Encephalartos eugene-marais</i>	Waterberg cycad	Tree	Roots, Corms	Zamiaceae
	<i>Mondia whitei</i>	White's ginger, tonic root	Herb	Roots, leaves	Apocynaceae
	<i>Ocotea bullata</i>	Black-stinkwood, Cape laurel	Tree	Bark	Lauraceae
	<i>Warburgia salutaris</i>	Pepper-bark tree	Tree	Root-bark, stems	Canellaceae
Vulnerable	<i>Alepidea amatymbica</i>	Giant alepidea, larger tinsel flower	Herb	Rhizomes	Apiaceae
	<i>Bowiea volubilis</i>	Climbing Green Lily, Climbing Onion	Herb	Bulbs	Hyacinthaceae
	<i>Ceropegia cimiciodora</i>	Parasol flower	Herb		Asclepiadaceae
	<i>Cucumis humifructus</i>	Aardvark pumpkin	Herb	Fruit	Cucurbitaceae
	<i>Dioscorea sylvatica</i>	Forest Elephant's Foot	Herb	Bulbs	Dioscoreaceae
	<i>Ocotea kenyensis</i>	Bastard stinkwood	Tree	Bark	Lauraceae
	<i>Prunus africana</i>	Red stinkwood	Tree	Bark	Rosaceae
	<i>Rhynchosia vendae</i>		Herb		Fabaceae
Near Threatened	<i>Adenia fruticosa</i>	Green-stem	Herb	Bulbs	Passifloraceae
	<i>Clivia caulescens</i>	Stem clivia	Herb	Bulbs	Amaryllidaceae
	<i>Curtisia dentata</i>	Assegai	Tree	Bark	Curtisiaceae
	<i>Drimia sanguinea</i>	Rooislangkop	Herb	Bulbs	Hyacinthaceae
	<i>Elaeodendron transvaalense</i>	bushveld saffron	Tree	Bark	Celastraceae
	<i>Gasteria batesiana</i>	knoppies-beestong	Herb	Leaves	Asphodelaceae
	<i>Lithops lesliei</i>	Living stones , Stone plants	Herb		Aizoaceae
Declining	<i>Adenia gummifera</i>	Monkey Rope, Snake-climber	Herb	Roots	Passifloraceae
	<i>Aloe cooperi</i>	Cooper's aloe	Herb	Leaves	Asphodelaceae
	<i>Ansellia africana</i>	leopard orchid	Herb	Bulbs	Orchidaceae
	<i>Balanites maughamii</i>	Green Thorn, Torch Wood, Torch-fruit	Tree	Roots and bark	Zygophyllaceae
	<i>Boophone disticha</i>	century plant, poison bulb, sore-eye flower	Herb	Fresh leaves	Amaryllidaceae
	<i>Callilepis leptophylla</i>	Wild Daisy	Herb		Asteraceae

Conservation category	Plants species	English common name	Habit	Part harvested	Family
	<i>Cassipourea malosana</i>	Onionwood	Tree	Bark	Rhizophoraceae
	<i>Crinum macowanii</i>	river crinum, river lily	Herb	Leaves, bulbs	Amaryllidaceae
	<i>Crinum stuhlmannii</i>	Candy-striped crinum	Herb	Bulbs	Amaryllidaceae
	<i>Cryptocarya transvaalensis</i>	Mountain Wild-Quince	Tree	Bark	Lauraceae
	<i>Drimia altissima</i>	Tall White Squill	Herb	Bulbs	Hyacinthaceae
	<i>Elaeodendron croceum</i>	saffron; saffron wood, forest saffron	Tree		Celastraceae
	<i>Eucomis autumnalis</i>	pineapple flower, pineapple lily	Herb	Bulbs	Hyacinthaceae
	<i>Eulophia speciosa</i>	Large Yellow Eulophia	Herb		Orchidaceae
	<i>Gunnera perpensa</i>	Pumpkin, Wild Rhubarb	Herb	Roots	Gunneraceae
	<i>Hypoxis hemerocallidea</i>	Star-flower, Yellow Star	Herb	Roots	Hypoxidaceae
	<i>Ilex mitis</i>	African holly, wild holly, water tree	Tree		Aquifoliaceae
	<i>Pterocelastrus rostratus</i>	Red Candlewood, White Pear	Tree	Barks	Celastraceae
	<i>Rapanea melanophloeos</i>	Cape Beech,	Tree	Roots, barks	Myrsinaceae
Rare	<i>Euphorbia sekukuniensis</i>	Sekhukhune Candelabra Tree	Tree		Euphorbiaceae
Least Concern	<i>Alsophila capensis</i>	Cape Tree Fern, Forest Tree Fern	Tree		Cyatheaceae
	<i>Boscia albitrunca</i>	Shepherd's tree	Tree	Roots, Leaves and Fruits	Capparaceae
	<i>Capparis sepiaria</i>	Wild caper bush	Shrub	Roots, Bark	Capparaceae
	<i>Catha edulis</i>	Bushman's tea	Tree	Leaves, Bark and Roots	
	<i>Encephalartos transvenosus</i>	Modjadji cycad	Tree	Roots, Bark and Leaves	Zamiaceae
	<i>Pittosporum viridiflorum</i>	Cheesewood	Tree	Roots, Stem-bark	Pittosporaceae
	<i>Securidaca longepedunculata</i>	Violet tree	Tree	Root-bark	Polygalaceae

9. CONCLUSION

B. zanguebarica an indigenous and endemic plant to Thengwe village in Vhembe District municipality, popularly known and well reputed for its medicinal and magical properties is critically endangered according to the listing of the IUCN. Review of literature revealed the frequent traditional uses and a variety of biological activities found in this plant have built a heavy pressure on the remaining population of this plant in the reserve. Considering the restricted distribution of *B. zanguebarica* in South Africa as well as throughout Africa, the species has received less attention and a few scattered studies have been conducted by researchers. However, most of the studies have been directed to the medicinal aspects of this plant. But, there are still many important challenges that need to be overcome in order to discover new potent compounds and further investigations for the biological activities of this plant species. This review summarized for the first time some aspects of *B. zanguebarica* so that this information gathered together in one place can be used as a guide to understand the present situation of this species. Therefore, there is significant necessity to continue doing research in various aspects of this species. Knowledge on the reproduction biology of this endemic plant species is strongly needed, because the availability of such information is crucial to develop a significant conservation strategy.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Investigation of current threats to the existence of *Brackenridgea zanguebarica* in a small geographic area in Vhembe, Limpopo Province, South Africa

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Abstract. *Tiawoun MAP, Tshisikhawe MP, Gwata ET. 2019. Geometric morphometry of pupae to identify four medically important flies (Order: Diptera) in Thailand. Biodiversitas 20: 1504-1509. Brackenridgea zanguebarica* is one of the most threatened plant species in South Africa, found only in Thengwe village, Vhembe District Municipality, Limpopo Province, South Africa. Due to the high traditional use of its stem bark and root, the species is now facing the threat of extinction. It has been assessed in accordance with the IUCN Red List of South Africa as a critically endangered species. The aim of this study was to investigate the impact of current threats to the population in the Brackenridgea Nature Reserve, in order to improve its conservation measures. The study was carried out in the reserve, where 10 belt transects of 50 m long and 20 m wide were laid at regular intervals of 5 m to investigate the population of *B. zanguebarica*. Individuals were counted and the structural parameters, e.g. tree height and stem diameter size of each were measured, while the impact of plant harvesting was estimated using a sliding scale of 0 to 5. The structure of the populations in terms of the stem diameter size classes was dominated by juvenile plants that showed the bell shape pattern. In addition, 59.9% out of the total plants recorded presented signs of plant parts extraction, with the stem bark the main part extracted. The population status of *B. zanguebarica* was unstable and under severe threat due to the destructive harvesting of the mature tree parts, leading to poor regeneration of individuals. It is thus recommended that in order to improve its conservation measures.

Keywords: *Brackenridgea zanguebarica*, conservation, endemic plant, harvesting, population status

INTRODUCTION

The extinction and declining populations of many rare plant species worldwide are increasing at an alarming rate. This escalating biodiversity crisis is an indication that the current diversity of nature is not capable of supporting the pressure that the growing humanity is placing on the planet. Overexploitation of the plants' roots and bark has endangered the survival of the mature trees as these are unable to fully recover, hence, eventually hindering fruit production but rather causing poor or slow seedling recruitment (Dhillion and Gustad 2004). The continued decline, coupled with the plant's endemism status and the small geographic range where this tree species occurs, make it particularly vulnerable to natural catastrophes. Rare species restricted to one or to a few populations have a greater chance of becoming extinct (Van Dyke 2003). In this scenario, *B. zanguebarica* has been classified as a protected tree in the Red List of South Africa. In terms of the IUCN criteria, this plant has been listed under the category of critically endangered plant in South Africa (Williams and Raimondo 2008).

Brackenridgea zanguebarica, a small deciduous tree, belongs to the family, Ochnaceae. It has a restricted geographic distribution and its natural distribution zone is known from only one population confined to a small area

within Thengwe, in the Vhembe District of Limpopo Province, therefore, endemic to the Vhembe District. The species is habitat-specific as many other rare and endemic plant species have only one or few ranges of distribution. It grows naturally in stony, light gray and shallow sandy loam soil, at low altitude, in open areas with low grass cover (Mutshinyalo 2011). In South Africa, *B. zanguebarica* is considered a critically endangered plant species according to the South African National Biodiversity Institute (SANBI) Red List categories (Raimondo et al. 2009), mainly due to severe harvesting from traditional herbalists, and commercial harvesting of its bark and roots to meet growing market demand. It is an important indigenous medicinal plant used by the Venda people, mostly for magical purposes (Tshisikhawe and Van Rooyen 2012).

Due to the high anthropogenic pressure, harvesting has depleted the population outside the Brackenridgea Nature Reserve as well as within the reserve, although to a lesser extent (Williams and Raimondo 2008); not a single plant seems to be available outside the reserve. Nowadays, this species only has a small population and is very threatened across its extremely narrow range of distribution. The number of individuals have fluctuated over time, however, the remaining population within the reserve it is not a guarantee for the survival of this species due to the destructive harvesting that is taking place within the reserve.

This species has been listed as protected since the establishment, in 1987, of the Brackenridgea Nature Reserve by the Provincial Limpopo Department of Economic Development, Environment and Tourism (LEDET). Despite its protection, this tree species still faces a high risk of extinction over its small geographical range due to unsustainable harvesting of its stem bark and root. The population density of *B. zanguebarica* inside and outside the reserve was investigated in 1990 and 1997. In this regard, Todd et al. (2004) reported that uncontrolled harvesting has resulted in an 86% decline in density from 140 trees/ha in 1990, to 25 trees/ha in 1997. The reserve, obviously, is not performing its role of *in situ* conservation of biological diversity confirming the prediction which states that, if the intensity of collecting this plant continues at this rate, this species will become extinct in South Africa (Mutshinyalo 2011). Action, therefore, must be taken for the sustainable conservation of this plant species by setting up an effective management scheme.

The conservation of this endemic tree species is hampered by the dearth of information on its population biology and ecology. Because of this, the present study was undertaken to understand how the current threats affect the population biology and ecology of this endemic tree species in the reserve. The specific objectives of the study

were, to (i) Assess the population density and structure of *B. zanguebarica*; (ii) examine the harvesting impact on the population structure and the natural regeneration of *B. zanguebarica*. The findings of this study might contribute to useful information for the planning of efficient conservation strategies for this critically endangered plant species in the Brackenridgea Nature Reserve by LEDET.

MATERIALS AND METHODS

Study site

The study was conducted in The Brackenridgea Nature Reserve (BNR) also known as the Mutavhatsindi Nature Reserve (MNR). The reserve is currently 110 ha in size and the conservation state of this species in this site is not satisfactory due to limited reserve size (Tshisikhawe et al. 2013). The soil is mainly stony and thin with sandy loam (Mutshinyalo 2011). The reserve is within the savanna biome and the vegetation in and around the reserve is classified as Vhavenda Miombo (Mucina and Rutherford 2006), and it is situated in the Vhembe District Municipality of the Limpopo Province (Figure 1).

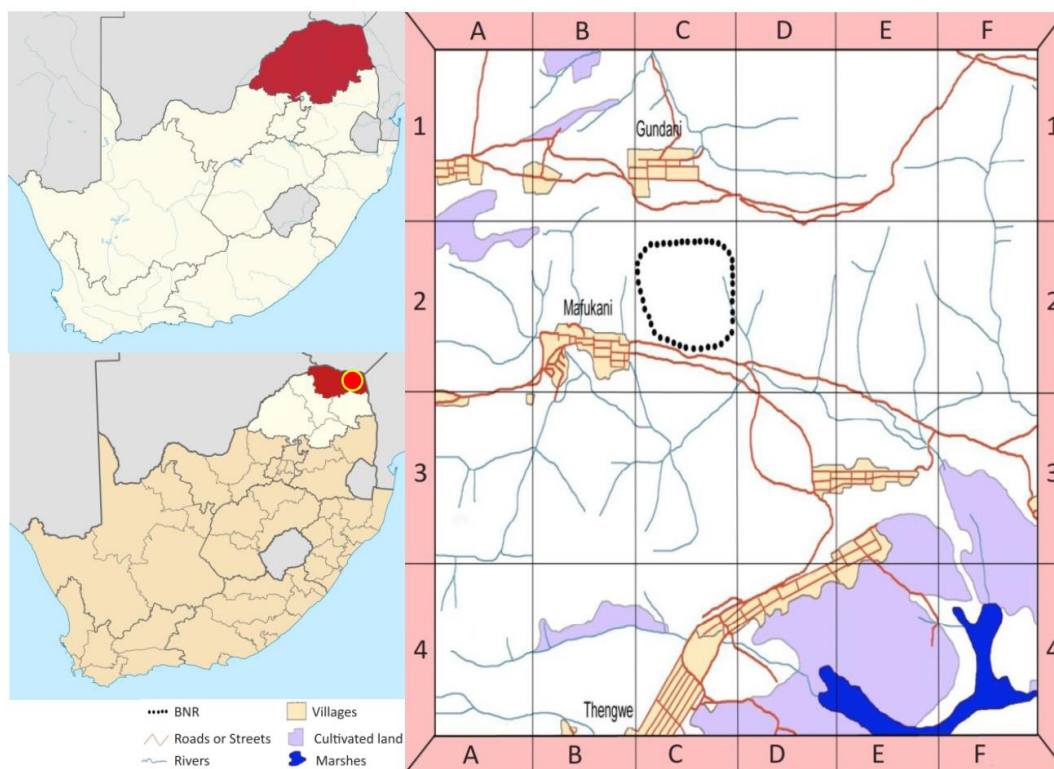


Figure 1. Map of the Thengwe village showing the Brackenridgea Nature Reserve (BNR), in Vhembe District Municipality, Limpopo Province, South Africa (Tshisikhawe et al. 2013; Wikipedia)

Its coordinates are 22° 24' 0.0'' -23° 36' 0.0'' S and 29° 12' 0.0'' and 31° 12' 0.0'' E. The reserve is at an average elevation of 600 meters above sea level. The mean annual rainfall is about 350 mm, but it occasionally varies with the annual mean maximum and minimum temperature being 26.5°C and 16°C, respectively (Mzezewa et al. 2010). The temperature, however, does not vary much over the seasons. Rainfall occurs mainly during summers, from October to March, and mild winters occur from April to September (Mucina and Rutherford 2006). The vegetation of the region is dominated mostly by *Colophospermum mopane*, *Terminalia sericea*, *Grewia flava* and *Combretum apiculatum* (Venter and Witkowski 2010). The dominant ethnic population group in the surrounding area of the BNR is the Vhavenda who survive mainly on farming.

Field data collection

A field survey was undertaken and samplings collected within the BNR in Thengwe village from November to December 2016. The population sampling was conducted through the vertical belt transect method of Michael (1990). This method is appropriate for recording the presence of as many taxa as possible, in the study area to produce detailed information on the population status of this plant species. In order to examine the population status of *B. zanguebarica*, it is necessary to record the presence of as many trees as possible that will be representative of the population. Ten belt transects of 50 m long and 20 m wide were laid at regular intervals of 5 m in the study area. Each belt transect was divided into two sub-belts of 50 m x 10 m for ease of sampling and five quadrats of 10 x 10 m were laid in each sub-belt as replicates. Ten quadrats were derived from each belt transect. One hundred quadrats of size 10 x 10 m, therefore, were sampled in the study site covering an area of 1 ha. The coordinates of all quadrats were recorded using a Global Positioning System (GPS).

All the *B. zanguebarica* trees, from seedlings to adult plants that were encountered within each quadrat were counted and different parameters, such as the height and stem diameter were measured. In addition, the evidence of anthropogenic bark and root harvested were estimated and scored.

Population density and structure

In each quadrat, the diameter of all samples of *B. zanguebarica* was measured and, the number of individuals in different diameter size classes was enumerated to assess the population structure. The diameter of seedlings and saplings were measured using tree caliper at the base of the plant, while larger individuals with DBH \geq 5 cm were obtained by measuring their circumference at 1.3 m from the ground level with a tape measure. In order to study the stem size class distribution, seedlings were regarded as samples with a stem diameter \leq 0.5 cm and height < 50 cm, whereas saplings were samples with a stem diameter between 0.5 and 1.99 cm and with height >50 cm. The DBH of larger individuals were recorded and grouped into eight DBH classes assigned at 4 cm DBH increments: 2.0-5.9 cm, 6.0-9.9 cm, 10.0-13.9 cm, 14.0-17.9 cm, 18.0-21.9 cm, 22.0-25.9 cm, 26.0-29.9 cm and 30.0-33.9 cm (Vesa et

al. 2015). These DBH classes were then, separated into three arbitrary categories based on the age stage for easy analysis: juveniles were those with 2.0 cm \leq DBH < 5.9 cm and 6.0 cm \leq DBH < 9.9 cm ; sub-adults: 10.0 cm \leq DBH < 13.9 cm and 14.0 cm \leq DBH < 17.9 cm and adults: DBH \geq 18.0 cm.

Plant parts harvested

Because of only some plant parts of this species were being over-collected, two major anthropogenic harvesting practices (root and bark) were investigated. The occurrence of any evidence of bark removal and root excavated was recorded for each sample; also chopped stems were noted as well. In order to investigate the impact of harvesting practices on the population structure of this species, all samples were divided into different size classes based on the stem diameter and harvesting practices were measured by classifying the proportion of extracted bark or root.

The intensity of bark harvesting was estimated and recorded in a sliding scale following the visual classification described (Cunningham 1993). Cunningham (1993), used the visual method to classify the levels of damage intensity into seven categories based on the proportion of bark collected from each sample. This method was applied in this study and interpreted by using the terms of Tshisikhawe et al. (2012) with slight modification as follows: 0: No damaged (Null); I: Individual with \leq 10 % damaged (Trace); II: Individual with 11-25% damaged (Light); III: individual with 26-50% damaged (Moderate); IV: Individual with 51-75% damaged (Severe); V: Individual with 76-100% damaged (Very severe) (Table 1 and 2).

Root excavated was assessed according to the level of disturbed surface area at the base of the stem as applied in the study of *Catha edulis* (Botha 2004). Roots excavated were recorded and scored in five classes as follows¹⁵: 0: no harvesting; I: <10 %; II: 11-25 %; III: 26-50 %; IV: 51-75 %; V>76 %, and then, interpreted by using the above terms of Tshisikhawe et al. (2012).

Natural regeneration

In each quadrat, the number of seedlings and saplings were counted and at the same time, all cut stumps with sprouting ability were recorded.

Data analysis

The numbers of the individual samples in different diameter size classes and distribution were used to create Bar graphs of the population structure. Data were entered into Microsoft Excel Spreadsheet 2010 and analyzed using SPSS version 24. Kruskal-Wallis Test (Nonparametric ANOVA) was performed to check for significant differences in the number of individual samples with signs of collection in different diameter size classes and to test if the proportion of trees harvested is diameter-dependent.

RESULTS AND DISCUSSION

Population density and structure

Two hundred and forty-seven *B. zanguebarica* individuals, in different diameter size classes, were recorded in 1 ha of the study area; this corresponded to a density of 247 individuals/ha. Based on the assessment of diameter size class, the population structure of *B. zanguebarica* showed that individuals are represented in all diameter classes that were arbitrarily selected. The highest number of individuals was observed in DBH (2-5.9 cm) (Figure 2.A). All individuals in different DBH were arbitrarily grouped into five tree age stages (seedlings, sapling, juveniles, sub-adults, and adults). Out of the total samples of this plant species, 32 were found in the seedlings category, 21 were grouped as saplings, 120 represented the juvenile categories; sub-adults and adults were 57 and 17, respectively (Figure 2.B). About half (48.6%) of individuals per hectare were concentrated in the juveniles classes (2.0-5.9 cm and 6.0-9.9 cm) followed by the sub-adults classes (10.0 cm \geq DBH \leq 25.9 cm) with 23% of the total tree species. Seedlings contributed to 13% and saplings represented 8.5% of the total tree; however, the adult category was under-represented at 6.9%.

Few individuals were recorded for both the lower and the higher stem diameter, whereas a high number of individuals were noted in the medium class. The population structure of *B. zanguebarica* according to the number of individuals per hectare showed a bell-shaped trend (Figure

2.B). The number of individuals increases with increasing of stem diameter up to a certain point, then decreases with increase in stem diameter.

Proportion of plant parts harvested

A total of two hundred forty-seven individuals of the species *B. zanguebarica* was recorded in the study area of which hundred (40.5%) presented some signs of bark collection, twenty-nine (11.7%) showed evidence of roots excavated while nineteen (7.7%) had cut stumps which were clearly visible. Overall, one hundred and fifty-three plants (60%) out of the total distributed in a different size class and recorded in this study, presented some signs of plant parts extraction. The number of plants showing a different proportion of bark and root extracted is presented in Tables 1 and 2, respectively.

Bark harvested

Based on the bark collection, the number of plants and the proportion of bark removal varied for different diameter size classes. One hundred forty-seven individuals (59.5%) out of the 247 found in this population had no evidence of bark extraction, whereas 100 plants (40.5%) showed some signs of bark extraction. The extent of bark removal ranged from simple scaling (trace) to the entire removal of the bark from the tree. All size classes presented signs of bark harvested except the lowest diameter class (seedlings \leq 2).

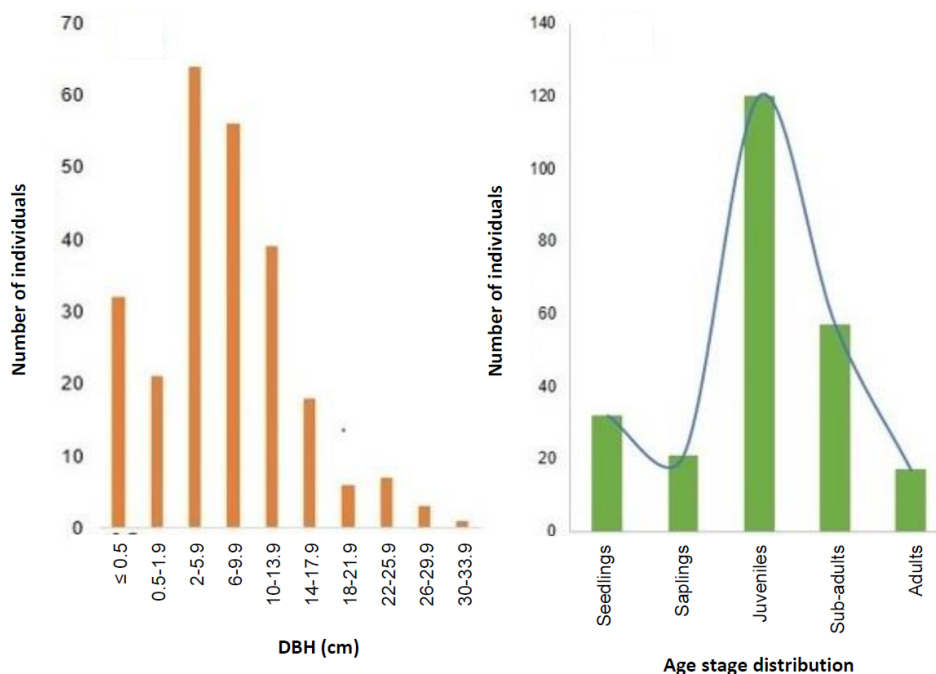


Figure 2. Illustration of population of *Brackenridgea zanguebarica* in the Brackenridge Nature Reserve. A. Distribution of *B. zanguebarica* in various diameter size classes; B. Patterns of age stage distribution of *B. zanguebarica*

Table 1. Number of individuals with the sign of bark extraction and proportion of bark harvested in different diameter size classes of *Brackenridgea zanguebarica*

DBH class (cm)	Total number of individuals sampled	Proportion of bark removal					Total number of individuals with bark harvested	
		0 (Null) 0%	1 (Trace) 1-10%	2 (Light) 11-25%	3 (Moderate) 26-50%	4 (Severe) 51-75%		5 (Very severe) 76-100%
Seedlings ≤ 0.5	32	32	0	0	0	0	0	0
Saplings 0.5-1.9	21	20	0	1	0	0	0	1
2-5.9	64	53	4	1	3	1	2	11
6-9.9	56	31	3	7	9	3	3	25
10-13.9	39	8	4	5	10	7	5	31
14-17.9	18	3	0	2	4	5	4	15
18-21.9	6	0	1	0	1	4	0	6
22-25.9	7	0	1	0	2	3	1	7
26-29.9	3	0	0	0	1	1	1	3
30-33.9	1	0	0	0	0	0	1	1
Total	247	147	13	16	30	24	17	100

Of those with signs of bark harvested, the highest number (30 plants) was moderately harvested (26-50%), 24 showed signs of severe bark removal (51-75%) while, 17 presented very severe signs of bark damaged (76-100%). Sixteen plants showed evidence of bark lightly exploited (11-25%) and the lowest number (13) were found in the trace category (1-10%). The greater the DBH of the tree, the more it was harvested and the higher the proportion of bark removal (Table 1). Kruskal-Wallis analyses revealed a significant relationship between the number harvested and the proportion of bark harvested in the diameter size class ($p < 0.05$).

Concerning the proportion of bark removal for each diameter class, none of the seedlings showed signs of bark removal. The sapling class had 1 plant where 11-25% of the bark had been collected. In the juvenile category (2-5.9 cm and 6-9.9 cm), 36 plants (30%) out of the 120 in this category showed signs of bark collection with different percentages. Among these 36, 5 had 76-100% of bark damaged, 4 and 12 plants were found with 51-75% and 26-50% of bark extraction, respectively. Eight plants showed 11-25% and 7 had 1-10% of their bark extracted. In the sub-adult category (10-13.9 cm and 14-17.9 cm), 46 (80.7%) out of 57 plants presented bark extraction. Four plants presented traces of bark damaged, and seven were lightly harvested. Fourteen individuals had moderately extracted bark while 12 were found with severe levels of bark removal. Nine plants were recorded in the highest proportion (76-100%) of bark extracted. In the Adult category (18-21.9 cm to 30-33.9 cm), 17 plants (100%) showed signs of bark damage (Table 1).

Figure 3 shows the percentage of plants with bark removal in different age stage distribution. In the adult categories, all the plants showed signs of bark extraction from trace to very severe, whereas, in sub-adults' categories, 80.7% of the plants presented signs of bark removal. 30% and 4% of plants were found with signs of bark harvested in the juvenile and seedlings categories. Almost all individuals of the five largest diameter classes that were found within this population, clearly, showed

signs of bark removal (Table 1). The majority of the larger stems had been intensely harvested in the past; hence, the larger the stem, the higher the percentage of bark removal (Figure 3). According to the Kruskal-Wallis Test, the number of individuals with the sign of bark harvested is considered insignificant between diameter size classes; P value of 0.5382 is considered insignificant ($p < 0.05$).

Root harvested

Among the total recorded plants (247) in all the diameter size classes, 29 plants (11.7%) showed some signs of root excavated as opposed to 218 (88.3%), which had no sign of root excavated. Of the 29 plants presenting signs of root damage, 23 (79.3%) had 1-10% (trace) and 11-25% (light) of their root extracted. Two plants presented moderate root extraction, 2 others had been severely harvested, while two showed more than 76% of root collection (Table 2).

Table 2 shows the number of plants with signs of a root collection and the proportion of root harvested in different diameter size classes. The two first classes (seedlings and saplings) showed no sign of root damage. In the juvenile category (2-5.9 cm and 6-9.9 cm) 4 samples had 1-10% of root extracted, 2 indicated light root removal and 2 showed 51-75% of root damage. Fifteen samples were found with evidence of root extracted in the sub-adult category (10-13.9 cm to 14-17.9 cm); of these, 8 presented trace of root damage, 4 showed slight proportion and 2 plants' roots had been moderately collected. In the adult category, 6 plants showed signs of root damaged of which 2, 3 and 1 had 1-10%, 11-25% and 76-100% of their roots removed, respectively (Table 2). The number of individuals with signs of root collection, in different diameter size classes, according to Kruskal-Wallis is significantly different ($p < 0.05$) between diameter size classes. Figure 4 shows that the larger the DBH class, the higher the percentage of plants with roots excavated. 35.3% of the samples in the adult categories showed signs of roots collection followed by 26.3 % in sub-adult categories and 6% in juveniles.

Table 2. Number of individuals with the sign of root collection and proportion of root harvested in different diameter size classes of *Brackenridgea zanguebarica*

DBH class (cm)	Total number of individuals sampled	Proportion of roots harvested						Total number of individuals with roots harvested
		0 (Null) 0%	1 (Trace) 1-10%	2 (Light) 11-25%	3 (Moderate) 26-50%	4 (Severe) 51-75%	5 (Very severe) 76-100%	
Seedlings ≤ 0.5	32	32	0	0	0	0	0	0
Saplings 0.5-1.9	21	21	0	0	0	0	0	0
2-5.9	64	61	0	2	0	1	0	3
6-9.9	56	51	4	0	0	1	0	5
10-13.9	39	31	4	3	0	0	1	8
14-17.9	18	11	4	1	2	0	0	7
18-21.9	6	4	0	2	0	0	0	2
22-25.9	7	5	1	1	0	0	0	2
26-29.9	3	2	1	0	0	0	0	1
30-33.9	1	0	0	0	0	0	1	1
Total	247	218	14	9	2	2	2	29

Cut stump

In this study, stem harvested were recorded only in five DBH size classes; from size class 2-5.9 cm up to size class 18-21.9 cm. In the study area (1 ha), the total number of individuals with their stem cut off was nineteen out of two hundred and forty-seven (7.7%) of which 8 (42.1%) occurred in the diameter size class (10-13.9 cm). Four plants were observed with their stems chopped off in each of the diameter size classes 6-9.9 cm and 14-17.9 cm. In the juvenile category, 5% of plants presented chopped stems, whereas only 1 (5.8%) was noted with stems cut off in the adult category. Overall, the majority of the samples presenting chopped stems were concentrated in the sub-adult category (21%). In smaller diameter classes (seedlings and saplings) stems chopped off were not observed (Figure 5).

Natural regeneration

The low number of seedlings (32) and saplings (21) (Figure 2.B) encountered in this study showed low rates of regeneration. Figure 2.B indicated a few individuals from the lower (32 individuals) and higher (16 individuals) stem diameter. Sprouting from few cut stumps (19) was recorded only in the Juvenile, Sub-adult and Adult categories (Figure 5). Only 3 out of 19 (15.8 %) cut stumps showed coppice ability (Figure 6).

The main threat to *Brackenridgea zanguebarica*

The results of this study revealed that the stem bark was the most collected plant part with about 40.5% followed by root (11.7%) and cut stump (7.7%). Overall, 60% of individuals showed signs of plant part extraction and 40% of this species showed no damage.

Discussion

Population density and structure

The results in terms of DBH size distributions of *B. zanguebarica* population revealed that the majority (48.6 %) of the plants were in the juvenile categories, although, few plants in both lower and higher diameter size classes were also observed. This result indicated that the low number

(6.9 %) of adult trees is probably due to the destructive harvesting of bark and root. The higher number of individuals in the juvenile stage could be due to the harvesting status of these plants; they are considered not mature enough to be intensely extracted by the harvesters. The higher number of individuals in the juvenile stage could further be due to irregular recruitment episodes when conditions were favorable (Tsheboeng and Murray-Hudson 2013).

The gradual decrease in density from the sub-adult to adult stages is due to past anthropogenic activities that targeted mature plants for bark and root collection, which might have led to reduced reproduction. Similar results have also been reported that populations of species of anthropogenic importance are often characterized by a decrease in occurrences in larger size classes (Botha et al. 2004). Williams et al. (2007) revealed that harvesters tend to select plants in the larger size classes. The structural population pattern of *B. zanguebarica* in this study contradicts that of Tshisikhawe and Van Rooyen (2012) from the same study area, who reported that the population structure of *B. zanguebarica* exhibited the inverse J-shaped curve, indicating a healthy population structure. This contradiction might be associated with the difference interval of the diameter size classes utilized in each study.

Plant parts harvested

Bark harvesting. A large number (40.5%) of plants showed evidence of bark extraction. In almost all diameter size classes, signs of stem bark collection were noted, which highlights the growing market demand for this multipurpose tree in the area. Despite, the collection of stem bark in almost all the diameter size classes, there was a tendency for more harvesting from trees belonging to the larger diameter classes. This is comprehensible because trees with a large stem diameter have more available bark to harvest than the small ones. These results correspond with other studies, such of *Garcinia lucida* Vesque (Guedje et al. 2007), *Anadenathera colubrina* (Soldati et al. 2011) and *Myracrodruo nurundeiva* (Lins Neto et al. 2008).

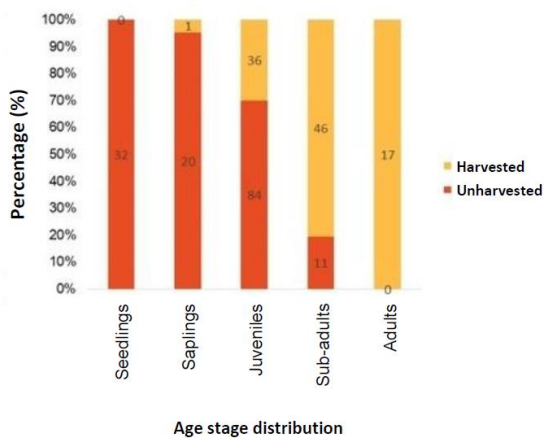


Figure 3. Percentage of individuals with bark removal in different age stage

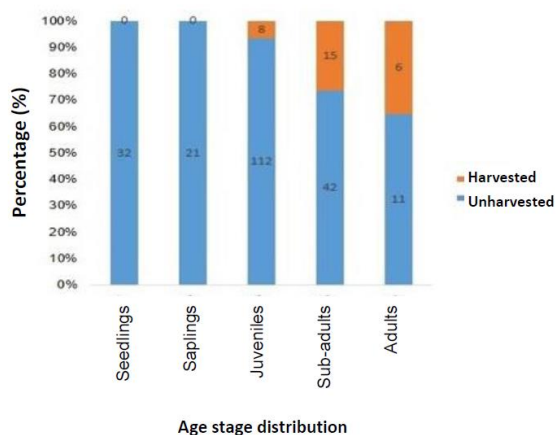


Figure 4. Percentage of individuals with root excavated in each age stage

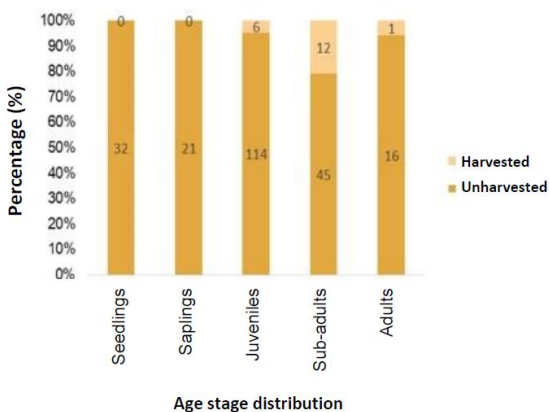


Figure 5. Percentage of individuals with a cut stump in each age stage distribution



Figure 6. Regeneration from axillary bud (coppice shoot) of *B. zanguebarica* in its natural habitat.

Bark extraction from large plants could affect the survival of reproductive trees as they will not be able to fully recover and will eventually hinder fruit production (Dhillion and Gustad 2004); this will lead to poor seed production. The poor reproduction of this tree species probably increases with the intensity of bark extracted.

The intensive bark collection of *B. zanguebarica* has caused serious damage to its population because harvesters seem to have no preference about size class. This result supports the findings of the study that revealed the non-size class effect on the extent of bark and foliage harvest of *Azelia africana* and *Pterocarpus erinaceus* in Eastern Burkina Faso (Nacoulma et al. 2011). If the bark is collected from the small-size class trees, it affects the growth and development of this species. Bark harvesting from small plants was reported in some studies such as the study of people's knowledge and extractivism of *Stryphnodendron rotundifolium* Mart. in Northeastern of Brazil (Feitosa et al. 2014).

The extraction of bark from this multipurpose tree, either from small size classes or from bigger reproductive trees, have had a detrimental impact on the population structure. This species, therefore faces a serious threat from the random collection of bark, which has eventually led to a critically endangered plant. If this trend continues to occur, it is predicted that the population will be extinct in the next years to come.

Root harvesting. In this study, few trees showed evidence of root extraction. A similar situation of root extraction was revealed by Todd (1999), with the number of the tree with root harvested concentrated in the higher diameter classes. Harvesters seem to focus their activities on higher-diameter size classes. The low proportion of root harvested of this tree species may reveal that, this plant part seems not to be the most preferred by harvesters. However, the harvesting impact of root might be responsible for the death of some individuals of this tree species.

Cut stump

The variation in the number of cut stumps in the different diameter classes reveals the dominance of plants in the intermediate size class. The high number of cut stumps in the medium diameter class may be due to high

density of medium diameter resulting in a large probability to be found and cut. Few cut stump in this category showed the sprouting ability, which is an important reproduction mechanism to maintain a population viable. Even though many factors influence the ability of a stump to sprout, some studies reveal that one of the most important factors in terms of coppice regeneration is the age of the tree (Johansson and Hjelm 2012).

Natural regeneration

The majority of individuals found in the medium diameter class of this tree may be due to irregular recruitment and mortality of trees of the larger diameter size classes. The *B. zanguebarica* status showed a bell-shaped pattern with fewer individuals in the seedling, sapling, sub-adult and adult stages compared to the juvenile stage (Figure 2.B). Species with such regeneration pattern is an indication of an unstable population (Helm and Witkowski 2012), and which are under threat due to lower recruitment levels (Colling et al. 2002).

Few numbers of cut stumps observed only in juvenile and sub-adult categories showed the sprouting ability. This may indicate that natural regeneration by coppicing of this species is influenced by the stem diameter size. These findings are also in accord with those of Sangeda and Maleko (2018) who stated that *Brachystegia boehmii* and *B. spiciformis* of large size diameter classes did not show much vegetative regeneration. The number of shoots per stump and how long they can start to sprout were not investigated in this study. However, this study showed that *B. zanguebarica* could regenerate vegetatively and this could assist in increasing the regrowth rates of the species.

In conclusion, the overall findings of this study show that the population of *B. zanguebarica* that occurs in the BNR is facing many stresses from human activities which need the reinforcement of certain conservation measures. The harvesting impact on population structure and regeneration showed that *B. zanguebarica* is severely overexploited in the reserve. The destructive harvesting of large individuals influenced the natural regeneration of this plant. The poor natural regeneration is characterized by few seedlings and saplings. Bark harvesting for medicinal and magical purposes is the main threat to the decline of *B. zanguebarica*. The bell-shaped size class distribution pattern characterizes the population structure of *B. zanguebarica* due to the high number of medium-sized trees with a poor number of individuals in both lower and higher DBH size classes. This indicates that the population of *B. zanguebarica* in the reserve is unstable and under threat. The ongoing anthropogenic activities are influencing the population structure and the regeneration status of this tree species. The species has become a critically endangered plant due to the intensive and improper collection of plant parts to supply the growing market demand. This trend may reduce the capacity of this tree species to maintain its population in the coming years. Hence, proper conservation measures have to be formulated to protect the remaining population of *B. zanguebarica*, taking into account not only its multiple values but also for future research. This study is an

important step towards the conservation of this threatened plant species. Hence, the following points are made as recommendations: (i) Protection and continuous inventory of the existing populations should be conducted, and it needs the participation of local people. (ii) Conservation through sexual and asexual propagation is urgently needed for overcoming the challenges of natural regeneration. (iii) Regular overnight patrols to reduce illegal harvesting should be implemented by the manager of the reserve. (iv) *Ex situ* conservation and plantation of *B. zanguebarica* are necessary to expand the new production area in the region.

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