Ethnobotany and conservation biology of *Warburgia salutaris* (G.Bertol.) Chiov., a threatened medicinal plant in southern Mozambique

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A thesis submitted in fulfilment of the requirements for the degree

of DOCTOR OF PHILOSOPHY

Department of Environmental

Science Rhodes University

Makhanda, South Africa

February 2021

Abstract

Warburgia salutaris, locally known as "chibaha" (Ronga, Tsonga), "isiBhaha" (Zulu) and "siBhaha" (Siswati), is one of the most highly-valued medicinal plant species in southern Africa. Its bark has long been employed to treat bacterial and fungal infections, and it is often a front line herbal treatment for malaria. The species is popular in rural and urban areas due to its lower price compared to commercial pharmaceuticals and because of its long-standing spiritual and symbolic significance. Consequently, *W. salutaris* is threatened in Malawi, South Africa and Swaziland and is believed to be extincted in the wild in Zimbabwe. In Mozambique, where the tree is confined to the southern part of the country, it is considered vulnerable due to high local demand and cross-border export. Although there is limited research, further work is urgently needed to provide the basis for options and strategies to achieve desired outcomes. This study aimed to explore different approaches for the conservation of *W. salutaris* in the Lebombo Mountains (LM), the Tembe River (TR) and the Futi Corridor (FC) areas, in southern Mozambique. For this, potential distribution, socio cultural factors that influenced sustainable management of the species, demography, harvest impact, genetic diversity and population differentiation were considered.

The kuenm package in R, 2 846 occurrence and 11 environmental data were used to model the potential current and future distribution in 2050 and 2070 using four general circulation models (CESM1(BGC), ACCESS1.3, FIO-ESM and IPSL-CM5A-MR) under representative concentration pathways (RCPs) 4.5 and 8.5. The extent of occurrence (EOO) and habitat suitability (HS) were also determined. Stratified random semi-structured interviews with 182 heads of households were carried out to explore cultural and socio incentives of use, compared knowledge distribution, determine local management practices and explore local ecological knowledge related to the species in the three study areas. In addition, 17 focus group discussion were conducted to clarify issues raised during household interviews. One hundred and twenty four quadrants were used to determine the abundance and population structure. The Simpson index of dominance, permutation index and quotient between consecutive classes were used to assess population stability, and an eight point scale was used to evaluate the extent of bark damages. Genomic deoxyribonucleic acid (gDNA) (n=48) was used to assessed genetic diversity and population structure through the newly developed single sequence repeats (SSR) molecular markers.

Model selected was statistical significant for prediction of potential distribution of *W. salutaris* in southern Mozambique (partial ROC =0.00, omission rate = 0.04 and delta AICc = 0.00). The current environmental conditions mostly explained by climatic predictor, indicated in general, the potential distribution on areas of occurrence observed during the field work in southern Mozambique. Future project suggested an increase of climatic suitable areas in all the general circulation models in both conservative and pessimistic climatic scenarios (RCPs 4.5 and 8.5, respectively) in 2050 and 2070, and consequently the increase of HS and the EOO. Results of this work suggests *W. salutaris* would not extinct at least in the coming 50 years due to climate change but other threats such as overexploitation of the species need to be addressed. With increase of climatic suitable areas, conservation and management of this species could focus on reintroduction and cultivation for recovery of the species, with the help of time.

W. salutaris was used for medicinal purposes to treat 12 health problems, with the bark being the most commonly used part (92% of informants). Knowledge of the species uses differed significantly between the three study areas but not with respondent gender or age. Informants also mentioned a considerable number of alternative species of *W. salutaris* (37 species in LM, 33 species in TR and 17 species in FC). Local ecological knowledge on the ecological niche of the species also varied substantial in the three areas with both types of knowledge generally higher in the Tembe River area. Local management practises were revealed mostly on harvesting through vertical bark stripping cited by 100% of informants in TR, 61.6% FC and 51.7 LM (71% of all informants). Yet most (64.3% of all informants, corresponding to 98.4% in TR, 51.7% in Fc and 41.7% LM) used non-traditional harvesting tools (machete) bark removal. For sustainable use of the species, consideration should be given to combined conservation strategies such as the use of alternative species with the same application, substitution of bark by leaves, and increases in alternative sources of plant material through cultivation.

The densities of alive stems were not statistically different in the three areas but was higher in LM (mean of 4500.3±9209.8 stems/ha). Equally the mean densities of recruits (diameter ≤2 cm) were not considerable different but was higher in the FC (3296.8±9116.7 stems/ha). The regression analyses for the three study area revealed negative slopes, indicating more recruits and fewer individuals in larger classes. The Simpson indexes of dominance, permutation indexes and quotients between the consecutive size classes suggested some levels of population instability in the three areas. Harvesting took place in the eight point scale, all size classes were harvested but preference was higher on intermediate (15.1-20 cm) or larger (>20 cm) size classes in all the study areas but gatherers avoided harvesting bark from recruits. Although the mean densities of harvested stem was not significant this work indicated higher levels of stem destruction in FC. Harvested dead stems ware significant in the three areas and FC had the higher of (39.5±85.9 stems/ha) compared to 7.6±19.1 and 2.1±4.3 in TR and LM, respectively. Equally, the density of physiologically stressed stems differed substantially in the three areas with higher in FC 20.5± 75.5 stems/ha compared to 18.7±82.9 stems/ha in TR and 3.8±11.8 stems/ha in LM. This results suggests implementation of protection measures such as the inclusion of the species in the national CITES list to regulated cross-border trade and strengthening of the law enforcement.

Genetic diversity, structure and gene flow in the exiting populations of southern Mozambique revealed that despite fragmentation and overexploitation, this species maintains a relatively high level of genetic diversity supporting the existence of random mating. Two genetic groups were found corresponding to the northern (LM and TR) and southern (FC) areas. The study suggests that, if local extinctions occurred in Mozambique, the pepper-bark tree persisted in sufficient numbers to retain a large proportion of genetic diversity. Management plans should concentrate on maintaining this high level of genetic variability through both *in* and *ex-situ* conservation actions.

Key Words: Species distribution models, climate change, extent of occurrence, habitat suitability, folk medicine, local ecological knowledge, quantitative ethnobotany, threatened species, trade, bark damage, demography, genetic diversity, population structure

Declaration

I, **Annae Maria Senkoro**, hereby declare that the work contained in this thesis is my own original work and that all other sources used or quoted have been fully acknowledged and referenced. The work has not been submitted before for the award of any other degree at any other university.

Signature: Date:

Dedication

This thesis is dedicated to my husband and children for inspiration, support, sacrifice and incessant love,

To the Senkoro family for their encouragement, support and prayers

and

In the memory of my father Hakiel Mbwambo Senkoro who departed at the early stage of my formal education.

Acknowledgement

This project would have not been possible without the support and funding from different entities. In particular, I would like to express my profound appreciation to the Organization for Women in Science for the Developing World (OWSD), to the Swedish International Development Cooperation Agency (SIDA), the Russell E. Train Education for Nature Program (World Wildlife Fund) through Agreement # SS20 and the Foundation for Science and Technology (Fundação para a Ciência e a Tecnologia) through the research unit UID/AGR/04129/2013 (LEAF) for funding. My sincere gratitude is also extended to Rhodes University for covering my tuition expenses during the period of the OWSD fellowship. I am equally indebted to the Mohamed bin Zayed Species Conservation Fund for supporting the initial Project, "Conservation of the pepper-bark tree in Mozambique" through Project # 0925806 that lead to this PhD research project.

I wish to express my deepest gratitude to my supervisor Prof. Charlie Shackleton for his thoughtful guidance, advice, encouragement, motivation and patience, which have contributed greatly to the completion of this endeavour. Prof., thank you for your invaluable support in raising the funding to undertake this research work, for sharing your extensive knowledge, and for finding time for the field trip in southern Mozambique and for providing me with the autonomy to learn. I am forever thankful to my co-supervisor Dr Ana Ribeiro for the privilege of having worked with her for more than 10 years. Thank you for mentoring me, encouraging me to further my studies, for introducing to me and for training me in the field of molecular biology, for the fundraising efforts, patience and words of encouragement. I would also like to extend my deep appreciation to my co-supervisor Prof. Robert Voeks for encouraging me to pursue this line of study. It was a great privilege for me to work under your guidance in ethnobotany research in southern Mozambique. I really appreciate your support throughout this research project until its final stage.

Clearly, it would have not been possible to pursue this research in molecular biology without the support from experts and specialists. Therefore, my sincere gratitude also goes to Dr Fernanda Simões for her valuable contribution during the optimisation of the protocol for DNA extraction, training in bioinformatics and for sharing her laboratory. I am greatly indebted to Dr Isabel Marques for her immeasurable support in bioinformatics and for her suggestions, which has improved the content of the genetics chapter. I am deeply grateful to Dr Pedro Talhinhas for conducting flow cytometry data analysis, to Dr Paula Batista-Santos, Ms Ivete Maquia and Ms Ana Gomes for their support during the laboratory work. My appreciation is also extended to the staff at ISA-ULisboa, especially Dr José Romalho, for sharing space in the laboratory at *Quinta de Marquês*, Oeiras, and Ms Isabel Moura for sharing her knowledge in Biotechnology.

I would like to express my gratitude to Mr Firmino Guiliche for his support in refining the questionnaire and for organizing the data. I would like to thank Mr Hugo Mabilana for developing the map of the study area and for generating the preliminary distribution model, and Prof. John Carroll for devoting his invaluable time to generate the preliminary model. I am also indebted to Dr David Draper Munt for sharing his insights on modelling and conservation experience, and for generating the final distribution model.

Similarly, this work would have not been possible without the support from the communities in the study areas and colleagues. Therefore, I would like to extend my special appreciation to *Nkulo* Massale Tembe and *Régulo* Francisco Xavier Tembe, traditional leaders who facilitated this work with the local communities. I thank field guides/assistants namely: Mr António Singuiza, Mr António Tembe, Ms Cristina Manhisse, *Nkulo* David Mazia, Mr Ernesto Bíe, Mr Filimone Cossa, Mr Formoso Chembene, Mr Lionel Thusse, Mr Luís Cossa, Mr Mabendane Mafumo, Mr Muzamane Tovela, Mr Ricardo Mulhovo, Mr Jorge Chachuaio, Mr Jorge Novela, *Nkulo* Joseph Tembe, Mr Rafael Tchavela, Mr Sifiso Masuku, Mr Silvestre Melembe, Mr Viandro Barrosoa and Mr Zeferino Mabjaia. I would also like to express my gratitude to my colleagues Mr Armindo da Silva, Mr Aurélio Bechel, Mr Domingos Maguengue, Mr Ernesto Boane and Mr Jotamo Mazive, for their support during the exhaustive and extensive search for *W. salutaris* and during the interviews. I thank all the community members for reserving their precious time for interviews and for sharing their knowledge.

I would also like to reiterate my appreciation to the people in the Department of Environmental Science, especially Ms Kate Benyton, a former staff member of the department for logistics support who assisted with my integration at Rhodes University and Ms Kathy Cassidy for her support during my research visits. To my friends in DES especially Angelina Martins, Baa Ojong Onokwenwa, Buhle Francis, Chenai Murata, Deo Kujirakwinja, Esther

Ekua Amoako, Gisele Sinnason-Koupamba, Precillia Ngome-Tata and Soumya Veeranna, thank you for the time we shared in the course of this work. I would also like to thank my colleagues from the Department of Biological Science, University of Eduardo Mondlane, especially Prof. Salomão Bandeira, Prof. Cornélio Ntumi for sharing their vehicles. To Alice Massingue, Filomena Barbosa Valdares Mulungo and Joaquim Campira, thank you for your friendship, support and encouragement. Rita Caré, I will never forget your hospitality during my stay in Lisbon.

My special gratitude also goes to my family, notably my mother Mrs Elinida Mwivano Senkoro, for being a role model throughout my life. I sincerely thank my husband, Saide Dade, as if it were not for his support, I would have not made to this stage. Thank you for your unconditional love. To my daughter Sofianna and sons Saide Júnior and Yossuf for being such an inspiration and for their braveness during my absence. I am also grateful to my brothers Noel and Isack for all their support, and to my nephews and niece for all their love and encouragement. I also thank my *Baba* Serage Anfai for helping me with figures layouts and representation and my cousin Perpetua Armando for her encouragement and love. My heartfelt gratitude is indeed extended to all persons and entities that unwittingly I might have omitted and that made a contribution to this work. You are all profoundly appreciated.

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ACCESS	The Australian Community Climate and Earth System Simulator
FIO-ESM	The First Institute of Oceanography-Earth System Model
IPSL-CM5A-MR	Institut Pierre Simon Laplace Model CM5A-MR.
CGIAR	Consultative Group on International Agricultural Research
METI	The Ministry of Economy, Trade, and Industry of Japan
AIST	The National Institute of Advanced Industrial Science and Technology of Japan
CENACARTA	National Center for Catography and Remote Sensing
DNA	Deoxyribonucleic acid
IUCN	International Union for Conservation of Nature
SSC	The IUCN Species Survival Commission
ASTER	Advanced Spaceborne Thermal Emission and Reflection Radiometer
NASA	The National Aeronautics and Space Administration
ROC	Receiver operating characteristics
AICc	Akaike information criterion adjusted for small samples
Qth	Quadratic, threshold and hinge
BGC	Biogeochemical model
UV	Ultraviolet

Chapter One: Introduction

1.1 Introduction

The reliance of rural communities on wild sources of plants has been documented worldwide (e.g. Fandohan et al. 2010; Luckert et al. 2014; Silva et al. 2018; Specht et al. 2015). Plants offer a variety of benefits, including the provision of goods such as food (wild vegetables and fruits), beverages, fuelwood, construction material, weaving, domestic utensils and medicinal plants (cf. Ghorbani et al. 2012; Joos-Vandewalle et al. 2018; Shackleton and Shackleton 2004). Traditionally, goods derived from plants are used at the household level which allows for cash saving for other needs (cf. Shackleton and Shackleton 2004, Shackleton et al. 2007). For example, the mean annual consumption of fuelwood in western Arunachal Pradesh (India) is 3 720±1 447 kg per household providing an average cash saving of 8 705±819 Rs/year (Saha and Sundriyal 2012). Concurrently, commercialisation of plant products generates cash income for many rural people (Ghorbani et al. 2012; Shackleton et al. 2007; van Wilgen et al. 2013). Wild plants also serve as safety-nets, especially for poor households, during hard times (Dewees et al. 2010; Djoudi et al. 2015; Brobbey et al. 2019; Shackleton et al. 2007). For example, a highly traded *Bertholletia excelsa* Bonpl. (Brazil nut) acted as safety-net to the local communities in Bolivia, involving advance payment especially during the harvesting period (Zenteno et al. 2013). Wild plants also offer other indirect benefits such as ecosystem services, including water and disease regulation, carbon sequestration and nitrogen fixation (cf. Djoudi et al. 2015; Mittermeier et al. 2003; Ribeiro-Barros et al. 2018; Shackleton et al. 2007).

People in the developing world rely on medicinal plants, a long standing health care involved in treating a numerous ailments (Abdullahi 2011). For example, 80%-99% of the people in Benin, Ivory Coast, Congo Gabon, Mali Papua New Guinea, and Myanmar use traditional medicine for their basic health care (WHO 2019). Other countries such as Chad, Ethiopia, Ghana, Mozambique Uganda and Tanzania have slightly lower percentages (60%-79% of the people) (WHO 2019) but the overall trends still indicates high dependency on traditional medicine among the people in the developing world. Ingrained in traditional medicine is the use medicinal plants, recognised for its value to treat different ailments (cf. Issa et al. 2018; Panmei et al. 2019; Ribeiro et al. 2017; Yaseen et al. 2015) and potentially use for discovery of new drugs (Mbele et al. 2017). For example, 122 compounds found from 94 plant species are used as modern drugs and 80% of these were reported to have equal or associated ethnomedicinal applications (Fabricant and Farnsworth. 2001). A diversity of medicinal plants has been reported to play vital roles in the livelihoods and health of rural communities (c.f. Abdullahi 2011; Ghimirre 2008; Giday et al. 2003; Phumthum and Balslev 2019). For example, 245 ethnospecies are recorded for medicinal use in rural Brazil (Albuquerque et al. 2011). Bussmann et al. (2006) identified 39 medicinal species used to for treating different ailments with common uses for dental hygiene, malaria and wound treatment in Kenya. Some households are also involved on the trade of medicinal plants (cf. Cunningham and Mbenkum 1993; Huber et al. 2010; Ouarghidi et al. 2012) with tendencies of increasing trade of these species (cf. Cunningham 1993; Cunningham and Mbenkum 1993; Ghimirre 2008; Hamilton 2004; van Andel et al. 2015).

The effects of commercial harvesting of natural populations of medicinal species is well studied (cf. Botha et al. 2004; Baldauf et al. 2013; Delvaux et al. 2010; Ghorbani et al. 2012; Hilonga et al. 2019). A common concern with commercial harvesting is the possibility undermining the ecological sustainability of wild harvested species, the main source of traded material, by increasing harvest efforts to maximise economic returns (c.f. Botha et al. 2004; Hilonga et al. 2019 Maroyi 2013; Peck and Christy 2006). However, this is complex because responses to harvesting of different plant lifeforms and plants parts are incomparable. For instance, numerous trees species cannot resist bark, stem, and apical meristem harvesting, but can survive when reproductive structures (fruits and flowers) are harvested (Ticktin 2004). Equally, overharvesting of underground parts and whole plants for trade can substantially affect populations (Ghorbani et al. 2012; Hilonga et al. 2019; Subedi et al. 2013). Most commonly, overharvesting increases mortality rates and lowers sexual reproductive success (lower flower, fruit and seed production) (Lopez-Toledo et al. 2018; Silva et al. 2018). The short term impact of overharvesting is a gradual loss of individuals that will later reflect at the population level (Ghorbani et al. 2012) and in the long term it may lead to failure to persist and consequently local extinction (Keller and Waller 2002)

1.1.1 Description, distribution and importance of the species under study

Warburgia salutaris (G.Bertol.) Chiov., previously known as *Chibaca salutaris* Bertol.f. and *Warburgia breyeri* Pott (Verdcourt 1990), belongs to the Canellaceae (cinnamon family). Locally it is known as “chibaha” (Ronga and Tsonga) (de Koning 1993), “siBhaha” (Siswati)

(Hutchings et al. 1996), “isiBhaha” (Zulu) (van Wyk and Gericke 2000) and elsewhere in southern Africa as “muranga” (Shona) (Veeman et al. 2014b), “peperbasboom” (Afrikaans), and “pepper-bark tree” (English) (Coates-Palgrave 2002). The species is an evergreen tree, usually five to 10 meters tall, but may reach 20 meters. The outer bark is brown and rough and reddish on the inner side. The leaves are simple, lanceolate or elliptic assembling in a spiral at the end of branches, shiny green above and paler below. Flowers are white or green and the fruit is a green, globose berry that turns black with maturity (Coates-Palgrave 2002). A specific characteristic of *W. salutaris* is a strong peppery taste in all parts of the plant (van Wyk and Wink 2004).

The species was reported as native to eastern and southern Africa (van Wyk and Wink 2004), but recent research suggests the occurrence of *W. salutaris* in southern Africa, and two other species from the same genus, *W. ugandensis* Sprague and *W. stuhlmannii* Engl. in eastern Africa (Muchugi et al. 2008; Naidoo and Lamb 2006). It occurs in evergreen forest, wooded ravines and bushveld (Coates-Palgrave 2002). In South Africa, wild populations have been reported in Limpopo, Mpumalanga and KwaZulu-Natal provinces (Coates-Palgrave 2002). In Eswatini (formerly Swaziland), the species occurs in Malolotya (and possibly in Lomati Valley) (Dlamini and Dlamini 2002), but recently, Dludlu et al. (2017) reported 14 new areas of occurrence totaling 18 areas in this country. In Malawi, it occurs in the southern part of the country (Msekandiana and Mlangeni 2002) and in Zimbabwe, in Chipinge district (Mapaura and Timberlake 2002; Verdcourt, 1990). In Mozambique, it is confined to the southern part of the country, particularly in the south-east and south of Maputo province (Jansen and Mendes 1990) and a possible occurrence was reported in Chimanimani, Manica province (Veeman et al. 2014a). *W. salutaris* was also reported in Kenya (Bussmann et al. 2006), Zambia, Botswana, Lesotho, Namibia and Tanzania (Orwa et al. 2009).

W. salutaris is a popular species used for medicinal purposes due to its relatively low price compared to commercial pharmaceuticals, and because of its long-standing spiritual and symbolic significance (Botha et al. 2004). It is one of the most highly-valued medicinal plant species in southern Africa, exemplified by the species name, *salutaris*, meaning ‘healthy’. The inner bark (from the stems and the roots) has long been employed to treat several ailments. The medicinal potential of *W. salutaris* is attributed to different active ingredients found in the bark, including drimane sesquinterpenoids such as warburganal and polygodial, which are effective in treating bacterial and fungal infections (van Wyk and Wink 2004). In southern Africa, the species is mostly used to treat respiratory tract ailments including coughs, colds and chest complaints (Jansen and Mendes 1990; Maroyi 2014; van Wyk and Wink 2004). It is also used in the treatment of ailments such as rheumatism, headaches, toothache and influenza and gastric ulcers (van Wyk and Wink 2004). It has also been considered as a frontline herbal for treating malaria (Bussmann et al. 2006).

1.1.2 Markets and economic value of *W. salutaris* in southern Africa

High demand for certain species such as *W. salutaris* bark has encouraged its trade in traditional medicine markets throughout southern Africa. For example, in South Africa, the species was reported to be traded in Durban, KwaZulu Natal (Mander et al. 2006) and in Johannesburg (including in the Faraday Street traditional medicine markets), Gauteng (Williams et al 2014b). Correspondingly, *W. salutaris* is traded in urban Harare (Mbare, Machipisa and Highfield traditional markets), between Harare and Birchenough (Sukubva traditional market) and in Birchenough area in Zimbabwe (Veeman et al. 2014a). In Mozambique urban medicinal plant markets of Maputo especially in Xipamanine, Adelino (also known as Mazambane) and Xiquelene (Barbosa et al. 2020; Krog et al. 2006).

Due to scarcity of a high demanded *W. salutaris*, even small portions of the bark can demand relatively high prices in informal markets. For example, a decade ago the price was estimated between 17 to 44 Rands per kg and the consumption was estimated at 27 tons in KwaZulu-Natal and Gauteng (Mander et al. 2006). In Mozambique, a relatively small bundle of the bark costs approximately 0.5 USD and it was one of the most purchased species (Krog et al. 2006). Currently a small piece (approximately 15 cm) is sold at 1.62 USD in the same markets and it is still among the high priced and most traded species medicinal plants (Barbosa et al. 2020). In Zimbabwe, price of piece of bark was between 0.30 USD (11 Z$ at the exchange rate of 1 US$ to 38 Z$) and 1.4 USD (52 Z$) and varied from 25.20 USD (959 Z$) and 36.90 USD (1 404 Z$) per kg of bark (Veeman et al. 2014a). However, the traded material is was wild harvested with countries such as Mozambique and South Africa as illegal suppliers of the bark to Zimbabwe (Mukamuri and Kozanayi 2014; Veeman et al. 2014b) and Mozambique to South Africa (Krog et al. 2006; Mander et al. 2006). Urban and cross-boundary demand for the species are hence a significant threat, given that the activity becomes attractive to people because of i) high demand of the species; ii) widespread knowledge on the potential collection sites and iii) widespread of poverty and need for cash income (Krog et al. 2006).

Studies of the economic value of *W. salutaris* are lacking across the distributional range of the species. The only existing work was developed by Veeman et al. (2014b) in Zimbabwe that presumed 24 years of planning under cut and resprout harvesting system. It was anticipated that growing trees (from seedlings) would be harvested after 8, 12, 16, 20 and 24 years after growing and would yield 6 kg of bark at the first harvest and 4 kg in the subsequent four harvests after resprouting. Pricing was deduced for pessimistic medium and optimistic scenarios to be 5 Z$, 25 Z$ and 50 Z$, respectively for a kg of bark. Market for leave was presumed to initiate on the fourth year at 0.66 US$ per kg for healers and 0.23 US$ for farmer. With all the production of *W. salutaris* inputs (except part subsidised for seedlings), harvesting expenditures and cut-rate, at the pessimistic scenario yield per tree the amount benefited at the full yield at the first harvest (6 kg) and the subsequent years (4 kg) would be 17.9 US$ for healer and 7.6 US$ farmers. For the medium scenario benefits was estimated at 42.02 US$ and 24.2 US$ for healers and farmers, respectively and for the optimistic scenario 72.6 US$ for healers and 45 US$ for farmers. At half and quarter of the yield the profit will be slightly lower. Yet, *W. salutaris* production was seen as profitable and a way to improve household income of farmers in rural southeast Zimbabwe.

1.1.3 Conservation status and threats – the southern African context

W. salutaris has a limited distribution and a low density which makes it susceptible to anthropogenic pressures (Maroyi 2013). Scholars (e.g. Botha et al. 2004; Dludlu et al. 2017; Krog et al. 2006) have reported unsustainable harvest for commercialisation to be the major threat to wild populations. Throughout its distribution, wild populations, particularly larger individuals, have been heavily debarked and devastated for trade (Coates-Palgrave 2002). Due to the high demand for *W. salutaris*, it is increasingly threatened throughout most of its range. According to the IUCN Categories and Criteria (IUCN 2001), *W. salutaris* is listed as Endangered in Malawi (EN A1acd) (Msekandiana and Mlangeni 2002) and South Africa (EN A4acd) (Botha et al. 2004). It was thought to be Critically Endangered in Eswatini (CR A1bcd) (Dlamini and Dlamini 2002) and was recently proposed as Endangered (EN B2ab (iii, v)) (Dludlu et al. 2017). In Zimbabwe, it is Critically Endangered (CR A1d B1B2abcdeC1C2a D) (Mapaura and Timberlake 2002) and it is believed to be extinct in the wild (Maroyi 2013). In Mozambique, it is considered Vulnerable (VU A2cd) (Izidine and Bandeira 2002) and globally it is considered as Endangered (Hilton-Taylor 1998).

Although *W. salutaris* has remarkable resilience to bark removal, frequent debarking reduces growth rates and life span of individuals (Cunningham 2001). Trees affected by constant harvest become susceptible to fungal infection, resulting in increasing mortality. Similarly, individuals suffering from repeated fires are vulnerable to fungal infection and further present shrubby growth (Botha et al. 2004). Besides the mentioned threats, land clearing for agriculture and settlement (Halafo 1996) and infrastructure construction are also pressures on some *W. salutaris* populations (Botha et al. 2004). Natural recruitment is very low, aggravated by frequent fires that contribute to diminishing the potential of sapling recruitment (Botha et al. 2004). Furthermore, seeds are often affected by parasites, resulting in loss of viability (Hannweg et al. 2015), and recalcitrant, and thus unable to survive for long periods (Kioko et al. 2003).

1.1.4 Tree conservation assessment

Under the threats of climate change and intensive harvesting, the assessment of the diversity, distribution, and abundance of sought after species is a crucial stage in conservation planning to define priorities that mitigate against the extinction of vulnerable species (Tali et al. 2015). According to IUCN (2001), conservation assessment integrates data on the decrease of populations, estimations of geographic range, proportion of mature individuals and probabilities of species extinction. This approach allows allocation of species into appropriate Categories and Criteria and subsequently the interventions needed for any management plan (Tali et al. 2015). The assessment can be further complemented through the analysis of the extent of damage to species populations (Cunningham 2001) and market surveys through quantitative inventories (cf. van Andel et al. 2015; Williams et al. 2014a, 2014b).

Medicinal tree species in high demand with a limited distribution are priority species for conservation assessment (Krog et al. 2006). Perceptions of shortage of specific medicinal plants amongst market vendors and gatherers can be a starting point to the assessment of the status of such species (van Andel et al. 2015; van On et al. 2001; Williams et al. 2013). For example, van Andel et al. (2015) conducted a quantitative market inventory with a species distribution model (involving ground thruthing) and reclassified a tree species, *Okoubaka aubrevillei* (Pellegr. & Normand), as Vulnerable A3cd. In Eswatini, *W. salutaris* was reclassified from Critically Endangered (CR A1bcd) (Dlamini and Dlamini 2002) to Endangered B_2ab (iii,v) (Dludlu et al. 2017) after such a assessment. However, the underlying knowledge of the conservation status of specific species is often limited (Hilton-

Taylor 2000). On the African continent, the majority of medicinal plant studies have been centered on pharmacology and phytochemistry, with relatively little attention to their conservation status (Moyo et al. 2015; Maquia et al. 2019). Exceptions are found in countries such as South Africa, where red listing revealed that 0.4% (82 species out of 20 456) are threatened with extinction (Williams et al. 2013). In Mozambique, 300 plant species in the national flora were red listed (Izidine and Bandeira 2002). Yet, several authors have urged for the assessment of the conservation status and for the need to understand the population dynamics (e.g. Krog et al. 2006; Moyo et al. 2015; Williams et al. 2014a). This is because levels of exploitation are high and current quantitative data are lacking to update their status (IUCN 2015).

Another dimension in assessing the conservation status of a species is to determine the extent of damage or harvest intensity (Cunningham 2001). Several studies have demonstrated that the levels of bark harvest can affect the health of a plant and that the physiological response is directly proportional to the extent of debarking (Botha et al. 2004; Delvaux et al. 2010; Guedje et al. 2007; Stewart 2009). For example, high rates of bark stripping resulted in higher death rates of *Garcinia lucida* Vesque (Guedje et al. 2007). Also, Botha et al. (2004) reported that frequent bark harvest appeared to be one of the expounding factors of higher mortality rates of *W. salutaris.* Additionally, the pressure on individuals arising from overharvest can contribute to a loss of allelic diversity (Baldauf et al. 2013) or compromise sustainable harvesting quotas (Delvaux et al. 2010; Stewart 2009). Therefore, reflection on the incorporation of genetic diversity studies and research to ascertain potential harvestable quantities to establish sustainable harvesting quotas are important.

1.1.5 Strategies for the conservation of bark harvested trees

High demand for bark for commercial trade has caused pressures in the wild to many important medicinal species. This includes *W. salutaris*, which is thought to be extinct in Zimbabwe (Mapaura and Timberlake 2002; Maroyi 2012; Veeman et al. 2014b). Others bark species such as *Prunus africana* (Hook.f.) Kalkman (Cunningham 2014, Cunningham et al. 2016; Stewart 2009), *G. lucida* (Guedje et al. 2007) and *Himatanthus drasticus* (Mart.) Plumel (Baldauf et al. 2013) are under similar pressure. At the same time, rural people depend on these resources. Therefore, conservation strategies are essential to design and implement short-term and long-term ecological, social and economic sustainability with integration of community participation (Geldenhuys 2004; Hongmao et al. 2002).

Sustainable management of bark extraction can potentially be achieved through a variety of conservation strategies. Several studies have reported strategies such as: i) *ex situ* and *in situ* conservation approaches, ii) establishment of alternative sources of bark through propagation and cultivation, iii) replacement of bark with other aerial plant parts, iv) substitution of target species with others, v) sustainable harvesting techniques, and vi) adopting international and national conservation regulatory frameworks (e.g. Cunningham 1993; Geldennhuys 2004; Kioko et al. 2003; Kowalski and Staden 2001; Zschocke et al. 2000).

1.1.5.1 *In situ* and *ex situ* conservation methods

An *in situ* conservation approach can be effective for conservation of many threatened species. The organisational infrastructure of protected areas (PA) provides conditions aimed to support the conservation of species (including medicinal plants). To avoid human intervention, buffer zones are defined to alleviate pressure on protected areas (Cunningham 1993). However, conservation in protected areas may not be simple because people living adjacent to them are often poor and rely on local resources for livelihoods. For example, people living adjacent to Mikumi National Park (MNP) Tanzania depend on hunting of wildlife for diversification of their income, and the presence of MNP raises conflict. In this case, the *in situ* conservation approach is likely to be effective with the incorporation of development concerns to minimise the overall socio-economic and opportunity costs of the PA (Vedeld et al. 2012).

Community-based natural resources management (CBNRM) is another useful approach of *in situ* conservation in southern Africa (Phuthego and Chanda 2004; Shackleton et al. 2002). The main assumption behind CBNRM is the shift from state or uncontrolled to community control over natural resources (Twyman 2000). However, strategies in this approach are often based on governmental or external interests and imposition with insufficient integration of local community knowledge and needs (Shackleton et al. 2002). For example, in Botswana, communities of the western Kalahari were against participation in CBNRM projects due to unsuccessful past experiences (unsustainable wildlife hunting) and fearing that new initiatives, apart from not bringing concrete benefits, would deplete the resources needed for their livelihoods (Twyman 2000). On the other hand, some CBNRM programs [e.g. Communal Areas Management for Indigenous Resources (CAMPFIRE) Zimbabwe] had effective community participation (Frost and Bond 2008; Murombedzi 1999), yet there was some disparity in income distribution between the communities and the district government

(Shackleton et al. 2002). Although CBNRM rarely deals specifically with medicinal trees, this approach may underpin the management of such species. For the success of CBNRM, transparency and community involvement are necessary from the beginning of the project, particularly on the definition of the objectives and priorities (Dyer et al. 2014; Twyman 2000).

Apart from *in situ* conservation, *ex situ* methods can be applied to conserve species, including overused medicinal species. The classic *ex situ* conservation methods are seed banking, botanical gardens (Cunningham 1993), tissue culture and cryopreservation techniques **(**Benson 1999**).** The Millennium Seed Bank Partnership is an example of global collaboration in the *ex situ* conservation of threatened species (Kew 2015). Heavily debarked medicinal plants, such as *W. salutaris* and *P. africana*, are likely to be included in this program.

Cryopreservation of plants is an important approach for long-term *ex situ* conservation of rare and threatened plant species. Plant materials such as nodal explants (Gonzáles-Benito et al. 1997), shoot tips (Sen-Rong and Ming-Hua 2009; Wilkinson et al. 2003) and seeds (Berjak and Pammenter 2008) are cryopreserved. This offers the opportunity to conserve stock plants to assist propagation programs (Kioko et al. 2003). Cryopreserved *W. salutaris* seeds from immature fruits were found to be among the alternatives of conserving material for future propagation programs (Kioko et al. 2003). Furthermore, shoot tips of *Cosmos atrosanguineus* (Hook.) Voss, another species of concern, produced plantlets with the same genotype as the initially preserved material (Wilkinson et al. 2003). In addition, *Emmenopterys henryi* Oliv. preserved shoots showed significant regeneration rates (75-85%) through *in vitro* propagation (Sen-Rong and Ming-Hua 2009). However, recalcitrant and orthodox seeds need further attention due to their nature, as desiccation induces death in recalcitrant seeds and loss of viability of orthodox seeds (Berjak and Pammenter 2008; Kranner et al. 2006).

1.1.5.2 Establishment of alternative sources of bark through propagation and cultivation

Identifying alternative sources of bark is crucial to avoid the mismatch between market demands and resource availability. Suggestions have been mooted to adopt massive production of *W. salutaris* through propagation and cultivation (Botha et al. 2004; Cunningham 1993; Geldennhuys 2004; Zschocke et al. 2000) and subsequently trade at competitive prices compared to bark harvested in the wild. However, the effectiveness of this strategy is likely to be constrained by the slow growth rates to attain sizes needed for bark harvesting (Cunningham 1993; Geldenhuys, 2004). Even with community adhesion to any reintroduction programs, the strategy does not offer immediate solutions and the opportunity cost to conserve the bark in the wild is high. For example, Cameroonian farmers adopted growing *P. africana* on their farms but it actually takes time for trees to attain harvestable size and some gatherers may still have strong ties to their collection locations (Franzel et al. 2014). Equally, cultivation of *W. salutaris* in Zimbabwe appeared to have the same issue as most of the traded material was acquired from South Africa and Mozambique (Veeman et al. 2014b; Mukamuri and Kozanayi 2014). However, propagation and cultivation of *W. salutaris* around Kruger National Park is likely to provide an alternative bark supply given that there is i) sufficient supply of the saplings to harvesters, ii) promotion of homestead cultivation of surrounding communities, and iii) massive propagation of the species (Hannweg et al. 2015). Thus, replication of this model in different countries could improve the conservation status of *W. salutaris* in the future.

1.1.5.3 Substitution with aerial parts

Replacement of bark with aerial parts is a possible strategy for the conservation of some threatened medicinal plants. The substitution of the parts traditionally used for medicinal purposes by others less sensitive could be an effective conservation strategy (Drewes et al. 2001; Geldennhuys 2004; Zschocke et al. 2000). For example, bark of some species, such as *W. salutaris*, could be substituted by leaves to ease the pressure on the species in the wild the active ingredients are present in both parts (Drewes et al. 2001; Zschocke et al. 2000). If users prefer the bark, interventions with traditional medical practitioners (TMP) could be implemented to promote the use of alternative parts. Although it may not be conventional to traditional users, changes can be engendered with education and awareness programmes (Geldennhuys 2004; Zschocke et al. 2000). It is therefore necessary to invest in phytochemical studies of aerial parts of bark species to examine active ingredients, in collaboration with TMP (Zschocke et al. 2000).

1.1.5.4 Sustainable harvesting techniques

Management of supply can be also optimised through suitable harvesting techniques, especially if species protection is not sufficient to guarantee survival in the wild (Geldenhuys, 2004). This could be done with the inclusion of prospective gatherers and users to adopt different methods that minimise impact to individual plants and populations (Botha et al.

2004; Geldenhuys 2004). In particular, for bark harvested species, harvest rates patterns and impacts should be scrutinised to determine sustainable methods (Delvaux et al. 2010; Gaoue and Ticktin 2007; Ticktin 2015). Strong community cohesion can also support the conservation of resources. For example, the strategy of establishing specific collection zones for bark and limitation of collectors has contributed substantially to reduce harvest pressure on *Rytigynia* spp. in Uganda (Kamatenesi et al. 2014). Furthermore, non-destructive harvesting approaches can optimise bark regeneration via vertical stripping (Pandey 2015). Indeed, the main concern regarding debarking of medicinal trees is the magnitude of stripping (Botha et al. 2004; Delvaux et al. 2010; Geldenhuys 2004). According to Delvaux et al. (2010), ring barking kills most trees and the likelihood of tree recovery decreases with the increasing extent of debarking, highlighting the importance of studies on recovery rates for sustainable harvesting quotas (Delvaux et al. 2010).

1.1.5.5 International and national regulatory frameworks

Regulatory frameworks are among the main tools for conservation of threatened plants (Scott et al. 2005; UN 1992). The most challenging issue in the conservation of bark harvested trees is the demand to supply growing human populations (both in rural and urban areas), driving illegal cross-border trade (Krog et al. 2006) and exports (formal trade) to other countries (Cunningham, 2014a; Stewart, 2009). For example, *W. salutaris* is traded in the three big medicinal plant markets of Maputo (Xipamanine, Xikelene and Adelino) and is further exported illegally to South Africa (Krog et al. 2006) and Zimbabwe (Maroyi 2012; Mukamuri and Kozanayi 2014; Veeman et al. 2014b). The species has been listed in the National Plant Red Data List (Izidine and Bandeira 2002) and is protected under the Environmental Law (GM 1997).Yet, these measures have failed to guarantee the survival of the species, particularly due to limitations in law enforcement (Senkoro et al. 2014; Stewart 2009). However, it remains a potential instrument to regulate traded species. For example, *P. africana*, another exported medicinal bark species, was listed in Appendix II, under the Convention on International Trade in Endangered Species of Wild Fauna and Flora as a management strategy. In response to this, the European community suspended imports from Cameroon where it was highly overexploited, until it was proved that exports would not compromise the viability of the species (Cunningham 2014). Therefore, listing of traded bark species can contribute to protection of potentially endangered species.

1.1.5.6 Local ecological knowledge and conservation

Local ecological knowledge (LEK) is the knowledge, practices, and beliefs related to interactions between people and ecological systems, accumulated, tested, adapted and transmitted orally (Brook and McLachlan 2008; Charnley et al. 2007). It is influenced by demographic variation such as ethnicity, age, gender, origin, economic conditions, and proximity to metropolitan areas (e.g. Souto and Ticktin 2012; Quinlan and Quinlan 2007). Yet, through the interaction with nature, people learn the insight for management of natural resources, in particular sustainable harvest practices (La Rochelle and Berkes 2003; McCater and Gavin 2014).

Several studies have reported that LEK has contributed substantially to the conservation of economically important species (La Rochelle and Berkes 2003; Schmidt and Ticktin 2013; Terer et al. 2012). For example, the limited time to harvest *Syngonanthus nitens* Ruhland (golden grass) promotes the sustainable harvesting of stalks, underpinned by the knowledge of the uprooting time and inappropriate harvesting period, but it does not limit quantities harvested in most areas. Harvesting during the uprooting time results in population reduction and has been observed among the new gatherers who lack LEK (Schmidt and Ticktin 2013). Another example is the collection of *Cyperus papyrus* L. (papyrus). Knowledgeable, older gatherers refrain from constant harvesting at the same site to allow the recovery of the species, contrary to the younger harvesters (Terer et al. 2012).

1.1.6 Important conservation interventions for *W. salutaris*

In response to the pressures referred above and given the limitations of seed propagation, *in vitro* techniques (*in vitro* culture and cryopreservation) may be a crucial option for conservation and management of *W. salutaris*, as is the case with some other rare and threatened species, such as *Primula scotica* Hook. (Benson 1999), *Aloe polyphylla* Schönland ex Pillans (Abrie and van Staden 2001) and *Cosmos atrosanguneus* (Hook.) Voss (Wilkinson et al. 2003). *In vitro* culture, in particular, is normally applied to enhance the number of individuals in the wild or provide alternative sources of plant material to avoid pressure in the wild. It is also an option for multiplying species that are hard to propagate, when conventional methods are difficult to apply (Pence 1999). The technique encompasses four stages, establishment (production of aseptic cultures and provision of *in vitro* conditions), multiplication (shoot formation), root formation that can be done *in vitro* or *ex vitro* and acclimatisation. The first and last stages are decisive for the success of *in vitro* operation because explants and plantlets have to adapt and survive extreme conditions, *in vitro* environment and *ex vitro* conditions, respectively (Hartmann et al. 1997). Other limitations include production of harmful compounds (e.g. polyphenols) that may kill explants in culture, high levels of contamination and the optimisation of the growing conditions which are usually species-specific (Lynch 1999; Pence 1999).

Kowalski and van Staden (2001) developed a tentative *in vitro* establishment protocol for *W. salutaris* explants. The main challenge was the exudation of a phenolic compound. The existing initiatives in Kruger National Park, on the other hand, managed to produce considerable numbers of *W. salutaris* to promote cultivation in homesteads as future sources of harvest material. Equally, development of a relatively affordable propagation kit for a wide range of stakeholders (including the local communities) was more recently accomplished (Hannweg et al. 2015).

Another approach to inform the design of conservation strategies is the assessment of genetic diversity (e.g. Bentley et al. 2015; Gepts 2006; Harris 1999; Noroozisharaf et al. 2015). Genetic variations and expression of living organisms are essential parts of natural evolution because they determine different levels of tolerance and adaptation of organisms to their environment. Through the application of DNA markers, the assessment of genetic diversity may be conducted at a community, species or gene scale (Harris 1999). Molecular marker (MM) studies have made significant contributions to i) the understanding of genetic diversity, taxonomy and phylogeny, ii) identification of ecogeographic races within domesticated or wild gene pools of plant species, iii) gene bank management (assessment of the level of redundancy within and between collections, assembly of core collections, assessment of the genetic integrity of the accessions during conservation) and, iv) Marker-Assisted Selection (MAS) for plant breeding (Edwards and McCouch 2007; Lanteri and Barcaccia 2006). Amongst others, MM-based techniques differ in terms of costs, technical requisites, execution time and difficulty, number of detectable polymorphisms and reproducibility (Edwards and McCouch 2007). To date there are no reports on such studies on *W. salutaris*.

1.2 Problem statement and justification

The current knowledge on *W. salutaris* in southern Africa is based on ethnobotanical surveys, including uses of the species (cf. Jansen and Mendes 1990; van Wyk and Wink 2004; Maroyi 2013, 2014), phytochemical properties and bioassay (e.g. Samie and Mashau 2013). A few studies have focused on the ecology, such as the demography and harvesting impact in South Africa (Botha et al. 2004), and on updating the distribution and the conservation status of the species in Eswatini (Dludlu et al. 2017). Others were based on the genetics of the species stressing the taxonomic classification of the *Warburgia* genus (cf. Muchugi et al. 2008; Naidoo and Lamb 2006). Krog et al. (2006) and Mander et al. (2006) studied the informal trade of *W. salutaris* in Mozambique and South Africa, respectively. Mukamuri and Kozanayi (2014) looked at institutional arrangements for management of important bark trees, including *W. salutaris* in Zimbabwe. Research on the economic value of *W. salutaris* was done in Zimbabwe (Veeman et al. 2014a, 2014b). The first considered markets for bark products and the second presented preliminary results of a cultivation project aimed to reintroduce *W. salutaris*. Further studies on the species included determination of bark available through a market survey (Williams et al. 2014a, 2014b) and propagation (cf. Kowalski and van Staden 2001; Hannweng et al. 2015, 2016). In the above context, the present work focuses on the conservation of *W. salutaris* in Mozambique. It employs an integrated, interdisciplinary approach to understand the dynamics and risks associated of the species, to inform prospective conservation actions and approaches.

Knowledge on the conservation status of a species is important to determine because it gears priority settings for conservation intervention and guide research, depending on the state of knowledge of a taxon (Marrero-Gomez et al. (2003). At the same time, species with a confined distribution may be associated with limited stocks and are vulnerable to overharvesting when demand is high (Cunningham 1993; Moyo et al. 2015; Veeman et al. 2014b). The conservation of such species requires multidisciplinary assessments. Biotechnology, through molecular biology, underpins characterisation of genetic diversity and assists *in situ* germoplasm collection and tissue culture technology allows the micropropagation of a species. Thus, integrating biotechnology into conservation programs is potentially a useful instrument in assisting plant conservation (Benson 1999). Furthermore, the inclusion of socio-economic, cultural and political dimensions into a management plan is essential because they influence resource exhaustion or augmentation. A good management plan will, therefore, require the scrutiny of these dimensions.

Ecological approaches such as the species distribution models (SDMs) (cf. Hipólito et al. 2015; Qin et al. 2017) allow determination of the distributional range of a species and reveal potential unidentified distributional areas (Hipolito et al. 2015). In particular, information provided on habitat suitability reveals potential areas of occurrence where the species may exist but not yet discovered, especially those with limited distribution, and provide suitable areas for cultivation and reintroduction that are important for conservation and management (Qin et al. 2017). Under climate change, trends in suitable habitat can affect a species distributional range leading to declines (cf. Pramanik et al. 2018; Remya et al. 2015; Sony et al. 2018; Yi et al. 2016), increases in range (Qin et al. 2020; Zhang et al. 2019) or shifts (Sen et al. 2016). Such information will support the development of suitable climate change adaptation strategies (Bobrowski et al. 2017). Further ecological assessments include demographic inventories of a species through the application of quantitative methods such as size class profiles to measure population vigour (Shackleton et al. 2005) and the assessment of harvesting intensity to appraise the extent of damage (Cunningham 2001).

1.3 Aims of the study

The aim of the study was to determine the distribution, demand, population structure and genetic diversity of *W. salutaris* in southern Mozambique to promote conservation measures for the rational use of the existing germoplasm. Specifically, the study aimed to (i) model the current distribution of *W. salutaris* as well as potential future distribution under climate change, (ii) explore the cultural and socio-economic factors at the household level that influence exploitation and sustainable management, (iii) determine the population structure and ascertain the extent of bark harvesting, (iv) assess the genetic diversity and structure through the application of microsatellite markers.

To respond to the conservation of threatened *W. salutaris* a sequence of research questions was addressed:

1. Which areas are currently potentially suitable for *W. salutaris* and how might they change with climate change? (Chapter 2)
2. Are the populations under decline in southern Mozambique? (Chapters 3 and 4)
3. How is the genetic diversity distributed within and among individuals across the geographic areas? (Chapter 5)
4. Is the genetic structure associated with the geographical distribution? (Chapter 5)

5. Is there any evidence of inbreeding or lack of gene flow between populations? (Chapter 5)
6. Which conservation strategies have more probability to secure the existence of *W. salutaris* in southern Mozambique? (Chapters 2-6).

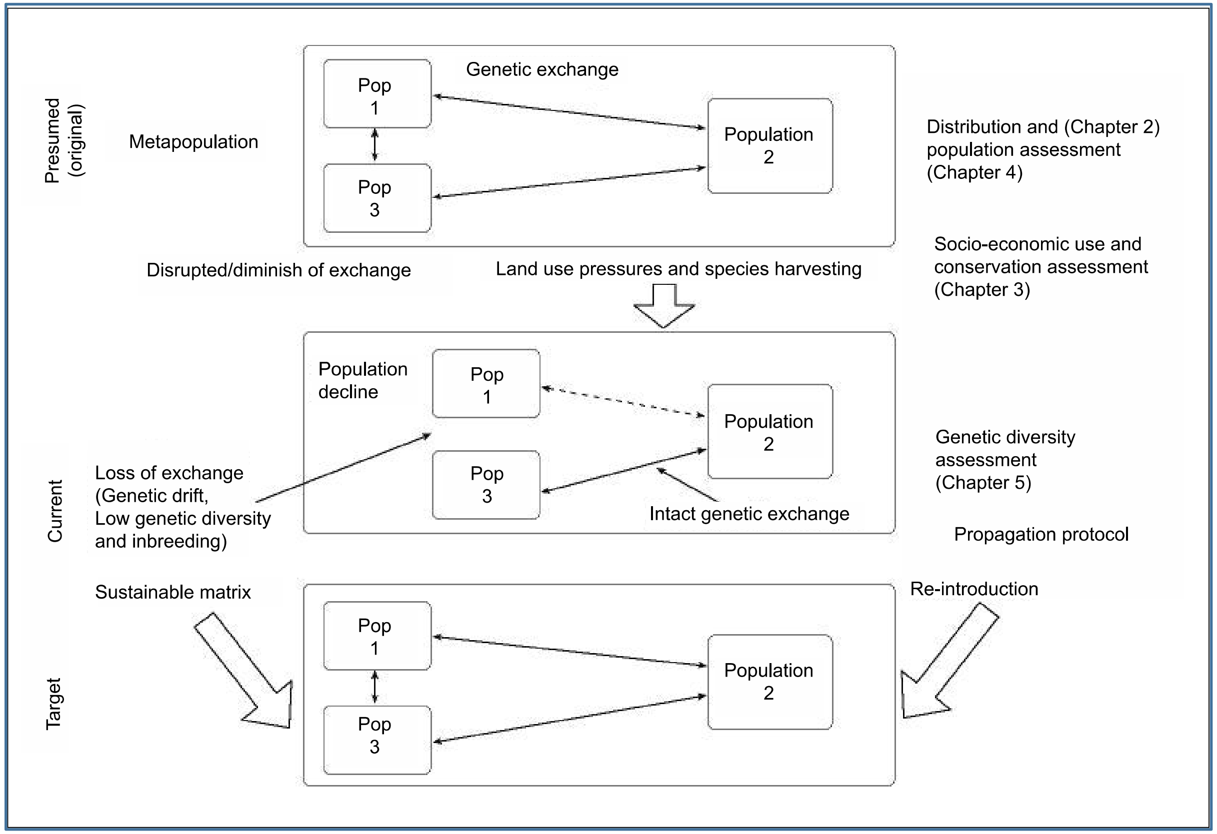
1.4 Conceptual and theoretical framework

The present study employed the metapopulation theory (MT) for analysis of the conservation status of *W. salutaris* in southern Mozambique. By definition, the concept considers spatially delimited populations that are linked by migration or gene flow (i.e., populations that are spatially separated but still interact), accounting for metapopulation persistence (Hanski and Gaggiotti 2004). It has been considered to have strength in (Hanski 2005):

i) dealing with reintroduction by adopting a range of strategies (e.g. frequency of reintroduction, numbers to be reintroduced, source of reintroduction) that maximised the overall metapolulation viability,
ii) addressing spatial dynamics (survival in landscape) through existing models capable of foreseeing the species dynamic in a particular landscape, that is, extinction (area and isolation) and colonisation context in a highly fragmented landscape,
iii) the patch area – isolation context has been helpful for studying of individual behaviour and movements and community structure. This provides knowledge on important aspects that can be integrated in conservation plans (e.g. migration rates, spatial variation rates, reproduction and mortality), and
iv) benefiting the management of habitat quality, habitat number, and connectivity to increase metapopulation persistence.

The weakness of MT is found in its focus on a single species rather than plant communities as a whole, resulting in limited knowledge and on the dynamics of other species (Hanski 2005). It is difficult to ascertain colonisation from recurrence of plants because seeds may have remained dormant in the site. Furthermore, plants are sessile but can move as pollen and seed. Their movement to other sites is, however, conditioned by other living organisms or environmental factors. It is, therefore, difficult to assess the provenience of seed and pollen and consequently understand genetic exchange and migration. Equally, the theory does not realistically report the drivers of recruitment of a species, i.e., whether they derive from dormant seed from *in situ* seed banks or brought by dispersal vectors making it difficult to affirm the origin of recruitment (Ouborg and Ericksson 2004).

Following similar studies (e.g. Bonnin et al. 2002; Hanski and Gaggiotti 2004; Wilcock and Neil and 2002) this work assumed that i) *W. salutaris* is a naturally fragmented species and has isolated populations, ii) small isolated populations may still be connected and allow genetic exchange and iii) others are far too isolated to allow gene flow through restriction of pollen reception, loads (pollination) and seed dispersal because most of pollinators and dispersal vectors have restricted ability to travel long distances. The framework developed, in this context (Fig. 1.1), shows four interventions required to ascertain the current status of a species by: i) mapping the potential geographic distribution of the species, ii) exploring cultural and social factors at the household level that influence exploitation and sustainable management techniques, iii) assessing the population structure and conservation status, and iv) understanding patterns of genetic diversity, structure and gene flow. These were examined in each of the three, spatial separated areas in southern Mozambique where *W. salutaris* was known to occur. A metapopulation approach was deemed useful. The three areas were 1) the Lebombo Mountains, ii) the Tembe River and iii) the Futi Corridor (see Section 1.11).



Pop 1- Lebombo Mountauns

Population 2 - Tembe River

Pop 3 – Futi Corridor

Figure 1.1. A metapopulation conceptual framework for the conservation of *Warburgia salutaris* in the three study area of southern Mozambique.

The potential geographic distribution of *W. salutaris* considered a habitat suitability framework which is important in metapopulation studies due to its capability to discriminate suitable and unsuitable habitats in the patch networks (Thomas and Hanski 2004). Habitat suitability models (HSM), simulate or link environmental predictors with the probability of species occurrence, thus predicting spatial occurrences while distinguishing environmental conditions associated with the species (Hirzel and Lay 2008; Rushton et al. 2004). Through the use of presence and absence data, HSMs have prompted studies of niche characteristics and have been considered as the functional or modern application of ecological niche (Hirzel and Lay 2008).

The niche theory in ecological studies (e.g. Chase and Myers 2011; Hirzel and Lay 2008; Pocheville 2015; Vandermeer 1972) emphasises i) the distributional area where a species can be detained by the basic and natural limitations that are subjected to gradual changes with time (the Grinnell niche) and ii) environmental conditions associated with the species and its link with “food” and “enemies” (Elton niche). In the niche concept, the first approach focused on potential areas of species occurrence without considering interspecific interactions, while the second considers the place of a species in the community. Later, Hutchinson (1957) established the idea of n-dimensional hypervolume, also known as the fundamental niche as all sites where environmental conditions would allow the occurrence of a species and realized niche, as the actual area occupied by the species. In the present work, the Grinnell niche definition was considered, because it is conceptually possible to rebuild a realised Grinnellian niche of a particular species using environmental data from areas of occurrence (Hirzel and Lay 2008).

The significance of HSM is found on their ability to extract corresponding environmental conditions for the species from occurrence data and ascertain the important environmental conditions for species (cf. Bobrowski et al. 2017; Freeman et al. 2019; Zhang et al. 2019). These data can subsequently generate a model with potential areas of occurrence unveiling different categories of suitable habitats (from less to very highly suitable areas), uncovering potential habitats for re-introduction initiatives (cf. Adhikari et al. 2012). The model can also uncovers unknown areas occupied by the species (cf. Hipólito et al. 2015), unrevealed areas during sampling and of this confined species that may have been left out during sampling and appropriate areas for introduction and cultivation of the species (cf. Qin et al. 2017).

The metapopulation concept also considers birth and death rates (equivalent to colonisation and extinction) and population growth bound with dispersal (Ticktin 2005; Valverde and Silvertown 1997). Considering the threatened status of *W. salutaris* (Botha et al. 2004; Dludlu et al. 2017; Izidine and Bandeira 2002; Mapaura and Timberlake 2002; Msekandiana and Mlangeni 2002) the present work also employed the framework to explore cultural and social factors that influence sustainable management of *W. salutaris*. This included cultural and social drivers of use, local management practices and LEK (e.g. knowledge on population trends) and existing threats to the species and its habitat.

Effective conservation and management of a species requires information on genetic diversity and population genetic structure (cf. Baldauf et al. 2013; Bossuyt 2007; Li et al. 2018). Genetic studies assess whether there is a lack of gene flow that may drive genetic differentiation between the metapopulations resulting in low genetic diversity (apart from random loss of genetic diversity due to changes in frequency of alleles, genetic drift). This is more accentuated in intra-population crossing, particularly in small and isolated populations (inter-population crossing results in more offspring vigour) (Bossuty 2007). The more isolated populations will be more susceptible to genetic drift and inbreeding depression. The later can result from self-pollination and pollination from closely related individuals diminishing heterozygosis within a metapopulation, causing accumulation of homozygosis and recessive genetic load (Bossuty 2007; Halsey et al. 2015; Keller and Waller 2002). This can be expressed as failure to seed set (presumably because of an increase in pollen incompatibility) (Kolb 2005), lack of seed germination, failure to persist and respond to stress. The relevance of this theory for the present work is to evaluate genetic diversity and structure, and population differentiations to ascertain the extent in which harvesting has affected populations of *W. salutaris* in the three study areas. As the continuous decrease in genetic diversity is a potential contribution to reduction of growth rates and increases extinction probabilities (Keller and Waller 2002).

From this work, it is expected that metapopulation persistence can be enhanced through re-connection of isolated fragments (Menz et al. 2011). This can be done through or promotion of a sustainable matrix across the landscape, by integrating environmental friendly agricultural practices (such as agroforestry and organic farming systems). The matrices produced may accommodate increased agricultural production and biodiversity conservation, along with the inter-patching transmissions of animals such as pollinators and dispersal vectors (Perfecto and Vandermeer 2008). Depending on the levels of isolation, considerations on offsetting of pollen from outside of the metapopulation through artificial pollination (genetic rescue) can be taken into account to enhance fruit set and quality. In this case, offspring fitness should be taken into account to avoid out-breeding depression (reducing of offspring vigor) (Bossuyt 2007). Further action to remediate any decreases of genetic diversity is the reintroduction (Halsey et al. 2015) underpinned by effective propagation techniques (Pence 1999) and restoration of pollinators using fast growing plant species (Menz et al. 2011). A well designed reintroduction program can enhance metapopulation viability, increase fecundity and projected span of its persistence. These can gradually contribute to successful reintroduction and restoration of a metapopulation dynamic (Halsey et al. 2015).

1.4.1 Population assessment through size class profiles

Habitat suitability models are the input in metapopulation studies that are coupled with metapopulation models for the study of population viability (Convertino et al. 2011). However, due to lack of long-term data the present work considered size class profiles for the study of population structure. Size class profile studies have been employed to assess population status with the assumption that it provides clues to size-related mortality (Cunningham 2001). Theoretically, higher population changes, results in a more steeply declining size distribution (Condit et al. 1998). Thus, the presence of more juveniles compared to adults in a population generally shows a healthy or a growing population, provided it is not truncated (Shackleton 1993), whereas fewer juveniles can be an indication of a declining population (Condit et al. 1998; Lykke et al. 1998). The most common is the steeper size distribution (e.g. Botha et al. 2004; Guedje et al. 2007; Martins and Shackleton 2017; Schumann et al. 2010; Shackleton et al. 2005; Ribeiro and Fernandes 2000) where more individuals are from smaller size classes, exhibiting an inverse J-shape curve (Fig. 1.2). In some cases, however, inverse J-shape curve represents a truncated form due to larger size classes having been lost, resulting in almost all individuals being in smaller classes (Shackleton 1993; Shackleton et al. 2005). Size distributions can also exhibit a flat (unimodal) or a belly curve (cf. Condit et al. 1998; Venter and Witkowski 2010) indicating few recruits due to sporadic reproduction (Cuningham 2001). Size class distributions can also be inferred by the slope obtained through regression analysis (e.g. Condit et al. 1998; Lykke et al. 1998; Obiri et al. 2002; Martins and Shackleton 2017; Venter and Witkowski 2010). Negative slopes suggests more recruits than adults, while a flat slope (slope = 0) presumes the same proportion of recruits and adults and positive slopes, a lack of recruits (Condit et al. 1998; Lykke et al. 1998; Obiri et al. 2002; Shackleton 1993).

Size class profiles can indicate different degrees of sensitivity of a species to harvesting. Species with a flat curve are more susceptible to overharvesting of recruits than those with a reverse J-shaped curve given the lack of recruits. Harvesting of some individuals from small classes will compromise regeneration of species with a reverse J-shaped curve because not all will transit to the subsequent classes (Cunningham 2001). Size class distributions are a partial solution for short-term studies for population assessment where long-term data is lacking to evaluate population trends. Yet, this approach provides basis for comparison of population trends for the monitoring process and conservation of the species (Cousins et al. 2013) and can be considered for the present study. The limitation is based on the exclusion of other demographic factors, including growth and survival rates that affect the size class distribution. Hence, population stability cannot be assessed through size class distributions alone (Condit et al. 1998). Cunningham (2001) pointed out that short-term studies should be treated with caution irrespective to the method employed.

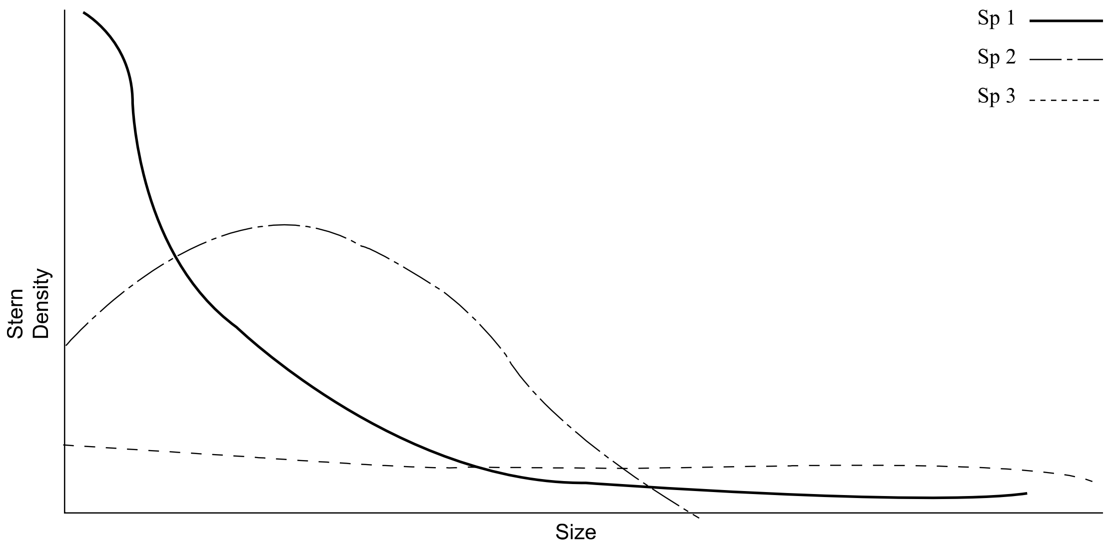


Figure 1.2. Population structure according to size class distribution. Sp 1 indicates a reverse J-shape curve with more recruits and fewer mature stems, Sp 2 a belly curve, Sp 3 a flat curve with low numbers of recruits due sporadic reproductive strategies (Cunningham 2001).

1.5 Structure of the thesis

The present thesis is composed of six chapters. One introductory chapter (Chapter 1) and four empirical research chapters (Chapters 2-5) prepared as individual papers. The first chapter outlines the importance of wild medicinal species and threats associated with their use. It also provide an overview of the importance of species assessment for conservation, and conservation strategies, with an emphasis on bark tree species. A description of the species under study, its distribution, ecological information, importance and threats are presented. The rationale behind and aims of this study are also provided in this chapter.

Chapter 2 is based on the application of ecological niche modelling to characterise the current and predict the future (2050 and 2070) distribution of *W. salutaris* in southern Mozambique under two pathways, the representative concentration 45 and 85 (2050 Rcp45 and 2070 Rcp45 and 2050 Rcp85, 2070 Rcp85). The distributional areas of *W. salutaris* were determined and trends of increasing or shrinking of suitable areas in the future are also exhibited, and the optimal habitat for the species are presented. The extent of occurrence is also calculated in the current and future distribution for *W. salutaris* to ascertain the conservation status of the species.

Chapter 3 identifies cultural and social drivers of uses, knowledge distribution, and determines local management practices for the species. It explores local ecological knowledge, and hence, perceptions on existing factors at the household level that determine uses and conservation of *W. salutaris.* For the sustainable uses of *W. salutaris* multiple conservation measures are suggested.

Chapter 4 presents a demographic study of *W. salutaris*. It employs different population parameters and size class profile for the scrutiny of the population structure. The chapter presents the application of different indices and evaluates population stability. It looks at bark harvesting impact through evaluation of the extent of harvesting. The chapter also establishes a baseline study for comparison with future research and monitoring of the species.

Chapter 5 presents the first Simple Sequence Repeats (SSR) markers developed for Canellaceae (cinnamon family), specifically for *W. salutaris*. It also determines the genetic diversity within the study areas, similarities and population differentiations between areas and population structure.

Chapter 6 is the synthesis and integration of the research from Chapters 2-5. It provides conclusions and recommendations for conservation and management of *W. salutaris.*

The empirical chapters (2-5) are written in the style of stand-alone papers for potential publication. Indeed chapter 3 has been published in *Economic Botany* and Chapter 5, in *Scientific Reports*. As typical in the natural sciences, there are other co-authors on the two published papers, including my academic supervisors. Whilst they are co-authors, my contribution to both papers was primary. I led the conception of the project, co-designed the methods, collected data, led the analyses and wrote first draft of the papers on which co-authors commented.

1.6 Study area

Using a metapopulation framing, the study was carried out in Maputo province, in Matutuine and Namaacha districts in three study areas: i) the Lebombo Mountains (LM) or western area, ii) the Tembe River (TR) or center and iii) the Futi Corridor (FC) or eastern area. The definition of the study areas followed the distributional data of *W. salutaris* found in herbarium vouchers of the National Herbarium (LMA) and the Herbarium of Eduardo Mondlane University (LMU), unpublished theses (e.g. Emanuelsson 2005; Halafo 1996; Sabíno 2007) and information from para-botanists, ecologists and collectors. These were finally compared with the existing published literature (Izidine and Bandeira 2002; Izidine 2003; Jansen and Mendes 1990; Verdcourt 1990) and plant databases (CJBVG and SANBI 2013). Villages in LM were Macanda, Goba Sede and Goba Fronteira; in TR were Manhihane, Djabula, Kazimat, Monucua and Porto Henrique and in FC were Huco, Massale, Phuza, Mussongue and Mabukutso.All the villages were areas of occurrence of *W. salutaris*, except Mussongue and Mabukutso. These two areas were added due to abandonment of Massale for the establishment in Mussongue by all except one household, to avoid human-elephant conflicts in FC, part of the Maputo Special Reserve linking this to the Tembe Elephant Reserve. Mabukutso was included because its villagers are undifferentiated with the others from the study area in gathering of species from Massale and Phuza forests. The

Lebombo Mountains are part of Namaacha district, FC Matutuine district and TR is shared by the two districts (Fig. 1.3).

Matutuine, with an area of 5 403 km² (INE, 2014), is located between 26° and 27° latitude south and between 32° and 33° longitude east. The district is delimited by Boane, Namaacha and Maputo city on the north, south by KwaZulu-Natal (Republic of South Africa), on the east by the Indian Ocean and to the west by the Kingdom of Eswatini (previously known as Swaziland) (MAE 2005a). With an area of 2 144 km² (INE, 2014), Namaacha is situated between 25° and 26º latitude south and 31º and 32º longitude east. It is limited on the north by Moamba district, on the west by Republic of South Africa and the Kingdom of Eswatini on east districts of Boane and Matutuine and south by Matutuine district (Fig. 1.3) (MAE 2005b).

Matutuine is predominantly characterised by the occurrence of coastal plains and along the river systems there are the main alluvial deposits. Inland is the Lebombo chain of volcanic origin, differing from the rest of the district (MAE 2005a). Namaacha district is constituted by highlands, the Lebombo chain complex extending from north to south of the district with its highest point at around 800 m. The climate in Matutuine is subtropical with two main seasons, wet from October to April and dry from May to September. The relative humidity is high, varying from 55% to 75% (MAE 2005a). Namaacha has a tropical, humid climate that changes with altitude. It has predominantly two main seasons, wet and hot (October to April) and cool and dry (April to September) (MAE 2005b). The average annual precipitation in Matutuine is 1 000 mm at the coast, decreasing towards inland to 600 mm (MAE 2005a). The mean annual rainfall in Namaacha is 750 mm with around 60% of the precipitation occurring between November and March (MAE 2005b). Temperatures are high in Matutuine, with an annual average value greater than 24 °C, but less in Namaacha with the mean annual of 21° C (MAE 2005b).

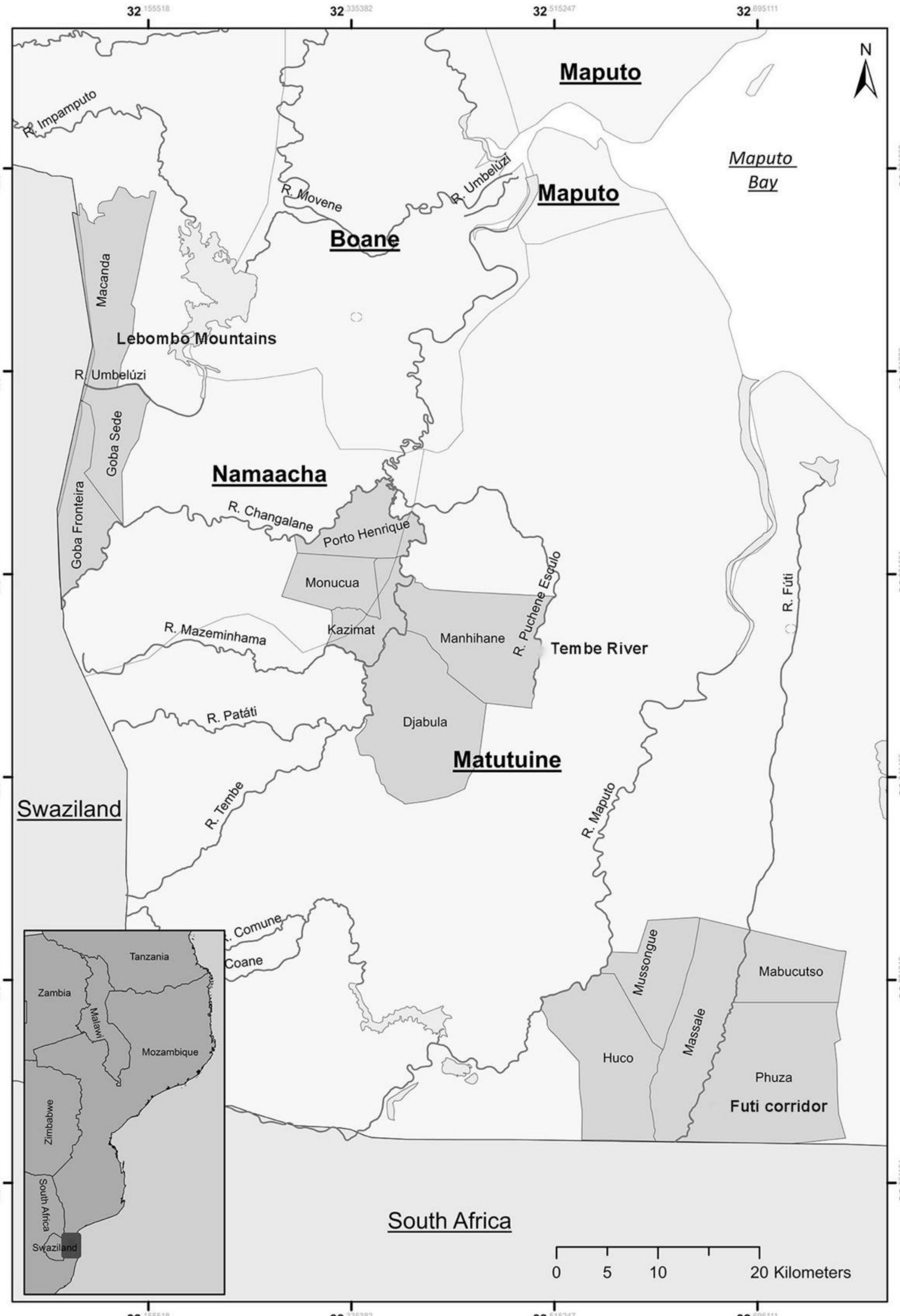


Figure. 1.3. Location of the Lebombo Mountains, Tembe River and Futi Corridor and their respective villages in Matutuine and Namaacha, southern Mozambique.

White sandy soils are the most predominant type in the Matutuine district. These soils are characterised by low fertility, low capacity of water retention and hence, low agricultural potential (INIA 1991). Along the Maputo River, alluvial dark greyish brown clay soils with high agricultural potential are found (GDM 2008). Namaacha district is composed by reddish brown soils of different depths and brownish clayey soils, both derived from basalt (INIA 1991). Soils of basaltic origin are generally fertile and enhance the agricultural potential of the district (MAE 2005b). In the Lebombo Mountains (encompassing the two districts), soils are yellowish brown sand clay loam that are shallow, derived from rhyolites of Karroo sequence. Adjacent to this, soils are very dark greyish brown clayey. Dark grey alluvial soils are also found along the Tembe River (shared by the two districts) and the Futi River, in the Matutuine district (INIA 1991).

Namaacha and Matutuine districts were described as part of the regional vegetation mosaic, with around 3 000 plant species of which about 40% are endemic including more than 20 genera of endemic woody species (White 1983). Matutuine district was also included as part of the Maputaland Center of Endemism due to its high flora and fauna diveristy (van Wyk 1996; van Wyk and Smith 2001). These areas were recently proposed as part of Maputaland sensu lato, which encompasses of Maputaland sense strito two sub centers, Lebombo Mountains and Inhambane has 114 strict endemic and near-endemic plant taxa in which 50 are endemic to the area (Darbyshire et al. 2019).

In specific the study areas, the vegetation associated with *W. salutaris* varies according to the location. In the Lebombo Mountains, *W. salutaris* occurs on rocky slopes, where the vegetation varies with topography, soil depth and aspect (Burrows et al. 2018). Common species includes *Acacia nigrescens* Oliv., *Acacia burkei* Benth. and *Combretum apiculatum* Sond. and on shallow soils, aloes, especially *Aloe marlothii* A.Berger., *Ficus* spp. and *Euphorbia* spp. *Olea africana* Mill. and *Combretum* spp. are among common species found on steeper and stony slopes (Kirkiwood 2014). In the Tembe River, *W. salutaris* is found in the Licuati Forest Reserve and adjacent areas associated with species such as *Pteleopsis myrtifolia* (M.A.Lawson) Engl. & Diels, *Cleistanthus schlechteri* (Pax) Hutch. and *Hymenocardia ulmoides* Oliv. (Izidine 2003). In the Futi Corridor *W. salutaris* is found near seasonal pans (MITUR 2002). The vegetation community structure is thicket related to termitaria encompassing of clay soils (Matthews et al. 2001), Common tree species include

Berchemia zeyheri (Sond.) Grubov, *Pappea capensis* Eckl. & Zeyh. and *Olea europaea* subsp. *africana* (Mill.) P.S. Green (van Rooyen et al. 1983).

Fire frequency is relatively low in Namaacha due to active involvement of community leaders (MAE 2005b), whereas it remains a major threats to herbaceous vegetation in some parts of Matutuine and for those depending on swamp herbaceous species for an alternative means of income (Mussagy 2006). In some parts of Matutuine fires are associated with poaching which is the major issue in conservation (GDM 2008). Additionally, persistent burning of vegetation across the Maputaland center caused by slash and burn agriculture and for improvement of fodder for livestock has contributed to transformation of vegetation through reducing forests and increasing of grasslands (Bruton 1980; Bruton et al. 1980).

The population of Matutuine is composed mainly of the Ronga ethnic group (whom are part of great Tsonga population) and comprise a Tembe clan which is believed to be one of the real Ronga of southern Maputo bay (MAE 2005a). Namaacha is mainly constituted by Swazi and Ronga ethnic groups, but currently there has been a trend of establishment of people from different clans (MAE 2005b). Languages spoken include Ronga, *siSwati* and in the area around the border with Eswatini and South Africa, *isiZulu* (Eberhard et al. 2019). According to the 2015 annual population projections, the population of Matutuine district was estimated to be 41 070 inhabitants, and Namaacha district estimated at 51 257 inhabitants (INE 2010). The population density in both districts was one of the lowest among the districts of Maputo province estimated at eight inhabitants/km^2 in Matutuine district and 24.4 inhabitants/km^2 in Namaacha district (INE 2015a).

According to the 2007 general population and housing census, the overall literacy rate was estimated at 58% for Matutuine with higher rates, with women being higher than men in all age classes (Fig. 1.4) (INE 2013b, 2014). The age class with the highest illiteracy rate is the population of 60 years and above of both genders (89% of women and 58% of men) and the minimum, age class 15 to 19 years old (15% of women and 11% men) (INE 2013a,2014). The overall literacy rate in Namaacha was estimated at 69%, with similar trends as Matutuine (Fig 1.4) (INE 2014). The most illiterate group was from age class 60 years and above, both for females (82%) and males (41%) while the least was group 15-19 years (7% for females and 8% for males) (INE 2013b). Formal education levels are very low in both districts. That is more people attain primary education but fewer continue with further education.

According to the analysis of 2014/2015 around 41% to 45% people (roughly 10.5 to 11.3 million people) in Mozambique live in absolute poverty. Maputo province contributes with 319 219 people (INE 2015b). The rural communities are primarily agrarian, with an average farm size of 0.9 ha per household in Matutuine district. The main crops grown include maize, cassava, cowpea, groundnut and sweet potato. Fruits comprise banana, mango, papaya, citrus, cashew nut and wild fruit (*Strychnos spinosa* Lam. and *S. madagascariensis* Poir.). Off-farm activities include trade of agricultural products and livestock, fuel wood (GDM 2008) and other non-timber forest products (NTFPs) such as palm wine (Martins and Shackleton 2018), medicinal plants (Nuvunga 1998; Sabíno 2007), wild food, art craft and fish for those living closer to water bodies (Mussagy 2006). Lack of employment also encourages labour immigration to neighbouring countries (South Africa and Eswatini), an important source of income for households (MAE 2005a).

Livelihoods of the majority in Namaacha also revolve mostly around on-farm activities with an average farm size of 1 ha. The main crops include maize, groundnut, cowpea, sweet potato, banana, cassava and sunflower), raising animals (predominantly cattle, goats, lamb, poultry and pigs) for family consumption and trade. The main off-farm activity is the production and trade of fuel wood. The geographical location of Namaacha favours other off-farm activities including labour migration to neighbouring countries (South Africa and Eswatini), informal and cross-border trade, soap manufacturing, commercialisation of products such as alcoholic beverages and ceramics. In addition, fishing and hunting of small mammals and birds contributes on provision of protein to the district communities (MAE 2005b).

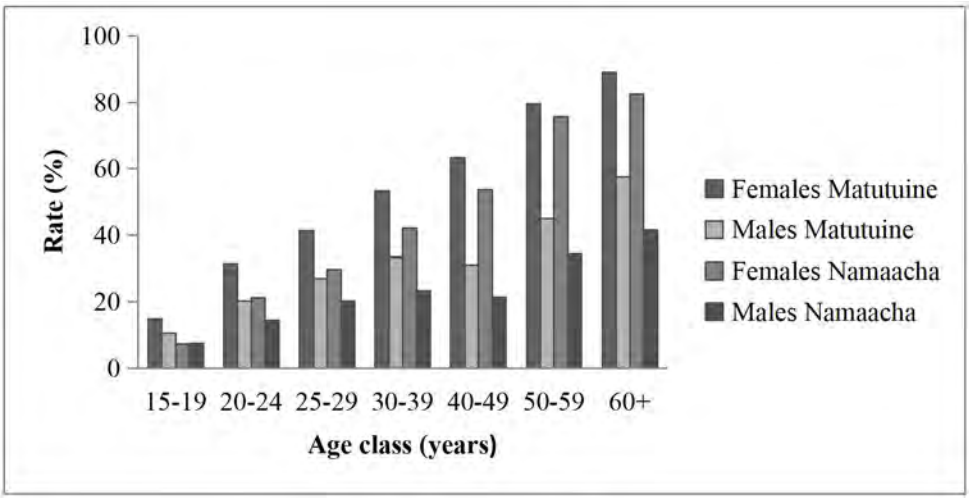


Figure 1.4. Rate of illiteracy of Matutuine and Namaacha district, 2007 (INE 2013a, 2013b).

Wild resources are indispensable for the people of the two districts. This include use of medicinal plants (e.g. *Salacia kraussii* (Harv.) Harv., *W. salutaris, Vernonia colorata* (Willd) Drake, *Kigelia africana* (Lam.) Benth.) (Jansen and Mendes 1990, 1991; Jansen et al. 2001), food plants (e.g. *Sclerocarya birrea* subsp. *caffra, Trichilia emetica* Vahl*, S. madagascariensis*. *Amaranthus* sp. *Mormodica balsamina* L.), fuel wood (e.g. *Acacia karroo* Hayne, *A. nigrescens*) (cf. Bandeira et al. 1999; Saide 2001), and construction material (e.g. *Androstachys johnsonii* Prain, *Pteroxylon obliquum* Thunb.) Radlk. (Bandeira et al. 1999). Additionally bushmeat from small mammals such as antelopes and rabbits and bird is important for dietary balance (MAE 2005a; 2005b).

Management of wild resources is done through indigenous practices encompassing protection of economic any important fruit trees, such as *S. birrea*, *T. emetica* and *S. spinosa* under traditional authorities (Nielsen et al. 2006). At the household level, productive fruit trees are preserved during land preparation for agriculture and some species that provide shade (e.g. *S. birrea*, *T. emetica* and *Afzelia quanzensis* Welw.) are spared for traditional ceremonies or community meetings (Saide 2001). Additionally, conservation of plant resources is supported by the restriction to certain forest patches where royal lineage has been buried, for example ancestors and Djabula (traditional leader of Djabula) in Licuati forest (Matutuine district) (Saide 2001) and the Mazie's family in the "Muntimu" secret forest in Goba (Namaacha district) (Moisés 2003). Formally, protection of threatened plants is through article 12(1) of the Environmental Law "are prohibited all activities that threaten the conservation, reproduction, quality and quantity of biological resources, especially endangered" and 12(2a) "special protection of plant species threatened with extinction …."(GM 1997a). In protection of medicinal plants objective (f) of the 2005 National Traditional Medicine policy affirming "to secure protection of biodiversity" and section 8.4 emphasises on cultivation intervention and through domestication of medicinal plants to alleviate pressures in the wild and enhance the productivity, and adoption of mechanisms to offset rural communities and traditional medicine practitioners with the provision of alternative income generation activities in place of jeopardising medicinal plants (GM 2004). Additional protection measures include national red listed plants species (Izidine and Bandeira 2002) and those benefiting indirectly of protection in the formal protected areas (cf. GM 2011).

The Constitution (Article 109) (GM 2004) and the 1997 Land Law [Article 3 (1-2)] (GM 1997b) states that "land is the State property" and it "must not be sold, or in any other way sold, nor mortgaged or pledged”. All citizens have the right to use and benefit from the land (Article 109 (3) unless the rights are conferred to others (Article 110 constitution). The State also defines conditions of land use and benefits and it secures the rights of land acquired through inheritance or occupation, except where there is a legal reserve or if the land has been legally assign to other entity or person (article 111 of the constitution). The only provision that gives the legal right to land use and benefits right is “*Direito do Uso e Aproveitamento da Terra* (DUAT) (Land use and Benefits Right) (Section III of Land Law), occurring when the State transfers tenure security. The land use and benefits rights in the rural is practically under the domain of local communities through DUAT gained from customary occupation. However, rural communities are lacking of land tenure security and are unaware of the existence of the Land Law especially for the women due to disadvantageous position they hold in social institutions (Veleta 2018). Similar to other places this insecurity may leads to over exploitation of species for fear that some community members would exhaust the species without others getting a chance to harvest and consequently, deplete the numbers of individuals of the species (cf. Robinson et al. 2018). Overall, the conservation of *W. salutaris* in both customary and formal land occupation is not efficient due to high demand for the bark for medicinal uses and weak perfomance of the law enforcement institutions to control overharvetsing of bark in the study areas (cf. Krog et al. 2006; Senkoro et al. 2019).

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Chapter Two: Predicting the current potential distribution and extent of occurrence of a threatened medicinal tree (*Warburgia salutaris*) in southern Mozambique under climate change

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Abstract

The present work addressed the following questions: Which areas are currently potential suitable for *Warburgia salutaris*? How might they change with climate change? Which conservation strategies have more probability to secure the existence of *W. salutaris* in southern Mozambique? The work aimed to model the current distribution of *W. salutaris* as well as potential future distribution under climate change. The potential current and future distribution of *W. salutaris* coupled with the extent of occurrence and habitat quality was done using maximum entropy (Maxent) species distribution modelling. Candidate models were created (2 953 models) and selected based on statistical significance (partial ROC< 5%), omission rate (≤ 5%) and delta AICc (≤ 2). Future simulations were done employing 2 846 occurrence data and 11 uncorrelated environmental (mostly climatic) variables for the years 2050 and 2070, under 4.5 and 8.5 Intergovernmental Panel for Climate Change's (IPCC) representative concentration pathways (RCPs) for four general circulation models [(CESM1(BGC), ACCESS1.3, FIO-ESM and IPSL-CM5A-MR]. A mobility-oriented parity (MOP) was conducted to identify extrapolation risk. Model selected was statistically significant (partial ROC =0.00), omission rate (0.04) and delta AICc (0.00). Major suitable and less suitable areas under current environmental conditions were found to be similar to that observed during the field survey. Future prediction by CESM1(BGC) and ACCESS1.3 indicated increase of highly suitable areas under both RCPs in southern Mozambique compared to the current distribution whilst FIO-ES under 4.5 showed a slight decrease and more less suitable areas in 2050 under RCP 8.5 and in 2070 under RCP 4.5 by IPSL-CM5A-MR. Overall, EOO and habitat quality in southern Mozambique increased steadily in 50 years compared to the current predicted distribution. Most of the modelled areas in southern Mozambique revealed potential suitable areas for the distribution of *W. salutaris*. Thus, climate change is not the major threat to the species for the coming 50 years. In favour of climate change coupled with an increase of EOO and habitat quality could be capitalised for re-introduction and cultivation of the species to provide alternative source of bark supply, starting from areas of current distributional range and gradually expand to new suitable areas, to alleviate pressure on natural populations.

Key words: Species distribution models, extent of occurrence, habitat quality, conservation

2.1 Introduction

For several decades, uses of many plant species, including medicinal ones, has shifted from subsistence to commercial exploitation increasing the risk of unsustainable harvesting (Cunningham 1993; Ghimirre 2008). In sub-Saharan Africa (SSA) for example, despite the reliance on medicinal plants from natural habitats for primary health care, trade has become a mean of subsistence for many people (Cunningham 1993; Moyo et al. 2015; van Andel et al. 2015), driven by the high demand for certain species due to their medicinal efficacies (Moyo et al. 2015), cultural preferences (Mukamuri and Kozanayi 2014) and lower cost of acquisition compared to modern pharmaceuticals (Botha et al. 2004; Cunningham 1993). Due to this, unsustainable exploitation of some medicinal plants has escalated (Botha et al. 2004; Mukamuri and Kozanayi 2014; Shackleton et al. 2005; Veeman et al. 2014b).

Much of the unsustainable, commercial demand is via informal and undocumented trade. Though it involves only a small number of rare and threatened species, quantities traded are significant (Krog et al. 2006; Mander et al. 2006; Moyo et al. 2015; van Andel et al. 2015; van Wyk and Prinsoo 2018; Veeman et al. 2014a). Nevertheless, information concerning the conservation status of many of these species is scarce especially on the African continent, and efforts to address the trade have faced a variety of challenges (Moyo et al. 2015). Assessment of the status of overexploitation of medicinal species in SSA is usually based on market surveys (cf. Krog et al. 2006; van Andel 2015; Williams et al. 2014a, 2014b), harvesting impacts (cf. Botha et al. 2004; Delvaux et al. 2010; Gaoue and Ticktin 2008; Guedje et al. 2007; Romero et al. 2014) or the demography of species (cf. Botha et al. 2004; Galabuzi et al. 2015; Kairu et al. 2013). Some works, however, have reported on the distribution and conservation status of species in high demand (cf. Dludlu et al. 2017; van Andel et al. 2015). Yet, precise estimates of the distribution of the majority of such species are limited (Moyo et al. 2015).

Interacting with overharvesting of species for trade are other direct drivers of population stress or declines, including land transformation (Botha et al. 2004; Giam et al. 2010; Tapia-Armijos et al. 2017), fire (Botha et al. 2004) and climate change (Dudley et al. 2019; Giam et al. 2010; Pramanik et al. 2018). Climate change in particular has caused the deterioration of habitat suitability for several threatened species (cf. Li et al. 2020; Pramanik et al. 2018). This has evolved to be the foremost threat to species distribution and viability reported across the globe (cf. Hipólito et al. 2015; Pramanik et al. 2018; Sen et al. 2016; Sony et al. 2018; Qin et al. 2017). It has changed biodiversity patterns in aspects such as phenology through seasonality alterations (Xu and Xue 2013) and the distributional range of species (Draper Munt et al. 2016; Zhang et al. 2019). The species distributional range has been reported to shift (Chen et al. 2011; Gray and Hamann 2013), decrease (Draper Munt et al. 2016; Pramanik et al. 2018; Remya et al. 2015), increase (Zhang et al. 2019) or in some cases, completely disappears as evidence through species extinctions (Thuiller et al. 2005). Hence, determining the effects of climate change on the distribution of species of conservation concern is crucial to provide early warning to mobilise the tools necessary for mitigation to avoid species extinctions (Draper Munt et al. 2016; Kakpo et al. 2019; Pramanik et al. 2018).

The assessment of the conservation status of species of concern, such as rare and those in high demand, is important for their protection (Moyo et al. 2015). Amongst different tools employed to assess the conservation status or classify the likelihood of species extinction is the IUCN Red List Categories and Criteria system. (IUCN/SSC 2012; Fourcade et al. 2013). Several researches have employed the IUCN red-listing approach (cf. Draper Munt et al. 2016; Fourcade et al. 2013; Jiménez-Alfaro et al. 2012; Sérgio et al. 2007). In particular, the application of the red-listing Criterion B, species geographic range through estimates of extent of occurrence (EOO) and area of occupancy (AOO) are necessary to assign species in threatened categories, i.e, Critical Endangered, Endangered and Vulnerable. This can be obtained through ecological niche modelling (Sérgio et al. 2007). A highly traded medicinal species in southern Africa is *Warburgia salutaris* (G.Bertol.) Chiov., which is predominantly wild-harvested. Overharvesting of the bark due high demand for trade has compromised the regeneration of *W. salutaris* leading to population declines and local extinctions (Botha et al. 2004; Maroyi 2013; Moyo et al. 2015; Mukamuri and Kozanayi 2014). Mozambique, for example, has been supplying (illegally) harvested bark to neighbouring countries (Krog et al. 2006; Mander et al. 2006; Mukamuri and Kozanayi 2014; Veeman et al. 2014b). Equally, Zimbabwe benefited from the illegal supply of the bark from South Africa (Mukamuri and Kozanayi 2014; Veeman et al. 2014b). Following the IUCN Categories and Criteria, the species has been classified as Endangered in Malawi (EN A1acd) (Msekandiana and Mlangeni 2002), South Africa (Botha et al. 2004) and Eswatini (EN B2ab (iii,v)) (Dludlu et al. 2017). It is Critically Endangered in Zimbabwe (CR A1d B1B2abcdeC1C1a D) (Mapaura and Timberlake 2002) where it was thought to be extinct in the wild (Maroyi 2013). In Mozambique, it was classified as Vulnerable (VU A2cd) (Izidine and Bandeira 2002) and is considered Endangered globally (Hilton-Taylor et al. 1998).

Ecological niche models or species distribution models (SDMs) have been recognised as important tools in ecology (Adhikari et al. 2012; Dubuis et al. 2011; Platts et al. 2010; Tarkesh and Jetschke 2012), evolution (Alexander 2013; Kumar et al. 2019), biogeography (Chatterjee et al. 2012; Guillera-Arroita et al. 2015; Hipólito et al. 2015) and conservation biology (Liu et al. 2019; Pramanik et al. 2018; Qin et al. 2017; Yi et al. 2016; Zhang et al. 2019). These models uses algorithms to predict the distribution of a species in a geographical space, employing occurrence data (presence, or presence and absence) and environmental data such as temperature, precipitation, soil, land cover, altitude, aspect and slope (cf. Fourcade et al. 2013; Hipólito et al. 2015; Phillips et al. 2006; Qin et al. 2017; Sony et al.

2018). Among the popular SDMs is maximum entropy (Maxent) which uses presence-only data. Maxent has been widely applied in modelling the potential distribution of species such as invasives (Bromberg et al. 2011; Costa et al. 2015; Jiménez-Valverde et al. 2011; West et al. 2016), threatened species of ecological, ornamental and medicinal importance and the relationship between species distribution and environmental correlates (cf. Adhikari et al. 2012; Li et al. 2020; Pramanik et al. 2018; Yi et al. 2016; Zhang et al. 2019).

Although *W. salutaris* is in a high demand throughout southern Africa (cf. Krog et al. 2006; Mander et al. 2006; Williams et al. 2014; Mukamuri and Kozanayi 2014) and is confined distributed (Jansen and Mendes 1990; Moyo et al. 2015; Veeman et al. 2014b), to my knowledge information on its precise distribution is scarce in southern Africa. Economic studies suggested illegal supply of bark from Mozambique to Zimbabwe (Mukamuri and Kozanayi 2014) and to South Africa (Krog et al. 2006) for trade in informal medicinal plants markets. Through markets surveys and by ascertaining available bark with corresponding harvested tree size, Williams et al. (2014b) suggested a reduction larger trees in the wild. The existing ecological studies related to the species suggested *W. salutaris* is resilient to bark harvest bark but it would not survive constant harvesting (Cunningham 2001). Botha et al. (2004) found high levels of tree destruction from bark harvesting in unprotected than protected areas in South Africa. Dludlu et al. (2017) reported on mass destruction of individuals of *W. salutaris* due to ring-barking coupled with declines of matured individual in Eswatini. The authors also uncovered new populations and ascertain the distribution of the species. However, the distribution of *W. salutaris* has never been studies in Mozambique to unveil possible unknown or unrevealed areas of occurrence and predict the potential future distribution.

Within this context, the present work aimed to (i) develop a spatial model based on environmental variables to predict the potential distribution of *W. salutaris* within its range in southern Mozambique, and (ii) assess the future changes in extent of occurrence (EOO) and habitat quality and iii) evaluate the implications of future changes for conservation of the species.. It sough to answer the following question: Which areas are currently potentially suitable for *W. salutaris* and how might they change with climate change? This work was developed for the first time to predict the impact of climate change on a threatened medicinal species in Mozambique and it is among the first for the species in southern Africa. Similar to other studies (cf. Draper Munt et al. 2016; Hipólito et al. 2015; Li et al. 2020; Qin et al. 2017), the current research provides knowledge on the distribution, habitat conditions and suitability that is crucial for the conservation and management of this threatened species

2.2 Material and methods

2.2.1 Species and target area

W. salutaris is a medicinal plant species with a confined distribution in southern Africa (Coates-Palgrave 2002; Dludlu et al. 2017; Maroyi 2013). It has long been used to treat respiratory problems such as cough, colds, chest complaints and oral thrush (Jansen and Mendes 1990; Maroyi 2014; Senkoro et al. 2019; van Wyk and Wink 2004). Other ailments treated using *W. salutaris* include rheumatism, influenza, gastric ulcers and malaria (Maroyi 2014; van Wyk and Wink 2004).The species was thought to occur in eastern and southern Africa (van Wyk and Wink 2004) but recent work indicated the its confinement to southern Africa (cf. Botha et al. 2004; Dludlu et al. 2017; Maroyi 2012; Senkoro et al. 2019, Veeman et al. 2014a, b; Williams et al. 2014a, b). In southern Africa, *W. salutaris* occurs in most parts of Eswatini (former Swaziland) (Dludlu et al. 2017), in parts of the Mpumalanga, Limpopo and KwaZulu-Natal provinces of South Africa, (Coates-Palgrave 2002; Botha et al. 2004), southern Malawi (Msekandiana and Mlangeni 2002) and in Chipinge district, southeast Zimbabwe (Verdcourt 1990; Mapaura and Timberlake 2002; Maroyi 2013), and perhaps an isolated occurrence in Chimanimani (Veeman et al. 2014a). In Mozambique, it occurs in the southeast and southern parts in Matutuine and Namaacha districts (Jansen and Mendes 1990; Senkoro et al. 2019).

Occurrence data were collected following the distributional data of *W. salutaris* based on herbarium vouchers of the National Herbarium (LMA) and the herbarium of Eduardo Mondlane University (LMU), unpublished theses (cf. Emanuelsson 2005; Halafo 1996; Izidine 2003; Sabíno 2007) and information from para-botanists, botanists, ecologists and collectors. These were compared with the existing published literature (Izidine and Bandeira 2002; Jansen and Mendes 1990; Verdcourt 1990) and plant databases (CJBVG and SANBI 2013). Subsequently a preliminary sketch map containing information on species occurrences and important features (e.g. roads and areas of natural vegetation) was produced for each of the 11 villages in the Tembe River area (Manhihane, Djabula, Kazimat, Porto Henrique and Monucua), the Lebombo Mountains (Macanda, GobaSede and GobaFronteira) and the Futi

Corridor (Huco, Phuza and Massale).The map was taken to respective village in a general community meeting to indicate where, within the delineated village boundaries, the species was found or not. The second round consisted of the same exercise with one to two groups per village composed by five to seven community members, identified by traditional leaders who knew the species occurrence, totalising 17 groups. Subsequently, one to two people who demonstrated more knowledge on the occurrence were invited to the systematic survey on the ground through transect walks and a geo-reference record was taken and saved in a geo-database. A raw dataset of 2 846 records were used as the base of this study.

2.2.2 Environmental data

Appropriate variables were extracted from the WorldClim database version 1.4 (Hijmans et al. 2005) as potential environmental predictors, excluding the four that combine temperature and precipitation (Bio_8, Bio_9, Bio_16 and Bio_17) (Escobar et al. 2014). Two future scenarios (representative concentration pathways; RCP 4.5 and 8.5) of four general circulation models with the lowest levels of interdependence (CESM1(BGC), ACCESS1.3, FIO-ESM and IPSL-CM5A-MR) were used to project future spatial changes on *W. salutaris*. Future data layers (2050 and 2070) were obtained from the CGIAR Research Program on Climate Change, Agriculture and Food Security database (Navarro-Racines et al. 2020) (available at http://www.ccafs-climate.org/data_spatial_downscaling/). A derived slope variable was accessed from the digital terrain model (NASA/METI/AIST/Japan Spacesystems and U.S/Japan ASTER Science Team 2009). As a measure of human influence a layer based on distance from human settlements was created from the data available from Mozambique National Cartography and Remote Sensing (CENACARTA available from http://www.cenacarta.com/) (Table 2.1).

All predictors were used at a spatial resolution of 30'. Correlation analyses were performed to reduce the set of variables, eliminating one variable per pair with Pearson correlations of $r \geq 0.8$ (Table 2.2). Jackknife processes in Maxent were used to select three distinct sets of variables (Cobos et al. 2019; Freeman et al. 2019) (Table 2.3) removing the variables with the lowest independent contributions. Duplicates were eliminated and reduced the effects of spatial autocorrelation by thinning records with a distance of 2 km (n=113). Occurrences were split randomly into 75–25% subsets for model calibration and testing, respectively.

Table 2.1. Uncorrelated environmental variables used to create the predictor sets for modelling the potential distribution of *Warburgia salutaris*.

Abbreviation	**Description**	**Unit**	**Source**
Isoth	Isothermality (Bio_2/Bio_7) (x100)	%	Hijmans et al. (2005)
T Seas	Temperature Seasonality (Standard Deviation x 100)	˚C	Hijmans et al. (2005)
Max TW Mth	Max Temperature of Warmest Month	˚C	Hijmans et al. (2005)
Min TC Mth	Min Temperature of Coldest Month	˚C	Hijmans et al. (2005)
T Ann Rng	Temperature Annual Range (Bio_5 - Bio_6)	˚C	Hijmans et al. (2005)
PPT Seas	Precipitation Seasonality (Coefficient of Variation)	%	Hijmans et al. (2005)
PPT W Qrt	Precipitation of Warmest Quarter	Mm	Hijmans et al. (2005)
DEM	Digital Elevation Map	Mm	NASA/METI/AIST/Japan Spacesystems and U.S/Japan ASTER Science Team (2009)
Slope	Slope	%	(derived from DEM)
VillageDistance	Distance from Villages	Km	CENACARTA
WBDistance	Distance to Rivers and Water Bodies	Km	CENACARTA

Table 2.2. Correlation matrix of preliminary variables (* indicate selected candidate variables to integrate the models), numbers in bold indicates highly correlated pairs of variables (r ≥ 0.8). Ann = annual, M = mean, T = temperature, D = diurnal, Rng = range, Isoth = isothermality, Seas = seasonality, C = cold, W = warmest, PPT = precipitation, Wet = wettest, Dri = driest, DEM = digital elevation map, WB = Rivers and Waterbodies.

Variable	Ann MT	MD Rng	Isoth	T Seas	Max TW Month	Min TC Month	T Ann Rng	MTW Qrt	MTC Qrt	Ann PPT	PPT Wet Mth	PPTDriMth	PPT Seas	PPT W Qrt	PPT C Qrt	DEM	Slope	Village Distance	WB Distance
Ann MT	1																		
MD Rng	**-0.83**	1																	
***Isoth**	-0.16	0.34	1																
***T Seas**	0.29	0.61	-0.25	1															
***Max TW Mth**	0.67	0.53	-0.18	0.61	1														
***Min TC Mth**	0.62	-0.63	-0.19	-0.48	-0.08	1													
***T Ann Rng**	-0.1	0.65	0.05	0.54	0.63	-0.62	1												
MTW Qrt	**0.98**	0	-0.24	0.44	**0.97**	0.49	0.05	1											
MTC Qrt	**0.94**	-0.41	-0.13	-0.03	0.43	**0.83**	-0.4	**0.87**	1										
Ann PPT	0.19	**-0.8**	0.1	-0.76	-0.45	0.78	**-0.87**	0.03	0.49	1									
PPT Wet Mth	0.29	-0.5	0.26	-0.52	-0.26	0.58	-0.6	0.16	**0.89**	0.79	1								
PPTDriMth	0.19	**-0.88**	-0.18	-0.69	-0.41	**0.81**	**-0.86**	0.04	0.46	**0.89**	0.73	1							
***PPT Seas**	-0.19	0.65	0.24	0.62	0.66	-0.69	0.52	-0.06	-0.43	-0.58	-0.3	-0.64	1						
***PPT W Qrt**	0.2	-0.52	0.37	-0.66	-0.41	0.58	-0.68	0.06	0.43	0.54	0.65	0.55	-0.36	1					
PPT C Qrt	0.29	**-0.92**	-0.26	-0.65	-0.35	**0.88**	**-0.89**	0.15	0.55	**0.87**	0.49	**0.97**	**-0.95**	0.52	1				
***DEM**	-0.24	0.19	0.72	-0.32	-0.3	-0.15	-0.06	-0.3	-0.16	0.22	0.43	-0.17	0.27	0.54	-0.23	1			
***Slope**	-0.01	0.04	0.42	-0.19	-0.11	0.05	-0.1	-0.04	0.05	0.13	0.24	-0.09	0.11	0.3	-0.09	0.78	1		
***Village Distance**	-0.07	0.07	-0.05	0.11	0.06	-0.07	0.09	-0.06	-0.1	-0.08	-0.33	0.04	-0.17	-0.29	0.02	-0.11	-0.06	1	
***WB Distance**	0.03	-0.25	-0.21	-0.08	-0.12	0.17	-0.2	0.03	0.08	0.12	0.19	0.17	-0.13	0.11	0.15	-0.14	-0.16	-0.03	1

Table 2.3. Candidate sets of environmental predictors tested during model calibration for *Warburgia salutaris*.

Environmental predictors	*W. salutaris*		
	Set 1	**Set 2**	**Set3**
Isothermality (Bio_2/Bio_7) (x100)	X	X	X
Temperature Seasonality (Standard Deviation x 100)	X	X	X
Max Temperature of Warmest Month	X	X	
Min Temperature of Coldest Month	X	X	X
Temperature Annual Range (Bio_5 - Bio_6)	X	X	
Precipitation Seasonality (Coefficient of Variation)	X	X	
Precipitation of Warmest Quarter	X	X	X
Digital Elevation Map	X		
Slope	X		
Distance from Villages	X	X	X
Distance to Rivers and Water Bodies	X	X	X

2.2.3 Ecological niche modelling

Ecological niche models were created using a maximum entropy algorithm (Elith et al. 2011; Phillips et al. 2006, 2017). A bias file was created to correct the sampling influence of geographical sampling bias following the procedures recommended by Syfert et al. (2013). The kuenm package in R was used (Cobos et al. 2019) (available at https://github.com/marlonecobos/kuenm#installing-the-package) for model selection, evaluation, projection to several scenarios and time periods and to identify extrapolation risks. Two thousand, nine hundred and fifty eight (2 958) candidate models were created following the default settings by combining three sets of environmental predictors (Table 2.3), 17 values of regularisation multiplier (0.1–1.0 at intervals of 0.1, 2–6 at intervals of 1, and 8 and 10), and all 29 possible combinations of five feature classes. Candidate models were evaluated by their performance based on significance (partial ROC, with 500 iterations and 50 percent of data for bootstrapping), omission rates (E_D 5%), and model complexity (AICc). Among models that were statistically significant and that presented omission rates below ≤5%, those with delta AICc up to 2 were selected as final models.

Final models were created with all the occurrences and the parameters defined by the previous procedures. Ten (10) replicates by bootstrapping were produced, with logistic outputs, and were projected to the same geographical area for current and future scenarios (see environmental data). The final model was evaluated according the partial ROC and omission rates (based on *E* D 5%) using the independent dataset. The median of all replicates was used across parameters to consolidate results for the species.

The mobility-oriented parity (MOP) analyses were performed to identify extrapolation risks in model projections (Owens et al. 2013; Escobar et al. 2014). The MOP analyses compare environmental values between the calibration area and the scenarios to which ecological niche models were projected. Result of MOP analyses produce layers with areas were strict extrapolation risks exist (in black) and similarity levels between the projection scenarios and the calibration area.

2.2.4 Future extent of occurrence and habitat quality trend of *W. salutaris* in southern Mozambique

The area the species may occupy was calculated as the equivalence of the extent of occurrence (EOO) (IUCN/SPC 2019) in each time period and the quality of the occupied habitat for the geographical target region. The EOO is defined as the area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the known, inferred or projected sites of present occurrence of a taxon, excluding cases of vagrancy (IUCN/SPC 2019). The major difference with this approach is that the assumption of the existence of non-continuous areas created by unsuitable environmental factors, make more biological sense than a minimum convex hull (Burgman and Fox 2003). Then the EOO is assumed as the sum of the area with values above a certain threshold that determines the presence of the species and the minimum convex polygon of this area (Cardoso et al. 2011). To define the current and future area of the species is mandatory to define a threshold which allows to pass from a logistic scale to a binary layer. But when dealing with presence only data it seems to be a reasonable choice using thresholds based on training omission such as in Pearson et al. (2006) but this method was applied for describing a "potential distribution" so may lead to over-prediction depending on the training omission error accepted. All records used to produce the models come from the field work, as validation of herbarium and unpublished records. The taxonomic status of *W. salutaris* is clearly defined as occurring in southern Africa while the remaning representatives of *Warburgia* in east Africa (cf. Coates-Palgrave 2002; Maroyi 2013; Muchugi et al. 2008; van Wyk and Wink 2004). Regarding these two considerations, the minimal predicted area (MPA), defined as the minimal suitable area predicted for 100% of the presences was used (Draper Munt et al. 2016; Engler et al.

2004; Guisan et al. 2006; Jiménez-Alfaro et al. 2012; Pearson et al. 2006; Sérgio et al. 2007), to transform the potential extent of occurrence by the species into estimates of presence and absence. After performing this reclassification, an adjustment was made in the pixel size to 2x2 km in order to perform the EOO calculations as recommended by IUCN/SPC (2019). Changes on the Extent of Occurrence (EOO) between current and 2050 and 2070 were based on the current MPA threshold and the minimum convex polygon (Cardoso et al. 2011), as it was assumed that species environment relationships remain unchanged during this time span.

The lack of correspondence between the time period of ten years to assess the IUCN criteria and the availability of RCP projections forced estimation of the future trend of EOO at different time periods than over a period of 10 years or three generations. Changes proportionally to the period between now and each of the projected periods were calculated. With such a scheme the Red List Categories were used, focusing on current EOO (criterion B1), but also on the criterion A3c defined as a "population reduction projected, inferred or suspected to be met in the future (up to a maximum of 100 years) based on a decline in extent of occurrence (EOO) and/or habitat quality"(IUCN/SSC 2012). The habitat quality of the occupied habitat is understood here as the average of the suitability values within the area of areas (Albert et al. 2004).

2.3 Results

2.3.1 Evaluation of model performance

The model selected, among the 2 958 candidate models, is defined by uses of Set 2, a regularisation multiplier of 2 and the combination qth feature classes (combination of quadratic, threshold and hinge). This model was selected based on statistical significance, omission rates, and AICc criteria (Table 2.4).

Table 2.4. Model performance under optimal parameters regarding regularisation multiplier (RM), feature classes (FC), and sets of predictors (Pred. Sets), for the models of *Warburgia salutaris*.

RM	FC	Pred. Sets	Partial ROC	Omission rate 5%	AICc	Delta AICc	Weight AICc	Number of parameters
2	qth	Set 2	0.00	0.04	1787.748	0.00	0.388	9

2.3.2 Suitable areas under current and future environmental conditions for *W. salutaris* in Matutuine and Namaacha districts

Under the current environmental conditions predominantly explained by climatic variables, the modelled distribution of *W. salutaris* indicated a major suitability from the Gumbe River (Namaaacha) and especially around Umbeluzi River extending southwards to the Changalane River (Changalane) and to the eastern part toward and bordering the Boane district. In Matutuine district, highly suitable areas were found from the Futi River inland to the Maputo River and some parts of the Tembe River. The model also identified low suitability in all of the southwest area of the Matutuine district (Catuane), Maputo Bay and adjacent areas including Bela Vista and in the coastal strip inland towards the Futi River, extending from Maputo Bay (Ndelane area) to the South African border (Zitundo). The northwest part of the Namaacha district and some of the western parts also revealed low suitability (Figure 2.1).

The results of suitable habitat for *W. salutaris* in the future under environmental conditions predicted by CESM1(BGC) and ACCESS1.3 identified highly suitable areas in almost all areas of both districts under both scenarios (RCP 4.5 and RCP 8.5). FIO-ESM in 2050 under 4.5 RCP showed a slight decline of highly suitable areas in some areas of the northernmost part of Namaacha district, Bela Vista and southwest of Matutuine district (Catuane). The less suitable areas were found by IPSL-CM5A-MR in the year 2050 under scenario RCP 8.5 and 2070 under RCP 4.5 around Umbeluzi River and in the southeast of Matutuine district and along the coastal strip from Maputo Bay to Zitundo (Figure 2.2).

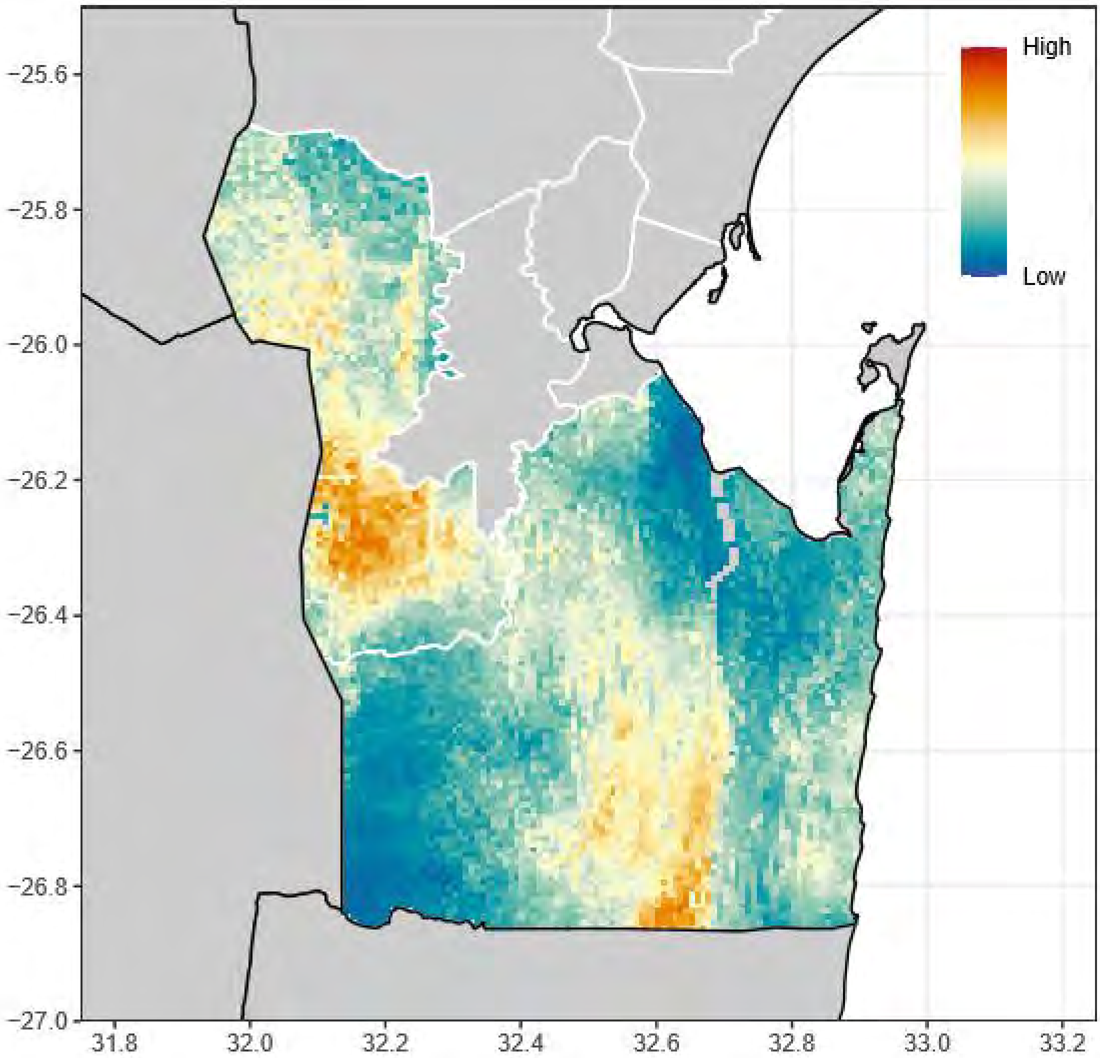


Figure 2.1. Suitability model of current *Warburgia salutaris* in Matutuine and Namaacha districts mostly predicted by climatic variables.

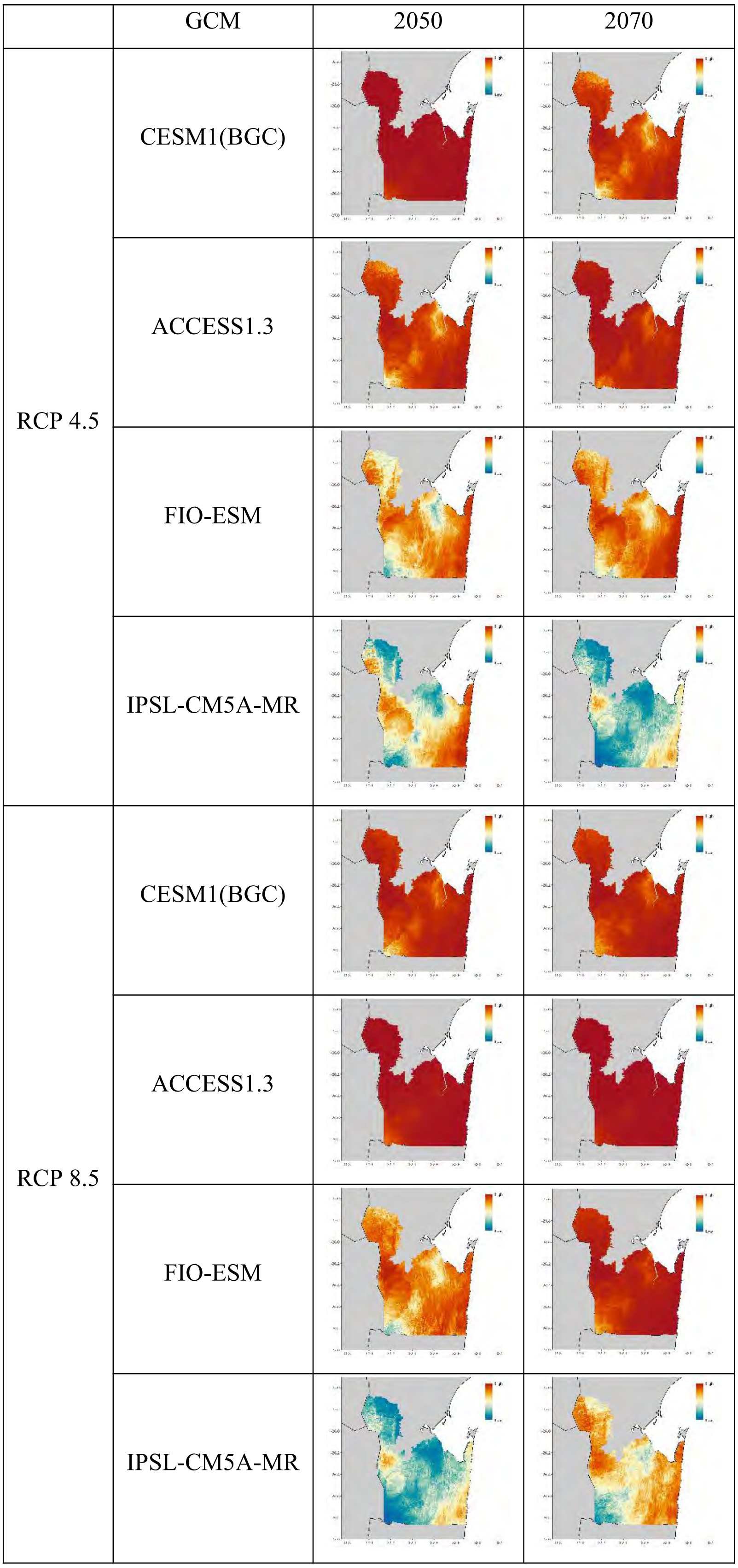
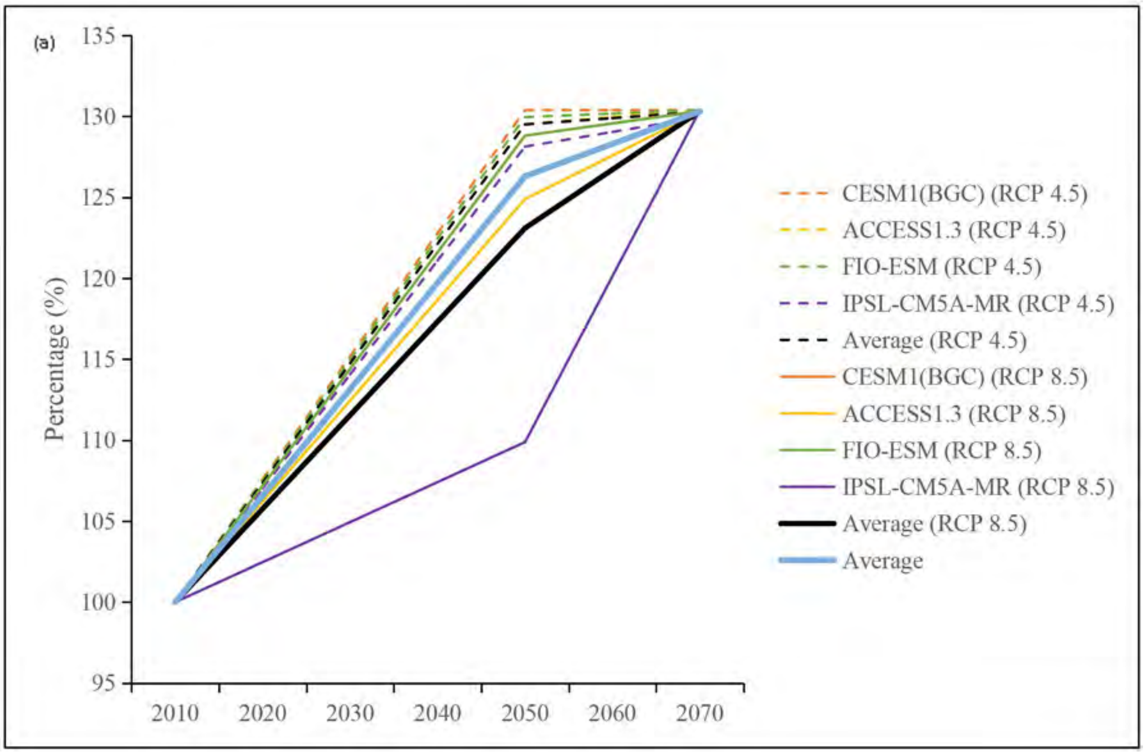


Figure 2.2. Projection of the suitability model under RCP 4.5 and RCP 8.5 for the four GCM (models were produced allowing extrapolation and clamping).

2.3.3 Habitat quality and the extent of occurrence

W. salutaris shows a favourable future trend both in terms of the amount of area occupied (Figure 2.3a) and the quality of the ecological space occupied (Figure 2.3b). Contrary to what happens with many species when exploring the influence of climate change, the species is likely to experience better conditions in the future. The results confirm an increasing trend for all the GCM considered and slightly better conditions under the RCP 4.5 scenario. Considering the periods analysed, it is found that rates are always increasing, although their growth slows down after 2050 and in some cases they even decrease.



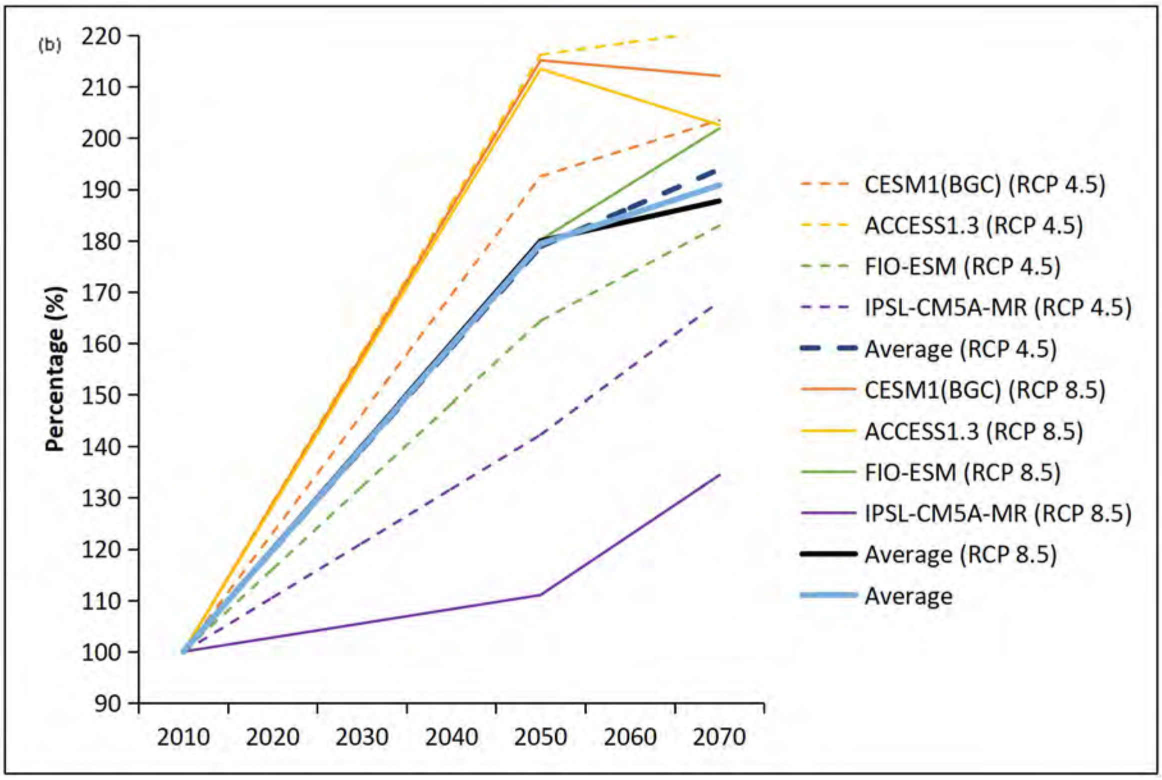
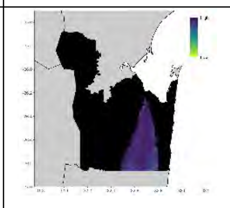
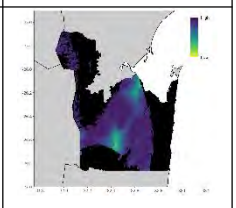
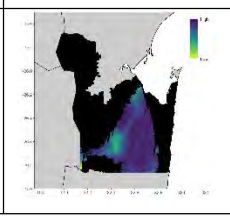
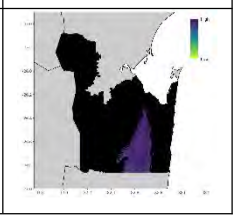


Figure 2.3. Expected trend of the (a) Extent of occurrence (EOO) and (b) Expected trend of the average habitat quality, of *Warburgia salutaris* throughout the analysed time intervals.

Despite the favourable trend in all the final models, caution must be taken when analysing future projections since the results of the MOP analyses indicate considerable areas of extrapolation (Figure 2.4) and little agreement between the different GMCs.

	GCM	2050	2070
RCP 4.5	CESM1(BGC)		
	ACCESS1.3		

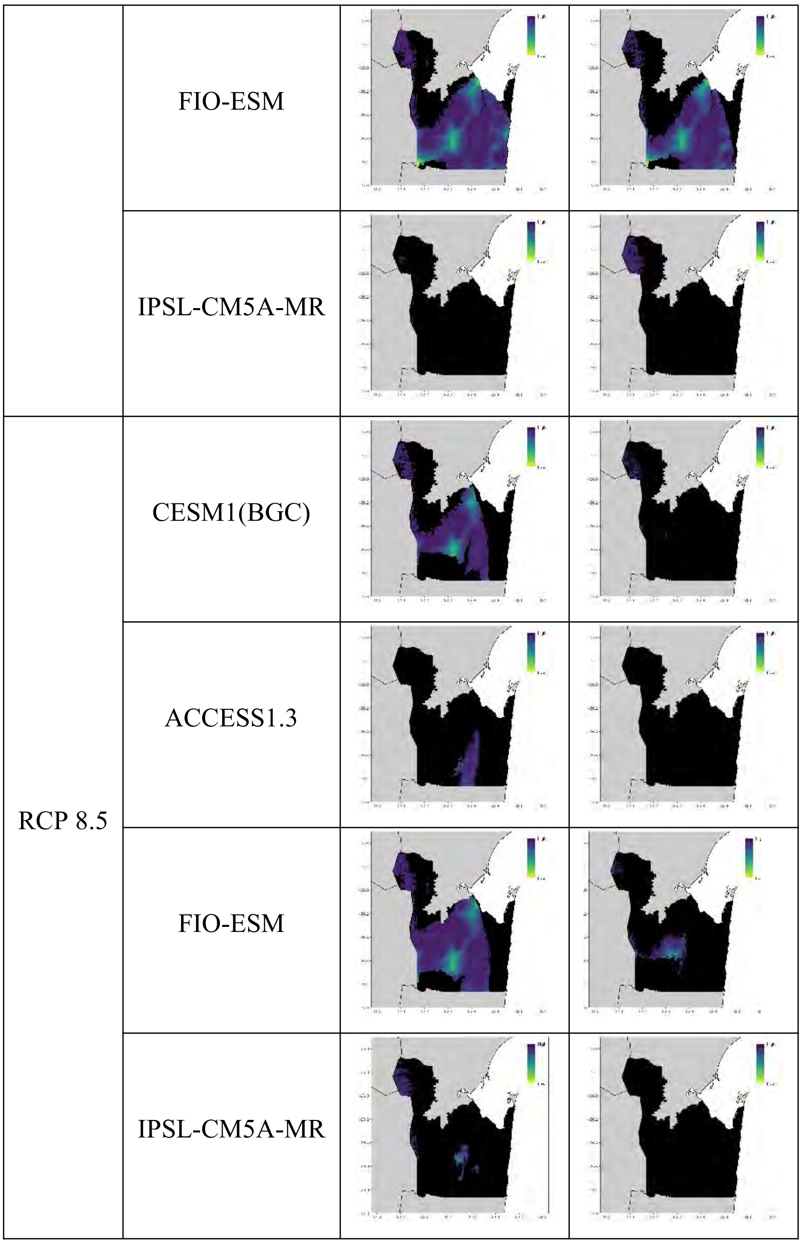


Figure 2.4. Extrapolation risk in future projections (MOP analysis) under RCP 4.5 and RCP 8.5 for the four used GCM (Black areas are those that are out of range for at least one of the environmental variables, such that model transfers to those regions will be unreliable; yellow areas are non-extrapolative, grading through green to blue areas, which are not out of range but are rather dissimilar to the conditions over which the model was calibrated).

2.4 Discussion

2.4.1 Model evaluation and validation

This work used the kuenm package (Cobos et al. 2019) for modelling the distribution of a threatened medicinal species *W. salutaris* in southern Mozambique. The kuenm package, in contrast to comparable packages (cf. Carpenter et al. 1993; Phillips et al. 2006; Stockwell and Peters 1999), presents a conscientious procedure of model evaluation such as partial ROC and computerises the selection of the most appropriate model based on statistical significance. Indeed, the partial ROC on the present work was 0.00 indicating that this model was statistically significant. Correspondingly, results of the omission rate (0.04) and delta AICc (0.00) qualified the selection of the present model. However, as suggested by Cobos et al. (2019), predictions should be interpreted cautiously due to the existence of areas of extrapolation, which was also observed in this work. Moreover, use of several GCMs employed in this work, enabled acquisition of a wide scope of the most likely projections for the species to capitalise the existing potentials of the SDMs for the conservation and management of species (Alkishe et al. 2020; Porfirio et al. 2014).

2.4.2 Suitable areas under current and future environmental conditions for *W. salutaris* in Matutuine and Namaacha districts

The results of the modelling prediction on highly suitable areas for the current distribution were, in general, in accordance with ground observation of the species distribution. However, a few locations were not recorded during the field survey. This could be due to the discovery of new areas within the potential suitable areas that may be revealed for rare and confined distributed species, giving unprecedented areas for species occurrence (Gogol-Prokurat 2011; Hipólito et al. 2015). For example, Qin et al. (2017) suggested, for the first time, additional potential occurrence localities for a rare species (*Thuja sutchuenensis* Franch.) in China. Similarly, Hipólito et al. (2015) identified new potential areas of occurrence of a poorly studied and restricted distribution *Aristolocha gigantea* Mart & Zucc in Brazil. In this study however, areas of occurrence were mapped through a participatory geographic information system (PGIS) with the local communities followed by confirmation in the field. It is most likely that unoccupied areas may been derived from lack of dispersal and not because they are less suitable habitat (Gogol-Prokurat 2011). However, new records reflected very highly suitable areas for *W. salutaris*. Therefore, it is worthwhile to consider confirmation of additional areas for conservation and management of this species.

Results of this work also suggest substantial increase of the current highly suitable area for *W. salutaris* in the Matutuine and Namaacha district in both conservative (RCPs 4.5) and pessimistic climatic (RCPs 8.5) scenarios for the coming 50 years. Compared to other works, several reveal the decrease in potential suitable habitat for target species (cf. Abdelaal et al. 2019; Jinga et al. 2020). For example, simulation indicated that the highly suitable area of *Rosa Arabica* Crép. in Egypt will declined by 47.5% and 60% in 2050 under 2. 6 RCP and RCP 8.5, respectively of the current area. Predictions for 2070 also suggested reduction of 60% for RCP 2.6 and 72.5% for RCP 8.5 (Abdelaal et al 2019). In contrast, other works indicate an increase in suitable habitat for the modelled species (cf. Adjonou et al. 2020; Jinga and Palagi 2020; Martins 2019). For example, the expansion of climatic suitable habitat for *Hyphaena coriaceae* Gaertn. increased by 30% for RCP 4.5 and 38.4% for RCP 8.5 in 2050. For 2070, the major suitable areas increased of its current distributional area by 40.3% and 30.3% under RCP 4.5 and RCP 8.5, respectively in southern Mozambique (Martins 2019). Correspondingly, the proportion of climatic highly suitable area for *Detarium microcarpum* Guill. & Perr. Increase by 5.6% under CNRH-CM model and 8.8% under HadGEM2-ES model under RCP 8.5 in 2050 in Benin (Agbo et al. 2019). In this work the climatically highly suitable areas for *W. salutaris* increase in all scenarios for the four general circular models. As with other threatened species (cf. Pramanik et al. 2018; Zhang et al. 2019), information on trends of habitat suitability under the changing climate is important to establish long-term conservation and management of the species

2.4.3 Habitat quality and the extent of occurrence

Several authors have argued that climate change is the major threat to the distribution of many species (cf. Anderegg et al. 2015; Pramanik et al. 2018). However, results of this work suggest positive trends of habitat quality and the extent of occurrence for *W. salutaris* in Matutuine and Namaacha districts in both 4.5 and 8.5 RCPs in 2050 and 2070. Thus, for the coming 50 years, *W. salutaris* distribution is unlikely to decline due to climate change. This could be explained by the ability of each species to react to climate stochasticity, depending on the plasticity possessed (Fenollosa and Munné-Bosch 2019; Jinga and Palagi 2020). Thus, the resilience to the changing climate and dispersal ability of the species influence the expansion of distributional range (Nogués-Bravo et al. 2014). This trends have been reported in species such as the dry miombo (*Brachystegia spiciformis* Benth., *B. boehmii* Taub. and *Julbernardia globiflora* (Benth.) Troupin) whose area was predicted to increase by 17.3% and 22.7% in 2050 and 2070, respectively in south-central Africa countries such as Angola, Malawi, Mozambique, Tanzania, Zambia and Zambia (Jinga and Palagi 2020). Correspondingly, the overall higher suitable area of *Oxytenanthera abyssinica* (A.Rich.) Munro was predicted to expand at the maximum of 123% and 166.1% in 2050 and 2070, respectively, under RCP 8.5 in Ethiopia (Gebrewahid et al. 2020). In the current study, the expansion of climatic suitable area for *W. salutaris* could perhaps be associated with physiological traits also reported in other species elsewhere (cf. Augustine and Reinhardt 2019). Interesting, however, is that respondents from the three study areas knew the resilience of *W. salutaris* to the existing episodic drought, in comparison with other co-occurring native species (Senkoro et al. 2019), suggesting the existence of physiological trait against drought in *W. salutaris* individuals. Nevertheless, habitat suitability is also determined by several factors beyond climate, including ecology of the species, site attributes, site history and anthropogenic influences (cf. Bobrowski et al. 2017; Deb et al. 2017a, b; Phillips et al. 2006). Indeed, the model supports this concept because the distribution of *W. salutaris* has been affected mainly by unsustainable harvesting of the bark for trade throughout its southern African range (Botha et al. 2004; Dludlu et al. 2017; Krog et al. 2006; Mander et al. 2006; Mukamuri and Kozanayi 2014; Senkoro et al. 2019; Williams et al. 2014b). Other factors that might have affected the distribution of the species include early fall of fruits and seed parasitism (Hannweg et al. 2015; Muatinte and Cugala 2014), land use transformation (Botha et al. 2004; Halafo 1996), wildfires and overexploitation of the species for charcoal production (Senkoro et al. 2019). These issues should be addressed and integrated in the conservation and management of the species.

An increase in habitat quality and EOO can also be capitalised for conservation and management of the species (Liu et al. 2019). Results of SDMs can assist conservation and management of species including restoration (Gelviz-Gelvez et al. 2015; Liu et al. 2019; Swart et al. 2018; Wei et al. 2018; Yang et al. 2013; Zhang et al. 2018), re-introduction (Adhikari et al. 2012; Yang et al. 2013) and cultivation (Li et al. 2020; Tshabalala et al. 2020; Zhang et al. 2019). For example Qin et al. (2017) identified additional suitable areas for *T. sutchuenensis* that can be used for different purposes, including cultivation, in China. Pramanik et al. (2018) found suitable areas from modelling of the current and future distribution that could support pilot programmes of restoration and re-introduction of *G.*

indica in India. Correspondingly, Adhikari et al. (2012) revealed areas for re-introduction of *Ilex khasiana* Purk. in India that would also support restoration of its habitat and populations. The same was done for recuperation of *W. salutaris* in Zimbabwe (Maroyi 2012; Mukamuri and Kozanayi 2014). Equally, in the Kruger National Park, South Africa, a project engaged in the production for re-introduction of *W. salutaris* to the communities and in the wild (TreeSA 2020). In southern Mozambique, the decline of many *W. salutaris* populations led Senkoro et al. (2019) to suggest the need for re-introduction. In support of suitable areas and the length of the favourable period, this intervention may be adequate in areas once occupied by the species.

2.5 Implication for conservation of *W. salutaris*

Previous work indicated that *W. salutaris* is confined distributed in southern Mozambique (cf. Jansen and Mendes 1990; Krog et al. 2006; Halafo 1996; Senkoro et al. 2019), with the local communities relying heavily on agriculture and harvesting of NTFPs for their livelihood (GDM 2008; INE 2013a, 2013b). Among the highly traded NTFPs from southern Mozambique is *W. salutaris* which is believed to have reduced its distributional range due to overharvesting (cf. Hilton-Taylor et al. 1998; Mapaura and Timberlake 2002; Maroyi 2008). Nevertheless, the precise distribution of the species was lacking to define its effective management plan. Results found in this work established the current distribution areas. Future simulations suggested that suitable habitats for *W. salutaris* are expected to increase compared to the current distribution due to climate change. Therefore, I suggest the re-introduction of the species in areas previously occupied by the *W. salutaris*. Re-introduction of the species has also been reported elsewhere for threatened species (cf. Adhikari et al. 2012; Pramanik et al. 2018). For *W. salutaris*, probationary re-introduction was conducted in southeast Zimbabwe involving small-scale farmers (Veeman et al. 2014b). Such initiative could be used for the re-introduction of the species in southern Mozambique as part of the recovery plan of the species. With the expectation of increase of suitable areas for *W. salutaris* due to climate change, the initial effort could be invested in areas of high suitability within the current distribution and gradually extended to other suitable areas.

Use of *W. salutaris* bark for medicinal purposes has been the major cause of the decline of individuals of the species. This cause can be mitigated by creation of alternative source of bark supply through cultivation (cf. Botha et al. 2004; Cunningham 1993; Moyo et al. 2015) to ease the pressure in the wild. Different cultivation initiatives for *W. salutaris* have been considered in southern Africa. For example, the Silverglen Nursery (South Africa) initiative integrating traditional medicine practitioners and herbalists (Xaba and McVay 2010) and in a small- scale plantation in KwaZulu-Natal (van Wyk 2011). Cultivation was also reported in several botanical gardens in South Africa (cf. Nyaba et al. 2018; Soyingbe et al. 2018), Zimbabwe (Veeman et al. 2014b) and in the Mutema Highlands with seedlings from the Vumba Botanical Garden (Truscott 2019). In southern Mozambique, cultivation is done at the Eduardo Mondlane University Botanical Garden (personal observation) and is rarely done in home gardens by the communities in the study areas (Senkoro et al. 2019). Similar to re-introduction, in the predicted potential suitable areas, preliminary cultivation *in situ* could be done in small-scale and in home gardens and later, increase to the maximum potential of climatically suitable areas involving local communities, including traders and traditional medicine practitioners. Source of suitable material for re-introduction is important but challenges could be the source of plantlets for cultivation. Therefore, the effective implementation on re-introduction and cultivation, in southern Mozambique would require regional cooperation.

This work suggest that climate change will not interfer with the growth of *W. salutaris.* However, re-introduction and cultivation alone are unlikely to enhance the conservation of *W. salutaris* unless other threat to the species are addressed. Senkoro et al. (2019) revealed cutting of the species for charcoal production, wildfires and clearing of land for settlement affect individuals of *W. salutaris*. Muatinte and Cugala (2014) found infestation of *W. salutaris* fruit by fruit flies that may be affecting germination potential of the species. For effective conservation, these threats could be integrated in the management plan of the species.

2.6 Conclusion

The present work employed SDMs to develop a spatial model for the prediction of the potential distribution of *W. salutaris* in southern Mozambique under climate change. Modelling results, mostly explained by the climatic variables, revealed highly suitable areas, most of which coincide with areas of occurrence observed during the field survey. New areas of occurrence from the models, require additional field work for confirmation. Considering the need to anticipate the effects of a changing climate in the future for conservation planning

(Chatterjee et al. 2012; Sony et al. 2018), predictions by the four general circulation models suggest a likely considerable increase of potentially very highly suitable areas in 2050 and 2070 under conservative (4.5) and pessimistic (8.5) RCPs conditions in southern Mozambique, compared to the current situation. Modelling results also indicated favourable trends in habitat suitability and extent of occurrence in the coming 50 years. With the ongoing pressure on the species, primarily resulting from unsustainable harvesting of the bark for trade couple with the increase of climatic suitable area, re-introduction in areas once occupied by the species (cf. Pramanik et al. 2018) is crucial to enhance the populations in the wild. Regional collaboration through experience sharing for re-introduction and mass production of plantlets is also important.

Author contributions

A.M.S., C.M.S., R.A.V., and A.I.R.B. contributed to the conception and design of work. A.M.S., C.M.S., R.A.V. performed the field survey and collection of occurrence data. A.M.S. and C.M.S. contributed to data analysis and interpretation. D.D.M. contributed with methodological design, extraction and selection of environmental data, generation of the model and production of tables and figures. A.M.S. D.D.M. and C.M.S. wrote the first draft. All authors corrected and reviewed the paper.

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Chapter Three[1]: Uses, knowledge, and management of the threatened pepper-bark tree (*Warburgia salutaris*) in southern Mozambique

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Abstract

The present work sought to answer the following questions: Are the population under decline in southern Mozambique? Which conservation strategies have more probability to secure the existence of *W. salutaris* in southern Mozambique? It aimed to explore the cultural and socio-economic factors at the household level that influence exploitation and sustainable management. Stratified random, semi structured interviews were conducted (182) to heads of household complemented by 17 focus group discussions in the three study areas. *W. salutaris* was used medicinally to treat 12 health concerns, with the bark being the most commonly used part (92%). Knowledge of the species varied between the three areas, but not with respondent gender or age. Harvesting was mostly through vertical bark stripping (71% of informants). Overall, interviews revealed that populations of *W. salutaris* in southern Mozambique declined in the past due to bark trade and it was foreseen as the potential threat for the future of the species. The work also revealed a considerable numbers of substitute species (51 species) for *W. salutaris* and possible substitute of bark by leaves for the treatment of ailments. Although people knew sustainable harvesting procedures, its

[1] This chapter has been published in Economic Botany (2019), 73(3): 304–324.

implementation was doubtful because of continuous threat of *W. salutaris* due to high demand for the bark. To promote sustainable use of the species, multiple conservation approaches, including the use of alternative species with the same application, substitution of bark by leaves, and increases in alternative sources of plant material through cultivation were proposed. Additional information on species demography, harvest impact, and post-harvest bark recovery rate area is required. Information obtained in this work can contribute to management guidelines and plans for the species in Mozambique.

Key words: Folk medicine, gender, local ecological knowledge, quantitative ethnobotany, threatened species, trade.

3.1 Introduction

Lack of access to modern medical facilities and experts is one factor associated with the continued use of traditional medicine in developing countries. In sub-Saharan Africa, for example, the ratio of traditional medicinal practitioners to population is 1:500, compared to 1:40,000 for general physicians (Abdullahi 2011). Even where modern medical facilities are available, many medicinal plant species remain in popular demand for cultural, traditional, and financial reasons (Bach et al. 2014). Demand for such species is increasing with population growth due to their high cultural recognition and often relatively low cost of acquisition compared to contemporary pharmaceuticals (Cunningham 1993; Maroyi 2013).

Agrarian communities in developing countries often have unreliable sources of income and therefore engage in diverse livelihood strategies, including the use of wild species for subsistence, cultural observances, and income generation (Ghorbani et al. 2012; van Wilgen et al. 2013).The range of species collected and the amounts harvested depend on species availability and access (Silva et al. 2018), local and market preferences (Mukamuri and Kozanayi 2014), as well as traditions and culture (Franco-Maass 2019; Sylvester and Alvaro 2009). In some contexts, such dependence generates sustainable harvesting practices to maintain species populations, which thereby contributes to improving people’s well-being (Lima et al. 2013; Opperman et al. 2018; Shackleton et al. 2015; Stanley et al. 2012). However, in some instances, use of wild species translates to unsustainable consumption through overexploitation, often motivated by economic incentives from trade (Botha et al. 2004; Sylvester and Alvaro 2009).

Although in some settings medicinal plants are associated with home garden cultivation or other anthropogenic habitats (Voeks 2004), in sub-Saharan Africa most medicinal plants are harvested from natural habitats (Cunningham 1993), with very few derived from cultivated sources (Botha et al. 2004; Maroyi 2013). In some instances and under weak governance, harvesting from wild populations may pose conservation challenges for the targeted species, as well as co-occurring biodiversity, habitat integrity, and ecosystem processes (Kideghesho 2009; Shackleton et al. 2018). In Africa, resource use in the historical past was sustainable for most species, due to low human populations and low demand, along with traditional approaches based on social and cultural values (Cunningham 1993). However, growing human populations and commercial demand are undermining the viability of traditional practices for some species (Botha et al. 2004), especially those characterized by narrow distributions, small populations (Moyo et al. 2015), slow growth rates (Zschocke et al. 2000), constrained recruitment and sensitivity to land use pressures such as fire (Botha et al. 2004), and browsing (Kouki et al. 2004). Persistence of unsustainable practices will likely result in the local or regional loss of some medicinal species in high demand, thereby negatively impacting the very basis of primary health care for millions of people (Giday et al. 2003).

A number of strategies are available to manage and conserve species in high demand. In particular, for culturally important species such as medicinal plants, integration of local ecological knowledge (LEK) and practices into management strategies is important to foster common visions, goals, and compliance. Local ecological knowledge, defined here as information, practices, and principles, accumulated and transmitted through generations, based on observation and interaction with the natural environment, has been used to support community-based management of species and sites (Charnley et al. 2007). This includes a variety of approaches such as rotational harvesting to avoid overharvesting to allow for regeneration, selective harvesting of only mature individuals and allowing time for regeneration (Terer et al. 2012), and regulating harvesting time according to critical life phases of the species (Schmidt and Ticktin 2012). Additionally, substitution of species with the same application provides alternatives for treatment (Albuquerque and Oliveira 2007) and may ease the pressure on certain species. For example, the use of *Paris forrestii* (Takht.) H. Li. in place of *Paris polyphylla* var. *yunnanensis* (Franch.) Hand.-Mazz. for anti-cancer treatment and the replacement of a traditional papyrus, *Cyperus madagascariensis* (Willd.) Roem. & Schult. by invasive *Eichhornia crassipes* (Mart.) Solms for handicraft have been reported by Wang et al. (2018) and Rakotoarisoa et al. (2016), respectively. It is frequently assumed that the greater the reliance on a particular species, the greater will be the motivation for valuing the species and the deployment of local ecological knowledge to underpin sustainable uses (Ghorbani et al. 2012; Terer et al. 2012).

Warburgia salutaris (G.Bertol.) Chiov. (Canellaceae), commonly known as the pepper-bark tree, is a protected medicinal species in high demand in eastern and southern Africa (van Wyk and Wink 2004). The tree is generally 5 to 10 meters tall, but occasionally grows up to 20 meters. The species occupies evergreen forests, wooded ravines, and bushveld (Coates-Palgrave 2002). It is used to treat a number of ailments including the common cold, sinus, and chest complaints (van Wyk and Wink 2004), inflammation of the gums, throat problems, and mouth sores (Jansen and Mendes 1990). The active compounds (drimanes and sesquiterpenoides) are mostly found in the inner part of the stem and root bark. Additionally, the peppery aroma of *W. salutaris* leaves makes them an attractive condiment for different food dishes and beverages (Venter and Venter 1996). Due to the high demand for pepper-bark, it is regarded as highly threatened throughout its southern African range (Botha et al. 2004), driven by local subsistence uses and high commercial demand in urban centres.

Despite the high demand for the species, its ecology, local knowledge, and management are not well understood. Research has been centred on its medicinal uses (e.g.,Maroyi 2013, 2014; van Wyk and Wink 2004), on the potential of the chemically active compounds in treating fungal (Samie and Mashau 2013) and bacterial (van Wyk and Wink 2004) infections, and on informal trade (Krog et al. 2006; Mander et al. 2006). In comparison, assessments of population status and local knowledge are limited, and none have been done in Mozambique. Botha et al. (2004) compared the status of populations in protected and non-protected areas in Mpumalanga, South Africa. Mirroring the situation with many tree species that are subjected to high demand for bark, ring-barking (peeling of a bark strip embracing the whole stem circumference [Delvaux et al. 2010]) is common among *W. salutaris* populations. Recently, Dludlu et al. (2017) surveyed the status of populations throughout Swaziland, reporting widespread ring-barking, even in protected areas, but they also discovered several hitherto unknown populations. Mukamuri and Kozanayi (2014) attributed such destructive practices within communal lands to the ineffectiveness of institutional structures for the management of natural resources, including, but not limited to, lack of tenure and economic hardship in Zimbabwe. Veeman et al. (2014a, 2014b) focused on the economics of the species in Zimbabwe. The first work found few, isolated, and small-scale markets for medicinal bark species, including *W.* s*alutaris*. The second study suggested that under high bark production due to re-introduction of the species and favourable prices, the species would be able to sustain high economic returns. Moreover, Williams et al. (2014a) determined the bark area, volume, and mass traded for six overharvested medicinal bark species, including *W. salutaris*, in South Africa, and concluded that bark mass (available bark) was directly proportional to the size of the stem. Through data on bark thickness from a market survey in Johannesburg, South Africa, Williams et al. (2014b) reported a decrease between 1995 and 2001 in the number of trees harvested for bark supply and changes in availability of bark from large individuals. There have been, however, no studies of the LEK of *W. salutaris* nor of the local perceptions of factors that influence its population status.

Conservation initiatives are unlikely to succeed without the cooperation of local communities on whose land the populations are found. Thus, communities need to be integrated into strategies for managing supply and demand for pepper-bark. Long-standing cultivation initiatives in this region include a) the supply of numerous cuttings for cultivation by traditional medicine practitioners and herbalists from the Silverglen nursery (Xaba and McVay 2010), and b) research to identify efficient propagation methods for the species in and around Kruger National Park, where over 40,000 plants from seeds were made available for distribution (Hannweg et al. 2015). Such initiatives in South Africa also resulted in the reintroduction of cultivated material from South Africa to Zimbabwe (Maroyi 2012), and similar initiatives could also be established in southern Mozambique. With this in mind, the present work sough to address the following questions: Are the population under decline in southern Mozambique? Which conservation strategies have more probability to secure the existence of *W. salutaris* in southern Mozambique? It aimed to a) identify the uses of *W. salutaris*, b) assess variations in use, knowledge and LEK within the study areas and by respondent attributes, and c) explore local management practices of *W. salutaris* in Mozambique.

3.2 Methods

3.2.1 Study area

See detailed information of the study areas on section 1.6.

3.2.2 Data collection

Authorisation for the research was granted by traditional and local leaders, as well as ethics approval by the departmental committee at Rhodes University (November 2015). Prior informed consent was obtained from respondents before conducting interviews. A stratified random sampling procedure was employed to select people who knew the species. Household interviews were carried out in: 1) Tembe River (Manhihane, Djabula, Kazimat, Porto Henrique, and Monucua villages); 2) Futi Corridor (Huco, Mussongue, Massale, Mabucutso, Phuza); and 3) Lebombo Mountains (Goba Sede, Goba Fronteira, and Macanda villages). Sixty to 62 interviews per study area (182 in total) with the heads of households were conducted between April and September 2016. The questions asked respondents to: a) identify cultural and social aspects of *W. salutaris* use, including reasons for use and parts used, substitute species, restrictions, and demands for the species; (b) uses and knowledge by informant gender and age; c) local management practices; and d) the LEK, comprising habitat preferences and phenology. Past and expected trends in local abundance and status were considered: a) before the civil war (1977); b) post the civil war until the present (1992–2015); and c) into the future (2016+). Demographic information of each informant was recorded, such as age, gender, and origin (born in the region or immigrant). Additionally, one or two focus group discussions per village (17 in total) were conducted with five to seven key informants identified by local traditional leaders to explore in-depth knowledge of any pertinent issues that were raised during the household interviews.

3.2.3 Data analysis

Following Phillips and Gentry (1993) the species use value (UV) was calculated as the average number of uses identified by informants. Along with the use value, an informant's diversity index and informant's equitability index were computed (Byg and Balslev 2001; Monteiro et al. 2006), but as the three were highly correlated, the last two were not reported. Because the data were not normally distributed (Shapiro-Wilk test), non-parametric analyses were favoured. Kruskal-Wallis and Mann-Whitney U tests were used to evaluate the differences between respondents in the three study areas. The Spearman correlation coefficient (rho) was employed to analyse the relationship between the use value and the age of informants. Based on the information synthesis from the focus group discussions, variables relating to the ecological information provided by respondents were coded in three response groups: no knowledge (score 0), elementary knowledge (1), and profound knowledge (2)

(Ghimire et al. 2004; Ticktin and Johns 2002). A Kruskal-Wallis test was performed to evaluate the differences in LEK (reflected in the coded responses) between respondents in the three study areas. Statistical analyses were conducted using SPSS 20 and STATISTICA 13, at a significance level of 95%.

3.3 Results

3.3.1 Respondent profiles

The respondent households were largely agrarian, with income supplemented by charcoal production and trade in the Lebombo Mountains and Tembe River areas, and by palm wine production and sales in the Futi area. Formal education levels were low. The proportion of immigrants was low in the Futi Corridor, high in Lebombo Mountains, and intermediate in Tembe River (Table 3.1).

Table 3.1. Respondent characteristics in the three study areas. (LM= Lebombo Mountains, TR= Tembe River, FC= Futi Corridor). [1]The number of people who moved into the area during the civil war is in brackets.

Attribute	LM (n=60)	TR (n=62)	FC (n=60)	Mean
Age range (yrs)	23-72	22-72	22-76	46.2
Origin:				
• Indigenous	16	31	48	31.7
• Immigrants[1]	44(13)	31(6)	12(3)	29(7.3)
Major household activities:				
• Agriculture	56	62	45	54.3
• Charcoal production & sales	29	37	0	22
• Permanent job	35	7	6	16
• Palm wine production & sales	0	1	21	7.3
Education levels:				
• Illiterate	21	25	31	25.7
• Primary education	38	37	27	34
• Secondary education	1	0	2	1
Gender:				
• Female	29	24	12	22
• Male	31	38	48	38.7

3.3.2 Uses, demand, restrictions, and alternatives

3.3.2.1 Local uses for the species

All but one respondent (in FC, who said that local populations were too far away) reported using *W. salutaris* for medicinal purposes, treating one or more of 12 health concerns. These included respiratory tract ailments (cough, asthma, and the common cold), digestive tract ailments (mouth and throat sores, dysentery, stomach ache, and haemorrhoids), fortification of children, fevers, headaches, body pains, and malaria. *W. salutaris* was mainly used for coughs (71.4% of households) and for mouth and throat sores (61%) (Table 3.2). Out of the 12 ailments, 10 were mentioned in TR, nine in FC, and eight in LM.

Bark was the most widely used part of the tree (92.2% of households) (Table 3.2). Of the 182 respondents, 69.2% used only the bark and 14.3% used bark and leaves. Bark was employed in the treatment of 11 ailments except for fortification of children. Leaves and roots were each used for the treatment of nine ailments (including the recurring ailments in the study areas), but were not commonly used (22% households used leaves and 12% roots) (Table 3.2).

Table 3.2. Plant parts used for specific health concerns by respondents in the three study areas. % Resp: refers to the percentage of respondents except in the No. of ailments and No. of parts used; No: number.

Health concern	Bark (% Resp.)	Leaves (% Resp.)	Roots (% Resp.)	Branches (% Resp.)	Trunk (% Resp.)	No. of parts used
No. of ailments	12	9	9	1	3	-
Households using that part	92.2	22	12	0.5	0.5	-
Asthma	4.9	2.2	1.1			3
Common cold	1.1	0.5				2
Body pains	3.2	0.5	0.5	0.5		4
Cough	71.4	9.9	4.4		0.5	4
Dysentery	1.1					1
Fever	1.1	7.1				2
Fortification			1.1			1
Haemorrhoid	14.3	1.6	2.2		0.5	4
Headache	5.5	1.6	0.5			3
Malaria	1.1		0.5			2
Mouth and throat sores	61	4.4	3.8		0.5	4
Stomach ache	3.8	1.1	1.1			3

3.3.2.2 Alternative species

Fifty-one species were mentioned as possible substitutes for *W. salutaris*, each treating from one to four ailments. The LM area had the most alternative species (37), followed by TR (33 species), and FC (17 species). The most mentioned alternative species in the LM were *Sclerocraya birrea* subsp. *caffra* (A.Rich.) Hochst. and *Citrus limon* (L.) Osbeck. In the TR they were *Terminalia sericea* Burch. ex DC, *S. birrea*, *Eucalyptus camadulensis* Dehnh., and *Anacardium occidentale* L., and in the FC, “macuene” (an unidentified species) and *Ficus* sp. All the health problems mentioned had plant substitutes for treatment, and the most common health problems had several alternative species (e.g., cough 44 species, mouth and throat sores 19, and haemorrhoids 15).

3.3.2.3 Harvesting restrictions and demands

Almost equal proportions of respondents said that there were (39%) or there were not (43%) any restrictions regarding who can collect pepper-bark. Households in the LM and the FC harvested less due to the long distances to collection grounds, i.e., 0.2 to 5.1 km in LM and 0.5 to 14.2 km in FC, compared to 0.006 to 2.6 km in TR, where the species was mostly found relatively close to homes. The presence of elephants in the Futi Corridor was an additional concern to harvesters. About 71% of households harvested *W. salutaris* themselves (98.4% TR, 63.3% FC, and 50% LM), whereas 16.5% acquired it from other community members (33.3% LM, 15% FC, and 1.6% TR), and the rest used a combination of procurement means, i.e., a) markets or other community members, b) markets or self-harvesting, and c) other community members or self-harvesting.

Most respondents said that there was no specific season (66.5% in rainy season and 65.9% dry season) or time of the day (71.4%) for harvesting. Approximately half (51.1%) indicated there were no areas where harvest is restricted, whereas 19.2% said there are some zoning restrictions. The remaining households were unaware of spatial harvesting restrictions. Quantities harvested by most households (72.5%) were very small, as were the quantities used, mostly a finger-sized piece of bark (56%), and harvesting was infrequent.

Of the 182 respondents, only 2.2% reported currently selling pepper-bark. However, 10.4% did so in the past, but are no longer selling it (Table 3.3). Those who were currently selling were from FC (6.7%), and those who sold it in the past were from the TR (14.5%), FC (10%), and LM (6.7%). However, 12.1% of respondents were not sure about the involvement of other household members in trade of the species (8.3% of LM respondents, 8.1% of TR, and 20% of FC). The existing trade was done more than twice a week by traditional medicine practitioners (1.1% of FC respondents), and of the remaining less than twice a year. The amount of bark currently sold varies from one teaspoonful (3% of the FC respondents), four pieces (equivalent to a palm size piece), and three bags of 50 kg, in each case cited by a single format.

Slightly less than one-third (30.8%) of respondents said that they knew someone who used to be involved in the trade (that is 35.5% TR respondents, 31.7% FC, and 25% LM), but 56% claimed not to know anyone that traded in the past. Approximately half of the respondents (51.1%) stated that currently, no outsiders from other communities come to collect *W. salutaris* bark in their area TR (67.7%), LM (60%), and FC (25%). The presence of collectors from outside the communities cited by 30.2% of respondents was largely mentioned in FC (56.7%). Those who pointed to external collectors (24.2% of respondents) knew their provenance (46.7% from FC, 21% from TR, and 5% from LM). Of those who reported knowing the provenance of outside collectors, 21.7% mentioned that people were coming sometimes (once in three or more months per year) to collect in FC, 16.1% from TR, and 1.7% from LM. Those who reported regular collections by outsiders (once a month) all were from FC (20%), and those who came rarely (once in two or more years) were few (varying from 3.3% in LM to 8.1% TR) in the study areas (Table 3.3). People coming to collect in LM and TR were reported to be family members from adjacent communities.

3.3.2.4 Use variation according to area, gender, and age

The average number of uses identified by informants (use value) for pepper-bark differed significantly between the three areas ($H=17.3$; $p<0.05$), mostly between the Tembe River and the Futi Corridor. On average, households in the TR listed 2.3±0.9 uses for *W. salutaris*, whereas those in the LM and FC mentioned 1.9±0.7 and 1.6±0.7 uses, respectively. The mean use value also differed between women in the three areas ($H=14.24$, $p<0.05$). Generally, female respondents in TR reported the highest values of 2.3±1.0 and the FC the lowest (1.3±0.5). Equally, the use value varied between men ($H=9.3$, $p<0.05$) in the three areas, with the highest value in the TR (2.2±0.9) and the lowest in FC (1.7±0.8). Within study areas, men had higher use values than women (2.1±0.7 men and 1.9±0.7 women in LM, 2.2±0.9 and

2.3±1.0 TR, 1.7±0.8 and 1.3±0.5 in FC), but the difference was significant only in the LM ($p<0.05$).

There was no statistical correlation between informant's age and the use value ($p>0.05$) in the three areas. The Spearman correlation coefficient (rho) was 0.212 in the LM, -0.002 in FC, and -0.116 in TR.

Table 3.3. Involvement in bark collection and trade in the three study areas. (LM= Lebombo Mountains, TR= Tembe River, and FC= Futi Corridor). 1 Refers to the provenance of non-local harvesters.

Description	Response	% respondents			
		All (n=182)	LM (n=60)	TR (n=62)	FC (n=60)
Current trade	Yes	2.2	0	0	6.7
	No	97.8	100	100	93.3
Past trade	Yes	10.4	6.7	14.5	10
	No	77.5	85	77.4	70
	Do not know	12.1	8.3	8.1	20
Community members (past traders)	Yes	30.8	25	35.5	31.7
	No	56	60	59.7	48.3
	Do not know	13.2	15	4.8	20
	N/A	0	0	0	0
Other communities (collectors)	Yes	30.2	10	24.2	56.7
	No	51.1	60	67.7	25
	Do not know	18.7	30	8.1	18.3
	N/A	0	0	0	0
Origin[1] (other communities)	Yes	24.2	5	21	46.7
	No	3.8	1.7	3.2	6.7
	Do not know	2.2	3.3	0	3.3
	N/A	69.8	90	75.8	43.3
Frequency of collection	Regularly	6.6	0	0	20
	Sometimes	13.2	1.7	16.1	21.7
	Rarely	5.5	3.3	8.1	5
	Do not know	1.6	0	0	5
	N/A	73.1	95	75.8	48.3

3.3.3 Harvesting Methods and Management

Three harvesting practices were reported. These included discontinued vertical strips (bark stripping on the length of trunk) mentioned by 71.4% of respondents (100% from TR, 61.7% FC, and 51.7% LM), ring-barking (0.5% corresponding to 1.7% of LM respondents), and cutting of branches (1.1%). Most people only used a machete (64.3%), corresponding to 98.4% from TR, 517% FC, and 41.7% LM, while other instruments (axes, stones, and knives) separately or together, were rarely used (ranging from zero to five percent of respondents). Bark was most typically harvested from the middle of the tree trunk.

Most respondents (58.8%) knew methods of bark harvesting that reduced damage to the plant, especially in the Tembe River (Table 3.4), including taking non-continuous vertical strips (17.8%), harvesting only small quantities (16.1%), and superficial wounds (13.9%). Less frequently mentioned harvesting approaches included not harvesting at the base of the stem (3.3%), rotational harvesting (1.1%), and using a knife (0.5%).

Approximately two-thirds (64.8%) of respondents could identify harvesting approaches that result in significant damage to plants, ranging from 50% in the Lebombo Mountains and the Futi Corridor to 91.1% in the Tembe River(Table 3.4). The method that was considered to be most damaging was ring-barking (54.8%). Other procedures mentioned less often included deep wounds (8.8% of respondents), harvesting large quantities (13.7%), repeat harvests (1.1%), and harvesting from small trees (1.1%). Even though conservation of *W. salutaris* was viewed as important by almost all of the respondents (93.4%), many (41.2%) reported an absence of specific actions to conserve the species (Table 3.4). Equally, very few households (6.6%) cultivated the species in their home gardens. However, some households (38.5%) mentioned existing measures related to the conservation of the species, including harvesting approaches (stripping small quantities, non-continuous vertical stripping) strictly for household use, avoiding uncontrolled fires, and investing in on-farm conservation (varying from 6% to 11% of respondents). Most of these (59.7%) were from the TR (Table 3.4).

Table 3.4. Harvesting and conservation practices for pepper-bark tree (*Warburgia salutaris*) (% of respondents). (LM= Lebombo Mountains, TR= Tembe River, FC= Futi Corridor).

Description	Response	% of respondents			
		All (n=182)	LM (n=60)	TR (n=62)	FC (n=60)
Less damage (methods)	Yes	58.8	48.3	75.8	51.7
	No	1.1	3.3	0	0
	Do not know	18.7	6.7	24.2	25.0
	N/A	21.4	41.7	0	23.3
More damage (method)	Yes	64.8	50.0	91.9	51.7
	No	1.1	1.7	1.6	0
	Do not know	12.6	6.7	6.5	25.0
	N/A	21.4	41.7	0	23.3
More damage (season)	Yes	18.7	13.3	19.4	23.3
	No	36.8	30.0	66.1	13.3
	Do not know	21.4	15.0	14.5	35.0
	N/A	23.1	41.7	0	28.3
Specific conservation action	Yes	38.5	26.7	59.7	28.3
	No	41.2	35.0	40.3	48.3
	N/A	20.3	38.3	0	23.3
Cultivation	Yes	6.6	6.7	1.6	11.7
	No	93.4	93.3	98.4	88.3
Conservation importance	Yes	98.9	100.0	100.0	96.7
	No	0	0	0	0
	Do not know	1.1	0	0	3.3

3.3.4 Local Ecological Knowledge

Knowledge related to the various ecological niches favoured by *W. salutaris* varied significantly between the three areas. In almost all cases (except non-habitat characteristics and reasons behind changes in abundance after the civil war), the Tembe River communities demonstrated more knowledge than the other two areas (Table 3.5).

Respondents mentioned 17 characteristics that described the favoured habitats for *W. salutaris*. The variation in knowledge was mainly in terms of soil texture, colour and fertility, occurrence near water bodies, shady areas, rocky substrates, lowlands, mountains, and termite mounds. Many households (50.5%, ranging from 21.7% in LM to 77.4% in TR) mentioned clayey soils as the main characteristic, followed by areas situated near water courses (22.5 % corresponding to 25% from FC, 24.2% TR, and 18.3%LM), and black soils (19.8%), mainly from TR (41.9%). Eleven conditions were mentioned as being avoided by the species, but

43.9% of the informants had no knowledge in this regard. The main characteristic reported was sandy soils (34%), mainly by the FC informants (60%).

Table 3.5. Mean scores (+ SD) of local ecological knowledge of *Warburgia salutaris* in the three areas (n=182). (LM= Lebombo Mountains, TR= Tembe River, FC= the Futi Corridor) and comparison of scores between the respondents in the three areas (p-value).

Variable	All (scores mean±SD)	LM (scores mean±SD)	TR (scores mean±SD)	FC (scores mean±SD)	p-value
Habitat characteristics	0.8±0.5	0.7±0.7	1.0±0.3	0.8± 0.4	0.0017
Non habitat characteristics	0.5±0.5	0.4±0.5	0.3±0.5	0.7±0.5	0.0004
Flowering month	0.2±0.6	0.1±0.4	0.4±0.8	0.0±0.3	0.003
Pollinators	0.3±0.7	0.2±0.6	0.7±1.0	0.1±0.4	<0.0001
Status (post-civil war)	1.1±1.0	0.8±1.0	1.5±0.9	1.0±1.0	0.0001
Reasons (abundance post-civil war)	0.7±0.8	0.5±0.7	0.8±0.4	0.9±1.0	0.0039
Future trends	1.1±0.9	0.7±0.9	1.6±0.7	1.1±1.0	<0.0001
Reason (future trends)	0.7±0.6	0.5±0.6	0.9±0.5	0.7±0.7	0.0001
Drought sensitivity	1.4±0.9	1.0±1.0	1.8±0.6	1.4±0.9	<0.0001

Very few respondents (8.2%) in the three study areas had knowledge of the flowering time of *W. salutaris,* with the highest response from TR. In addition, potential pollinators, mostly bees, were identified by very few people (16.5%), also with the highest response from TR.

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More than half of the respondents (54.4%) across the three areas stated that the abundance of *W. salutaris* had declined in their areas (ranging from 38.3% in LM to 75.8% in TR). Four drivers were identified as underpinning this perceived decrease, namely the bark trade (30.8%), cutting for charcoal production (22.5%), wildfires (12.6%), and opening up land for construction (0.5%). Approximately one-third of the respondents (37%), mostly from TR, felt that the abundance of the species was reduced by harvesting for charcoal and the bark trade. Additionally, 14.3% of respondents felt that the abundance was likely to decrease in the future, largely as a consequence of the bark trade (12.1%). Other reasons mentioned included prolonged drought (1.6%), exploitation for charcoal (0.5%), absence of harvest norms in the community, and scarcity of the species (0.5%).

Most people (70%) perceived *W. salutaris* to be drought tolerant relative to other tree species as a baseline for comparison. The TR communities were more knowledgeable than others (90.3%), while FC had 68.3% and LM 50%. This was based on the prolonged drought during the sampling period where some species lost leaves, and others died, but *W. salutaris* remained evergreen throughout the year even during a drought.

3.4 Discussion

3.4.1 Uses, demand, restrictions, and alternatives

3.4.1.1 Local uses for the species

The results revealed 12 local medicinal uses for pepper-bark tree, including for common ailments such as colds, chest complaints, coughs, malaria, and headaches. The use of *W. salutaris* to treat these health problems has been widely reported in southern Africa (Jansen and Mendes 1990; Maroyi 2013, 2014; van Wyk and Wink 2004). In Kenya *W. salutaris* is among the most valuable medicinal species for malaria treatment, and is also used for chest complaints or pneumonia (Bussmann et al. 2006). Medicinal uses mentioned in this work corresponded to 36.7% of those reported for *Warburgia* genus in southern and eastern Africa (Maroyi 2014). The results did not reveal any non-medicinal uses for the species (e.g., fencing and for food) (Venter and Venter 1996), or ethnoveterinary remedies for treating livestock (Grande et al. 2009).

Almost all parts of the plant were used, with bark being the most preferred, as has also been reported elsewhere in southern Africa (cf. van Wyk and Wink 2004). High demand for the bark in the region is regarded as the main cause for the decline of the species (Botha et al. 2004; Coates-Palgrave 2002). For example, out of 15 applications recorded in Zimbabwe, all but one used bark or roots, whereas in South Africa, 38 uses employing *W. salutaris* all used bark, in some cases in combination with leaves or roots (Maroyi 2014). Some authors argue that the substitution of bark by leaves could contribute to the conservation of the species (Zschocke et al. 2000). Generally, the use of leaves by the respondents was low, but they did cover a considerable number of ailments, including common ones. Consequently, as recommended by Zschocke et al. (2000), analysis of the chemical compounds and relative efficacy in different organs of the plant is required. Until then, it is worthwhile considering substitution of bark by leaves as one possible conservation strategy for *W. salutaris* in southern Mozambique. Involvement of traditional medicine practitioners will facilitate adoption of the strategy if they prescribe leaves instead of bark (Zschocke et al. 2000).

3.4.1.2 Alternative species

Respondents reported many substitute species for *W. salutaris,* as has been reported for several other medicinal species of concern in different countries. For example, *Cryptocarya* spp. are used as substitutes for *Ocotea bullata* (Burch.) Baill. in South Africa (Zschocke and van Staden 2000), and in New York City, a number of species have been substituted in the traditional healing flora of Candomblé adherents (Fonseca and Balick 2018). The availability and use of alternatives is a potentially important conservation strategy to avoid or limit further population declines (Wang et al. 2018; Zschocke and van Staden 2000). However, challenges might be encountered because of people's beliefs around specific species which may constrain their willingness to adopt the measure. For example, the conviction of people in Zimbabwe about the superior medicinal value of *W. salutaris* makes it difficult to promote substitute species (Mukamuri and Kozanayi 2014), even though some alternative species may not necessarily be of lower quality. For example, *Cryptocarya* spp. (including rarely used species within the genus) are more effective than *O. bullata* and were qualified substitutes (Zschocke and van Staden 2000). Equally, in a list of substitute species for medicinal *Myracrodruon urundeuva* Fr. All., and in consensus of communities, *A. occidentale* was selected as a better substitute by two semi-arid rural communities of northeastern Brazil

(Monteiro et al. 2006). Our results revealed a considerable number of species that can be used to replace *W. salutaris*, e.g., *S. birrea* in LM, *T. sericea* in TR, and *Ficus* sp. in FC.

3.4.1.3 Harvest restriction and demand

The results indicated that there were no local restrictions in the three areas on who collects *W. salutaris*, the plant parts collected, harvesting season, and gathering time of day. However, more than half of the people in the Futi Corridor mentioned the existence of spatial harvesting restrictions in their area. The disagreement between the respondents on restriction of collectors could be explained by the long distance from the homestead to collection grounds in the Lebombo Mountains and Futi Corridor areas. Collection in these areas was normally done by men due to the long distance to collection sites, similar to the situation for men in the Caura River Basin (Venezuelan Guayanas) communities, who are working away from home (Souto and Ticktin 2012). However, in this study, this seems to be a case of spatial division of labour, having men working far from home in natural areas (Voeks 2007), rather than a restriction. Furthermore, human-elephant conflict is a problem in the study area, which also constrains access to species populations. Thus, only braver people with an understanding of elephant movement along the Futi Corridor might harvest NTFPs in that region. In addition, community members travelling in vehicles on their way home from Phuza Fair (occurring twice a week) or from South Africa are reported to stop at times and harvest *W. salutaris*.

Spatial restriction of harvest was only mentioned in the Futi Corridor, which is a protected area established as an extension of the Maputo Special Reserve (GM 2011), with the aim of enhancing the protection of local wildlife, especially elephants. Although community members were aware of harvest restrictions, they have long been collecting in the area because *W. salutaris* is mostly confined to the immediate area. Some legally protected areas, like the Futi Corridor, serve to limit people's rights of access to traditional resources, even those who use traditional harvesting methods (Ward et al. 2017). On the other hand, credit needs to be given to informal institutions promoting and regulating sustainable use practices by encouraging voluntary compliance (Colding and Folke 2001). With the absence of cultural restrictions, the existence of spatial restrictions could be capitalized upon for protection of multiple species, including the target endangered species. However, it is challenging to spatially restrict harvesting for resources that are deeply embedded in local cultures and livelihoods (Kideghesho 2009). Further work on the population status and dynamics of *W. salutaris* could cast light on the effectiveness of the Futi Corridor as a protected area.

The results revealed that most respondents harvested only small amounts of plant material, and relatively infrequently. Very few confirmed their own involvement or knew community members who were involved in the past or present trade of *W. salutaris* bark. However, previous work in southern Africa (e.g., Botha et al. 2004; Krog et al. 2006; Mander et al. 2006) has reported extensive bark harvesting and trade networks. Mozambique is known to supply *W. salutaris* bark to urban medicinal markets of Maputo province (Krog et al. 2006) and to neighbouring countries (Mander et al. 2006; Veeman et al. 2014b). I acknowledge that there might have been some under-reporting by the respondents in the survey as some may have worried about us informing authorities. Not surprisingly, under-reporting and denial of involvement is common when investigating practices that are non-compliant with formal or informal regulations or laws (Jann et al. 2012; Tourangeau and Yan 2007). One way of investigating illegal activities is through asking questions in an indirect manner (Nuno and St. John 2014). For example, in this study, when exploring LEK, most respondents mentioned the bark trade as one of the primary drivers of current and likely future decline of the species in the study areas, but did not admit to personal involvement. This suggests that the bark trade is still practiced and is a potential threat in the study areas. Another possibility is that the bulk of the bark harvested and sold in the three areas is done by only a few individuals, representing only a very small proportion of the population and the sample. It is noteworthy that some respondents were involved in the trade in the past, but currently the bark trade is reported only in the Futi Corridor. I suspect that past involvement was a partial consequence of the civil war in Mozambique, which undermined normal agricultural activities and livelihoods, and thus many households turned to whatever means possible to survive, including trade in high value resources. It appears, however, that illegal trading still persists in Futi Corridor. A study on local livelihoods and incomes and harvest impact (bark damage) are required to shed more light on this.

3.4.2 Harvesting methods and management

Bark was the most frequently harvested part of the pepper-bark tree, normally through vertical strips removed from the middle of the stem using a machete. Most of the households could identify harvesting methods that they believed limited damage to the plant, as well as the opposite, i.e., harvesting procedures that could result in more damage to the harvested tree. Most respondents regarded ring-barking as the most inappropriate harvesting method as opposed to vertical stripping. This is because ring-barking results in removal of all the cambium, impeding the regrowth of living tissue around the wound that results in bark regeneration. With the removal of the inner bark, transportation of photosynthates from leaves to the roots is blocked, ultimately causing plant death (Delvaux et al. 2010). Appropriate harvesting techniques can limit damage to the inner bark, allowing for bark regeneration (Pandey 2015; Stewart 2009). For example, regeneration of six medicinal tree species in India was improved if the inner bark was not harvested (Pandey 2015), as was the case with twelve species in Benin (Delvaux et al. 2010). This is because shallow damage to the bark is unlikely to affect the cambium (Baldauf et al. 2014; Romero 2014), and thus still allows the flow of sugars from leaves to the roots.

An alternative approach was the harvesting of only small areas of bark. This allows bark to regenerate because only a small portion of the cambium is damaged. For this type of harvest, Chen et al. (2014) reported the formation of new periderm and wound cambium from callus, and the wounded cambium subsequently forms new phloem. The presence of cambium determines the survival of the tree, and the applied harvesting practices allow bark regeneration through preservation of the cambium. Therefore, work on the recovery rates post-bark harvesting is important for recommendations of sustainable harvesting approaches (Delvaux et al. 2010; Pandey 2015).

Harvesting of other parts of *W. salutaris* was uncommon. However, according to Zschocke et al. (2000), bark and underground parts are some of the most used in South Africa. The authors suggested substitution by aerial parts, such as leaves, to reduce the negative effects of harvesting. This would first require validation of the therapeutic efficacy of the leaves.

Although *W. salutaris* was viewed as an important resource by the surveyed households, there were very few deliberate actions to maintain trees or populations, and cultivation in home gardens was uncommon. Home gardens are most often used to produce fruit trees, vegetables, and herbaceous medicinal plants, rather than perennial medicinal trees (cf. Panyadee et al. 2018; Williams et al. 2018). Notable exceptions are usually multipurpose trees such as the drumstick tree (*Moringa oleifera* Lam.), the African baobab (*Adansonia digitata* L.), and African mahogany (*Khaya senegalensis* [Desv.] A.Juss.) (Agundez et al.

2018; Gandji et al. 2018; Gaoue and Ticktin 2007). The *in situ* cultivation of *W. salutaris* practiced by a few community members in the study areas is done through transplanting suckers (root sprouts). For species of conservation concern, the use of suckers may be a strategy for widespread propagation and distribution, although it would need to be from multiple populations to maintain genetic diversity. Vegetative propagation of *W. salutaris* is also possible through tissue culture, as demonstrated in a large program in neighbouring South Africa (Hannweg et al. 2015; Mbambezeli 2004). Additionally, *W. salutaris* is been cultivated *ex situ* in South Africa (Mbambezeli 2004), Zimbabwe (Veeman et al. 2014b), and in Mozambique (personal observation), which would provide strong bases for possible cultivation. Moreover, the experience of reintroduction into its natural habitat in Zimbabwe (Veeman et al. 2014b) could also be capitalized for *in situ* home gardening in southern Mozambique. As elsewhere in the world, home gardening of native species for people living in close proximity to relatively intact habitats is uncommon because people can acquire products easily in the wild (Kujawska et al. 2018). Nevertheless, it is a potential practice for conservation of many species of concern that are important for subsistence or culture (Barbhuiya et al. 2016; Das and Das 2015; Huai et al. 2011). And the practice of cultivating medicinal wild species has been recommended elsewhere for species in high demand (e.g., Maroyi 2012; Moyo et al. 2015) including *W. salutaris*.

3.4.3 Local ecological knowledge

In this study focus group answers were used as a benchmark to evaluate LEK between the three study areas rather than comparing it with scientific knowledge (Chalmers and Fabricius 2007; Steele and Shackleton 2010). Overall, levels of LEK were higher in the Tembe River for almost all the measures, followed by the Futi Corridor and the Lebombo Mountains. Although previous studies have revealed that different factors, such as age, gender, origin, and occupation underpin variations in LEK (cf. Naah and Guuroh 2017; Souto and Ticktin 2012), that was not the case in this study. Rather, higher LEK is likely the result of increased frequency of contact with the species by community members in the Tembe River area, due to the higher concentration of *W. salutaris* there. Similar findings were reported in the northern Ukraine for *Viburnum opulus* L. due to its occurrence in nearby forests (Pieroni and Soukang 2018).

Half of the respondents from TR were immigrants from other parts of the country. They nevertheless exhibited similar levels of knowledge of *W. salutaris* as indigenous people. LEK of non-indigenous people was likely acquired by transmission during social interaction with indigenous neighbours and friends (Souto and Ticktin 2012), as has been similarly reported for Latino immigrants among Mexico's indigenous Mayas (Atran et al. 2002). Local ecological knowledge acquisition is a lifetime learning process, and older members are often more knowledgeable than younger people (Agbani et al. 2018; Quinlan et al. 2016). In the case of *W. salutaris*, the similarity of LEK exhibited by indigenous and non-indigenous residents may well be a function of the high commercial value and overall cultural salience of this valuable tree species (Voeks 2018).

Local ecological knowledge can be a useful tool in the development of conservation and management strategies (Turvey et al. 2013). In Madagascar, for example, local people provided reliable information on wildlife harvesting patterns, suggesting that LEK can contribute to the species monitoring process (Jones et al. 2008). In Mexico, Hellier et al. (1999) reported the decline of useful plants due to overharvesting, and proposed the value of traditional knowledge in monitoring trends in local biodiversity. Thus, understanding of the depth and diversity of LEK, such as the information provided by respondents in this study, can offer valuable and necessary recommendations for conservation and management of overused species (Ghimire 2004; Turvey et al. 2013).

3.4.4 Use variation according to area, gender, and age

The results revealed that knowledge differed significantly between the Tembe River and the Futi Corridor, as well as between men and women in these areas. This may be due to the long distances from their homesteads to *W. salutaris* collection grounds in the Futi Corridor and the Lebombo Mountains areas. In addition to being time-contingent, as noted earlier, ethnobotanical knowledge is also space-contingent, and close proximity of forests to homesteads facilitates greater opportunities for interaction between people and plants (cf. Byg and Balslev 2001). These results are supported by the application of optimal foraging theory in ethnobotany, which suggests that increasingly rare or distant plant resources will be used less than near and abundant resources (Gaoue et al. 2017) if for generalist uses.

In the case of southern Mozambique, large parts of the population migrated to South Africa and Swaziland during the civil war and stayed for many years, where they had improved access to modern medical facilities. In other cases, lack of employment opportunities in the study area prompted labour migration to regional urban areas, where they similarly had improved access to biomedicine. Access to modern medicine has been widely reported to undermine people's use and knowledge of medicinal plant species (Voeks 2018, 237–238). In Oaxaca, Mexico, for example, knowledge and use of medicinal plants persists, but it has declined in importance with increasing access to modern medicine (Pérez-Nicolás et al. 2017).

A gendered division of knowledge of medicinal plant species is common in traditional societies, particularly where there are strong divisions of labour and space (Voeks 2018). In southwest Ethiopia, for example, where most medicinal plants come from home gardens, women are more knowledgeable than men because they work in the home gardens (Hunde et al. 2015). Similarly in Brazil, where most medicinal species are associated with anthropogenic habitats, women know the medicinal properties of more plant species than men (Voeks 2007). This is not universally the case, however. In South Africa, for instance, men are reported to be more knowledgeable about medicinal species than women (Dovie et al. 2008). However, contrary to these opposing patterns, the results indicated relatively few gender or age differences. This may be a consequence of the multi-directional and efficacy of knowledge transmission among community members (Lozada et al. 2006). Almost all the people in the study areas, young and old, male and female, non-indigenous and indigenous, used *W. salutaris*. This suggests that information on the species is widespread and acquired though vertical knowledge transmission from parents and grandparents along with horizontal transmission from neighbours or friends (Mathez-Stiefel and Vanderbroek 2012).

3.5 Implications for the sustainable use of *W. salutaris*

Most households in the study areas relied on agriculture and NTFP collection for the bulk of their cash and non-cash income (GDM 2008; INE 2013a, 2013b). This is a result of the limited job opportunities in the area, long distances to urban centres, and the generally low levels of formal education. *W. salutaris* is a key NTFP within local livelihoods, supporting the health of most households as well as providing cash incomes for some through their involvement in trading *W. salutaris* bark locally and farther afield (Veeman et al. 2014b). However, trade has been identified as the main cause of decline of this species in many parts of southern Africa due to high demand (cf. Botha et al. 2004; Krog et al. 2006). I believe that *W. salutaris* is at this time sustainably exploited in some parts of the study area, but not all (see chapter 4) but that during the civil war this was likely not the case. To ensure that the species is sustainably exploited into the future, considerations should be given to activities that encourage conservation and contribute to people's livelihoods. As suggested by Berkes (2004), access to natural resources is of utmost importance to the livelihoods of people living in natural environments, and any action that impedes access is likely to be disapproved by the local communities (Berkes 2004). Therefore, I propose that the limited number of existing *W. salutaris* traders in the three areas should be permitted to continue harvesting bark, with assistance and supervision provided by relevant resources agencies. Limiting the overall wild harvest of the species and at the same time encouraging its cultivation would benefit the local communities economically and contribute to conservation of the species. However, the viability of this small enterprise would require institutional support, as has been demonstrated elsewhere (Tewari 2012). For example, a NTFPs enterprise established for production and commercialisation of wild honey was created to respond to people's needs and to support forest conservation in Ethiopia. Through assistance from the Netherlands Development Organization, Ethiopia is exporting wild honey to the European community (Lowore et al. 2018).

To promote early detection of unsustainable uses of *W. salutaris* in the study area, the regular inventory of the populations is essential. Additionally, determination of the rates of bark recovery in relation to plant, site, and harvesting approaches would be useful in contributing to local management guidelines (Botha et al. 2004; Delvaux et al. 2010). Monitoring of population trends can be done using simple methods such as size class profiles that will inform changes in mortality and recruitment rates (Cunningham 2001; Venter and Witkowski 2010). These studies could be integrated into the management plan of the species.

3.6 Conclusion

This study examined the uses, knowledge distribution, management practices, and local ecological knowledge (LEK) of the pepper-bark tree (*W. salutaris)* in southern Mozambique. The species is employed to treat 12 health complaints, and is mainly used for coughs, mouth, and throat sores. Gender and age did not influence knowledge and use of the species, suggesting that acquisition occurs through vertical and horizontal transmission from kin and neighbours. Bark is the most used part of the plant, but leaves are also used to address a number of common ailments. Although less often used by respondents, leaf use could support *W. salutaris* conservation through substitution for bark. Furthermore, a considerable number of substitute species for *W. salutaris* were reported, and these could be used in lieu of *W. salutaris*. There are no reported prohibitions relating to *W. salutaris* harvest in the study areas, but there are some collection restrictions in the Futi Corridor, a formal protected area linked to the Maputo Special Reserve.

Outside of the Futi Corridor, very few respondents admitted to involvement in the bark trade, now or in the past. Similarly, confirmation of non-community members coming to collect *W. salutaris* was mostly reported for the Futi Corridor. Nevertheless, given the large quantities of *W. salutaris* bark of Mozambican provenance that has been and is flowing into nearby countries, it is most likely that: a) informants were or are involved in the bark trade and did not want to implicate themselves, b) were or are not involved in trade but knew who commercial harvesters were and avoided implication of others, or c) the longer distances to collection grounds in the Futi Corridor and possibly Lebombo Mountains favoured commercial harvesting of the species. Some trade clearly still persists in southern Mozambique, but to ascertain which areas are involved, harvest impact and size class profile studies are needed.

Local management practices for *W. salutaris* are mainly through bark harvesting procedures, but avoiding wild fires, on-farm conservation, and cultivation were also mentioned. Cultivation of *W. salutaris* to create alternative sources of plant material has been recommended in the region as a primary tool for management of the species (Botha et al. 2004; Maroyi 2012; Veeman et al. 2014b), which would require regional collaboration. Long-term studies of post-harvest bark recovery are also necessary in order to recommend sustainable bark harvesting techniques for *W. salutaris*.

Finally, variation of habitat characteristics for *W. salutaris* in southern Mozambique revealed through LEK could inform possible reintroduction programs. Likewise, the ways that local people see the population trends in different periods will assist monitoring programs. Local people's views of the driving forces causing any decline in of *W. salutaris* should be integrated into the management plan of the species. The results of the present study could contribute to the design of an appropriate and locally sensitive conservation strategy for this important medicinal species in southern Mozambique.

Author contributions

All authors contributed to the conception and design of the study. A.M.S. C.M.S. R.A.V. contributed to data collection, analysis and interpretation and wrote the first draft of the manuscript. All authors corrected and reviewed and approved manuscript submission to *Economic Botany* and the submitted revisions.

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Chapter Four: Population structure and extent of bark harvesting on the threatened pepper-bark tree (*Warburgia salutaris*) in southern Mozambique

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Abstract

The present study investigated the following research questions: Are the population under decline in southern Mozambique? Which conservation strategies have more probability to secure the existence of *W. salutaris* in southern Mozambique? The present study aimed to determine the population structure and ascertain the extent of bark harvesting. Size class distribution and bark damage evaluation scale were employed to assess population stability and harvesting impacts of *W. salutaris*, respectively. For this, all individuals of the known populations of *W. salutaris* in the three study were sampled in a total of 124 plots (14 Lebombo Mountains, 56 in the Tembe River and 54 in the Futi Corridor. The mean density of alive stems was higher in LM (4500.3±9209.8 stems/ha) compared to other areas. All the three study areas had negative slopes showing more recruitment than individuals adults, also revealed by their mean densities. The Simpson index of dominance, the Permutation index and Quotient between successive size classes revealed some degree of population instability in all the study areas. The results also indicated bark harvest preference was for individuals (stems) in larger classes, reflected by significantly lower densities of reproductive individuals. Higher mean density of harvestable stems were found in LM and higher mean density of harvested stems in TR but more stem

destruction was recorded in FC compared to other study areas. Bark harvest for trade was done in the past in all the three study areas. However, evidence indicated that FC is currently an active source of bark supply for trade in southern Mozambique. For the conservation of *W. salutaris,* work to determine factors affecting sexual reproduction is required. Monitoring of population trends using this work as a baseline is crucial. Multiple conservation strategies such as listing of *W. salutaris* under CITES to regulated transboundary trade of the species and strengthen the existing law enforcement institutions for protection of *W. salutaris* need to be considered. Mass cultivation of the species to provide alternative source of the bark to alleviate pressure in the wild, plant substitution and plant part substitution strategies are also of utmost importance.

Key words: Demography, size distribution, bark damage, coppice/coppicing stems, trade, conservation

4.1 Introduction

The importance of trees as a source of non-timber forest products (NTFPs) has been well documented across the globe (Lins Neto et al. 2010; Sinasson et al. 2017; Specht et al. 2015). A range of products provided by trees have long been used to sustain people's livelihoods (Dadjo et al. 2012; Fandohan et al. 2010; Luckert et al. 2014), such as food and beverages (Agúndez et al. 2018; Fandohan et al. 2010; Luckert et al. 2014), medicines (Albuquerque 2006;de Wet et al. 2010, Fandohan et al. 2010; Giday et al. 2003), firewood (Fandohan et al. 2010; Silva et al. 2018; Specht et al. 2015), fibre (Binnqüist et al. 2012; Luckert et al. 2014) and cork (Cunningham 2014; Seth 2004). However, in some places high demands on trees as sources of NTFPs, especially for trade, has compromised the sustainability and population viability of some species (Botha et al. 2004; Krog et al. 2006; Shackleton et al. 2005).

Harvesting of different parts of trees can influence the structure and dynamics of populations (Baldauf et al. 2013; Guedje et al. 2007; Shackleton et al. 2005). The risk is accentuated for species with multiple uses because of the combined effect of harvesting different parts of the same plant (Gaoue and Ticktin 2007; Houehanou et al. 2011). For example, exploitation of *Garcinia lucida* Vesque seeds and bark for palm wine production and traditional medicine in West Africa (Guedge et al. 2007). Equally, extensive uses of three multipurpose tree species, *Khaya senegalensis* (Desr.) A. Juss., *Afzelia africana* Smith ex Pers. and *Pterocarpus* *erinaceus* Poir. contributed to their threatened status in Benin (Houehanou et al. 2011). Of the species mentioned, the last three are widespread in tropical Africa (Gérard and Louppe 2011) but *G. lucida*, has a limited distribution (Bamp 1970). However, overharvesting, especially with preference to large size classes, has been reported to affect population structure by reducing reproductive potential (Gaoue and Ticktin 2007; Guedje et al. 2007; Martins and Shackleton 2017). In the tree bark harvested species, the magnitude of harvest determines the capacity for bark regeneration. Such capacity can be evaluated by conducting post-harvest bark recovery experiences that will provide information including appropriate harvesting season and time taken for wound closure. It is crucial to understand harvesting rates, patterns, and impacts to develop comprehensive and appropriate tools for the management of NTFP species (Botha et al. 2004; Gaoue and Ticktin 2007).

Demographic inventories of size class distributions can be used to assess trends in populations, including those subjected to harvesting (Cunningham 2001; Martins and Shackleton 2017; Venter and Witkowski 2010). Size class distributions can indicate the dynamics of populations by illustrating the likelihood of plant survival from one size class to the next (Cunningham, 2001), which also indicates if it is stable, expanding or declining. Thus, a large number of recruits compared to adults, represented by an inverse J-shape curve or a negative slope, is a clear indication of a stable population. The inverse, indicated by a positive slope or unimodal SCD curve, suggests poor recruitment, which could be a sign of population decline (Condit et al. 1998). Nevertheless, the size class profile alone should not be seen as the only predictor of population health because other factors, such as low growth rates of young plants and low survival rates, may also influence the size class distribution (Condit et al. 1998). However, it is an effective allocation of time and financial resources to appraise the status of a population (Cunningham 2001).

Size class distributions can be combined with other indexes useful in studies of population dynamics (Botha et al. 2004; Martins and Shackleton 2017; Shackleton et al. 2005; Wiegand et al. 2000). These include the permutation index, Simpson's index of dominance, and quotient between size classes (Wiegand et al. 2000). The permutation index, for example, deals with the ranking of the size classes relative to each other. The Simpson's index of dominance is centred on the numbers of individuals, while the quotient considers the evenness of the ratios of individuals in the successive classes. The combined application of the three indexes is worthwhile in demographic studies because they capture complementary, yet separate quantitative measurements (Shackleton et al. 2005).

Warburgia salutaris (G.Bertol.) Chiov. (Canellaceae) is a native species valued in folk medicine throughout southern Africa (Maroyi 2013; Veeman et al. 2014b). In Mozambique, *W. salutaris* is found in Maputo province (Izidine and Bandeira 2002; Jansen and Mendes 1990) and possibly in the mountainous region of Chimanimani in Manica province (Veeman et al. 2014a). The species is used in the therapy of different health complaints, including common colds, sinus and chest complaints, throat and mouth sores (Jansen and Mendes 1990; Senkoro et al. 2019; van Wyk and Wink 2004). The bark is the most valued part of the plant and is in high demand (Krog et al. 2006; Mander et al. 2006; Mukamuri and Kozanayi 2014; Senkoro et al. 2019). Consequently, most populations are threatened and the species is considered as endangered at the global level (Hilton-Taylor et al. 1998).

With a limited distribution, *W. salutaris* has been under pressure from unsustainable bark harvesting for trade (Jansen and Mendes 1990). The wild populations in Mozambique are the source of bark for urban areas in Maputo and in neighbouring countries including South Africa (Krog et al. 2006; van Wyk and Gericke 2000; Williams et al. 2014b) and Zimbabwe (Mukamuri and Kozanayi 2014; Veeman et al. 2014b). Although, *W. salutaris* has a strong resilience to bark removal, repeated removal of the bark diminishes the growth rate and lifespan of individuals (Cunningham 2001). Botha et al. (2004) studied the harvest impact of the *W. salutaris* in protected and non-protected areas in Mpumalanga and Limpopo provinces (South Africa) and found greater damage to individuals in non-protected areas compared to the protected ones. They also reported low natural recruitment, which was exacerbated by frequent fires. In Zimbabwe, the species is believed to be extinct in the wild due to overharvesting (Maroyi 2013). The seeds are readily parasitized resulting in rapid loss of viability (Hannweg et al. 2015). Harvested individuals subjected to constant fires exhibit shrubby growth and are susceptible to fungal infections (Botha et al. 2004). Dludlu et al. (2017) reported a considerable numbers of ring-barked individuals in Swaziland, suggesting the possible decline of mature individuals. Through a market survey, Williams et al. (2014a) determined the available bark (bark mass) for trees species including *W. salutaris* in South Africa. From their work the authors could determine the size of tree from the bark. Further work concluded that a lack of bark from large trees in medicinal plants markets in South Africa was an indication of a reduction in large specimens in the wild (Williams et al. 2014b).

Despite a growing number of demographic studies on important NTFP trees on the African continent, very little has been done on *W. salutaris,* and none in Mozambique, despite it being the main source of bark throughout the region. Thus, there is a need to assess the ecological sustainability of bark supply to avoid local extinction. This entails an insight into harvesting approaches and associated ecological impacts. Ideally this requires long-term studies such as assessment of bark harvesting intensities and post-harvest bark recovery (Ticktin 2015). However, with limited resources, short-time studies are a necessary first step (Cunningham 2001). Size class profiles, static data from short-term research, have been employed in different ecological studies to analyse population trends of different species in southern Africa (cf. Botha et al. 2004; Martins and Shackleton 2017; Shackleton et al. 2005). In addition, quantitative data obtained will inform the need (or not) for further research or monitoring of species (Botha et al. 2004). Thus, adding and/or strengthening on existing knowledge of the species from Botha et al. (2004). The present work aimed to a) determine the density, b) assess the population structure and c) ascertain the extent of bark harvesting of *W. salutaris* in three study areas in southern Mozambique. It seeks to answer the following questions: Are the population under decline in southern Mozambique? Which conservation strategies have more probability to secure the existence of *W. salutaris* in southern Mozambique?

4.2 Methods

4.2.1 Study area

See detailed information of the study areas on section 1.6.

4.2.2 Data collection

In each area known populations of *W. salutaris* were sampled. A population was taken as a group of individuals (except in one case where there was just a single tree) within a clearly defined area and distinctly spatially separated from adjacent populations. All visible *W. salutaris* plants within a population were included in the sample plot (varying from 1 to 3 332 stems). Consequently, the size of the sample plot varied in accordance with the size of the population, ranging from 0.75 m^2 (for the single tree) to 5 779 m^2. The plot area was calculated by multiplying the longest access and perpendicular distance and the product by two. All *W. salutaris* individuals in the plot were counted and marked to avoid double counting. Fourteen plots were sampled in the Lebombo Mountain area (approximately 2.2 ha), 56 in the Tembe River area (45.2 ha), and 54 in the Futi Corridor area (24 ha), totalling 124 plots, corresponding to 71.4 ha.

The basal diameter and height of each rooted stem was measured at 30 cm above ground level. The extent of bark damage was assessed up to the height of 2 m on every individual in the plot using an eight point scale (Fig. 4.1) (Botha et al. 2002, 2004; Cunningham 2001). The status of each stem (dead, stressed, or alive), the number of coppice shoots for each individual chopped, and fire evidence through fire scars were recorded.

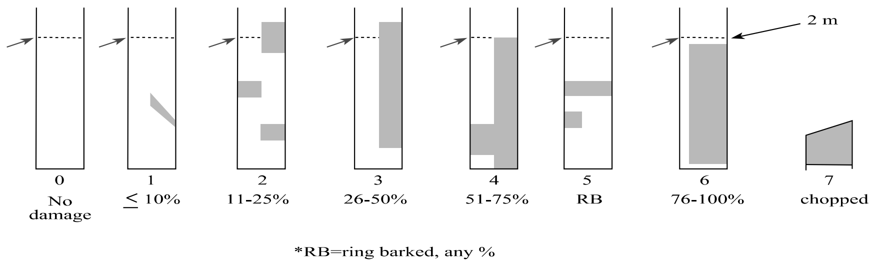


Figure 4.1. Bark damage evaluation scale (Botha et al. 2002; 2004, Cunningham 2001).

4.2.3 Data analysis

Population parameters were assessed in terms of the densities of live, stressed, dead, burnt and reproductive stems. These were computed as mean stem numbers per hectare of the species in each study area. In addition, the mean stem height was calculated for each area. The stem basal diameter was used to construct size class distributions. Six diameter classes were defined, the smallest (≤2 cm) was termed recruits (young plants). Four successive classes were categorized into 5 cm increment classes and the last size class included all individuals with a diameter over 20 cm. The densities of individuals in each size class were determined.

The Simpson Index of Dominance, Permutation Index and Quotient between consecutive classes were used to assess population stability (Botha et al. 2004; Martins and Shackleton 2017; Venter and Witkowski 2010; Wiegand et al. 2000). The Simpson´s Index of Dominance was used to measure size class evenness (Equation 1). This index reports the probability of two randomly drawn stems from the same community, are equal in size. Values greater than 0.1 indicate that the size class ratio is steeper than would be predicted from an exponential decrease in a population, and values beneath 0.1 show more equitably distributed size classes (Botha et al. 2004; Wiegand et al. 2000):

$$C = \frac{1}{N(N-1)} \sum_{i=1}^{6} N_i (N_i - 1) \qquad (1)$$

where C is Simpson's Index of Dominance, N is the overall number of stems and N_i is the sum of stems in class i.

The Permutation Index was used to calculate divergence from a monotonic reduction of a presumed ideal population (Equation 2). For this, ranking is equal to numbering the stem size classes from the smallest (most frequent) to the largest (less frequent). If a size class distribution is discontinuous, i.e., increase of numbers in larger size classes than preceding, the numbering of ranking changes, causing a pronounced Permutation Index (Botha et al. 2004; Wiegand et al. 2000)

$$P = \sum_{i=1}^{6} | J_i - i |; \qquad J_i = 1,2,...,6 \qquad (2)$$

where P is the Permutation Index, J_i is the rank of size class i (i = 1 for the smallest stems), with the maximum rank (J_i = 1) given to the most frequent size class.

Quotients between succeeding size classes were calculated and presented graphically. Unsteady quotients between successive size classes indicate unstable populations, while constant quotients illustrate population stability (Botha et al. 2002, 2004; Martins and Shackleton 2017; Venter and Witkowski 2010). Quotients were computed following equation 3 (Harper 1977; Meyer 1952):

$Q = N(i – 1)/N_i(3)$

where N(i – 1) is the number of stem in the preceding size class and N_i is the number of stems in size class i.

To analyse the population structure of *W. salutaris*, size class distribution (SCD) was scrutinized using a method defined by Condit et al. (1998), Martins and Shackleton (2017), Obiri et al. (2002), and Venter and Witkowski (2010). A least square regression was performed between the mid-point of each size class (independent variable) and the mean density of stems in each class (dependent variable). Since data in the two variables contained no zeros, no transformation was required. Interpretation of the SCD slope followed Obiri et al. (2002) and Venter and Witkowski (2010). The steepness of the slope was employed to investigate trends of recruitment. Negative slopes indicate adequate levels of recruitment by presenting more individuals in lower size classes than higher size classes, while flat slopes show relatively even distribution of individuals in lower size classes and higher. Positive slopes express lower recruitment with fewer individuals in smaller classes and more individuals in larger classes (Venter and Witkowski 2010).

All sampled plots were pooled per population to analyse harvesting damage and subsequently, information was synthesized and presented graphically (Botha et al. 2004). The preference ratio (inclination to harvesting a particular size class) was computed as the percentage of harvested stems in a class over available individuals (in percent) in the class. The preference ratio expresses the disjunction between demand (harvested) and accessibility of individuals for harvest. Preference ratios above one indicate preference for the size class, roughly one, indicates random harvesting and below one, avoidance of a particular size class (Shackleton et al. 2003).

The harvestable sizes of stems in the three populations were considered as those stem size classes that were being harvested presently (Botha et al. 2002). The mean densities of harvestable, dead, alive and stressed stems were calculated and summarized. So were the mean densities of harvested, single stemmed, coppicing stumps and coppice for each population.

Data normality was tested through the Shapiro-Wilk test, and non-parametric tests were applied (not normally distributed data). Population parameters, namely size of the three populations, height, densities of alive, stressed, dead, harvested, burnt and reproductive stems in the three populations, were compared using Kruskal-Wallis tests. Equally, the test was used to compare between harvestable, harvested and unharvested stems, single stemmed, coppicing stumps and coppice, and so were differences of densities in the size classes between the three populations and within each population. Statistical analyses were performed using STATISTICA version 13.

4.3 Results

4.3.1 Population parameters

There were 24 654 stems of *W. salutaris* in the 124 sampled plots. More than half of the stems were found in the Tembe River area (13 818 stems), 37.9% (9 349 stems) in the Futi Corridor and 6% in the Lebombo Mountains (1 487 stems). The mean density of matured stems differed significantly between the three areas ($H = 30.3$, $p < 0.05$) and was greater in LM (1513.0±4401.4 stems/ha) and least in FC (916.6±4401.4 stems/ha). Equally, the mean stem height varied significantly between the three areas ($H=1121.9$, $p<0.05$), being highest in LM (2.6±2.1 m) and lowest in the FC (1.3±1.2 m), with TR intermediate between these two (Table 4.1).

Table 4.1. Population parameters of *Warburgia salutaris* in the three areas. (LM= Lebombo Mountains, TR= Tembe River, FC= Futi Corridor). Unlike superscripts in rows denote significant differences (Kruskal-Wallis test $p < 0.05$) and [1]represents matured individuals.

Parameter		All study areas (n=124)	Lebombo Mountains (n=14)	Tembe River (n=56)	Futi Corridor (n=54)
Height (mean ±SD) (m)		1.7±1.6	2.6±2.1[a]	1.8±1.6[b]	1.3±1.2[c]
Population density[1] (Stems/ha)		1216.7±3379.2	1513.0±2079.3[a]	1432.0±2385.2[a]	916.6±4401.4[b]
Status (Stems/ha)	Alive	3785.9±9522.5	4500.3±9209.8[a]	3195.0±5254.7[a]	4213.4±12660.7[a]
	Dead	81.3±251.1	10.3±17.1 [a]	69.1±236.5[a]	112.3±292.9[b]
	Recruits	2569.1±7014.2	2987.2±7857.2[a]	1763.0±3757.2[a]	3296.8±9116.7[a]
	Harvested	358.4±1312.5	323.5±887.4[a]	552.9±1848.3[a]	165.9±426.3[a]
	Stressed	20.6±78.6	3.7±11.7[a]	19.4±82.9[a]	26.2±84.1[b]
	Burnt	78.9±312.2	6.4±10.4[a]	115.4±435.2[a]	59.9±162.7[a]
Status (%)	Alive	92.5	98	92.7	91.4
	Dead	7.5	2	7.3	8.6
	Recruits	72.3	63.7	74.4	85.3
	Harvested	7.6	5.5	7.7	7.6
	Stressed	1	0.4	0.6	1.8
	Burnt	7.4	1.7	10.2	3.8

More than 90% of the stems were alive (22 811 stems), most of which were recruits (basal diameter ≤2 cm) (17 813 stems). Less than 10% of stems were dead (1 843 stems), and less than 1% (86 stems) showed reproductive signs (flowers or seeds on or under the tree). The density of dead stems differed significantly between the three areas, with the highest in FC and the least in LM. However, the densities of live, mature stems, and recruits were not statistically different between the three areas (live stems $H=5.1$, $p>0.05$; recruits $H=1.7$,

$p>0.05$) (Table 4.1). Stems with reproductive structures were rare in all the areas, and most common in the TR (TR with 291±1792.9 stems/ha, LM 2.7±6.8 stems/ha and FC 0.1±0.5 stems/ha).

Approximately 8% (1 875) of the stems showed evidence of harvesting, regardless to their status (alive, stressed, or dead). However, those that were harvested and alive represented 5.4% of all stems (1339 stems). In both cases, recruits were excluded. One percent (254 stems) demonstrated signs of stress (alive but with signs of rotting) and roughly 7% (1 831) showed burn scars. The densities of stressed stems were significantly different in the three areas ($H = 12.5$, $p < 0.05$), being higher in FC than the other two areas. However, the densities of harvested and burnt stems were not statistically different between the three areas (harvested $H = 3.8$, $p>0.05$; burnt $H=1.7$, $p>0.05$). In both cases, the TR had higher densities and the LM, the least (Table 4.1).

4.3.2 Size class distribution

The mean densities of *W. salutaris* in all the size classes, except recruits, varied substantially across the three areas, as within individual areas. Overall, the TR had the highest stem density in almost all size classes, except for 10.1-15 cm class and recruits. These latter two were highest in the FC. In general, the mean density decreased steadily with increasing size class. Thus, the most common size class in all areas was ≤2 cm, with 68.7% of all stems in LM, 74.4% in TR and 85.3% in FC (Table 4.2), corresponding to more than 70% of all stems registered in the three areas.

The Simpson's index of dominance was above 0.1 in the three areas, indicating uneven size class distributions. The permutation index was five in all cases, illustrating that each area had an equal level of discontinuity in size class distributions. The three areas had a negative slope, showing more recruitment and fewer individuals in large size classes. The steepest slope was apparent in LM, demonstrating a better recruitment than the other two areas (Table 4.3). The quotients in the three areas demonstrated fluctuations, indicating some degree of instability of population transition between successive size classes (Fig. 4.2).

Table 4.2. Mean density and the percentage of individuals of *Warburgia salutaris* among the six size classes in the three areas. Small, unlike superscripts in rows and uppercase in columns illustrate significant differences.

Diameter class (cm)	Lebombo Mountains		Tembe River		Futi Corridor	
	stems/ha	%	stems/ha	%	stems/ha	%
≤2	2987 ± 7857.2^{aA}	68.7	1763 ± 3757.2^{aA}	74.4	3296 ± 9116.7^{aA}	85.3
2.1-5	1294 ± 2058.2^{aA}	19.8	591 ± 1223.1^{abA}	9.8	523 ± 2292.8^{bA}	9.2
5.1-10	201 ± 332.7^{abAB}	7.5	353 ± 581.5^{bA}	7.5	217 ± 1100.3^{aB}	3.9
10.1-15	12 ± 27.3^{aBC}	3	100 ± 176.9^{bBC}	2.6	165 ± 1087.8^{aBC}	0.8
15.1-20	2 ± 4.8^{aC}	0.5	281 ± 1777.7^{bB}	1.7	6 ± 20.3^{aC}	0.4
>20	4 ± 9.4^{aC}	0.5	108 ± 391.4^{bC}	4	4 ± 15.4^{aC}	0.4

4.3.3 Harvesting and selection

Harvesting of *W. salutaris* was recorded in all the three areas. In the LM area stems were harvested at all levels defined in the eight point scale (from ≤10% bark removal to chopped) and was evident for all the diameter classes. Very few stems in the smaller size class were harvested, mostly at levels ≤10% (three stems) and 26-50% (one stem). Chopping took place in all size classes, except recruits, and ring-barking was also evident on all classes excluding ≤2 cm, 15.1-20 cm and >20 cm (Fig. 4.3a). Harvesting also occurred on all basal diameter size classes in TR. Recruits were harvested at ≤10%. Ring-barking did not occur on small individuals (≤2 cm) and 15.1-20 cm size class, but was evident for all larger size classes. There was some chopping of individuals across every size class (Fig. 4.3b). Similar patterns were observed in the FC. Harvesting also took place in all size classes. Recruits were harvested at all the levels except 51-75%. Thus, ring-barking was evident for almost all size classes (except 15.1-20 cm) and chopping occurred in all size classes (Fig. 4.3c).

Table 4.3. Simpson's Dominance Index, Permutation Index and slopes of basal diameter size class distribution of *Warburgia salutaris* in the three areas of southern Mozambique (n=124).

Parameter	Lebombo Mountains	Tembe River	Futi Corridor
Simpson's Dominance Index	0.52	0.57	0.74
Permutation Index	5	5	5
R^2	0.36	0.24	0.25
Slope	-0.43	-0.15	-0.19
p-value	0.21	0.32	0.31

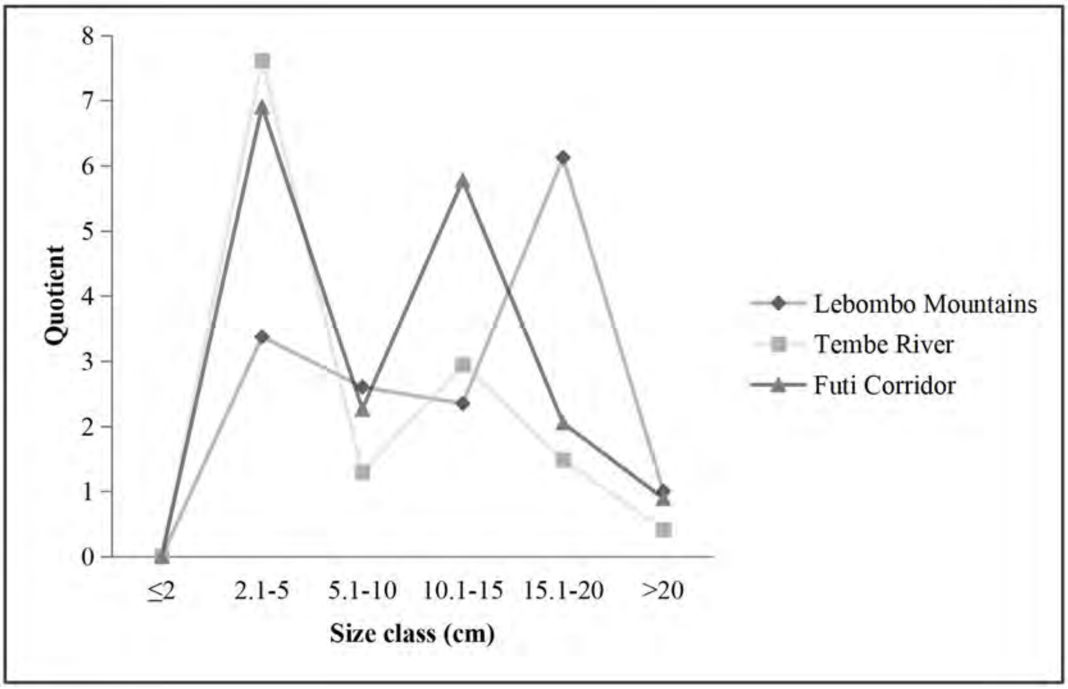
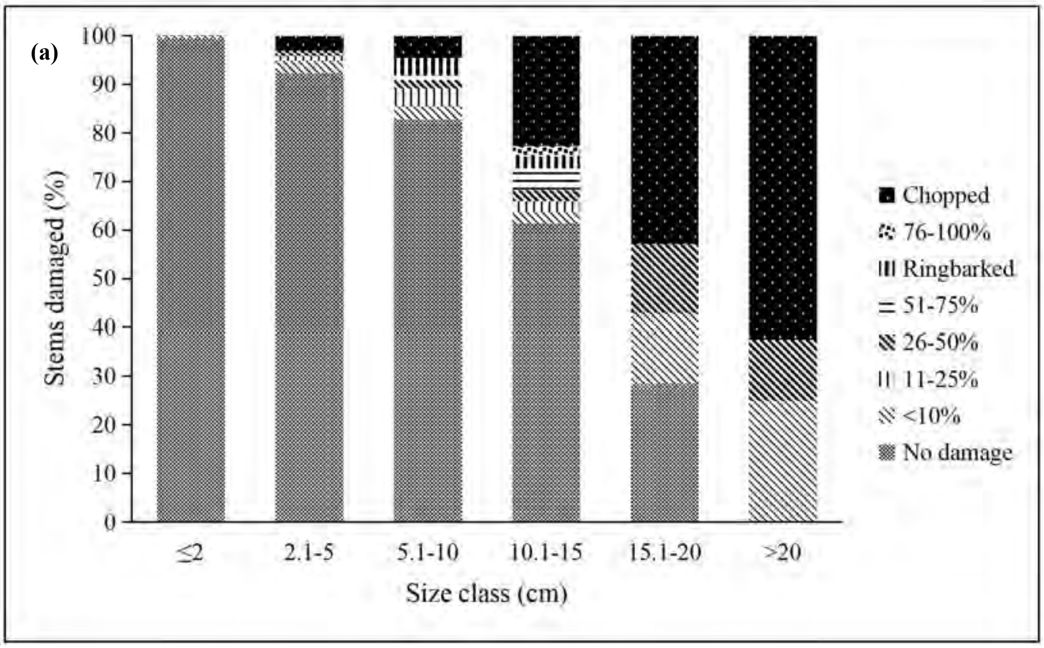
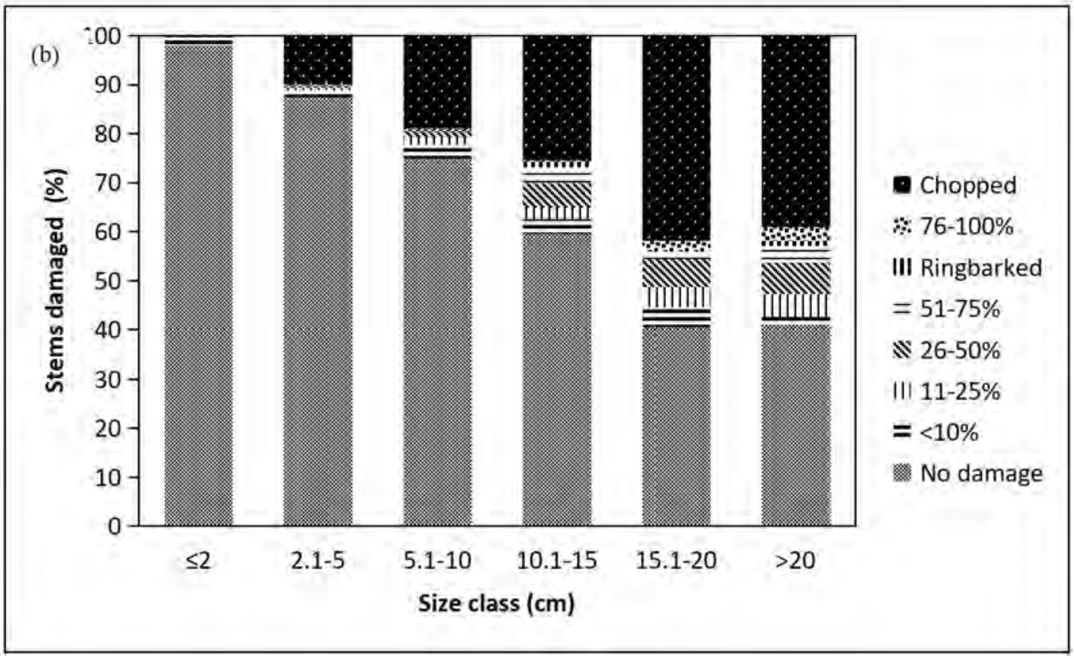


Figure 4.2. Quotients between the number of individuals in successive diameter size classes of *Warburgia salutaris* in the three areas.





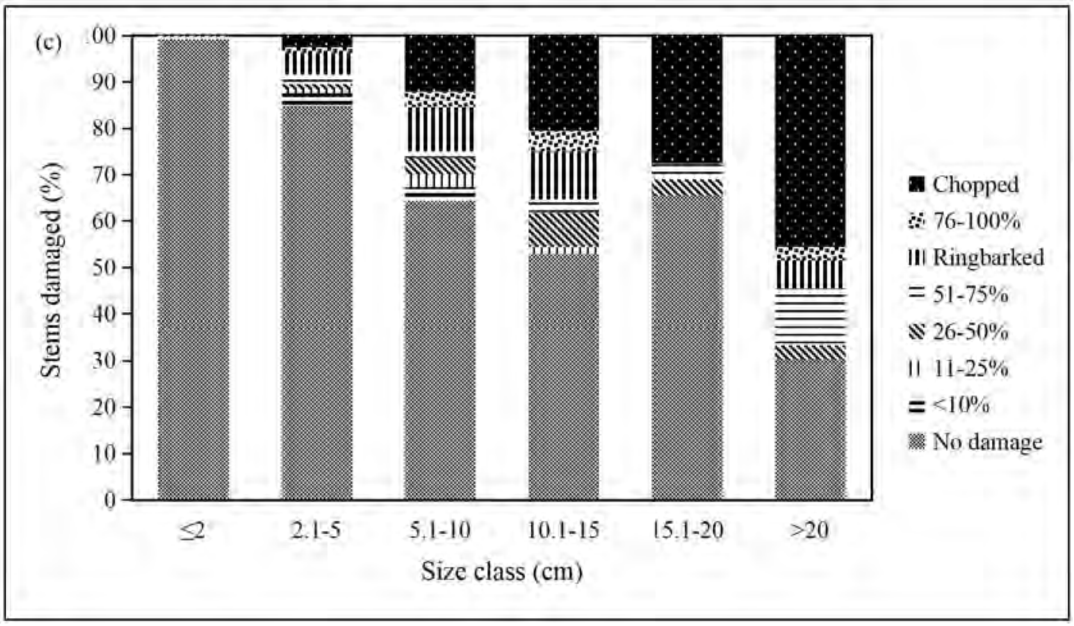


Figure 4.3. Extent of harvest damage in the six basal diameter size classes (cm) of the three areas (a) the Lebombo Mountains, (b) the Tembe River and (c) the Futi Corridor.

The preference ratio was greater than one for all size classes, except recruits, in all study areas, and it increased with stem size. It was highest for intermediate, or large-sized stems in each area, i.e. 15.1-20 cm (12.6) in the LM, 15.1-20 cm and >20 cm (6.5) in the TR and >20 cm (8.3) in the FC. Harvesters of *W. salutaris* throughout the study area avoided gathering of bark from recruits (≤2 cm).

The mean densities of harvestable stems differed significantly between the three areas (H=30.3, p<0.05). On average, the LM presented the highest density of harvestable stems (1513.0±2079.3 stem/ha), while the FC had the least (916.6±4401.4 stems/ha). The mean densities of harvested stems were not statistically different in the three areas (H = 3.8, p >0.05). The TR, had the highest mean number (552.9±1848.3 stems/ha) and the FC, the least (165.9±426.3 stems/ha) (Table 4.4).

Similarly, the status of stems post-harvest (alive, stressed or dead) varied considerably between the three areas (alive H = 18.2, p<0.05; stressed H = 13.7, p<0.05; dead H = 21.9, p<0.05). The TR presented the greatest density of harvested stems that were alive (526.6±1844.4 stems/ha, equivalent to 91.1% of harvestable stems in the area) compared to FC which had the least (105.9±395.6 stems/ha, 41.1% of FC harvestable stems). Although the FC had fewer harvested stems compared to other areas, the former had high levels of destruction, with more harvested and dead stems (39.5±85.9 stems/ha, corresponding to 40.7%) and 18.2% stressed stems (20.5±75.7 stems/ha). In both cases, LM had few cases (3.8±11.8 stems/ha harvested stems and 2.1±4.3 stems/ha dead stems) (Table 4.4).

Table 4.4. Comparison of the average densities, percentages and status of harvested stems of *Warburgia salutaris* between the three areas. [1]Refers to the percentages of stem category over the total stems within individual areas and [2] the proportion of harvestable stems harvested within individual areas.

Category of stems	Lebombo Mountains	Tembe River	Futi Corridor	p-value
Harvestable (Stems/ha)	1513.0±2079.3^{a}	1432.0±2385.2^{a}	916.6±4401.4^{b}	<0.001
Harvested (Stems/ha)	323.5±887.4^{a}	552.9±1848.3^{a}	165.9±426.3^{a}	0.15
Harvested, alive (Stems/ha)	317.6±888.9^{ab}	526.6±1844.4^{a}	105.9±395.6^{b}	<0.001
Harvested, stressed (Stems/ha)	3.8±11.8^{a}	18.7±82.9^{a}	20.5±75.7^{b}	0.001
Harvested, dead (Stems/ha)	2.1±4.3^{a}	7.6±19.1^{a}	39.5±85.9^{b}	<0.001
Harvestable (%)[1]	30.7	23.8	13.4	N/A
Harvested (%)[2]	18.6	32.4	57.7	N/A
Harvested, alive (%)	83.5	91.1	41.1	N/A
Harvested, stressed (%)	9.4	4.4	18.2	N/A
Harvested, dead (%)	7.1	4.5	40.7	N/A

The densities of single stemmed individuals was statistically different in the three study areas (H = 26.1; p <0.05). On average, LM had the higher density of single stemmed (1501.8±2085.8 stems/ha) while FC had the lower (899.9±4402.5 stems/ha, 40.7% of FC adults stems). However, the densities of coppicing stems and coppice were not statistically different between the three areas (coppicing stemmed $H = 3.2$, $p > 0.05$; coppice $H = 4.4$; $p > 0.05$) although the two groups varied considerably between areas. The TR had the highest average density of coppicing stems of 81.8±203.3 stems/ha and LM had the lowest of 11.2±20.2 stems/ha. Thus, singled stems were more represented in the three areas compared to the coppicing stems (92.1% LM, 88.2 FC and 77.7 TR single stemmed as opposed to 7.9% LM, 11.9% FC and 22.3 TR coppicing stems). Equally, TR had greatest density of coppice (493.4±1172.7coppices/ha) and LM the least (54.0±94.6 coppices/ha) (Table4.5). Stems were cut at an average height of 0.01 to 0.02 m.

Table 4.5. Harvesting and coppicing stumps in the three areas of *Warburgia salutaris* in southern Mozambique. In brackets are the percentages of stem category over the total in the three areas and out of brackets, within individual areas.

Parameter	Lebombo Mountains	Tembe River	Futi Corridor	p-value
Single stemmed (Stems/ha)	1501.8±2085.8^{a}	1112.6±1708.7^{a}	899.9±4402.5^{b}	<0.001
Coppicing stems (Stem/ha)	11.2±20.2^{a}	81.8±203.3^{a}	16.6±34.1^{a}	0.203
Coppice (Coppice/ha)	54.0±94.6^{a}	493.4±1172.7^{a}	76.0±201.2^{a}	0.114
Single stemmed (%)	92.1	77.7	88.2	N/A
Coppicing stems (%)	7.9	22.3	11.8	N/A

4.4 Discussion

4.4.1 Population parameters

The densities of matured stems were statistically different in the three areas and were considerably high, especially in LM. Comparing population densities in the commercial harvested (259±96 trees/ha) and protected (56±19 trees/ha) in South Africa (Botha et al. 2004), the present work recorded higher densities. For *W. ugandensis* Sprague in Malabigambo and Kaiso forests, Uganda, the density was 2 individuals/ha (Galabuzi et al. 2015), which is far lower than recorded in the current work. However, preference of bark from large individuals reported for some bark tree species (cf. Delvaux et al. 2010; Guedje et al. 2007), including *W. salutaris* (Coates-Palgrave 2002; Dludlu et al. 2017), has been accounted for loss of larger specimens due to unsustainable harvesting. Thus, although the densities of *W. salutaris* in the study areas was higher than those reported in South Africa (Botha et al. 2004) and Uganda (Galabuzi et al. 2015), the prospective density could have been even higher if the demand for the species was lower. Hence, size class profile inventory and monitoring are of outmost importance to understand trends of mature individuals.

The densities of dead and stressed stems were significantly greater in the FC than the other areas. Thus, along with lower stem heights, FC revealed higher mortalities than other areas. The higher densities of dead and stressed stems could be explained by heavy harvesting or inappropriate harvesting techniques employed to harvest large quantities of bark (cf. Delvaux et al. 2010). For example, Botha et al. (2004) recorded higher mortality rates for *W. salutaris* in commercially harvested populations compared to protected populations. Kairu et al. (2013) also observed greater numbers of dead trees derived from complete debarking of *W. ugandensis* individuals in Kenya. Guedje et al. (2007) reported 70% mortality of large trees in *G. lucida* population severely exploited for bark in Cameroon. High mortality rates recorded in 12 species during the work on bark recovery post-harvest in Benin, revealed ring-barking and harvesting of large quantities as causes of death (Delvaux et al. 2010).This is a common practice amongst harvesters who seek to maximize harvesting efforts for financial gains (Peck and Christy 2006). Mortality is usually a result of either ring-barking (Delvaux et al. 2010) or frequent harvesting that makes the plant vulnerable to fungal infection (Botha et al. 2004). The first obstructs phloem vessels and the cambium layer which is responsible for phloem regeneration and hampers transportation of food from leaves to roots, causing the death of plants (Delvaux et al. 2010; Romero 2014). The later results in rotten stems which gradually leads to death of plants (Botha et al. 2004). Hence, high impact levels in the three study areas leads to the assumption that, in recent years, FC could be the most active source of bark supply for trade of *W. salutaris* in southern Mozambique.

This work revealed a scarcity of reproductive individuals in the three study sites, especially in the Futi Corridor. The reduction in densities of large individuals affects the potential for sexual reproduction. For example, in KwaZulu-Natal province (South Africa), high debarking intensities on *W. salutaris* diminished the number of mature individuals, and increased the number of non-reproductive coppice stems (Johnson et al. 1995). Similarly, limited fruit set was also observed in *K. senegalensis* in the Sudano-Guinea regions due to overharvesting of bark (Gaoue and Ticktin 2007). With overharvesting, it is possible that the overall reproductive success decreases, that is, the low production of inflorescences results in lower fruit set and hence, low seed production. In the study areas, *W. salutaris* reproduced asexually by suckering. Lack of seedlings have been related to early fruit fall observed in the TR area.

Although fruit infestation was not in the scope of this work, Muatinte and Cugala (2014) reported infestation of fruit fly (*Ceratitis cosyra* (Walker)), which negatively effects seed viability (Johnson et al. 1995). Similar failure in sexual reproduction and the occurrence of clonal reproduction was reported in KwaZulu-Natal province (Scott-Shaw 1999). Clonal reproduction has long-term implications for population persistence because it lowers genetic diversity (Silvertown 2008). Therefore, studies of factors limiting the reproduction of *W. salutaris* are of importance and should be integrated into local management plans for the species (Botha et al. 2004).

The density of live, recruits, harvested, and burnt stems did not vary across the three areas. The higher densities of live stems were, however, strongly influenced by the numbers of recruits (which will be discussed further) corresponding to 72.3% of the total stems recorded in the three areas out of which 85.3% were from FC, 74.4% TR, and 63.7% LM. A high density of burnt stems was present in the TR, reflecting the frequent wildfires in the area. With frequent fires, individuals of *W. salutaris* reveal shrubby growth or damaged bark, exposing the trunk to fungal infections (Botha et al. 2004). Frequent fires from slash and burn and for maintenance of grasslands are common in the region (Moll 1980).

Results of this work revealed significant differences in the mean stem height in the three areas, with the FC having the lowest mean height compared to other areas. Botha et al. (2004) found 4.0 ± 0.1 m (mean ± S.E.) and 2.3± 0.2 m for protected and non-protected populations, respectively, which were higher than those found in TR and FC. For species of the same genus, *W. ugandensis*. Tesfaye et al. (2002) reported a high proportion of individuals in the lowest height classes and a lack or decrease in the number in the mid and taller classes in Harenna forest, southeastern Ethiopia. This could be explained by harvester selection for larger individuals, as reported for other NTFPs (cf. Dludlu et al. 2017; Gauoe and Ticktin 2007; Guedje et al. 2007). It is plausible that short heights, in particular in the FC, could indicate a greater harvesting pressure in the area. Average heights presented in this work and that reported on the non-protected populations (Botha et al. 2004) are far below common height (5-10m) reported for the species in southern Africa (Coates-Palgrave 2002). Anthropogenic disturbances, including overharvesting for trade, have been reported for *W. salutaris* throughout its distribution (Botha et al. 2004; Krog et al. 2006; Veeman et al. 2014b).

4.4.2 Size class distribution

The densities of *W. salutaris* in all adult size classes were significantly different across the three areas. Within each area, in general, smaller classes had greater densities than larger classes. The SCD slopes in the three areas were negative, indicating more individuals in the smaller classes, but it was steeper in the Lebombo Mountains indicating a better recruitment relative to other areas. This is contrary to the densities of young plants in most *W. salutaris* populations in Mpumalanga and Limpopo that showed constrained recruitment (Botha et al. 2004). Similarly to Mpumalanga and Limpopo, the densities of young plants in the second class were higher than the lowest class for *W. ugandensis* in the Kaiso and Malabiambo forests, Uganda (Galabuzi et al. 2015). Further similarities that can be drawn between this study and Botha et al. (2004) is that harvesting did not affect the density of stems in the smallest classes. The large numbers of small stems is a reflection of asexual reproduction through suckering, resonating with observations in KwaZulu-Natal (Scott-Shaw 1999). Furthermore, fires in the TR area promote clonal reproduction, thus boosting the number of recruits (L. Cossa, Pers. comm., 11 February 2017) which is contrary to protected and non-protected populations in South Africa (Botha et al. 2004). Another limiting factor on seedlings and sapling establishment has been the loss of habitat due to infrastructure development (Botha et al. 2004).

The reversed J-shape SDC represents a stable population with steady replacement of older individuals and a growing population (Condit et al. 1998). A negative slope is associated with an inverse J-shape curve, representing adequate regeneration, that is, higher numbers of recruits and fewer individuals in large size classes (Venter and Witkowski 2010). The results of this work indicated that *W. salutaris* exhibited the inverse shape SDC curve in the three study areas, and slopes were negative but relatively steep in the LM compared to other areas. Botha et al. (2004) found a reverse J-shape SDC curve for the species in Mpumalanga and Limpopo, South Africa. The same curve was exhibited by *W. ugandensis* in southern Uganda (Galabuzi et al. 2015; Ssegawa and Kasenene 2007). I suspect that the reverse J-shape curve is typical for *Warburgia*, but in some instances, however, the densities of recruits could be affected by habitat disturbances (cf. Botha et al. 2004; Galabuzi et al. 2015). Other limitations to recruits are sexual reproduction constrains (Muatinte and Cugala 2014) and loss of seed viability (Johnson et al. 1995). Amongst the adult size classes there was a sharp drop of individuals in all the succeeding classes recorded in all areas. This could be due to preference for bark collection from large individuals (cf. Coates-Palgrave 2002; Gaoue and Ticktin 2007; Tesfaye et al. 2002) resulting in their overexploitation. Overexploitation is commonly associated with unsustainable harvesting techniques including constant harvesting of the same individuals and ring-barking (Botha et al. 2004; Dludlu 2017). Unavoidably, some trees die before attaining maturity (Lykke 1998), but constant harvesting of bark increases the risk of fungal infections (Botha et al. 2004) and ring-barking starves the roots (cf. Delvaux et al. 2010; Romero 2014). Both lead to death of larger individuals and hence a decrease in numbers of individuals in these size classes.

This work revealed, on the one hand, that harvesting pressure is quite high (37.5% of adult trees) and it increases with increasing tree size. On the other hand, the size-class profiles suggest that the populations are stable because of the reverse J shape, with the linear decline in abundance with increasing size class. This contradiction is, however, questionable because the species in the larger context has been in high demand for many years, and is under pressure or extinct in many other regions (cf. Krog et al. 2006; Mukamuri and Kozanayi 2014; Veeman et al. 2014b). Although *W. salutaris* is resilient to bark harvesting (Botha et al. 2004), it is more likely that the size-class profile for the species is truncated. A truncated size class profile was also found for *Ziziphus mucronata* Willd. subsp. *Mucronata* Willd in the semiarid Lowveld of South Africa (Shackleton et al. 2005) and for *Lantana camara* L. in the communal land in the eastern Transvaal Lowveld (Shackleton 1993). This was due to lack of stems in upper size classes of the useful species, with most stems found in smaller classes (Shackleton et al. 2005). Williams et al. (2014b) found the absence of bark from larger trees of *W. salutaris* in medicinal plants markets in Johannesburg. Coates-Palgrave (2002) reported loss of larger specimens of the species throughout its range due to unsustainable harvesting of the bark for medicinal purposes. Preference and high demand of bark from larger specimen could have caused loss of matured individuals that were long gone and did not exist during field survey for the present work. Thus, SCD studies are useful to determine population trends but they do not show everything unless there are repeat surveys of the same populations through time.

Fluctuating quotients between consecutive classes in the three areas indicated population instability. The Permutation index was above zero, demonstrating gaps of size class distribution and the Simpson's index of dominance was above 0.1, representing unbalanced size class distribution. The results of three indexes indicate some instability in population transitions into subsequent size classes in the three areas. In their work on the same species, Botha et al. (2004) reported similar trends in all the three indexes. For the Permutation index and quotient, previous work suggests that discontinuities of some size classes was due to climatic oscillations, herbivory, and anthropogenic disturbance (cf. Botha et al. 2002; Helm et al. 2011; Venter and Witkowski 2010).

4.4.3 Harvesting and selection

All size classes were harvested in the three study areas. The preference ratio, however, revealed a positive selection for larger classes. Dludlu et al. (2017) reported a decline in mature individuals in Swaziland due to bark harvest from larger classes. Botha et al. (2004) recorded the decline of *W. salutaris* in commercially harvested populations and protected populations but with more intensity in the former in South Africa. Through market surveys, Williams et al. (2014b) revealed the absence of bark from large specimens in the medicinal plants markets of Johannesburg. For *W. ugandensis*, Tesfaye et al. (2002) found an absence of large individuals in the study in southeastern Ethiopia, indicating strong selection of these size classes. For other bark species, Guedje et al. (2007) recorded selection of bark harvesting on mature individuals, the same for *K. senegalensis* in Benin (Gaoue and Ticktin 2007). Preference for large individuals is probably related to harvesting efficiency because a greater quantity of bark can be harvested and in shorter time than from many small trees (Johnson 2017). In southern Africa, however, traditional practitioners urge that preference for large individuals is related to medicinal efficacy of some species which increase with plant age (cf. Kamatenesi et al. 2014; Sparg et al. 2005; Zschocke et al. 2000). Community members in the study area also believed that bark from older individuals was more effective in treating health problems than from younger individuals (R. Mulhovo, Pers. comm., 13 April 2019). However, due to the limited distribution and popularity of *W. salutaris* in traditional medicine (Krog et al. 2006; Veeman et al. 2014b), the demand for the bark is high and thus represents a potential threat to sustainable harvest of the species. Increment in the extent of harvest, and short time between collections in the same plant may ultimately cause the loss of matured individuals given preference of bark from large specimens. This will ultimately lead to lower production of bark due to high mortality rates (Guedje et al. 2007) apart from the earlier mentioned decline in fruit set and consequently reduced regeneration through sexual reproduction (Gaoue and Ticktin 2007). Avoidance of harvesting small size classes was recorded in the study area, similar to bark tree medicinal plants in South Africa (Geldenhuys

2004). However, with few large specimens remaining, there is some evidence of shifting to smaller classes (cf. Dludlu et al. 2017). It is important to highlight that unsustainable harvesting of the bark was one of the main threats to *W. salutaris* populations in southern Africa (Botha et al. 2004; Dlamini and Dlamini, 2002; Izidine and Bandeira 2002; Mapaura and Timberlake 2002). Therefore, monitoring of population trends, a study of post-harvest bark recovery to inform sustainable harvesting techniques and quotas, are of crucial importance (Botha et al. 2004).

The highest density of harvestable stems and single stemmed individuals was in LM. On average, high densities of harvested and harvested-alive stems were found in TR, compared to other areas. Similarly, higher density of coppicing stems was recorded in TR, resulting on a considerable numbers of coppices. This may be due to high resilience found in *W. salutaris* in response to bark harvest (Botha et al. 2004). As suggested by Luoga et al. (2004) and Shackleton (2000), increasing the height of cutting promotes coppicing, which could have been the case of TR. Botha et al. (2004) found similar results in commercially harvested populations in Mpumalanga.

4.5 Implication for conservation

In many regions, traditional institutions that once used to regulate harvesting to prevent overexploitation are now increasingly less able to prevent it, especially for species in high demand by urban populations (Cunningham 1993). Even under legal protection, formal institutions and regulations have failed to halt the illegal trade of *W. salutaris* bark in southern Africa (cf. Botha et al. 2004; Veeman et al. 2014b). In Mozambique, *W. salutaris* is included in the National Plant Red Data List (Izidine and Bandeira 2002) and it has protection through article 12(2b) of the Environment Law that requires the State to offer special protection to plant species that are threatened with extinction (GM 1997). The Forestry Law (GM 1999) could potentially secure protection of the species in the Maputo Special Reserve (integrating the Futi Corridor) and Licuati Forestry Reserve (a reserve within the Tembe River area). These reserves, however, have a specific species focus, the first for elephant protection and the latter for protection of pod mahogany (*A. quanzensis*). Even with the existing environmental law, rangers in the Futi Corridor cannot adequately protect *W. salutaris*. Furthermore, harvesters in Mozambique have been trading the bark of *W. salutaris* to neighbouring countries (Krog et al. 2006; Mukamuri and Kozanayi 2014; Veeman et al. 2014b), yet the species is not in the National List of CITES. This suggests cross-border trade requires the inclusion of the species in the CITES national and the law enforcement institutions.

In the face of high demand and dependence on bark from the wild populations for informal trade of *W. salutaris* (cf. Geldenhuys 2004; Krog et al. 2006; Mander et al. 2006), extensive cultivation of the species has been recommended (Botha et al. 2004; Veeman et al. 2014b). Thus, cultivation material is required to augment or even substitute harvests from wild habitat (Cunningham 1993). Large quantities of plantlets for cultivation and a permanent source to supply the cultivated material until establishment of the species is required. With existing capabilities of propagation *W. salutaris* from cuttings (Xaba and McVay 2010) or through tissue culture techniques (Moyo et al. 2015), lessons learnt from reintroduction programs in southeast Zimbabwe (Maroyi 2012; Veeman et al. 2014b) and collaborations from other institutions involved in propagation and cultivation of the species in southern Africa, cultivation of the species is likely to succeed.

For the long term persistence of *W. salutaris* in the study areas, work to ascertain limiting factors to sexual reproduction is required. In parallel, monitoring of populations is required to evaluate population trends using the results of this work as a baseline. Furthermore, a plant part substitution strategy (Botha et al. 2004; Zschocke et al. 2000) and a plant substitution strategy (cf. Zschocke and van Staden 2000) should be considered to alleviate pressure on the species. Underpinning the first strategy is the similarity in active ingredients found in the bark and the leaves (Zschocke et al. 2000). Although not common, leaves are used to treat the most recurrent ailments in the study area (Senkoro et al. 2019). The involvement of traditional medicine practitioners licensed under the Association of Traditional Medics of Mozambique (AMETRAMO) to promote the adoption of the strategy is required. For the latter strategy, available alternative species for *W. salutaris* in the study areas (Senkoro et al. 2019) could also be used to lessen harvesting pressure of the species.

4.6 Conclusion

This work determined the density, assessed the population structure and ascertain harvest impacts of *W. salutaris* in the three study areas of southern Mozambique. Results revealed higher densities of recruits in all the study areas than individuals from larger classes. The same was disclosed by the negative slopes and a small number of reproductive individuals.

Findings also suggested some levels of *W. salutaris* population instability in all the study areas. Harvesters had preference of bark from larger individuals, compromising the sexual reproduction, translating to long term implication to offspring rigour of *W. salutaris* individuals. Results also uncovered possible scenarios of bark past trade in all the study areas but currently, FC appeared to be the only active source of *W. salutaris* bark for trade from southern Mozambique.

Author contributions

A.M.S., C.M.S., R.A.V. and A.I.R.B. contributed to the study conception and design. A.M.S. and C.S. contributed to data collection, analysis and interpretation and wrote the first draft. All authors corrected and reviewed the paper.

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Chapter Five[2]: The genetic legacy of fragmentation and overexploitation in the threatened medicinal African pepper-bark tree, *Warburgia salutaris*

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Abstract

Different questions were raised for the genetics study: How is the genetic diversity distributed within and among individuals across the geographic areas? Is the genetic structure associated with the geographic distribution? Is there any evidence of inbreeding or lack of gene flow between populations? Which conservation strategies have more probability to secure the existence of *W. salutaris* in southern Mozambique? The also study aimed to assess the genetic diversity and structure through the application of microsatellite markers. Simple sequence repeats molecular markers were used to evaluate DNA samples for comparison for the Lebombo Mountains, the Tembe River and the Futi Corridor. Genomic DNA was extracted from fresh from 48 individuals of *W. salutaris* in the three study areas. Genomic library were constructed followed by Illumina Hiseq 2500. Potential SSRs were screened and based sequence found, primers were designed. Fourteen representative samples from all the study areas were used to test microsatellite amplification and 10 that presented >20% of polymorphism were used in the study. The

[2] This chapter has been published in Scientific Reports (2020) 10: 19725

number of alleles varied from three to nine with an average of 5.8±2.3 alleles per locus, with a sum of 58 alleles in all loci. The average observed heterozygosis per loci varied from 0.299±0.186 to 0.852±0.062 and for expected heterozygosis from 0.249±0.109 to 0.812±0.062. A sum of 156 alleles were found in 48 individuals of *W. salutaris*. The Shannon's diversity index, observed and expected heterozygosis were higher in LM than other study areas. The average values of polymorphism information index (*PIC*) were higher while the inbreeding coefficients were low in all study areas. STRUCTUE and principal component analysis suggested two populations one composed by LM and TR and the second in FC. Moderate levels of population differentiation were found between FC and TR, between LM and FC but lower levels between TR and LM. This study showed that, despite fragmentation and overexploitation, *W. salutaris* maintains a relatively high level of genetic diversity supporting the existence of random mating. The study suggests that, if local extinctions occurred in Mozambique, *W. salutaris* persisted in sufficient numbers to retain a large proportion of genetic diversity. Management plans should concentrate on maintaining this high level of genetic variability through both in and ex-situ conservation actions.

Key words: Microsatellites, simple sequence repeats (SSR), genetic diversity, population structure, genome size

5.1 Introduction

Medicinal plants have been used worldwide since ancient times, being particularly relevant in the developing world where *ca.* 80% of the population rely on these resources to fulfil their basic health care needs (Bandaranayake et al. 2006; Bodeker et al. 2005; Mukherjee 2002; WHO 2012). Additionally, at the global level the importance of bio-based compounds continues to grow and phytochemical research towards the identification of new active compounds of medical and nutritional importance is among top research priorities (e.g. Du et al. 2015; Gechev et al. 2014; Idris et al. 2017; Iqbal et al. 2017; Lalisan et al. 2014; Mangal et al. 2013; Moyo and Makunganyama 2015; Mulaudzi et al. 2011; Munodawafa et al. 2013; Wangchuck et al. 2012).

Sub-Saharan Africa harbours a vast repository of plant biodiversity, with 45,000 known vascular plant species (Linder 2014), many of which are used in traditional medicine (Alaribe et al. 2019; Burlando et al. 2019; Moura et al. 2017; Moyo et al. 2015; Ribeiro et al. 2010. However, efforts to safeguard this biodiversity are often compromised by anthropogenic pressures, with proximal drivers being land transformation, synergistic impacts of fires, grazing, climate change and harvesting (cf. Botha et al. 2004; Dludlu et al. 2017; Dudley et al. 2019; Feng et al. 2017; Giam et al. 2010; Kouki et al. 2004; Moyo et al. 2015; Pramanik et al. 2018), and growing commercialisation of medicinal plant in high demand (cf. Krog et al. 2006; Moyo et al. 2015; Mukamuri and Kozanayi 2014, van Andel et al. 2015). The last is motivated by preferences for certain species due to cultural identity, traditions, and lower costs in comparison with modern pharmaceuticals, even under circumstance of access to modern medical facilities (Botha et al. 2004; Cunningham 1993). On the other hand, the conservation status of many endemic and native species is poorly understood (Maquia et al. 2019; Ribeiro-Barros et al. 2019) and many natural populations may be at risk. Current exploitation rates, often in tandem with other pressures like fire, invasive species, browsing and land transformation, threaten wild populations unless management methods are established, including community-based approaches (Botha et al. 2004; Cunningham 1993; Moyo et al. 2015).

Under the current scenario of climate change and human population growth, the use of genomic tools is valuable to understand species evolution and adaptation in natural ecosystems (Baldauf et al. 2013; Scribner et al. 2006). The importance of phylogenetic data, genetic diversity, and population structure analyses to characterise the biodiversity of wild species has been well-established in numerous studies (e.g. Bessega et al. 2019; Braun et al. 2019; de Sousa et al. 2020; Hu et al. 2012; Maquia et al. 2013). Microsatellites (Single Sequence Repeats, SSR) are amongst the most efficient and widely used markers for these studies as they are codominant and highly polymorphic loci (Zane et al. 2002). Although these markers are species specific, the increasing accessibility to next-generation sequencing (Jackson et al. 2011) has enabled the development of SSRs for the so-called orphan, neglected or wild crop relative species (e.g. Camacho et al. 2017; Dantas et al. 2019; Eom et al. 2019; Mercantil et al. 2019), although sequencing large plant genomes still remains a challenge (Kyriakidou et al. 2018).

The pepper-bark tree, *Warburgia salutaris* (Bertol.) Chiov. (Family Canellaceae) is one of the most widely used and traded medicinal plants in southern Africa. This slow growing species is part of an early diverging group of basal angiosperms, thought to be native to eastern and southern Africa (van Wyk and Wink 2004). However, subsequent studies confined the distribution of *W. salutaris* to only a sub-region of southern Africa, i.e. South Africa (Botha et al. 2004; Hannweg et al. 2016), Eswatini (previously known as Swaziland) (Dhlamini and Dlamini 2002; Dludlu et al. 2017), Zimbabwe (Maroyi 2012; Mukamuri and Kozanayi 2014; Veeman et al. 2014a, 2014b), Malawi (Msekandiana and Mlangeni 2002) and Mozambique (Jansen and Mendes 1990; Krog et al. 2006; Senkoro et al. 2019). This species is commonly used to treat several ailments such as common colds, throat and mouth sores, or coughs (Senkoro et al. 2019; van Wyk and Wink 2004).

In the past, sustainable harvesting of medicinal plants was regulated through traditional practises such as taboos, restrictions and harvesting tools (Cunningham 1993). However, with commercial demand increasing, *W. salutaris* groves were repeatedly raided by harvesters that often debarked the whole tree, especially mature plants (Coates-Palgrave 2002). That resulted in high tree mortality in many areas and in the extinction of many local populations (Botha et al. 2004; Dludlu et al. 2017; Hilton-Taylor et al. 1998) and consequently, *W. salutaris* is considered threatened throughout its range (Botha et al. 2002; Dlamini and Dlamini 2002; Izidine and Bandeira 2002; Mapaura and Timberlake 2002), and listed as an *Endangered Species* in the IUCN Red List (Hilton-Taylor et al. 1998). The most extreme case is that of Zimbabwe, where the species is listed as extinct in the wild (Maroyi 2008; Mukamuri and Kozanayi 2014). That resulted in the import of bark supplies in the late 1990s from Mozambique and South Africa (Veeman et al. 2014b) being later trafficked from the same countries (Mukamuri and Kozanayi 2014). For instance, in South Africa, 43% of *W. salutaris* bark in the Johannesburg main market originated from Mozambique, with annual traded amounts estimated at 500 – 1 000 kg (Krog et al. 2006). As a result, populations of *W. salutaris* in Mozambique are currently restricted to fragmented patches in the Lebombo Mountains, Tembe River and Futi Corridor (Fig. 1.3) (Senkoro et al. 2019). According to the Red List classification for Mozambique, this species is considered *Vulnerable VU A2 cd* (Izidine and Bandeira 2002). Despite this critical situation, only a few studies on the populations dynamics of *W. salutaris* are available; of the 60 research and review papers available in the Web of Science on *W. salutaris* on 05 February 2020, only seven address this topic (Botha et al. 2004; Dludlu et al. 2017; Hanngwen et al. 2015; Muchugi et al. 2008; Senkoro et al. 2019; Williams et al. 2007) while the vast majority are focused on the medicinal applications of this species. Nevertheless, amplified fragment length polymorphisms (AFLPs) have been used to solve genetic relationships between *W.*

ugandensis Sprague, *W. salutaris* and *W. stuhlmanni* Engl. showing a high degree of genetic variation among individuals within populations as well as between populations (Muchugi et al. 2008).

In this work, SSRs markers for *W. salutaris* were developed to investigate the genetic legacy of exploitation in this slow growing species and to contribute to future re-introduction actions. For that, its best known area of occurrence in Mozambique was used (Fig. 1.3) to addressed the following questions: (1) How is genetic diversity distributed within and among individuals across geographical areas?; (2) Is the genetic structure associated with the geographical distribution?; and (3) Is there any evidence of inbreeding or lack of gene flow between populations?

5.2 Methods

5.2.1 Study area

See detailed information of the study areas on section 1.6.

5.2.2 Population sampling, DNA extraction, genome size value, and SSR development

Based on the areas of occurrence (Senkoro et al. unpublished data), 48 individuals of *W. salutaris* were sampled: 19 individuals from LM, 15 from TR and 14 from FC (Table 5.1). Fresh, young undamaged leaves were collected for each individual plant and frozen at -80°C until DNA isolation. Total genomic DNA was extracted from 50 mg of ground leaves using the InnuSPEED Plant DNA Kit (Analytik Jena Innuscreen GmbH, Germany) according to the manufacturer's protocol. The average yield and purity were assessed spectrophotometrically by OD230, OD260 and OD280 readings (Nanodrop 2000, Thermo Fisher Scientific, Waltham, MA, USA) and visualized by electrophoresis in 1% agarose gels under UV light. Normalized DNA from five individuals of each population was used to develop the SSR markers at CD Genomics (cd-genomics.com/hi-ssrseq.html).

For the development of the markers, the nuclear DNA content of *W. salutaris* was first estimated by flow cytometry using fresh young leaves that were chopped using a razor blade together with an internal standard in a Petri dish containing 1 mL of Woody Plant Buffer

(Loureiro et al. 2007) following the protocol described in (Guilengue et al. 2020). *Solanum lycopersicum* L. 'Stupické' (2C = 1.96 pg) (Doležel et al. 1992) was used as internal standard. The nuclear suspension was then filtered through a 30 µm nylon filter, and 50 µg/mL of propidium iodide (PI; Sigma-Aldrich, St. Louis, USA) and 50 µg/mL of RNase (Sigma-Aldrich) were added to stain the DNA only. The fluorescence intensity of nuclei was analysed using a CyFlow Space flow cytometer (Sysmex, Kobe, Japan). Four independent replicates collected from Kazimat (TR) were measured. Conversion of mass values into numbers of base pairs was done according to the factor 1 pg = 978 Mbp (Doležel et al. 2005). The mean 2C-value of *W. salutaris* was found to be 2.91 pg (± 0.068), corresponding to an average genome size of 2845 Mbp. Samples had an average coefficient of variation of 4.18%.

Genomic libraries were constructed using the KAPAHyper prep kit and sequenced by Illumina Hiseq 2500. Firstly, SSRHunter1.3 was used to screen the potential SSRs from the sequenced data that had at least five repeats (penta-) for 3-5 bp units. Based on the obtained sequences, primers were designed with Primer Premier 5.0 software (Table 5.2).

Table 5.1. Sampled accessions and locations of *Warburgia salutaris* sorted by geographical area. LM= Lebombo Mountains, TR= Tembe River, FC= Futi Corridor.

Accessions	Location	ID	Lat	Long
GF1 (1)	Goba Fronteira	LM	-26.23266	32.09810
MAC13 (2)	Macanda	LM	-26.03522	32.12181
MAC14 (3)	Macanda	LM	-26.03577	32.12150
MAC15 (4)	Macanda	LM	-26.03778	32.12730
MAC16 (5)	Macanda	LM	-26.03692	32.12772
MAC17 (6)	Macanda	LM	-26.05158	32.11803
MAC18 (7)	Macanda	LM	-26.05159	32.11565
MAC19 (8)	Macanda	LM	-26.81118	32.64545
MAC20 (9)	Macanda	LM	-26.04696	32.11979
MAC22 (10)	Macanda	LM	-26.04508	32.11982
MAC24 (11)	Macanda	LM	-26.03521	32.12181
GF2 (12)	Goba Fronteira	LM	-26.26867	32.10719
GF5 (13)	Goba Fronteira	LM	-26.23250	32.09818
GF6 (14)	Goba Fronteira	LM	-26.23241	32.09815
GF12 (15)	Goba Fronteira	LM	-26.23240	32.09822
GS09 (16)	Goba Sede	LM	-26.23238	32.09822
MAC11 (17)	Macanda	LM	-26.04509	32.11983
GF3 (18)	Goba Fronteira	LM	-26.26879	32.10747
GF4 (19)	Goba Fronteira	LM	-26.23233	32.09818
KZT6 (22)	Kazimat	TR	-26.41303	32.36338
KZT7 (23)	Kazimat	TR	-26.41190	32.36422
KZT9 (24)	Kazimat	TR	-26.40960	32.36578
KZT14 (25)	Kazimat	TR	-26.40414	32.35073
KZT15 (26)	Kazimat	TR	-26.38806	32.35008

Accessions	Location	ID	Lat	Long
KZT16 (27)	Kazimat	TR	-26.40994	32.35490
KZT21 (28)	Kazimat	TR	-26.40391	32.36711
KZT22 (29)	Kazimat	TR	-26.40059	32.35109
KZT24 (30)	Kazimat	TR	-26.40206	32.36188
KZT28 (31)	Kazimat	TR	-26.36735	32.37323
KZT35 (32)	Kazimat	TR	26.36737	32.37266
KZT40 (33)	Kazimat	TR	-26.36873	32.37078
KZT41 (34)	Kazimat	TR	-26.36929	32.37334
KZT46 (35)	Kazimat	TR	-26.36935	32.37321
MON47 (36)	Monucua	TR	-26.36952	32.32288
Huc16 (44)	Huco	FC	-26.85013	32.60338
Huc17 (45)	Huco	FC	-26.86159	32.60604
Huc18 (46)	Huco	FC	-26.86169	32.60353
Huc19 (47)	Huco	FC	-26.86129	32.60282
Huc20 (48)	Huco	FC	-26.86025	32.60309
MSL32 (49)	Massale	FC	-26.83979	32.88339
MSL33 (50)	Massale	FC	-26.86458	32.60790
MSL34 (51)	Massale	FC	-26.80948	32.64368
MSL36 (52)	Massale	FC	-26.80590	32.63823
Pz41 (53)	Phuza	FC	-26.78824	32.67368
Pz42 (54)	Phuza	FC	-26.78817	32.67434
Pz43 (55)	Phuza	FC	-26.78814	32.67383
Pz44 (56)	Phuza	FC	-26.78760	32.67419
Pz45 (57)	Phuza	FC	-26.81144	32.66415

Table 5.2. Characteristics and genetic diversity statistics of the 10 polymorphic microsatellite markers developed for *Warburgia salutaris*. For each loci, the repeat motif, Genbank accession number, primer sequence, and size range (bp) is indicated. Na refers to the number of alleles, Ho to observed heterozygosity (mean ± SE) and He to expected heterozygosity (mean ± SE).

Locus	**Repeat motif**	**Accession number**	**Primer Sequence5′–3′**	**Size Range**	***Na***	***Ho***	***He***
1-N1002135	(ATG)5	MT515706	F: TATGTTGGGAGAGGGTGAGG R: GTTTAACGACTGCATCATCCCA	132-174	6	0.487±0.139	0.394±0.101
7-N1082598	(AAT)9	MT515707	F:GTTGATCATAGACACGCCAAGG R: GTCGTGCAACCTAGAGGTCC	161-182	7	0.633±0.085	0.700±0.029
10-N1110523	(TTA)9	MT515708	F: AACCATTGGCACCTCAAGTC R: GTTGAAGTTGAGGGAAGGGATG	244-262	7	0.852±0.060	0.786±0.023
12-N1126672	(TTG)7	MT515709	F: GTTAAATCTGGACCCACTTGCC R: GGGTGAATTAGTGAACGTCTTG	161-180	7	0.805±0.125	0.718±0.074
13-N1132836	(AAG)7	MT515710	F: GTTCCTGCTCCGAGACCTAGAA R: TCATGAAGAAATCGCAACCA	138-144	3	0.304±0.087	0.296±0.086
16-N1150626	(TGG)5	MT515711	F. GTCTTTGGCGAAATCAGTTGGT R: GAAGGTTTCCAGGTTGGTGA	149-159	3	0.299±0.186	0.249±0.109
18-N1173706	(AAG)6	MT515712	F: GAGCTGCCTCGATATGGACT R: GTTATCCAATGGCCAAGAAACC	164-170	3	0.398±0.105	0.421±0.078
31-N2284857	(TTC)12	MT515713	F: GTCTCTTGCTATCATGCGGTCA R: CAGATTGGAGAATCCAGACCA	207-263	9	0.771±0.138	0.812±0.078
33-N3477883	(TGA)6	MT515714	F: GTACAAGATTCATGTGACCGGC R: GCAAGGCATCATATTCACGA	184-200	4	0.550±0.171	0.472±0.124
43-N1009973	(AT)10	MT515715	F: GTTGCGCTCATCGATCTGTA R: GTGCGAACTATGATCGGACGAA	146-185	9	0.439±0.102	0.778±0.027

Fourteen geographically representative samples of *W. salutaris* (LM, TR and FC; Fig. 1.3) were first used to test microsatellite amplification and to troubleshoot amplification conditions. Amplifications were performed in 15 µl reactions containing: 1.25U TaKaRa Hot startTaq polymerase, 1X Buffer I, 1mM dNTPs, 5 µM Primer F and R and 100 ng DNA. The PCR amplification conditions were run as follows: 95 °C for 5 min, 94 °C for 30 s, 30 cycles of 56 °C for 30 s, 72 °C for 30 s, 94 °C for 30 s, 10 cycles of 53 °C for 30 s, 72 °C for 30 s and final extension at 60 °C for 30 min. Ten markers that presented >20% polymorphism were considered, which were used to amplify all samples within this study (Table 5.2). The amplified fragments were analysed on a 3730x1 gene analyzer (Thermo Fischer Scientific) and examined manually for microsatellite peaks. Allele sizes were determined using GeneMapper 3.2 (Applied Biosystems).

5.2.3 Estimates of genetic diversity

For each microsatellite locus, genetic polymorphism was assessed in 48 individuals by calculating the number of alleles (*Na*), observed heterozygosis *(Ho*), expected heterozygosis (*He*), Shannon's diversity index (*I*), and inbreeding coefficient (F_{IS}) using GenALEX software version 6.5 (Peakall and Smouse 2006). The polymorphic information content (*PIC*) was calculated as $PIC = 1 - \Sigma P_i^2$, where P_i is the allele frequency for each SSR marker locus (Benor et al. 2008; Chesnokov et al. 2015; Tonguç and Griffiths 2004). Values of *PIC* above 0.5 were considered highly informative, between 0.5 and 0.25 moderately informative, and below 0.25 less informative (Botstein et al. 1980).

5.2.4 Population genetic structure and differentiation

The Bayesian program STRUCTURE v.2.3.4 (Pritchard et al. 2000) was used to infer the population structure and to assign individual plants to subpopulations. Models with a putative numbers of populations (K) from 1–5, imposing ancestral admixture and correlated allele frequencies priors, were considered. Ten independent runs with 50 000 burn-in steps, followed by run lengths of 1 000 000 interactions for each K, were computed. The number of clusters in the data was estimated using STRUCTURE HARVESTER (Earl and vonHoldt 2012), which identifies the optimal K based both on the posterior probability of the data for a given K and the ΔK (Evanno et al. 2005). To correctly assess the membership proportions (q values) for clusters identified in STRUCTURE, the results of the replicates at the best fit K were post-processed using CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007). GenALEX software version 6.5 (Peakall and Smouse 2006) was used to calculate the Nei's genetic distance (Nei 1978) among individuals. A Principle Coordinate Analysis (PCoA) (Gower 1966) was performed to detect genetic variations between *W. salutaris* individuals. POPULATION 1.2 (Langella 2000) was used to construct an unrooted neighbour-joining tree with 1000 bootstrap replicates. The Wright's F_{ST} value was computed to estimate population differentiation (Peakall and Smouse 2006). Lower genetic differentiation was considered for F_{ST} below 0.05, moderate from 0.05 to 0.15 and high genetic differentiation above 0.25 (Wright 1978).

5.3 Results

5.3.1 Genetic diversity

For each locus, the numbers of alleles varied from three (13-N1132836, 16-N1150626 and 18-N1173706 locus) to nine (31-N2284857 and 43-N1009973 locus) with an average of 5.8±2.3 alleles per locus and a total of 58 alleles considering all loci (Table 2).The average observed and expected heterozygosis per loci varied from 0.299±0.186 (16-N1150626) to 0.852±0.062 (10-N1110523), and from 0.249±0.109 (16-N1150626) to 0.812±0.048 (31-N2284857), respectively.

From the three sampling areas of *W. salutaris* 156 alleles were found in the 48 individuals sampled, being the number of alleles higher in LM than in the other two areas (Table 5.3). The average Shannon's diversity index (*I*) was also higher in LM than in TR and FC. Observed and expected heterozygosis had similar average values in LM and TR being slightly lower in FC. The polymorphic information content (*PIC*) had high average values while inbreeding coefficients (F_{IS}) were low and showing negative values in the three sampling areas.

5.3.2 Population genetic structure and differentiation

The Bayesian clustering program STRUCTURE found the highest LnP(D) and ΔK values for K = 2 (Fig. 5.1). One cluster was predominantly found across LM and TR areas, while a second one characterized the FC. Nevertheless, some individuals in this last area showed signs of genetic admixture between the two genetic groups (* indicated in Fig. 5.1).

Table 5.3. Genetic diversity of *Warburgia salutaris* in the three study areas. Na refers to the number of alleles, I to Shannon's diversity index, Ho to observed heterozygosity (mean ± SE), He to expected heterozygosity (mean ± SE) and PIC to polymorphic information content.

Locus	**Lebombo Mountains (LM)**					**Tembe River (TR)**					**Futi Corridor (FC)**				
	Na	*I*	*Ho*	*He*	*PIC*	*Na*	*I*	*Ho*	*He*	*PIC*	*Na*	*I*	*Ho*	*He*	*PIC*
1-N1002135	3	0.809	0.579	0.499	0.499	3	0.840	0.667	0.491	0.491	2	0.340	0.214	0.191	0.191
7-N1082598	7	1.457	0.526	0.672	0.672	9	1.764	0.800	0.758	0.758	8	1.516	0.571	0.671	0.671
10-N1110523	9	1.942	0.895	0.832	0.832	5	1.480	0.733	0.762	0.762	7	1.649	0.929	0.763	0.763
12-N1126672	7	1.716	0.842	0.795	0.795	7	1.739	1.000	0.789	0.789	4	1.061	0.571	0.569	0.569
13-N1132836	2	0.576	0.421	0.388	0.388	2	0.245	0.133	0.124	0.124	2	0.562	0.357	0.375	0.375
16-N1150626	3	0.455	0.158	0.234	0.234	2	0.637	0.667	0.444	0.444	2	0.154	0.071	0.069	0.069
18-N1173706	3	0.942	0.579	0.564	0.564	3	0.680	0.400	0.407	0.407	2	0.469	0.214	0.293	0.293
31-N2284857	14	2.429	0.947	0.895	0.895	9	1.884	0.867	0.816	0.816	6	1.487	0.500	0.727	0.727
33-N3477883	4	0.954	0.526	0.517	0.517	3	0.468	0.267	0.238	0.238	3	1.090	0.857	0.661	0.661
43-N1009973	11	1.980	0.632	0.801	0.810	9	1.827	0.400	0.798	0.798	5	1.438	0.286	0.724	0.724
Average±SE	6.300± 1.274	1.326± 0.213	0.611± 0.075	0.620± 0.068	0.621± 0.216	5.200± 0.952	1.156± 0.203	0.593± 0.089	0.563± 0.081	0.563± 0.256	4.100± 0.722	0.977± 0.175	0.457± 0.089	0.504± 0.080	0.504± 0.252

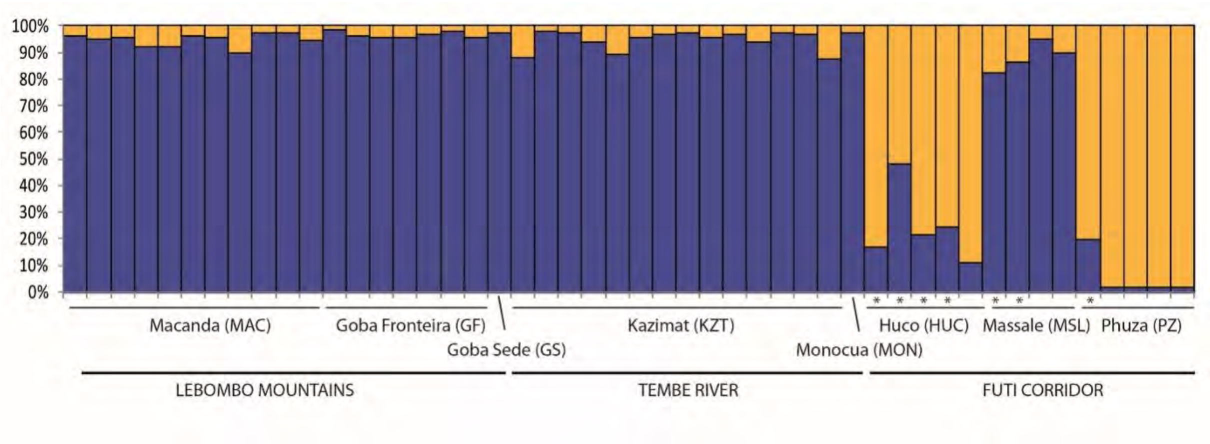

Figure 5.1. Population structure of *Warburgia salutaris* based on 10 SSRs and using the best assignment result retrieved by STRUCTURE ($K = 2$). Each individual sample is represented by a thin vertical line divided into K coloured segments that represent the individual's estimated membership fractions in K clusters. Populations and main geographical areas are indicated below following Table 5.1. Asterisks indicate individuals with a probably of membership lower than 90% to the main genetic cluster, as revealed by STRUCTURE.

The first two coordinates of the principal coordinate analysis (PCoA) explained 22.9% of the total variation, and populations were spatially separated into the two main groups found by STRUCTURE (Fig. 5.2). The neighbour-joining tree revealed several small clusters although mostly with a very low support (<30% BS) and overall, with no association between the clusters found and the three geographic areas (Fig. 5.3) as reported in the other analyses. However, a clear cluster grouped all the FC geographical area.

The pairwise population F_{ST} values varied from 0.049 (TR *vs*. LM) to 0.114 (FC *vs*. TR) revealing moderate levels of genetic differentiation between FC and TR and between FC and LM and lower levels between TR and LM (Table 5.4).

Table 5.4. Pairwise population FST values for *Warburgia salutaris* in the three study areas.

Population	Lebombo Mountains	Tembe River	Futi Corridor
Lebombo Mountains	0.000		
Tembe River	0.049	0.000	
Futi Corridor	0.084	0.114	0.000

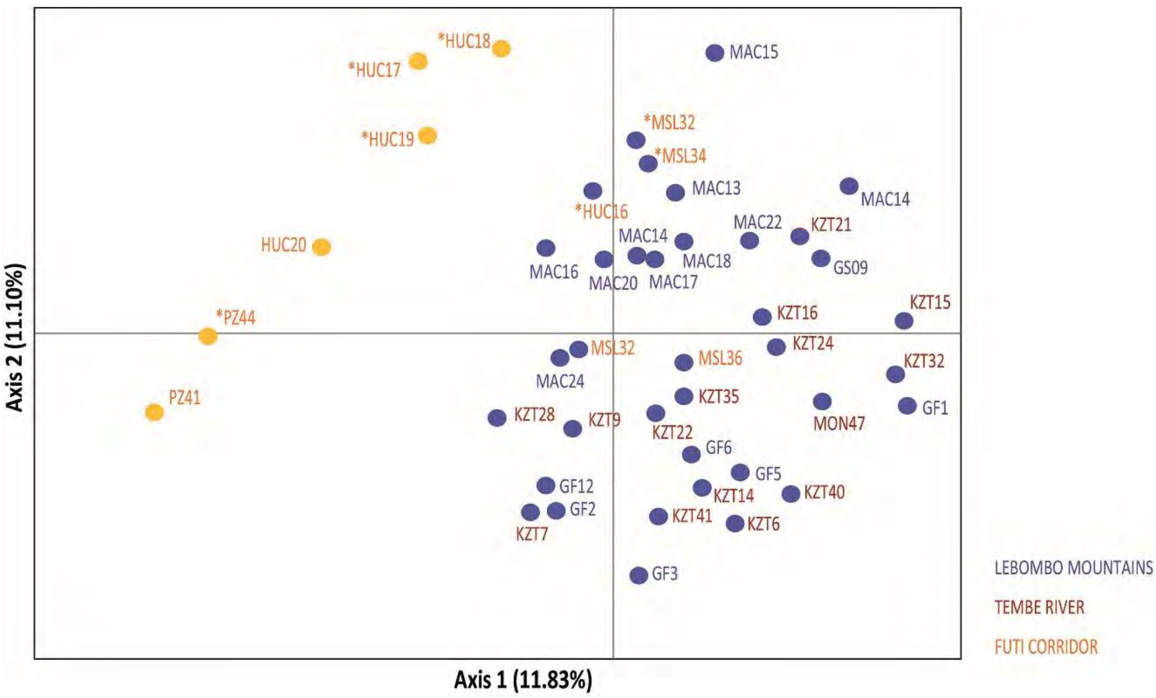

Figure 5.2. Principal Coordinate analysis (PCoA) of the studied *Warburgia salutaris* using the scored SSRs markers. Percentage of explained variance of each axis is given in parentheses. Population labels follow Table 5.1. Colour of symbols (circles) indicate the two genetic groups identified by STRUCTURE. Colour of labels followthe three main geographic areas as depicted in Figure 1.1. Asterisks as in Figure 5.1.

5.4 Discussion

5.4.1 High genetic diversity and admixture in *W. salutaris*

Assessment of genetic diversity is critical to understand the ability of a species to cope with changing conditions and environments, specifically for threatened species (Paliwal et al. 2016; Tian et al. 2016; Tonguç and Griffiths 2004; Zhao et al. 2012). In this study, for the first time the development of Single Sequence Repeats (SSR) markers in *W. salutaris* by employing next generation sequencing (Illumina platform) was reported.The 10 SSRs markers were validated and found to be highly polymorphic, similar to threatened species such as *Acer miaotaiense* P.C.Tsoong (*PIC* = 0.604) (Li et al. 2018) or *Corylus avellana* L. (*PIC* = 0.778) (Martins et al. 2015). These markers are now available to extend *W. salutaris* population studies to a regional level. Additionally, the SSRs developed during this work might potentially be suitable to study genetic diversity in other species within the genus *Warburgia*, since only a limited number of studiesis available and based on Amplified Fragment Length Polymorphism (AFLP) (Gacheri et al. 2016; Muchugi et al. 2008), a time-consuming and costly technique. To the best of our knowledge, the present study represents the first genome size estimation of *W. salutaris* and only the second within the Canellaceae family having a genome size 4×smaller than *Winterana canella* L. (*Canella winterana* (L.) Gaertn.) [2C= 11.7 pg] (Leitch et al. 2002; Soltis et al. 2003). The relatively small genome size of *W. salutaris* is within the range of the non-expanded genomes of currently known magnoliids and may facilitate future genomic initiatives although further analyses are needed to determine its ploidy level.

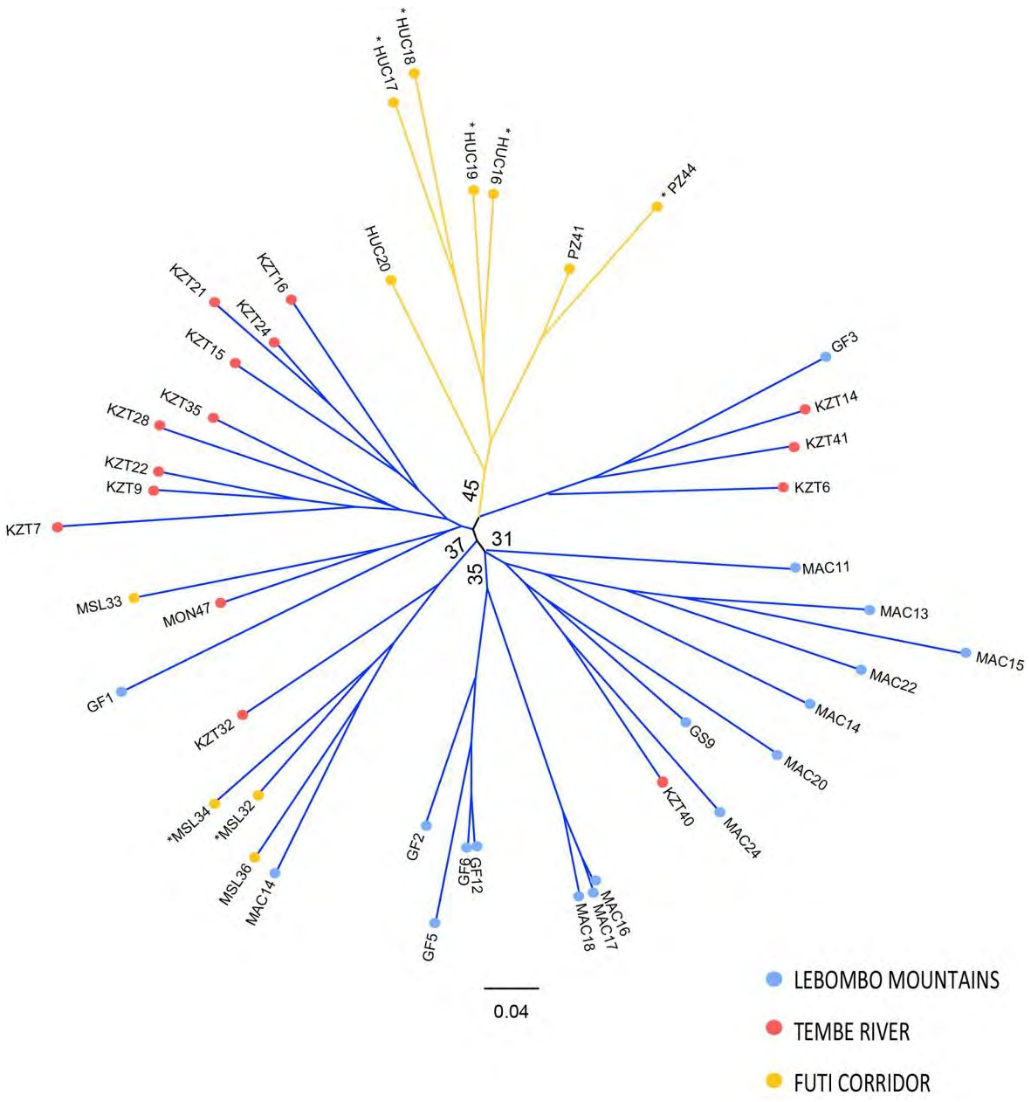

Figure 5.3. Unrooted neighbour-joining tree of the studied *Warburgia salutaris* based on Nei's Da genetic distance. Numbers associated with branches indicate bootstrap values (BS) based on 1000 replications. Only BS above 30 are shown. Colours of branches indicate the two genetic groups identified by STRUCTURE. Colour of circles near each label indicate the three main geographic areas as depicted in Figure 1.1. Asterisks as in Figure 5.1.

Due to the heavy harvesting pressure to which *W. salutaris* is subjected in Mozambique (Krog et al. 2006; Senkoro et al. 2019), genetic diversity levels were expected to be low. However, high levels of genetic diversity found in the three surveyed areas in comparison to other narrowly distributed species, as for instance, the tropical tree *Paypayrola blanchetiana* Tul. (*Na:* 2-5 alleles per locus; *Ho:* 0.063-0.563 in the two populations; *He:* 0.063-0.567in the first population and 0.063-0.627

in the second) (Braun et al. 2019). However, genetic diversity indices of *W. salutaris* were similar to other species where bark has been heavily-exploited, such as *Cinchona officinalis* L. (*Na:* 5.2-7.6 alleles per locus; *Ho:* 0.580-0.680; *He:* 0.616-0.717) (Cueva-Agila et al. 2019) or even lower than *Himatanthus drasticus* (Mart.) Plumel (*Na:* 6-24; *Ho*: 0-0.847, *He*: 0-0.864) (Baldauf et al. 2013).

High levels of heterozygosis may be due to factors including the reproductive system such as self-incompatibility of pollen (Yang et al. 2019) or high gene flow George et al. 2009; Zhao et al. 2012). Results from this work revealed a range of the inbreeding coefficient of -0.492 (TR) to -0.363 (LM), which is much lower than those found in e.g. *H. drasticus* (0.248-0.303) (Baldauf et al. 2013), *Calotropis gigantea* (L.) W.T.Aiton (0.167), *C. procera* (Aiton) W.T.Aiton (0.177) (Muiriira et al. 2019), or *Phoenix theophrasti* Greuter (0.9) (Vardareli et al. 2019). The negative inbreeding values found here suggest the existence of random mating (Sun et al. 2016) among individuals of *W. salutaris* and might also explain the levels of heterozygosis found here. Indeed, the related species *W. ugandensis* Sprague has a mixed mating system being predominantly outcrossing (Muchugi et al. 2008). Additionally, insect pollinators of *W. salutaris* such as bees are probably able to travel over the large agricultural blocks separating the three geographical areas studied here, promoting gene flow. Genetic admixture between sites might also be facilitated by frugivorous birds that often eat the berries thereby facilitating the dispersion of seeds. In accordance, high levels of genetic admixture found between populations with only two genetic clusters being found, one grouping the northern populations and the other one, the southern populations.

Our study suggests that, although some local populations might have been severely affected by harvesting, the pepper-bark tree might have persisted in sufficient numbers in Mozambique to allow outcrossing between sites, retaining a large proportion of genetic diversity. Although there are no records of the historical distribution of this species, the studied populations could be relicts of once much larger populations that persisted in specific locations. In addition, recent conservation efforts might have diminished trade in Mozambique, avoiding severe barking in these populations. Further research should focus on understanding the factors limiting the regeneration of *W. salutaris* trees.

5.4.2 Population differentiation between geographic areas

Population differentiation of endangered species is variable. For example, low differentiation was found between populations of *Platanthera leucophaea* (Nutt.) Lindl. ($F_{ST} < 0.02$ over distances <2 km (Paul et al. 2013) while in *H. drasticus* the differentiation levels were high (F_{ST} from 0.036 to 0.077 over short distances) (Baldauf et al. 2013). In contrast, the endangered *Paeonia rockii* (S.G.Haw & Lauener) T.Hong & J.J.Li ex D.Y.Hong revealed a high differentiation between populations (F_{ST} varied from 0.780 to 0.982) (Yuan et al. 2011). Despite the narrow distributional area of *W. salutaris* in Mozambique, this study revealed a high genetic differentiation between the northern populations located in LM and TR and the southern populations located in FC (Fig. 1.1). Pairwise F_{ST} comparisons showed lower genetic differentiation between LM and TR (0.049), which are separated by only 28km, than either between LM and FC areas (0.084, separated by 81 km) or between TR and FC (0.114, separated by 49 km). STRUCTURE analyses also found a distinct genetic cluster in the FC area, which was also supported by PCoA analyses and the NJ tree. Contrary to LM and TR areas, where *W. salutaris* occurs in slopes and forest patches, in the FC area this species occurs near seasonal pans in thicket vegetation associated with termitaria on clay soils (MITUR 2002; Matthews et al. 2001). This might imply differences inreproductive ecology, particularly regarding flowering phenology and the activity of pollinators, which would affect gene flow with the other sites, explaining the genetic structure and population differentiation found between the studied sites. Thus, the differentiated FC genetic clusters could be harbouring novel and important alleles and should be given priority in *in situ* and *ex situ* conservation strategies (Ge et al. 2005; Muriira et al. 2019; Zheng et al. 2008) in Mozambique.

5.5 How to conserve a species widely exploited and needed?

Several populations of *W. salutaris* are threatened by fire from slash and burn agriculture, as they occur in adjacent patches or in agricultural lands (Senkoro et al. 2019). Equally, burning of natural vegetation to improve livestock fodder, poaching, and opening of new areas for settlements are also potential threats to the species (e.g. GDM 2008; Halafo 1996; MAE 2005). Vegetative propagation of *W. salutaris* is possible through tissue culture (Hannweg et al. 2015) although expensive. This species is being largely cultivated *ex situ* in South Africa (Mbambezeli 2004) and in small scale in Zimbabwe (Maroyi 2012; Veeman et al. 2014b) and Mozambique (unpublished data), to encourage the sustainable use of the species. Home gardening would also be important for this species although that requires the involvement of local communities and understanding their perceptions towards the conservation of this species.

Considering the confined distribution and threatened status, the long-term persistence of *W. salutaris* should be secured by conserving the maximum genetic diversity of the species. As it is impossible to designate every natural wild plant habitat as a protected area, nurseries could be implemented to ensure production stability. The disclosure of genetic variation and understanding of genetic relatedness within populations is useful for their sustainable uses (Pan et al. 2017). Knowledge of genetic diversity from other countries as the one reported here would also help to implement conservation strategies including re-introduction programs, selecting the most suitable material to be used. Understanding the degree of genetic variation between Mozambique and the neighbouring countries would facilitate transborder conservation actions. Further studies must also be conducted to detect and understand how reductions of natural regeneration or fitness are affected by harvesting. Finally, efforts to educate the local population and landowners on the importance of conserving the natural populations of *W. salutaris* should continue.

Author contributions

A.M.S., C.M.S., R.A.V., and A.I.R.B. conceived the work and the experimental design. A.M.S., C.M.S., and R.A.V. performed the field data survey and sample collection. A.M.S., P.B.S., F.S., and P.T. performed the laboratorial analysis. P.T. performed the flow cytometry data analysis. A.M.S., F.S., I.M. and A.I.R.B. performed the microsatellite data analysis. A.M.S., I.M., and A.I.R.B. wrote the first draft and final version of the manuscript, which has been thoroughly reviewed by all authors.

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Chapter Six: Synthesis and Recommendations

6.1 Introduction

The reliance on medicinal plants in Mozambique is based on traditional cultures of the people and limited access to modern health services. Traditional medicinal practitioners (TMP) are the first to be consulted and they provide the greatest primary health coverage in the country, estimated at 70% of the people with the ratio of one TMP to 200 inhabitants (MISAU 2013). The foundation of the traditional medicine health care system is the repository of plant species known for their medicinal value (MISAU 2013). Despite the use of medicinal species at the household level, increasing numbers of people are involved in the trade of medicinal species. This is due to high levels of poverty, lack of formal employment, low education levels (World Bank 2018) and low agricultural productivity (Malate 2013). For example, Fato (1995) reported that there were 106 traders in medicinal plant markets of Maputo city. Later Krog et al. (2006) recorded 198, corresponding to an approximately 87% increase in a decade. Most of the traded material in Maputo is acquired from the wild and adjacent provinces and is also traded to South Africa (Krog et al. 2006). Such trade provides cash to supplement household needs (cf. Shackleton et al. 2007), or can be the main source of income for some.

Among the wild harvested and highly traded medicinal species in Maputo's medicinal markets is *W. salutaris*. This species has a confined distribution and is overexploited due to high demand throughout southern African (Krog et al. 2006). For these reasons, the species is threatened throughout its range and it is red listed in Mozambique under the "threatened" category of IUCN (Vulnerable) (Izidine and Bandeira 2002) and globally as "Endangered" (Hilton-Taylor et al. 1998). Despite the concerning conservation status of *W. salutaris*, little is known about its ecology and genetics. Consequently, research is necessary to provide insight into factors affecting its conservation. Only ethnobotanical surveys reporting medicinal value (cf. Jansen and Mendes 1990) and markets for the species have been reported (Barbosa et al. 2020; Krog et al. 2006). Hence, additional research was crucial to inform stakeholders about the conservation status of the species and to propose appropriate conservation strategies. With this in mind, this thesis looked at four different dimensions for the conservation of *W. salutaris* in southern Mozambique. The first was to understand the distribution of *W. salutaris* in southern Mozambique. Using occurrence data and environmental predictors (mainly climatic variables due to lack fine-scale data), I simulated the potential current and future distribution of the species. I used four different general circulation models for a broader view of different results to project possible future distribution under pessimistic and conservative climatic change scenarios. Second, I identified drivers of *W. salutaris* uses, examined knowledge distribution and local ecological knowledge, and determined the existing local management practices of the species in the Lebombo Mountains (LM), the Tembe River (TR) and the Futi Corridor (FC) areas of southern Mozambique. Third, I determined the population structure and ascertained the extent of bark harvest on individuals of *W. salutaris*, and lastly, assessed the genetic diversity and differentiation in the three study areas of southern Mozambique.

6.2 Key findings

6.2.1 Potential current and future distribution

To determine the current and potential future distribution of *W. salutaris* in southern Mozambique, a spatial model was developed to predict the potential distribution. The conceptual framework showed that it is possible to reconstruct a realised niche for the species (areas that is occupied by the species) through a habitat suitability model using environmental predictors and species occurrence data. It was also projected that the distribution of *W. salutaris* is naturally fragmented. Modelling results suggested that the current distribution corresponded to the areas of occurrence observed during the field work but with a few additional localities. Results also indicated that *W. salutaris* is naturally fragmented with a limited distribution across the two districts of southern Mozambique (Namaacha and Matutuine). This distributional pattern was also confirmed throughout the southern African region (cf. Coates-Palgrave 2002; Jansen and Mendes 1990; Krog et al. 2006; Moyo et al. 2015). This means that wild harvested populations are more susceptible to threats such as overharvesting despite resilience conferred by its ability to respond to harvest by coppicing (Chapter 4; Botha et al. 2004) because of limited stocks in the wild (Cunningham 1993; Moyo et al. 2015; Veeman et al. 2014b). Furthermore, model simulations suggested a considerable increase in potentially climatically suitable areas as well as habitat quality and the extent of occurrence of *W. salutaris* for all four general circulation models in both conservative and pessimistic climatic scenarios over the next 50 years (up to 2070) (Chapter 2). Thus, consideration needs to be given to other threats to the species, in favour of the changing climate. The conceptual framework showed different anthropogenic threats that could result in loss of gene flow. Findings from this work indicated the main threats were unsustainable harvesting of the bark for trade (Chapters 3 and 4), harvesting for charcoal production (Chapter 3), wildfires (Chapters 3 and 4) and clearing of land for settlement (Chapter 3), the last two were rarely mentioned. Bark trade was still considered to be a potential threat for the species in the future (Chapter 3). These findings are also in consistent with previous works on the *W. salutaris* reported in the country (Halafo 1996; Krog. et al. 2006) and elsewhere in southern Africa (Botha et al. 2004; Coates-Palgrave 2002; Dludlu et al. 2017; Hilton-Taylor 1998; Mukamuri and Kozanayi 2014; Williams et al. 2014). This means that if overharvesting of bark for trade persists, it will affect the population structure, sexual reproduction and genetic diversity and subsequently lead to local extinction of the species (see section 6.2.3 and 6.2.4) (Keller and Waller 2002). Other threats include lack of regeneration through seeds due to early fall of fruits and seed parasitism that have diminished the potential of sexual reproduction through loss of germination potential (Scott-Shaw 1999), resulting in regeneration largely from asexual reproduction (also refer to sections 6.2.3 and 6.2.4).

6.2.2 Cultural and social factors that affect use and sustainable management

To identify cultural and social issues that impact use and sustainable management of *W. salutaris* this work considered different drivers of use, knowledge and local ecological knowledge and identified the current management practices for the species (Chapter 3). Results revealed that bark was the most demanded part of the plant, used to treat several health complaints and that leaves were rarely used although used for several important ailments. Nevertheless, researchers have reported the use of leaves by traditional healers elsewhere in the region (cf. Botha et al. 2004; Mukamuri and Kozanayi 2014). In this context it is worthwhile to consider adoption of leaves as an alternative to bark, to alleviate pressure on *W. salutaris* populations (Zschocke et al.

2000). In addition, a significant number of alternative species for many of the ailments were available in the study area. This is also a challenge that needs to carefully be addressed because *W. salutaris* is deemed indispensable for its medicinal value (Mukamuri and Kozanayi 2014).

The conceptual framework presumed the existence of local management practices, it also presumes the existing threats to the species and its habitat. Results of this work revealed bark harvesting approaches were the main local management practice for *W. salutaris* (100% TR, 61% FC, and 52% LM) and people were aware of harmful harvesting methods (91% TR, 52% FC and 50% LM). Results also showed that overharvesting of *W. salutaris* for trade as the main threat for the species, while habitat transformation for settlement was a threat to the habitat of *W. salutaris* although less mentioned (Chapter 3 and 4). Indeed the effect of bark trade has been recorded for the species throughout its southern Africa range (cf. Botha et al. 2004; Dludlu et al. 2017; Krog et al. 2006; Mukamuri and Kozanayi 2014). For example, due to unsustainable harvesting of the bark for trade, *W. salutaris* was thought to be extinct in the wild in Zimbabwe (Maroyi 2013). Interesting, however, is that informants knew appropriate harvesting methods but their implementation seemed to be doubtful, especially when continuous declines are recorded. I also expected that informants would provide information on bark trade to justify unsustainable consumption, but instead they declined and understated their involvement in trade. This can either be a frequent reaction among law transgressors (refer to Chapter 3) (cf. Jann et al. 2012; Tourangeau and Yan 2007) or the case of unidentified bark traders (community members or not), but both involved extracting unknown amounts for trade. Southern Mozambique (the only known area of occurrence of *W. salutaris* in Mozambique) has been a primary source of illegal bark supply for trade to the regional medicinal plant markets (Krog et al. 2006; Mander et al. 2006; Veeman et al. 2014b). This undermines the time needed between harvest to allow the recovery (cf. Costa and Oliveira 2015; Pandey 2015). Under these circumstances, it is important to develop post-harvesting bark recovery strategies to complement existing knowledge on appropriate harvesting methods by understanding sustainable harvesting quantities and time periods between consecutive harvests from the same individual (cf. Costa and Oliveira 2015; Pandey 2015). In addition, the extent of bark harvesting found in this work suggests FC is currently the main source sustaining most of bark trade

(Chapter 4). Perhaps it is under this circumstance that most informants were afraid to affirm their or other community members' involvement in the trade.

6.2.3 Population structure and harvesting impacts

To evaluate the population structure and assess the extent of bark harvesting, I investigated different population parameters, size-class distribution, population trends and harvesting impacts. Size class profiles used in the conceptual framework argued that an inverse J-shape, showing the presence of more juveniles than adults, indicates a stable population (Chapter 1). The results revealed this pattern. However, the three indexes evaluating population trends showed some level of instability in *W. salutaris* populations, in all three study areas. Nevertheless, the negative slope indicating more recruits and fewer individuals in larger size classes (refer to conceptual framework in Chapter 1) was found for *W. salutaris* in the present work. Thus, the mean stem densities were high for small size classes and low for large classes. These results are consistent with other *Warburgia* species (Galabuzi et al. 2015; Ssegawa and Kasenene 2007) and *Khaya senegalensis*, a bark harvested trees species in West Africa (Gaoue and Ticktin 2007; Guedje et al. 2007). Moreover, the mean densities of mature stems varied considerably in the study areas and evidence of reproductive structures was infrequent (mean densities of 0.1±0.5 stems/ha in FC to 291±1792.9 stems/ha in TR), resonating with other studies of intensively harvested species (cf. Lopez-Toledo et al. 2018; Silva et al. 2018). This means that overharvesting impacts reflect beyond the population structure such as commercially harvested populations in South Africa (Botha et al. 2004) to genetic diversity and structure (see section 6.2.5), as well as socio-cultural and economic dimensions of the species (Chapter 1). In addition, burnt stems were not significant in the three study areas, also verified during in household interviews (Chapter 3) and was confirmed in the Namaacha district (MAE 2005b; Section 1.6). However, fire remains as a challenge in some parts of the Matutuine district because of poaching and shifting cultivation (cf. GDM 2008; Mussagy 2006).

Bark harvesting took place on all size classes in all the study areas but with a preference for larger size classes (basal diameter size classes > 15 cm) and harvesters tended to avoid harvesting smaller stems (basal diameter ≤2 cm). This echoed the findings for *W. salutaris* in unprotected areas in South Africa (Botha et al. 2004) and in Eswatini (Dludlu et al. 2017). In general, densities of harvestable, harvested, harvested and alive stems were lower in FC compared to LM and TR but the densities of harvested and stressed, and harvested and dead stems were significantly different between the study areas and were highest in FC. Although *W. salutaris* is resilient to some effects of harvesting by readily coppicing, it would not withstand repeatedly harvesting and intensive ring barking (Botha et al. 2004). It is not surprising that high levels of stem destruction were recorded in FC. Interestingly, most communities in FC ascribed this to strangers encroaching their area to harvest the bark (Chapter 3). This is similar to the commercially harvested population and is contrary to the protected population found in South Africa (Botha et al. 2004). LM and TR were involved in bark trade in the past but FC (protected area) is the major and current supplier of bark from southern Mozambique. It is important to establish i*n situ* protection of *W. salutaris* with priority given to FC and strengthen the law enforcement institutions (cf. Dreyer 2013; Hannweg et al. 2015) by capitalising on its status as a protected area and monitor population trends. Consideration should also be given to regulate the transboundary commercialisation through the Convention of International Trade in Endangered Species of Fauna and Flora (CITES) of the bark to avoid further loss of *W. salutaris.*

Trade of highly demanded NTFPs can affect the population structure when sustainable harvesting approaches are not observed because harvesters seek to maximise harvesting returns which may be above the ecologically sustainable levels, for financial returns (Peck and Christy 2006). For example, tapping of individuals between size classes 101 to 150 cm tall of *Hyphaene coriacea* Gaertn. and *Phoenix reclinata* Jacq. for palm wine production in Mozambique affected the population structures of the two species (Martins and Shackleton 2017). Similarly, commercial harvesting of *W. salutaris* bark in unprotected populations altered the population structure of the species in South Africa by affecting large trees (Botha et al. 2004). Correspondingly, the overexploitation of larger individuals of *K. senegalensis* in certain populations for medicine (bark) and fodder (leaves) in Benin affected the population structure of the species (Gaoue and Ticktin 2007). In all cases selective harvesting with preference for mature individuals reduced the ability of the species to reproduce through seeds, lowering the numbers of seedlings and saplings. In some settings, high mortalities have been reported on mature individuals due to ring-barking (Delvaux et al. 2010) and harvesting all the available bark from the stem (Pandey 2015). It is, therefore, not surprising that some levels of population instability were observed in the current situation.

A limited number of seedlings and saplings is among the consequences of unsustainable harvesting. This is because overharvesting affects several reproductive parameters, commonly by reducing reproductive effort. For example, the short-term effect of bark harvesting in *H. drasticus* and *Stryphnodendron rotundifolium* Mart. resulted in lower production of most reproductive attributes of the species, notably, lower fruit set and seed production. It is presumed that plant photosynthate is diverted to the wound recovery and hence, less resource allocation to reproduction (Silva et al. 2018). Similarly, harvesting of *Brahea aculeata* (Brandegee) H.E.Moore leaves lowered the production of flowers and fruits, and consequently poor seeds production (Lopez-Toledo et al. 2018). Tapping of *Boswellia papyrifera* (Caill. Ex Delile) Hochst. reduced flower and fruit production, resulting in fewer and inviable seed (Rijkers et al. 2006). Indeed, results of this work indicated insignificant evidence of reproductive structures for *W. salutaris* in all three study areas. In addition, loss of seed viability was attributed to early fall of fruit and subsequent seed parasitism (Muatinte and Cugala 2014). The short-term impact of constrained reproductive success is likely to be restricted gene flow that results in crossing between close related individuals (Silva et al. 2018). This may elevate the levels of inbreeding, resulting in a lowered ability to persist and resist stress (Keller and Waller 2002).

6.2.4 Genetic diversity and structure

To evaluate the genetic diversity and population differentiation in the study areas, the work revealed a relatively high number of alleles and high values of observed and expected heterozygosis. The conceptual framework suggested that overharvesting could result in interrupted gene flow and postulated vulnerability to inbreeding due to isolation and crossing between closely related individuals (Bossuty 2007; Halsey et al. 2015; Keller and Waller 2002). Contrarily, low values of inbreeding were recorded, suggesting the existence of random mating and that the overexploitation of the populations had not compromised the genetic diversity (Sun et al. 2016). The opposite was observed for *H. drasticus,* a bark harvested tree in Brazil (Baldauf et al. 2013).

Interestingly, results of this work indicated population instability (Chapter 4), a decline of individuals in *W. salutaris* populations (Chapter 3), few mature individuals, a lack of reproductive structures and a high frequency of clonal recruits (Chapter 4). Clonal reproduction in general, is related to massive flower production, limited pollen dispersal and auto-pollination that can cause inbreeding and less success of pollen outcrossing (Vallejo-Marín et al. 2010) increasing homozygosis and hence, lowering genetic diversity. This means that beside the impacts of harvesting, fire, opening of land for settlement (refer to section 6.2.1) on populations in *W salutaris*, the species still has considerable levels of genetic diversity. Therefore, strategies such as *in situ* protection need to be developed as precautionary measures, to conserve the existing genetic diversity within populations of *W. salutaris*. In parallel, monitoring program to assess trends in genetic diversity and evaluate the effectiveness of *in situ* conservation is also of utmost importance. In addition, research aiming to determine factors affecting sexual reproduction such as overharvesting, early fall of fruit (e.g. hormonal causes, inefficiencies of pollination and seed parasitism) (cf. Piñero et al. 2010; Tatari et al. 2018; Tisné et al. 2020; Zeng et al. 2016) need to be considered.

STRUCTURE and Principal coordinate analysis differentiated two genetically distinct populations of *W. salutaris* in the study areas, LM and TR as one, and FC as another. STRUCTURE also distinguished signs of genetic constitution of the two groups among individuals in FC. In addition, levels of genetic differentiation were moderate between FC and the remaining areas (0.084 LM and FC, 0.114 TR and FC) but lower between LM and TR (0.049). The genetic study suggested the existence of novel alleles in FC and a relatively homogenous group in LM and FC. This means that harvesting intensity is currently more accentuated in the area harbouring unique alleles to those found in other areas. This will eventually affect people's social, cultural dimensions due to dependence on the species for their health (especially for common ailments) and consequently their income generation activities (e.g. agricultural production, charcoal and wine and production, and trade, trader in *W. salutaris* bark) (Chapter 3). However, the output of the conceptual framework is to secure the long-term persistence of *W. salutaris* and conservation of this genetic diversity. For this, interventions such as re-introduction and cultivation programs are essential. Indeed, Botha et al. (2004) and Moyo et al. (2015) suggested cultivation of *W. salutaris* with massive production of plantlets to establish a stable source for bark for trade. Maroyi (2012) and Veeman et al. (2014) reported the experience of re-introduction of *W. salutaris* in Zimbabwe. Zhao et al. (2012) proposed incorporation of different individuals on the production of plantlets to maximise the genetic diversity and simultaneously conservation of mature individuals for the existing diversity. Low fruit set, early fall of fruits and loss of seed viability through parasitism could constrain the propagation of *W. salutaris* (Botha et al. 2004). However, the number of plantlets required can only be achieved through sophisticated methods. Rout et al. (2000) indicated *in vitro* culture as the appropriate method for production of cultivation material. Hannweg et al. (2015) showed considerable production of *W. salutaris* through tissue culture in Kruger National Park for cultivation. Kikulwe (2016) established community nurseries for acclimatisation of banana disease free for posterior cultivation. Propagated material should also be made available to the communities (cf. Böhringer et al. 2003; Botha et al. 2004). Therefore, regional co-operation to propagate cultivated material could be done and collaboration from the community to acclimatise and establish community nurseries for subsequent cultivation of the species is proposed.

6.3 Limitation of the study

Modelling of *W. salutaris* distribution (Chapter two) lacked credible anthropogenic and additional environmental variable leading the climatic based simulations. Due to this, results unveil that there was apparently no spatial limitation in terms of future climatic conditions for *W. salutaris* to grow. Probably this distributional range could have been narrowed by additional variables, generating a more reliable and accurate prediction because climatic variable are not the only determinants of habitat suitability (cf. Bobrowski et al. 2017; Pramanick et al. 2018; Qin et al. 2017). Whereas, other variables that could explain future distribution (edaphic characteristics, hydrological characteristics, human pressure on populations of *W. salutaris*) were lacking projections on its future evolution or in other cases were assumed constant within the period considered (ca 70 years).

Chapter 3 explore the use knowledge and local ecological knowledge (LEK). The study failed to evaluate differences in knowledge between men and women in FC because the number of male informants was considerably higher than of women. Thus, the use knowledge and especially LEK was based on male's perceptions. This could have been addressed through conducting stratified sampling to increasing the participation of women who knew *W. salutaris* the rather than using stratified, random sampling in all the three study areas. The work also lacked information on communities' involvement on markets of *W. salutaris* because most informants refused to respond to the questions presumably because they avoided to incriminate themselves and other community members (Chapter 3). Indirect questions could have been used to surpass this gap (Nuno and St. John 2014). Additionally, study on the economics of. *salutaris* in southern Mozambique to appraise the contribution of the species in peoples livelihoods is required.

6.4 Conclusion

Studies advancing the conservation of highly traded medicinal species, especially those assessing the *in situ status quo* are limited in southern Africa. Hence, there is a need to consider developing such works to scrutinise different dimensions to support conservation and management of such species. Therefore, the aim this study was to determine the distribution, demand, population structure, genetic diversity, genetic population structure and population differentiation to gather evidence to inform stakeholders such as government ministries, non-governmental organisations, local government and other entities dealing with threatened and medicinal plants in Mozambique.

This work confirmed the current distribution of *W. salutaris* in southern Mozambique and suggested that climatically suitable areas will increase, favouring the expansion of *W. salutaris*. In the coming 50 years, climate change will not be a threat to the species but addressing other threats is crucial to safeguard this species. This study also confirmed the existence of population instability in all three study areas as a result of preference for bark from large stems for trade for medicinal use rather than aerial parts of the plant (e.g. leaves), affecting large size classes thereby constraining regeneration through seed. However, most informants did not admit to past or current involvement in bark trade or of others in their community. This suggests they might have been involved or knew people involved and sought to avoid incriminating themselves or others or they were genuinely unaware of people linked with bark trade, perhaps due to long distances from LM and FC to collection sites. Charcoal production, wildfires and land use transformation were also found to contribute to declines in abundance of *W. salutaris* in the study areas.

Local management practices were primarily through vertical and discontinuous bark harvesting and awareness among informants of harmful harvesting methods. Nevertheless, this did not effectively protect *W. salutaris* as evidenced by heavy debarking of large individuals was found throughout the study areas. This work suggests special attention needs to be given to FC, in particular, because of high levels of stem harvesting and mortality was confirmed, and the long distances to collection grounds from homesteads constrained collective community protection of *W. salutaris*. Indeed, this work considered FC as the current main source of bark supply for much of the *W. salutaris* trade in southern Mozambique.

The genetic study indicated high levels of genetic diversity despite high harvesting pressures on stems of reproductive size. The work also lead to the assumption that considerable levels of genetic diversity were found as a results of random mating between individuals of the species. However, prevalence of clonal reproduction due to failure to reproduce sexually will reduce levels of the existing genetic diversity if harvesting especially from of large specimens, is not halted. This could elevate levels of inbreeding resulting from mating between closely related individuals and low gene flow between populations and hence, reduce offspring vigour, increase the chances of species loss and extinctions.

6.5 Recommendations

Findings from this study indicated population instability with a substantial number of recruits and fewer individuals in larger classes for *W. salutaris* in southern Mozambique (Chapter 4). Therefore, future research should consider monitoring of population trends to inform all the stakeholders involved including government ministries (e.g. Ministry of Land and Environment and Ministry of Health), local governments (Governments of Matutuine and Namaacha districts), non-governmental organisation (e.g. Worldwide Fund for Nature and International Union for Conservation of Nature), local communities and others (e.g. universities and

Association of Traditional Medics of Mozambique). Short-term studies should consider using size class profiles to understand population structure (e.g. mature individuals, mortality rates and recruitment) for comparison with finding from the present study. Such an approach was used, for example, by Cousins et al. (2014) for 19 *Aloe plicatilis* (L.) Burm.f. populations in South Africa, reporting bell-shaped a curve as the common size class distribution for the species. However, in long-term studies giving more insight on population trends such as population viability analysis that predict the probability of extinction by combining species attributes and environmental variations, will be required.

The present study indicated low densities of stems with reproductive structures, lack of seedlings and dominance of clonal reproduction in *W. salutaris* (Chapter 4) suggesting failure of sexual reproduction. Future research is needed to ascertain factors that affect this kind of reproduction for the management responses. This includes the effect of harvesting (Botha et al. 2004) and fruit flies (cf. Hannweg et al. 2015; Muatinte and Cugala 2014) and other causes of early fruit drop, such as inadequate hormonal levels (Shaya et al. 2015), pollination (Tatari et al. 2018) and environmental variations (Tisné et al. 2020). Most studies suggest that some of these factors that can be managed to enhance fruit production (cf. Straka and Starzomski 2015; Tatari et al. 2018; Zeng et al. 2016) except for unsustainable harvesting (cf. Botha et al. 2004; Gaoue and Ticktin 2007, 2008; Lopez-Toledo et al. 2018). For example, a short-term experiment on the effects of bark harvesting revealed severe impacts on reproduction of *H. drasticus* by reducing reproductive attributes affecting the ability to reproduce through seeds (Silva et al. 2018). Furthermore, early fall of fruits and seed parasitism faced by *W. salutaris* in southern Mozambique and elsewhere in the region (cf. Botha et al. 2004; Johnson et al. 1995; Muatinte and Cugala 2014) needs to be addressed, preferably by employing methods such as trapping for pest management. For example, the use of different lure trapping methods enabled trapping the most important pests for citrus (genus *Bactrocera* and *Zeugodacus*, each with three species) in China (Xia et al. 2020). Similar methods (Biolure^{R}Fruit Fly traps) were used to trap fruit flies using *W. salutaris* in South Africa (Hannweg et al. 2015). Moreover, growth hormone problems can be surpassed to hamper early fall of fruits, but studies are needed to scrutinise inappropriate hormone levels for intervention. For example, it was determined that early fall of fruit of two different cultivars of *Diospyros kaki* L.f. was repressed by administration of 100 mg l^{-1} gibberellin (Shaya et al. 2019). In addition, supplementing pollen from the appropriate source into female flowers (avoiding crossing between closely related individuals), can enhance fruit production. For example, *Shepherdia canadensis* (L.) Nutt. doubled its fruit production after pollen supplementation, demonstrating that lack of accessibility and mobility of pollen can restrict fruit production (Bateman and Nielsen 2020).

The present work suggests that bark trade is the main reason for decline of *W. salutaris* populations in southern Mozambique, especially in the past and it is still a potential threat in the future (Chapter 3). However, most informants denied any involvement in bark trade, rather blaming unknown harvesters of encroaching into FC for bark collection (Chapter 3). Findings on harvest impact supported this result showing that FC as an active source of bark supply in southern Mozambique (Chapter 4). In addition, the results of the genetics study suggests that FC has a distinct population compared to the other areas that holds novel alleles (Chapter 5). To protect the existing population and genetic diversity, *in situ* conservation of *W. salutaris* future management should consider strengthening of the institutions of community and external law enforcement. The existing formal protected areas, the Futi Corridor (FC) and the Licuati Forestry Reserve (TR) focus on protecting elephants and *A. quanzensis*, respectively. In doing so, they fail to protect of other species including *W. salutaris*. Priority needs to be given to FC and equipped this with necessary means (e.g. staff and equipment) to protect threatened plant species, specifically *W. salutaris*. For example, protection of *W. salutaris* in the Kruger National Park where it was also highly poached for trade, is secured by armed rangers in the same way as rhino (Dreyer 2013).

From the results, I recommend the use of sustainable harvesting approaches with defined quotas and harvesting outside protected areas. Exceptions may be given to harvesting inside PAs when it is for household use. Harvesting approaches should promote the existing knowledge of vertical and discontinuous strips, but involve a limited number of authorised traders submitted under controlled bark harvest. The establishment of harvesting quotas should following guidelines developed for *W. salutaris* from post harvesting bark recovery studies. For example, the work of

Pandey (2015) revealed harvesting of six valuable medicinal species should be done through longitudinal strips of 5-7 cm within 25% of the stem circumference for stem with girth at breast height of less than 60 cm. The time established between harvests varied from one and a half years (*Terminalia arjuna* (Roxb. Ex DC) Wight & Arn., *Saraca asoca* (Roxb.) W.J. Wilde and *Bauhinia variegata* L.) to two years (*Holarrhena antidysenterica* Wall., *Litsea glutinosa* (Lour.) and *Oroxylum indicum* (L.). Similarly, bark of *Quercus suber* L. is strictly harvested when reaching circumferences of 70 cm and the time between harvests is at least nine years, with a defined harvesting amount, which is tree size dependent (Costa and Oliveira 2015).
The results also suggest that FC is likely to be currently supplying bark to neighbouring countries and effort should be made to halt this practice. For this, future management should consider the regulation of transboundary trade by listing *W. salutaris* under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Since this work also encourages the use of material from cultivated sources, CITES should consider distinguishing traded material of cultivated origin from wild harvested (cf. Cunningham 2014) while monitoring natural stocks. Implementation of this strategy should also consider regional collaboration of the law enforcement institutions. Different states should be given liability to execute actions against illegal trade of plant species as in wildlife (Phelp and Webb 2015). For example, *P. africana* was registered in Appendix II of CITES in 1995 to regulate international commercial trade of its wild harvested bark followed by the interdiction of bark imports by the European Union. Later, CITES also proposed a ban on trade in different countries to avoid further loses (Cunningham 2014).

The results indicate declines of *W. salutaris* wild stocks, the unique source of bark supply from southern Mozambique. Adding to this is preference of bark from large individuals that compromise sexual reproduction, and if not halted, may result in population extinction (Chapter 4), especially because of its confined distribution (Chapter 2). Due to this and similar research (cf. Botha et al. 2004; Cunningham 1993; Moyo et al. 2015), I also recommend the establishment of alternative and permanent sources of bark to alleviate pressure on wild populations. Thus, cultivated material should be promoted to meet the market demand (Cunningham 2014). For the effectiveness of this intervention, it is crucial to secure massive propagation of plantlets for cultivation. This may begin in areas previously occupied by the species and gradually extended to other climatically suitable areas (Chapter 2). The obstacle of for implementation of this strategy is the lag time for cultivated material to attain desirable sizes and the opportunity cost of collectors to abandon their collection sites (Franzel et al. 2014). Nevertheless, this strategy is achievable and has been done elsewhere in the region. For example, the Kruger National Park has been producing in mass, plantlets for cultivation in the adjacent communities and in the wild (TreeSA 2020). Equally, the Silverglen Nursery has been producing *W. salutaris* plantlets as well as assisting TMPs cultivate their own sources of supply through provision of propagation material and cultivation (Xaba and McVay 2010) and thus securing future alternative sources of bark.

A large number of alternative medicinal species for *W. salutaris* was recorded in the present work (Chapter 3). Due to over-exploitation of this species, I also recommend conservation through plant substitution to alternative to alleviate pressure on populations of *W. salutaris*. Challenges that may be encountered for the implementation of this strategy is the unwillingness of the people to adopt the use of these species because of different medicinal values of such species (cf. Mukamuri and Kozanayi 2014). Collectors may still have ties with their collection sites which may also lead to overharvesting of other species (Zschocke et al. 2000). Mander (1998) suggested that most alternatives were highly rejected by consumers in South Africa. However, it may be feasible in some species. For example, due to the shortage of one of the important medicinal plant in South Africa, *O. bullata*, different species of *Cryptocarya* are gradually becoming substitutes (Mander 1998; Zschocke and van Staden 2000).

The present work revealed that different parts of *W. salutaris* can be used for remedies, in particular leaves, although not commonly used, but it covers the most frequent ailments in the study areas (Chapter 3). I also recommend the uses of leaves as alternatives to bark to support the conservation of *W. salutaris*. This is because aerial parts of plants are less sensitive to harvesting compared to others (e.g. roots, bark and rhizomes). As an evergreen plant (Coates-Palgrave 2002), leaves will be available throughout the year. Promotion of the use of leaves as alternative of bark should be conducted given that both parts present the same active ingredients (Drewes et al. 2001; Zschocke et al. 2000). However, this strategy will require support from traditional medicine practitioners (TMPs) (Zschocke et al. 2000) especially, TMPs affiliated to the Association of Traditional Medics of Mozambique (AMETRAMO). According to Zschocke et al. (2000), this will motivate collectors to mainly harvest leaves as a substitute of bark. For example, some TMPs (8% to 15%, n=37) in KwaZulu Natal medicinal plant markets have adopted the same plant part use as a substitute (Mander 1998).

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Annexures

Annexure 1. Guide of household interviews on *Warburgia salutaris*

General information

Date___________ Site______ Village______________
District__________

A. Home Use & Collection

1. Do you know chibaha? Yes [ ] No [ ]

2. Do you use this plant at home? Yes [ ] No [ ]

3. Which parts do you use? Roots [ ] Bark [ ] Leaves [ ] Fruits [ ] Flowers [ ]

4. What do you use the different parts for?

Part	Use 1	Use 2	Use 3
Roots			
Bark			
Leaves			
Fruits			
Flowers			

5. For the uses that you have just mentioned are there any other plants that may be used instead of chibaha?
   Yes [ ] No [ ] Don't know [ ]

6. If so please name them:

	Use 1	Use 2	Use 3
Alternative sp 1			
Alternative sp 2			
Alternative sp 3			

7. When using chibaha do you usually buy it of harvest your own? Harvest own [ ] Buy [ ] Both [ ]

8. If sometimes or always harvest it, from where do you harvest it?
   ____
   ____
   ____
   ____
   ____
   ____

9. How close is the collection site?

____

10. Is collection restricted to certain people? Yes [ ] No [ ]

11. If yes, who and why?

____
____
____
____
____

12. How often do you usually collect the plant? *(tick which applies for* ***both*** *of rainy and dry season)*

Rainy season	>Twice/wk	Weekly	2-3 x /mth	Monthly	Every 2-3 mths	1-2 x/yr	Rarely
Dry season	>Twice/wk	Weekly	2-3 x /mth	Monthly	Every 2-3 mths	1-2 x/yr	Rarely

13. When was the last time you collected the plant?

____

14. When was the last time you used some chibaha?

____

15. How much did you use?

____

16. Is collection restricted to specific times? Yes [ ] No [ ] Don't know [ ]

17. If yes, when?

____

18. If yes, why?

____
____
_____

19. Do you remember who taught you about using chibaha? *(tick option that applies)*

Mother	Father	Grandmother	Grandfather	Aunt	Uncle
Sister	Brother	Friend	General knowledge	Cannot remember	

20. Do you know others who use chibaha? Yes [ ] No [ ]

21. Do you know what they are using it for? Yes [ ] No [ ]

22. If yes, please say:

____
____

____
____

B. Commercial Use & Collection

23. Do you ever collect chibaha for sale? Yes [ ] No [ ] *(if no go to Q28*)

24. If yes, how often do you sell chibaha? (*tick which applies for* ***both*** *of rainy and dry season)*

Rainy season	>Twice/wk	Weekly	2-3 x /mth	Monthl y	Every 2- 3 mths	1-2 x/yr	Rarely
Dry season	>Twice/wk	Weekly	2-3 x /mth	Monthl y	Every 2- 3 mths	1-2 x/yr	Rarely

25. Approximately how much to you sell each time?____

26. For how much do you sell it per unit?

____

27. Where or to whom do usually you sell it? ____
____

28. Even if not selling now, has anybody from your household sold any part or product of chibaha (bark, charcoal, etc.) in the past?
Yes [ ] No [ ] Don't know [ ]

29. If yes, how long ago, and why are they not selling anymore? ____
____
____
____
____

30. Are there people from other communities who come to collect the bark of chibaha in your area? Yes [ ] No [ ] Don't know [

31. If yes, do you know where they are coming from?
Yes [ ] No [ ] Don't know [ ]

32. If yes, from where?
____
____
______

33. How often do they come? Regularly [ ] Sometimes [ ] Rarely [ ]

C. Harvest Methods and Management

34. What tools do you use to harvest the bark?

____

35. How do you normally harvest bark from the tree?
Horizontal strips [ ] Vertical strips [ ] Ring barking [ ]

36. What is the position of the plant that you normally harvest (or strip) the bark? (*tick which apply)*

Bottom of stem	Top of stem	Whole stem	Anywhere	Other (pls specify)

37. Are there some ways of harvesting that cause **<u>less</u>** damage to the plant? Yes [ ] No [ ] Don't know [ ]

38. If yes, pls describe:

____
____
______

39. Are there some ways of harvesting that cause**<u>more</u>** damage to the plant? Yes [ ] No [ ] Don't know [ ]

40. If yes, pls describe:

____
____
______

41. Is there any time of year when harvesting is more damaging then other times? Yes [ ] No [ ] Don't know [ ]

42. If yes, when and why?

____
____
____
____

43. How long does it take for the bark to regrow over a harvested patch?

____

44. Do you do any specific things to help maintain chibaha plants in your area?
Yes [ ] No [ ]

45. If yes, pls describe what you do:

____
____

____
____
____

46. Do you know of any things that others do to help maintain chibaha in the area?
    Yes [ ] No [ ]

47. If yes, pls describe what they do:
    ____
    ____
    ____
    ____
    ____

48. Have you every planted chibaha? Yes [ ] No [ ]

49. If yes, where and when and how many?____

50. Do you know of others who have ever planted chibaha? Yes [ ] No [ ]

51. If yes, where and when and how many?____

52. Do you think it is important to conserve chibaha? Yes [ ] No [ ] Don't know [ ]

53. Please explain

____
____
____
____
____

54. Why do you think some harvesters ring-bark chibaha?
    ____
    ____
    ____
    ____
    ____
    ____
    ____
    ____

D. Local Ecological Knowledge

55. In which types of places is chibaha most common (e.g. soil types, near the river, rocky sites, etc.)?
    ____
    ____
    ____

56. Under which condition is it not found?

57. Do you know during which months chibaha plants flower?
Yes [ ] No [ ]

58. If yes, when?
Oct Nov Dec Jan feb Mar Apr May Jun Jul Aug Sep

59. Do you know how the flowers are pollinated? Yes [ ] No [ ]

60. If yes, what pollinates it? *(tick any answer/s they provide)*
Wind Insects Bats Birds Animals Other (*pls specify*)

61. Has the abundance of chibaha in your area changed after the civil war ended?
Yes [ ] No [ ] Don't know [ ]

62. If yes how? Increased [ ] Decreased [ ]

63. If yes, what has caused these changes?

64. What do you think about the abundance in the next ten years?
Will stay approximately the same [ ] Will increase [ ] Will decrease [ ]

65. Why?

66. **Relative** to other tree species in your area, how fast does chibaha grow?
Slower [ ] About the same [ ] Faster [ ]

67. **Relative** to other tree species in your area, how sensitive is chibaha to fire?
More sensitive [ ] About the same [ ] Less sensitive [ ]

68. **Relative** to other tree species in your area, how sensitive is chibaha to drougth?
More sensitive [ ] About the same [ ] Less sensitive [ ]

E. Respondent and Household Profile

We have come to the end of the questions about chibaha and its use. However, we would like to ask a few questions about you and your household. This is because it helps us understand who uses chibaha and who doesn't, and why. I can assure you again that the answers you provide are totally confidential and will not be shared with anybody else. If you feel any of the questions are too personal, you do not have to answer them.

69. Respondent profile:

Age		Home language	
Gender		Immigrant/native	
Highest education		No. of years lived in this area	
Marital status			

70. How many people usually live in your house? _______________

71. How many rooms does your house have?__________________

72. How many of the following domestic animals do you have?

Cow		Sheep	
Goats		Duck	
Pigs		Chicken	

73. How many of the following items does you house have?

Car/van		Bicycle		Television	
Tractor		Radio		Fridge	

74. Pls list the activities that you use to support your family? (*tick all that apply*)

Agriculture	Fishery	Art crafts	Apiculture
Hunting	Charcoal production	Gathering medicinal plants	Migrant labour
Permanent job	Others (pls specify)		

75. Pls list which of these you consider the most and second-most important:
Most _____________________ Second-most_____________________________

76. Do you sell any crops? Yes [ ] No [ ]

77. If yes, which ones?

____

____

____
____

78. Approximately how much do you earn for your activities per year/month__________

Additional comments
____
____
____
____
____
____
____

We have come to the end of the interview. THANK YOU for sharing your time and knowledge with us. Having heard my questions, do you have any questions that you might like to ask me?

Annexure 2: Checklist of questions for focal group interviews on local ecological knowledge

1. When is “chibaha” collected?
2. What size of plants do you collect the bark from?
3. When you cannot collect?
4. Where you cannot collect it and why?
5. How is it collected?
6. Who cannot collect?
7. How many populations do you know?
8. What is the population size?

9. In your opinion the abundance or availability and distribution of “chibaha” has changed or not comparing the periods before civil war and after civil war to now? What do you think about the abundance or availability and distribution in the future? Please explain.

Period	**Abundance or availability and distribution**	**Explanation**
1977- (before civil war)		

1992 to 2016 (post civil war to now)		
2016+ (in the future)		

10. In which type of habitat does "chibaha" occur? Or where is it collected?
11. At what time of the year does "chibaha" give flowers?
12. What pollinates the flowers?
13. In which time of the year does 'chibaha" give fruits?
14. Have you ever seen "chibaha"seed?
15. How does "chibaha" multiply itself in the bush?
16. What disperses it ?
17. Does the bark re-grow after harvesting?
18. How quick?
19. Does the stem re-grow after cutting?
20. Does fire impact chibaha tree?
21. Does anything eat it?
22. Is it drought tolerate?

Annexure 3: Population survey and site attributes

Data________________
Site (1-3)_______, Village_________, Patch Nr______, Aspect______˚, Slope (%)_____, Rockiness (%)_________, Tree cover (%)______,
Longest access_____m, Perpedicular distance_______m. Distance to homestead_____km

Plant Nr	BD (cm)	Height (m)	Bark damage (0-7)	Fire evidence (1-3)	Nr of coppice stems	Status of the stem (dead, alive or stressed)	Evidence of fruits or flowers	Height of stem cut (m)	Obs

Annexure 4: Ad hoc statistics of structure analysis of *Warburgia salutaris*

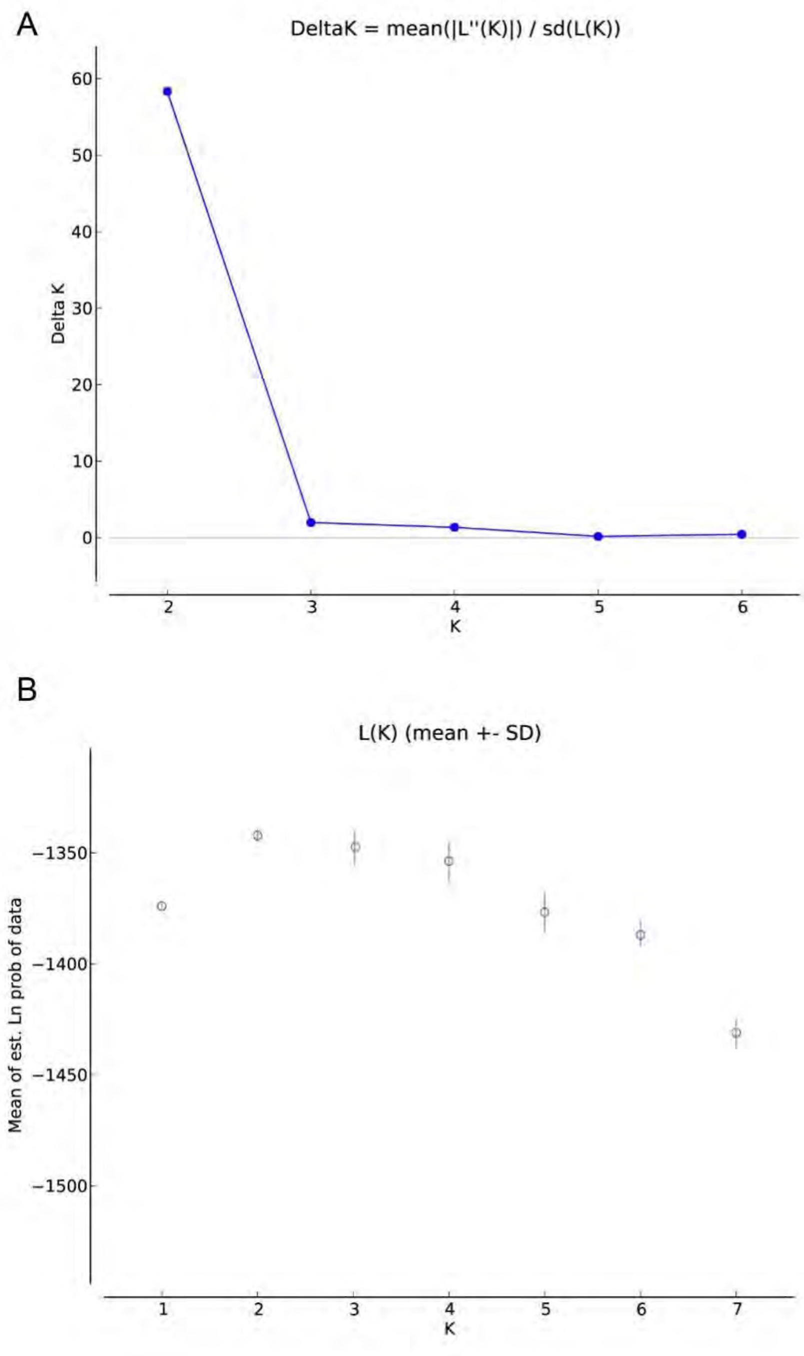

Figure S1.STRUCTURE analysis of *Warburgia salutaris* in Mozambique. (A) Evanno's ad hoc statistic; DK as a function of K. (B) Mean log probability of data LnP(D) over 10 runs for each K value as a function of K (error bars represent standard deviation).

Annexure 5: Flow cytometric analysis for determination of genome size of *Warburgia salutaris*

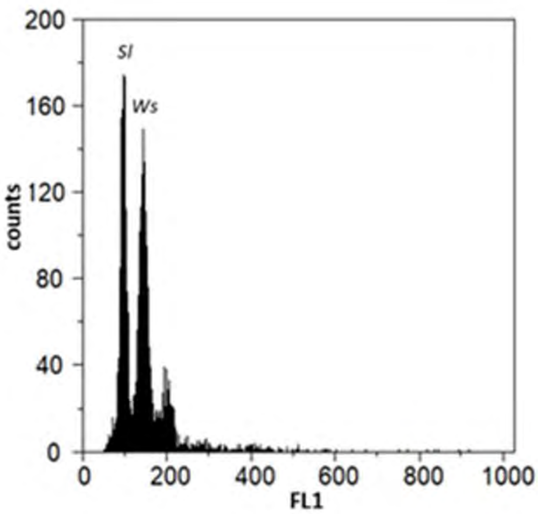

Figure S2. Histogram showing flow cytometric analysis of relative fluorescence intensities (FL1) of propidium iodide-stained nuclei simultaneously isolated from *Warburgia salutaris* (*Ws*) and *Solanum lycopersicum* 'Stupické' (*Sl*).

Annexure 6: Genome size of different Magnolids underscoring the Canellales

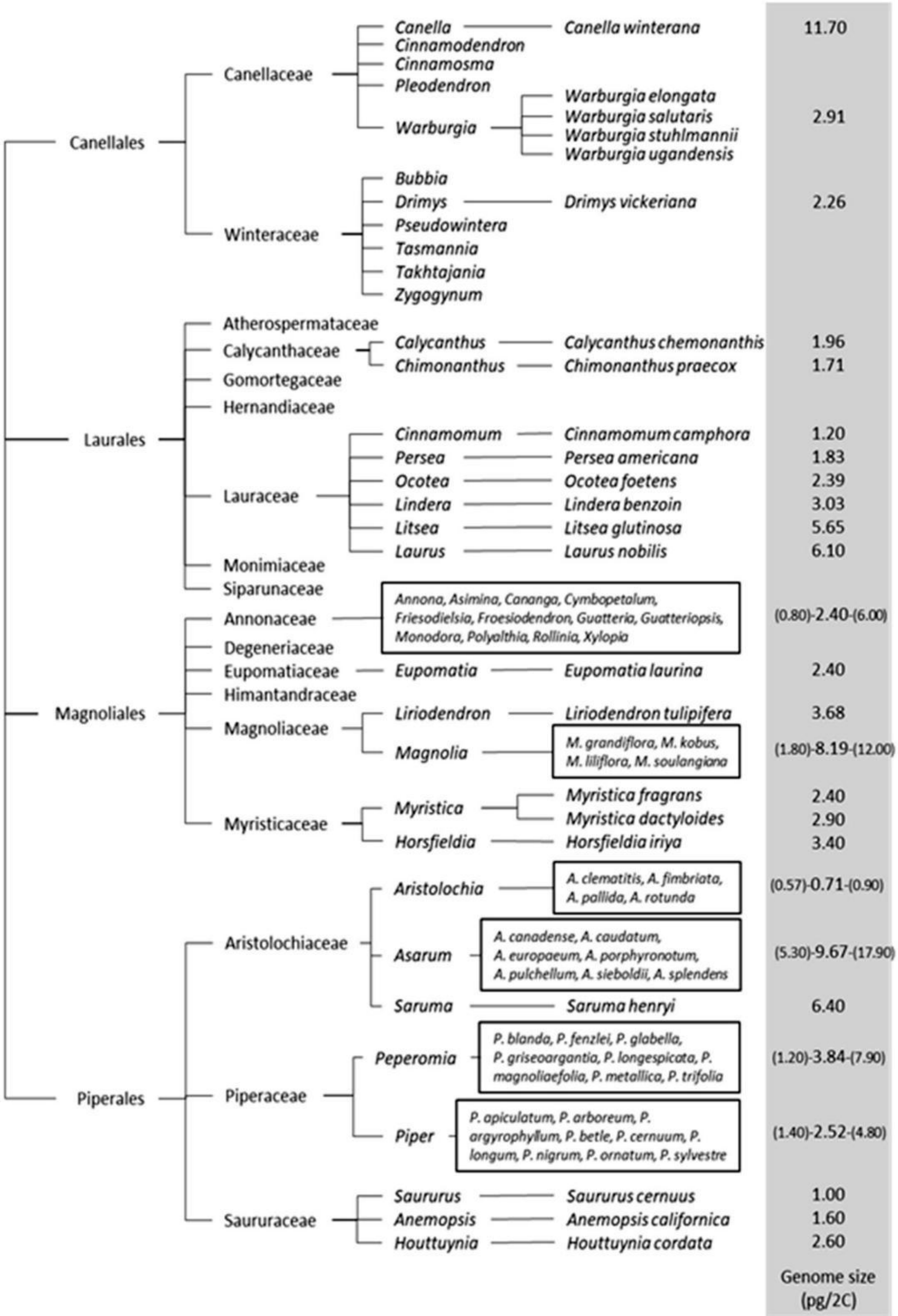

Figure S3. Genome size variation across the magnoliids, with emphasis on *Warburgia* and on the Cannelales. In each family, species (apart from the *Warburgia* genus) and Genera (apart from the Canellales) are only included if genome size values are available. Data retrieved from the Plant DNA C-values database (Royal Botanical Gardens Kew) and compiled (Leitch et al. 2002).