ANALYSIS OF POPULATION STRUCTURE, SOIL PHYSICOCHEMICAL PROPERTIES AND PHYTOCHEMICAL PROFILE OF *Prunus africana* (Hook.f.) Kalkman IN NYIKA NATIONAL PARK, MALAWI AND ZAMBIA.

MSc THESIS (FORESTRY AND ENVIRONMENTAL MANAGEMENT)

Paston Patani Simkoko

MZUZU UNIVERSITY

JULY 2024

ANALYSIS OF POPULATION STRUCTURE, SOIL PHYSICOCHEMICAL PROPERTIES AND PHYTOCHEMICAL PROFILE OF *Prunus africana* (Hook.f.) KALKMAN IN NYIKA NATIONAL PARK, MALAWI AND ZAMBIA.

Paston Patani Simkoko

BSc (Forestry), Dip. (Natural Resource Management)

A THESIS SUBMITTED TO THE FACULTY OF ENVIRONMENTAL SCIENCES, DEPARTMENT OF FORESTRY AND ENVIRONMENTAL MANAGEMENT IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE (MSc) IN FORESTRY AND ENVIRONMENTAL MANAGEMENT

MZUZU UNIVERSITY

JULY, 2024

DECLARATION

I hereby declare that this thesis titled**, '**Analysis of population structure, soil physicochemical properties and phytochemical profile of *Prunus africana* (Hook.f.) Kalkman in Nyika National Park, Malawi and Zambia**"** has been written by me and is a record of my research work. All citations, references, and borrowed ideas have been duly acknowledged. It is being submitted in fulfilment of the requirements for the award of the degree of Master of Science in Forestry and Environmental Management of the Mzuzu University. None of the present work has been submitted previously for any degree or examination in any other University. **Parts of the materials presented in this thesis have been published and appear as: "Report on the ecological status and abundance of African Cherry *Prunus africana* in Nyika National Park, Malawi and Nyika National Park, Zambia". www.nyika-vwaza-trust.org/research.**

Paston Patani Simkoko

_____ _____

Students name Date

CERTIFICATE OF APPROVAL

I, the undersigned, certify that this thesis is a result of the author's work, and that to the best of my knowledge, it has not been submitted for any other academic qualification within the Mzuzu University or elsewhere. The thesis is acceptable in form and content, and that satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on Date.

_____ _____

Major Supervisor: Date

Name (Associate Professor Lusayo Mwabumba)

_____ _____

Co- Supervisor: Date

Name (Mr Dominic Gondwe)

_____ _____

Co-Supervisor: Date

Name (Associate Professor John Kamanula)

ABSTRACT

Prunus africana is an important medicinal plant and its population is declining in most of its natural range because of over-exploitation. Its bark is processed by pharmaceutical companies in Europe to treat Benign Prostatic Hyperplasia (BPH). Little is known about the status of *Prunus africana* in Malawi. Therefore, the research was conducted to investigate the population structure, soil preference, and phytochemicals of *Prunus africana* in Nyika National Park, Malawi and Nyika National Park, Zambia. The data was collected using belt transects. Data on species name, height (m), dbh (cm), canopy cover, soil and tree bark samples were collected at random. The data was analysed using GenStat, past 4.03, Mintab and PC-ORD statistical software. The study recorded 5 trees/ha in both study sites. Juniper Forest had the highest mean density (14 trees/ha) while Manyenjere Forest recorded a density of (4 trees/ha). There were more mature trees than saplings and seedlings across the study area, and Manyenjere had no seedlings at all. This was attributed to the thick canopy cover in Manyenjere, as the species is a light demander. Soil analysis revealed that all essential elements (Ca, N, Mg, pH, and C) were the same in both sites except phosphorus which varied significantly ($p<0.05$) between the Juniper Forest (9.01±4.7 mg/kg) and Manyenjere Forest (3.83 ±3.4 mg/kg). Most phytochemical compounds known to treat BPH such as β-sitostenone**,** γ-Sitosterol, ursolic acid, and Apigenin 6-c--glucoside were detected. It was concluded that both sites are dominated by mature trees with very little or no recruitment at all which is due to limited disturbances (Geldenhuys and Venter 2002). It was, therefore, recommended that pruning in thick forest be promoted to allow light into the forest floor to trigger regeneration. Further research to assess the impacts of pests on the regeneration of *Prunus africana* is needed.

DEDICATION

I dedicate this thesis to my family, who accorded me support and encouragement during my studies more so to my wife Lydia, my mother, my daughters, and all my brothers for the financial and moral support offered to me.

ACKNOWLEDGEMENTS

I express my deepest heartfelt and profound appreciation to Nyika-Vwaza Trust UK , National Commission for Science and Technology (NCST), and Wilderness Welfare (WW) for the financial assistance towards this work.

The author is greatly indebted to Assoc. Prof. Lusayo Mwabumba, Associate Prof. John Kamanula and Mr Dominic Gondwe {May he continue resting in peace} (the thesis supervisors) for their untiring effort, valuable comments and criticisms made during the research proposal phase, through the data collection period up to the final writing up of the thesis document. Without their supervisory work, the project would not have been a success. My acknowledgements should also go to Maganizo Namoto for his great technical assistance throughout the period.

Mr. Edwin Kathumba (May he rest in peace) and Steve Mphamba should be thanked for their technical assistance in plant identification. Mzuzu University lecturers and students are recognised for their constructive contributions during proposal development. Dr. Dorothy Nyamai of Jomo Kenyatta University in Kenya is thanked for her assistance in phytochemical analysis. Without her contribution, this phytochemical analysis component would not have been possible considering the enormous challenges posed by Covid - 19.

My appreciation should also go to Madalitso Chimombo, Sarah Kanadi and Chiyamiko Mame from the Forestry Research Institute of Malawi (FRIM), for their assistance in laboratory work. Nyika National Park research rangers (Aliel Moyo, Timothy Chirwa and Mike Sisya) are thanked for showing me all the *Prunus africana* locations on Nyika Plateau.

ABBREVIATIONS AND ACRONYMS

ACQUITY UPLC-	Ultra-Performance Liquid Chromatography
ANOVA	Analysis of Variance
BPH	Benign Prostatic Hyperplasia
BEH	Ethylene Bridged Hybrid
C	Carbon
Ca	Calcium
CITES	Convention on International Trade in Endangered Species
DBH	Diameter at breast Height
DCM	Dichloromethane
DF	Degrees of Freedom
DNA	Deoxyribonucleic acid
DPNW	Department of National Parks and Wildlife
DRC	Democratic Republic of Congo
EDTA	Ethylene diamine tetraacetic acid
EPA	Extension Planning Area
ESI	Electrospray ionization
FRIM	Forestry Research Institute of Malawi

GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
GoM	Government of Malawi
GPS	Global Position System
IBA	Indole-3-butyric acid
IUCN	International Union for the Conservation of Nature
LC	Liquid Chromatography
LC-MS	Liquid Chromatography Mass Spectrometry
LSD	Least Significant Difference
Mg	Magnesium
MS	Mass Spectrometry
N	Nitrogen
NNP	Nyika National Park
P	Phosphorus
pH	Potential of hydrogen
Ppm	Parts per million
SABONET	Southern African Botanical Diversity Network
SCN	Suprachiasmatic nucleus

UPLC-MS	Ultra-Performance Liquid Chromatography Mass Spectrometry
UV-VIS	Ultraviolet–visible spectroscopy

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

1.1 Background information

Prunus africana, commonly known as the Red Stinkwood, African Cherry, or Bitter Almond, is a medicinal tree. It is known to treat Benign Prostatic Hyperplasia (BPH) a condition common in old men (Cunningham *et al.,* 1997; Fashing, 2004; Jimu, 2011; Nguta, 2012). Locally *Prunus africana* is known as Dadzi in Chichewa; Mzumira in Tumbuka and Msisita or Mkunu in Yao (Burrow and Willis, 2005). *Prunus africana* has been subjected to unsustainable harvesting of its bark for the international medicinal plant trade for many decades (Cunningham and Mbenkum, 1993; Cunningham *et al*., 1997; Fashing, 2004) and was listed as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix-II species in 1995 (Cunningham *et al*., 1997). The high demand for bark extracts of *P. africana* has caused serious damage to wild populations in most range states (Fashing, 2004; Nyamai *et al*, 2015).

1.2 Description of *Prunus africana*

The genus name "Prunus" is derived from a Latin word that refers to the plum family, and the scientific name "*Prunus africana*" refers to the species of African origin (Komakech *et al.*, 2017).

Prunus africana belongs to the subfamily Prunoideae within the Rosaceae family. There are around 400 species in the genus Prunus, divided into two sub-genera: Padus (deciduous) and Laurocerasus (evergreen). The Laurocerasus sub-genus includes *P. africana*, the only one found in Africa and Madagascar (Navarro-Cerrillo *et al.*, 2008; Nguta, 2012).

The genus Prunus comprises over 400 species, of which only 98 are of great importance (Nguta, 2012). *Prunus africana* is a species with a mature stem diameter of up to 1 m and a

height of more than 40 m with open branches and a blackish-brown bark (Hall *et al*., 2000). Leaves are simple, alternate, oval-shaped, shiny-deep green on the top side and lighter on the underside, with a conspicuous prominent midrib on the underside (Munjuga *et al*., 2000). Flowers are greenish or white and fruits are spherical, 7 mm long, 1.3 cm wide, pinkish-brown, and bilobed, with thin, dark red to reddish brown pulp when ripe (Komakech *et al*., 2017).

In the Equatorial zone, there is no specific flowering season and some individuals of the species flower almost every month of the year (Munjuga *et al*., 2000). In regions north of 5ºN, flowering takes place from November to January when the temperatures are low and there is lower rainfall (Munjuga *et al.*, 2000). In regions South of 5ºN, flowering takes place from April to October when the conditions are cool and dry. Fruiting of the species occurs 2-3 months after flowering and is associated with rainfall. In the northern zones, fruiting takes place in the relatively wet months. In the southern regions, fruiting occurs when the rains are starting or in the second half of the dry season (Sun *et al*., 1996).

1.3 Habitat preference

Prunus africana is indigenous to the montane regions. It is a highland forest plant that grows in humid and semi-humid conditions at an altitude of about 900–3,400 m above the sea level of Sao Tome' and Madagascar and Afromontane Forest islands in Africa with a mean annual rainfall of 890–2,600 mm and a mean annual temperature of 18–26°C (Komakech *et al*., 2017).

1.4 Uses of *Prunus africana*

This high-altitude species is of economic, social and scientific importance for the local people and the international community. Locally, it is a source of timber (Craft), firewood, income and it also contributes to the traditional pharmacy (Hall *et al*., 2000). Scientifically and

internationally, its bark is used by Western industries to treat Benign Prostatic Hyperplasia (Betti and Ambara, 2013; Awono *et al*., 2016).

Prunus africana is also valued for apiculture as the flowers have sufficient nectar and pollen for good bee forage. Some communities in the range states use leaves as an inhalant for fever or are drunk as an infusion to improve appetite. Water is added to pounded bark, and the red liquid is used as a remedy for stomach-ache; bark extract may be used as a purgative for cattle (Orwa *et al*.,2009). Additionally, other services include erosion control, shade, or shelter. It is also known to improve soil fertility and ornamental as it makes an attractive garden shade tree (Orwa *et al.*,2009).

1.5 Geographical distribution

Prunus africana is a monoecious tree and is native to 21 countries in sub-Saharan Africa. It is known to occur in Africa and mostly concentrated in the eastern part of the continent (Fig. 1). It is a mountain tree species of tropical Africa including Côte d'Ivoire, Bioko, Sao Tome, Ethiopia, Kenya, Uganda, South Africa, Madagascar, Congo, the Democratic Republic of Congo, Mozambique, Tanzania, Burundi, Cameroon, Zimbabwe, Nigeria, Equatorial Guinea and Malawi (Cunningham *et al*., 1997; Betti, 2008; Cunningham, 2005; Betti and Ambara, 2013; Awono *et al.*, 2016).

Figure 1: Distribution of Prunus aficana in Africa (Source: Betti and Ambara, 2013)

In Malawi, the distribution of *P. africana* was widespread across the country (Table 1). According to (Mphamba, 2019 pers. com) recent forest inventories have failed to locate *P. africana*, in most of the sites where it was previously sighted in the 1970s an indication that the species has disappeared in most of the sites in Malawi. This could be mostly attributed to deforestation, settlements and agriculture.

Table 1: Location of *Prunus africana* in Malawi in the 1970s

REGION	DISTRICT/SITE	SPECIFIC LOCATION	COORDINATES/ POSITION
South	Thondwe, Mpita estate	Edge of forest	15" 26'S, 35"13'E
South	Domasi, Katsonga	Near river bank	15"17'S,35"22'E
South	Thyolo, Mchima estate	Msikidzi river banks	5km SE of Thyolo
North	Chitipa, Chisenga	Misitu forest	SE of Chisenga 1950m
North	Chitipa, Misuku hills	Wilindi forest	09"41'S,33"28'E
Central	Dedza mountain	Evergreen patches	14"20'S,34"20'E
Central	Ntchisi	Chinthembwe mission	13"26'S,33"59'E
South	Mulanje mountain	Phwera water falls	16"04'S,34"44'E
South	Michesi mountain	Western slopes of Napolo Valley	Rocky slopes 1300m
South	Chiradzulu mountain	SE slopes above Boma	36LYT3364, 1550m
South	Mj. Likhubula valley	Source of Nansato stream	
North	Rumphi	Juniper forest	10"45'S,33"53'E
South	Thyolo	Mpeni tea estate	15"58'S,35"05'E
Central	Ntcheu - Kalichelo hills	On top of the hill	14"26'S,37"27'E
South	Phalombe	Phalombe river	15"46"S,16"03"E
South	Zomba mountain	South of Chingwe's Hole	15"20"S, 35"16"E
Central	Dedza – Chiwamba vg 1	Msitu wa Lengwe	14"10'S, 34"22"E
South	Neno Primary School	Mkulumadzi River	
South	Machinga Chikala Hills	Evergreen patches on top	
South	Mangochi Hills	Near the campsite, edges of evergreen forest patches	

Source: Adapted from Department of Forestry (FRIM-Zomba) Plant Manual Book

1.6 Statement of the problem

Prunus africana has been reported from 22 countries in Africa, mostly in the Eastern part of the continent (Navarro-Cerrillo *et al.,* 2008; Nguta, 2012). In 1966, the bark extracts of *P. africana* were found to be effective in the treatment of benign prostatic hyperplasia (Fashing, 2004). Since its discovery, the species has been declining from its natural range, and even

getting locally extinct from some of the ranges because of over-exploitation (Cunningham *et al.*, 1997; Ingram, 2007; Betti, 2008; Cunningham, 2005).

A number of studies on population structure, soil preference, species associations, propagation and phytochemical analysis have been done extensively in countries like Cameroon, Kenya, Uganda, South Africa, Zimbabwe and Rwanda (Betti, 2008; Nguta, 2012, Nyamai *et al.*, 2015; Koros *et al.*, 2016). Most studies have been done in Cameroon and Kenya where the rate of decline has been high (Farwig *et al.*, 2007; Nguta, 2012; Nyamai *et al.*, 2016). These studies provided essential information on population status important for monitoring, management and conservation of the species in these countries. For example, in Cameroon, a lot of cultivation and domestication initiatives of *P. africana* have been carried out and this was due to recommendations made by different researchers (Nguta, 2012).

In Malawi, the distribution of *P. africana* was widespread across the country. According to (Mphamba, 2019 pers. *com*) recent forest inventories have failed to locate *P. africana,* in most of the sites where it was previously sighted in the 1970s an indication that the species has disappeared in most of the sites in Malawi. This could be mostly attributed to deforestation, settlements and agriculture. Additionally, there has been no studies conducted on population structure, species association, soil preference and phytochemical analysis of *Prunus africana* in Malawi and Zambia even in sites where the species has been confirmed especially Nyika. This has remained a gap and affected conservation initiates for the species in both Malawi and Zambia.

It is against this background that a research was initiated to investigate population structure, soil preference and phytochemical analysis of *P. africana* in Nyika Malawi and Nyika Zambia National Parks. The generated information will help draw up overall conservation policies or making sound conservation and management recommendations.

1.7 Objectives

1.7.1 Main objective.

The main objective of the study is to analyse population structure, soil physicochemical properties, and phytochemical profile of *Prunus africana* species in Nyika National Park, Malawi and Zambia.

1.7.2 Specific objectives

i. To establish *P. africana* population structure in Nyika National Parks, in Malawi and Zambia;

ii. To examine soil physicochemical properties in Nyika National Parks, in Malawi and Zambia;

iii. To evaluate the phytochemical profiles of *P. africana* stem bark extracts sampled from Nyika National Parks, in Malawi and Zambia.

1.8 Justification of the study

Prunus africana is threatened with extinction but can be conserved by domestication initiatives (Cunningham *et al*., 2005). Domestication and reliance on planted trees will make the pharmaceutical future of *P. africana* bark and bark extracts more secure (Cunningham *et al.*, 1997). A lot of studies that have been done in Cameroon and Kenya on population structure, seed behaviour and phytochemical analysis have provided essential information on population status important for monitoring, management and conservation of the species in these countries (Farwig *et al.*, 2007; Nguta, 2012; Nyamai *et al.*, 2016). Hall *et al.*, (2000) reported that over 3500 small holder farmers were involved in cultivation and domestication of *P. africana* in western Cameroon and this response was largely attributed to a number of research activities on the species. In Malawi and Zambia, this study is the first of its kind. Therefore, the study will help generate information that will assist Malawi and Zambia Governments come up with

policies and strategies that will assist in the conservation of *Prunus africana* through cultivation and domestication and other conservation iniatives.

1.9 Hypotheses

Ho_1: Population structure of *P. africana* is not similar between Juniper and Manyenjere Forests.

Ho_2: Soil physicochemical composition is not similar between Juniper and Manyenjere Forests.

Ho_3: There are no chemical variations in *P. africana* stem bark from Juniper and Manyenjere Forests.

CHAPTER 2: LITERATURE REVIEW

2.1 Background

P. africana is classified by the International Union for Conservation of Nature (IUCN) as a vulnerable species, (Appendix II). *P. africana* was declared an endangered species in 1994 as documented by the Convention on International Trade in Endangered Species of Fauna and Flora (CITES) (Betti and Ambara, 2011). The over-exploitation of this species is due to its high demand on the international market. The species was discovered as a medicinal plant in 1966 in South Africa to treat BPH (Fashing, 2004).

2.2 Importance and potential threats of *Prunus africana*

According to Stewart (2003) Prunus *africana* has been used in the treatment of Benign Prostatic Hyperplasia and other disorders for decades. The bark, from which the treatment was derived, was entirely wild-collected.

2.2.1 Economic importance of Prunus africana

The major exporters of bark include Cameroon, Madagascar, Equatorial Guinea, and Kenya. Groupe Fournier of France and Indena of Italy produce 86% of the world's bark extract, both for their products and for the free market (Stewart, 2003). An admittedly rough retail value of the trade based on the 3,225 tonnes of bark processed annually would be US$ 220 million/year (Cunningham *et al.*, 1997).

Traditional healers across Africa use *P. africana* as a medicine to treat diarrhea, dysmenorrhoea, epilepsy, impotence, infertility, irregular menstruation, kidney disease, mental illness, eye disorders, fevers, obesity, pneumonia, arthritis, hemorrhage, hemorrhoids, hypermenorrhea, hypertension, prostate gland enlargement and as an antibiotic, antigonorrheic, antihelmeintic, anti-inflammatory, antimalarial, anti parasiticide,

antirheumatic and antitussive (Stewart, 2003). Additional to the medicinal value, the high strength and durability of *P. africana* timber makes it a useful tree throughout its natural range. In Uganda, timber is used to make mortars, pestles, and bee hive supports (Cunningham, 1996). Similarly, large trees are hollowed out to make ‘‘beer boats’’ and banana beer (Cunningham and Mbenkum, 1993; Stewart, 2003). In Cameroon, timber is used to produce window and door frames whilst in West Africa; it is used for truck bodies, chopping blocks, bridge decks, cabinets and furniture (Stewart, 2003). In South Africa, the wood is used for making wagons (Stewart, 2003). In Zimbabwe, it is used as general-purpose timber (Jimu, 2011).

2.2.2 Ecological importance of Prunus africana

The species plays an important ecological role, providing food and home for pollinators and rare fauna, and supporting vascular and non-vascular canopy epiphytes (Fashing, 2004). The species has hermaphrodite flowers pollinated by insects. According to Farwig *et al* (2006), fruits are dispersed by birds and monkeys. Fashing (2004) found out that Colobus Monkeys in Kakamega Forest in Kenya depend a lot on *Prunus africana* fruits and leaves as their main food.

2.2.3 Potential threats to Prunus africana

Worldwide exports of dried bark in 2000 were estimated at 1350–1525 metric tons per year, down from its peak of 3225 tons in 1997. In 2000, Plantecam, the largest bark exporter in Africa, closed its extraction factory in Cameroon, due to complex ecological, social, and economic factors (Stewart, 2003). Wild collection was and is no longer sustainable where harvesting seriously affects morbidity and mortality rates of harvested populations (Fashing, 2004; Cunningham *et al*., 2002; Nguta, 2012). Several studies have provided evidence of the adverse effects large-scale bark harvesting has on *P. africana* populations (Cunningham *et al*., 2002; Ndibi and Kay, 1997; Sunderland and Tako, 1999). For example, at Pico Basile,

Equatorial Guinea, Sunderland and Tako (1999) found that 68% of exploited *P. africana* were either dead or experiencing canopy dieback. In a study of *P. africana* near the most southerly end of its geographic distribution along the Bloukrans River Gorge, South Africa, Geldenhuys (1981) as reported by Fashing (2004) found that 47% of the standing stems ≥10 cm DBH were dead, but made no mention of bark harvesting. Instead, he attributed the mortality to increasing aridity in the Southern Cape region. Jimu (2011) reported bushfires, grazing and alien species invasion as major threats to the species in Zimbabwe. Similar threats face the Malawi *Prunus africana* population. The species is locally extinct in some areas like Viphya and some other areas in Malawi.

2.3 Cultivation and domestication of medicinal plants

In recent years, medicinal plants have been gaining immense popularity in developing and developed countries due side effects of synthetic drugs (Gupta *et al.*, 2003). Therefore, the demand for the basic raw material has been increased and forest areas cannot meet demand of industries. Given the aforesaid reasons, there is an urgent need to conserve and propagate some important medicinal plants species to save them from extinction. This is to ensure greater availability of raw material (Gupta *et al.*, 2003).

It is because of the threats facing medicinal plants, that the Medicinal Plant Specialist Group was founded in 1994, under the auspices of International Union for the Conservation of Nature (IUCN). The major roles of the commission are to increase global awareness of conservation threats to these plants; to promote conservation actions, and to enhance domestication (Kideghesho and Msuya, 2010).

In Malawi, an organization called the International Healers Council of Malawi (IHCM) was created in 1996 to manage medicinal plants. The council bought approximately 4.8 hectares of

indigenous forestland in Mwanza District to serve as a herbal botanic garden. The botanic garden was officially opened by the Minister of Natural Resources in 1996 (Compass, 2002).

2.3.1 Factors affecting medicinal plants cultivation and domestication in Africa

According to research done by Vodouhe and Dansi (2012) most communities in Benin domesticate wild plants based on usefulness, scarcity and high demand. He also reported that plant domestication is done by simple curiosity in a few cases or for dietary, medicinal, economic, or cultural reasons. Among these reasons, the most important is food security. Nyamai *et al*., (2015) argues that understanding plant growth factors such as height, diameter at breast height(dbh), chemistry, seed behavior, etc is important in making sure that cultivated or domesticated plants are successful in cultivated areas. Lack of knowledge in the propagation of plant species was identified as a major deterrent towards plant domestication by local communities in Malawi (Maghembe, 1998).

2.3.2 Cultivation of Prunus africana

Some countries like Cameroon, DRC, Tanzania, Kenya, Uganda and Madagascar have intensified effort to cultivate and domesticate *Prunus africana* to save it from local extinction (Cunninghan *et al.*, 1997). Many commercial plantations (Nguta, 2012) and small-scale farms have been established in most of these countries (Cunningham *et a*l., 2002). For example, by 2002, a total of 3500 farmers were involved in the cultivation of *Prunus africana* in North West Province, Cameroon. This resulted in 25,000 trees planted (Cunningham *et al.*,2002) According to Sunderland and Nkefor (1997) propagation of *P. africa* is very easy, especially through seeds.

2.3.3 Prunus africana seed behaviour

Seed storage behaviour refers to the capacity of seeds to survive desiccation (Gold and Hay, 2014). Seed behaviour has been divided into two categories i.e. Desiccation tolerant, or

orthodox, seeds that can be dried, without damage, to low moisture content (mc). Seed longevity increases with reductions in mc and temperature in a quantifiable and predictable way. Desiccation-sensitive, or recalcitrant, seeds do not survive drying to any large degree, and are thus not amenable to long-term storage, although the critical moisture level for survival varies among species (Berjak *et al*., 1990; Sacande *et al*, 2004; Daws *et al,* 2006; Gold & Hay, 2014).

Some studies suggest that *Prunus africana* is recalcitrant (Orwa, *et al*, 2009). Other studies describe the species as Orthodox (FAO, 2002; Daws *et al.,* 2006). This behaviour may be the species has diverse genetic characteristics (Cunningham *et al*., 1997; Sunderland & Nkefor, 1997; Nyamai *et al*, 2015). Seed behaviour knowledge for *Prunus africana* is fundamental to the domestication or cultivation of the species. Seeds are not expensive and convenient plant materials which do not require some levels of technical knowledge compared to other propagation methods such as grafting, layering and others (Cunningham *et al.,* 1997; Sunderland and Nkefor, 1997).

2.3.4 Seed germination of Prunus africana

Negash (2004) conducted studies on the germination characteristics of various seed groups, the extent of seedling and wildling establishment, as well as measurements on early seedling growth, on *Prunus africana*. Germination of seeds gathered *en masse* (together with soil and litter) from beneath *Prunus* trees was significantly greater compared to those collected in pure form from the ground (with or without fruit flesh) or those picked from the trees. Treating seeds with 10 or 100 mmol gibberellic acid (GA) showed better germination than the control, but the difference was not statistically significant. Gibberellic acid at 1000 mmol (10 M) inhibited germination significantly. Seedlings survived better in the glasshouse (90%) than in the nursery (87%). Whereas 60% of the 280 wildlings between 2.5 and 4.0 cm were

successfully established, only 10% of the 220 wildlings between 8.0 and 13.0 cm survived 3 months after these had been maintained in the glasshouse. Seedlings derived from the GA - treated seeds showed higher initial growth than the control owing to rapid hypocotyl elongation. Transferring seedlings to larger pots enhanced growth significantly after a lag period of about two weeks. Seedlings maintained in the glasshouse grew better than those in the nursery. The study found that germination of seeds/fruits (gathered *en masse* from beneath trees) on a seedbed is convenient, technically less demanding, and is cost-effective.

2.3.5 Other propagation techniques for Prunus africana

Tchoundjeu *et al*., (2002) conducted a series of nursery experiments to assess the effects of rooting medium (sawdust, sand and a 50:50 mixture of sand and sawdust), auxin concentration (0, 50, 100, 150, and 200 µg Indole-3-butyric acid (IBA), and leaf area (0, 5, 10, 20, and 25 cm^2) on rooting success of juvenile cuttings of *P. africana*. The percentage of cuttings rooted was significantly greater in sawdust (80%), than in sand alone (72%) or in a mixture with sawdust (71%). Leaf area also significantly affected the percentage of rooting. Leafless cuttings did not root and were all dead by week 6, but in leafy cuttings rooting ability increased proportionally with leaf area up to 20 cm^2 (79%). Larger leaf cuttings (25 cm^2) are rooted at the same level as those of 20 cm^2. The cuttings with the largest leaves also had the greatest mean number of roots per cutting (14 roots cutting–1), while those with the smallest (5 cm^2) leaf area produced the fewest roots (5 roots cutting–1). The application of auxin Indole-3-butyric acid (IBA) promoted rooting ($P < 0.05$) up to an optimum application of 100–200 µg IBA per cutting, but 300 µg was supraoptimal. It was concluded that *P. africana* is amenable to vegetative propagation. Komakech *et al.,* (2020) discuss other possible propagation methods in detail such as *in vitro* and micropropagation techniques. This was the first study to develop a protocol for the efficient micropropagation of *P. africana* from juvenile nodal shoot

segments obtained from 1-year-old *P. africana* plants. The explants were sterilized and the axillary shoots were induced, rooted, and acclimatized. This protocol proved suitable for use in large-scale *P. africana* propagation to meet the increasing demands for it in the global market.

2.4 Population structure of *Prunus africana*

The population structure is defined as the tree size class distribution in a forest/woodland (Geldenhuys, 2010).

2.4.1 Classification of Prunus africana population structure

Many studies have classified plant population structure based on diameter class distribution. Those with a height of less than 30 cm are considered seedlings while those with a height > 30 cm and DBH ≤ 5 cm are considered as saplings and outside these ranges considered trees (Nguta, 2012; Koros *et al.*, 2016). The inverse 'J' pattern exhibited by a population indicates that the population is stable (Jimu, 2011; Koros, 2016). This is the pattern that displays more seedlings than saplings and mature trees. Alternatively, 'J' shaped population patterns indicate an unstable population with more mature trees than saplings and seedlings (Jimu, 2011; Nguta, 2012; Koros, 2016).

2.5 Phytochemical analysis of *Prunus africana*

Millions of people worldwide traditionally use natural or herbal remedies for their primary health care since time immemorial. these natural products are readily available, environment-friendly, cheap and without any side effects (Mukul, 2007). Traditional medicine on the other hand, because of its decentralized nature, is easily and quickly available (Elliot *et al.,* 1986). The global demand for natural medicine is now growing day by day as almost 80% of the

human population in developing countries relies chiefly on traditional, largely natural medicine to meet their primary healthcare needs (Farnsworth *et al.,* 1985).

Medicinal properties in plants are mainly due to the presence of secondary metabolites which the plants need in their natural environments under particular conditions of stress and competition and which perhaps would not be expressed under monoculture conditions. Active-ingredient levels can be much lower in fast-growing cultivated stocks, whereas wild populations can be older due to slow growth rates and can have higher levels of active ingredients (Schippmann, *et al.*, 2006). This can be bottle-neck in terms of propagation and domestication of wild medicinal plants as herbalists will always select potent genotypes for cultivation and domestication.

According to Komakech *et al.,* (2017) *P. africana* anti-prostate cancer can be divided into three major categories based on their targets and pharmacological effects (i) phytochemicals that kill the tumor cells through apoptotic pathways, a common mode of action of chemotherapeutic agents against a wide variety of cancer cells (ii) phytochemicals that alter the signaling pathways required for the maintenance of prostate cancer cells, and (iii) phytochemicals that exhibit strong anti-androgenic and anti-angiogenic activities.

Prunus africana bark extract contains the presence of sterols, pentacyclic triterpenoids, several lipid-soluble substances, including fatty acids (C_{12} to C_{24}) and two linear alcohols, n-tetracosanol and n-docosanol (Cunningham *et al.*, 1997). Chemical analysis confirms that it is possible to distinguish between *Prunus africana* bark sourced from different localities in Africa and Madagascar (Cunningham *et al.*, 1997). Using high-resolution gas chromatographic (HRGC) analysis, the authors showed that bark extracts from different sources in Africa had characteristic HRGC profiles and that it was possible to distinguish between these using eigenvector analysis. Bark extracts from *Prunus africana* in Madagascar were distinguished

from *Prunus africana* bark extracts from the Democratic Republic of Congo, Cameroon, and Kenya by their high 3-0-acetyl oleanolic acid content. *Prunus africana* bark extracts from Cameroon and the Democratic Republic of Congo were far more difficult to distinguish apart, but were considered to differ due to variations in ursolic acid, β-sitosterol and 3-0-glucoside content (Cunningham *et al*., 1997).

Although costly and time-consuming, the different chemical profiles of the bark may offer an opportunity for CITES to profile bark according to country of origin in the same way that this can be done through isotope analysis of elephant ivory. This may be useful in cases when more complex trade routes (such as the export from Cameroon to Madagascar, then the re-export of bark extract to Italy) are followed (Cunningham *et al.*, 1997). It can be a tool to combat illegal trade. Additionally, chemical profiling can act as a marketing tool for the *Prunus africana* population and an incentive for the local community's participation in the cultivation of the species to ease pressure on the wild population (Cunningham *et al.*, 1997).

2.5.1 Qualitative phytochemical profile of Prunus africana

Mutuma *et al.,* (2020) conducted a phytochemical qualitative analysis using the standard protocols of *Prunus africana* bark extracts. The qualitative evaluation of stem bark extract illustrated the presence of tannins, saponins, flavonoids, alkaloids, quinones, cardiac glycosides, terpenoids, phenolics, and coumarins. They concluded that dichloromethane stem bark extract of the *P. africana* presented anti-inflammatory activity hence a possible candidate for extraction of active anti-inflammatory compounds (Table 2). Detailed use of the compounds has been thoroughly discussed in Komakech *et al.,* (2017).

Table 2: Qualitative phytochemical compositions of dichloromethane stem bark extract of Prunus africana

Carbohydrates	++
Tannins	++
Saponins	+
Flavonoids	++
Alkaloids	++
Anthocyanin and betacyanin	-
Quinones	+
Glycosides	-
Cardiac glycosides	+
Terpenoids	++
Phenols	+
Coumarins	+
Steroids	++

Adapted from (Mutuma *et al.*, 2020): Key: +=trace, ++=moderate, +++=intense, - =not presen

2.5.2 Quantitative Phytochemical analysis of Prunus africana

Studies to understand the profile and quantify the bark extracts of *P.africana* were conducted by (Kadu *et al.*, 2012; Nyamai *et al*., 2015). Kadu *et al* found that the average concentration [mg/kg w/w] in increasing order was: lauric acid (18), myristic acid (22), n-docosanol (25), ferulic acid (49), β-sitostenone (198), β-sitosterol (490), and ursolic acid (743). This study compared phytochemical compounds and quantities across the continent. Estimates of variance components revealed the highest variation among populations for ursolic acid (66%) and the lowest for β-sitosterol (20%). However, a comprehensive study conducted by Nyamai *et al*. (2015) in three different sites in Kenya indicate that *P. africana* trees from the wild,

domesticated stand and on-farm remnant habitats do not vary significantly in the concentration of most of the phytochemical compounds related to benign prostate hyperplasia (BPH) treatment. The main objective of the study was to evaluate and compare the growth characteristics and phytochemical profile of trees in the domesticated stand at Muguga, with reference samples from Kobujoi, a wild stand, and Karuri a remnant on-farm stand. Extraction of compounds was done using aqueous, hexane, dichloromethane, and methanol solvents. Phytochemical analysis was done using Liquid Chromatography and Gas Chromatography-mass spectrometry. Evaluation of the crude yields of organic extracts of the three populations showed no significant differences. From the three stands, bark sample essential oils were primarily composed of myristic acid, linoleic acid, lauric acid, methyl myristate, methyl laurate, and methyl linoleate. Campesterol, β-sitosterol, lup-20(29)-en3-one, palmitic acid, β-sitostenone, (3.β., 5.α)- stigmast-7-en-3-ol, stigmastan-3,5-diene and α-tocopherol were detected in dichloromethane and hexane extracts of the three populations(Table 3) (3.β., 5.α)-stigmast-7-en-3-ol, β-sitosterol and β-sitostenone increase urine flow and inhibit prostaglandin production in the prostate(Komakech *et al.,* 2017). Cyanidin-o-galactoside, cyanidin-3-o-rutinoside, procyanidin B5, and robinetinidol-(4-α-8) catechin-(6,4- α) robinetinol are believed to inhibit cell proliferation and have free radical scavenging activity on cancerous cells. Ursolic acid is believed to have anti-inflammatory, antioxidant, and anti-proliferative effects on BPH (Nyamai *et al.,* 2016).

Table 3:Concentrations of compounds in hexane extracts of Muguga, Karuri, and Kabujoi Prunus africana(mg/Kg).

Compound	**Muguga**	**Karuri**	**Kobujoi**
Campesterol	9.16 ± 1.93[a]	4.51 ± 3.75[a]	6.70 ± 3.29[a]
Lauric acid	9.16 ± 1.93[a]	4.84 ± 7.56[a]	0.72 ± 0.32[a]
β-Sitosterol	131.04 ± 31.34[a]	160.05 ± 3.91[a]	153.36 ± 13.01[a]
Lup-20(29)-en3-one	13.32 ± 2.81[a]	10.04 ± 5.29[a]	7.97 ± 3.61a
Palmitic acid	82.24 ± 30.049[a]	34.28 ± 19.83[a]	55.13 ± 58.46[a]
Squalene	34.25 ± 14.99[a]	26.72 ± 3.18[a]	28.34 ± 19.13[a]
β-sitostenone	36.92 ± 12.05[a]	19.79 ± 0.49[a]	27.80 ± 13.87[a]
3β,5α-Stigmast-7-en-3-ol	15.63 ± 4.71[a]	10.46 ± 4.64[a]	11.23 ± 6.01[a]
Stigmastan-3,5-diene	35.99 ± 11.50[a]	20.42 ± 2.49[a]	27.21 ± 16.94[a]
Myristic acid	7.21 ± 0.50[a]	5.65 ± 5.06[a]	2.02 ± 0.09[a]
α-Tocopherol	13.44 ± 2.71[b]	1.84 ± 1.15[a]	4.88 ± 1.3[a]

Adapted from (Nyamai *et al.,* 2015)

Beta-Sitosterol-β-sitosterol is a plant-derived sterol, also known as a phytosterol (Komakech *et al*., 2017). The major effects of *P. africana* on prostate cancer have been reported to be due to the presence of this chemical which is present in high concentrations in the plant (Nyamai *et al*., 2015; Komakech *et al*., 2017). Studies have shown that β-sitosterol affects the membrane structure and function of the tumor as well as the host tissue signal trans-duction pathways that regulate tumor growth, leading to an apoptotic condition (Kadu *et al.,* 2012).

CHAPTER 3: MATERIALS AND METHODS

3.1. Study Area

The study was done in Nyika National Park, Malawi and Nyika National Park, Zambia (Figure 2). Nyika National Park, Malawi is found in the northern part of Malawi and runs astride three districts namely Chitipa, Karonga, and Rumphi with half of the park in Rumphi District (GoM, 2003). Nyika National Park, Malawi occupies a tract of mountains plateau and associated hills and escarpments in northern Malawi. Its area is 3,200km^2, and it is centered upon 10^o 33'S, 33^{o}50'E. Part of its western boundary coincides with the Malawi-Zambia border, and a section is contiguous with the Zambia Nyika National Park (Figure 2).Nyika National Park, Zambia lies on the northeast of Zambia (in Chama District) on the western edge of the Nyika Plateau, which is one of the highest parts of the country (Zambia Tourism, 2021). Zambia Nyika National Park has an area of 70 km^2 (GoM, 2003).

3.1.1 Location

The study was conducted at the Juniper and Manyenjere Forests inside Nyika National Park, Malawi, and Nyika National Park, Zambia, respectively (Figure 2).

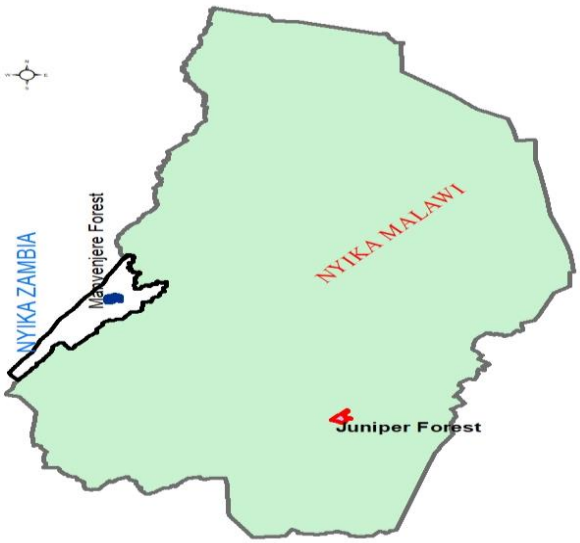


Figure 2: Location of the the study sites in Nyika Malawi and Nyika Zambia

3.1.2 Vegetation type

Mill (1979) distinguished four different types of plant communities: dambo, montane grassland, montane evergreen forest, and *Brachystegia* woodland. The following account incorporates data gathered by Dowsett-Lemaire (1985).

3.1.2.1 Dambo

This community occurs at the headwaters of drainage lines where impeded drainage gives rise to grass and sedge-dominated associations. There is usually a mixed herb mat giving partial ground cover. Grasses are often strongly tufted.

3.1.2.2 Montane Grassland

More than 90 percent of the high plateau (above 1 800 m) is covered with short, tufted grassland. The main dominants are *Loudetia simplex* and *Andropogon schirensis*. Local dominants include *Monocymbium ceresiiforme* and *Exotheca abyssinica*. There is a wide range of shrubs and many legumes. Bracken (*Pteridium aquilinum*) occurs in light stands throughout the grasslands, and there are a few very dense, almost pure stands of several hectares.

3.1.2.3 Montane Evergreen Forest

There are four main montane forest associations in the park (Chapman and White, 1970).

High Rainfall Sub-Montane Forest: Patches of this type are restricted to the high rainfall, eastern escarpment, and contain *Ocotea usambarensis* and *Ficalhoa laurifolia*. Their combined areas total 34 km^2, and the largest is Mwenembwe (13.5 km^2). These patches have been broken up by fire, and they may once have formed a single forest. The distribution of these patches ends abruptly at the plateau edge in an ecotone of *Agauria salicifolia* and *Philippia benguelensis*, both of which are fire-resistant.

Sub-Montane Forest: Patches of this type occur on the Malawian-Zambian border and are dominated by *Entandophragma excelsum*, *Aningeria adolfi-freidercii* and *Olea capensis*. The largest examples occur in the Zambian Nyika National Park. The most extensive Malawian example is Kasyaula Forest (40 ha) at 2 000 m elevation. From secondary growth and burnt remains Dowsett-Lemaire (1985) estimated its previous size to have been 120 ha. The Zovo Chipolo area, situated between the international border and Kaulime ridge to the east, is still fairly dense forested but the patches are small, the largest being 12 ha. The higher elevation and presumably lower rainfall, make these slightly impoverished compared with the Kasyaula Forest and Zambian examples. For example, the genera *Entandrophragma*, *Chrysophyllum*, and *Cola* do not occur.

Juniper (Juniperus procera) Forest: This is a comparatively minor association restricted to a few locations. Most of the surviving trees occur in two stands along the Uyaghaya Stream in the south-eastern plateau. The larger is about 9 ha. The smaller is still regenerating and should eventually cover twice the area (Dowsett-Lemaire, 1985). This was the first association to attract official protection, but despite this, extensive damage has been caused by fire. Several other patches that occurred nearby were destroyed.

Broad-Leafed Montane Forest: These are forest patches of poorer growth and species diversity that occur out on the high plateau. They are found at valley heads, along steep slopes, and on old landslide scars or near rocky outcrops. Chapman and White (1970) believed that they were relic patches of a formerly more extensive forest cover that had concentrated in response to fire. Repeated burning has led to thickets dominated by *Buddleja salviifolia*, *Kotschya recurvifolia,* and *Philipia benguelensis*. On the high plateau are a few dense mature forests where *Hagenia abyssinica* occurs on the edges, but the majority of patches appear to be in a young, non-climax stage, and contain *Hagenia* throughout. This suggests that the forests are

regenerating from secondary growth left by fire. *Hagenia* can only grow where there is strict fire protection. This regeneration appears to be a response to fire protection carried out since the 1950s.

3.1.2.4 Brachystegia Woodland

The deciduous Brachystegia woodlands are separated from the plateau grasslands by a transition zone of shrubs in which Protea bushes are conspicuous. The zone roughly follows the 1 800 m contour. *Brachystegia* spp. and *Julbernaria globiflora* are the dominant woodland species.

3.1.3 Climate

The elevation ranges in the park and its proximity to Lake Malawi influences the climate of Nyika National Park. Temperature and rainfall are variable, depending on the location within the park. The plateau (about 2000 to 2600m above sea level) is much cooler with average temperatures of about 14.3°C. In the warmest months, September to December, temperatures rarely exceed 26°C. Nyika National Park experiences four seasons (GoM, 2003), which are early wet from November to January, late wet from February to April, early dry from May to July and ate dry from August to October.

The rainy season in Nyika lasts from November through to mid-April. However, rainfall has been recorded in each month. The amount of rainfall received on the plateau ranges from 1700mm on the windward side to 1500mm on the leeward side.

3.1.4 Soil

The soils of the plateau are mainly acidic with a pH ranging from 4.0 to 5.5 (Burrow and Willis, 2005). These humic latosols are typical of the high plateau soils of the tropics (GoM, 2003)

3.2. Research design

Ecological surveys and laboratory analysis were conducted to achieve the study objectives.

3.3. Sampling framework and methods.

Several methods and techniques were required to achieve the three objectives looking at the nature of the study. The study is interdisciplinary and requires multi-disciplinary methods and techniques to critically evaluate it. Population structure requires ecological survey techniues whilst soil and phytochemistry require laboratory techniques.

3.3.1 Population structure

The sites were chosen based on the literature by Burrow and Willis (2005) who reported the presence of *Prunus africana* in these sites. A preliminary survey was undertaken to have a broad overview of the site. In the Juniper Forest, the distribution of *Prunus africana* was along the stream while in Manyenjere it was along the forest edge. To obtain representative samples, the area was stratified into Riverine (Riparian) and Non-riparian habitats. The belt transects of 50m by 500m divided into smaller plots of 25m x 25m were systematically applied and mid of the stream was taken as the mid-point of the belt (25m left and 25m right). Subsequent belt transects were obtained at an interval of 100m moving away from the previous belt transect along the same baseline (Figure 3). In Non-riparian habitat, a belt transects of 50m by 500m started on the edge of the forest to about 50m inside the forest (Figure 3). The initial point was randomly selected within the forests close to the forest edge. GPS Garmin eTrex touch 35 was used to collect location data. A map for both forests (Juniper and Manyenjere) was created by walking around the forests while setting the GPS on "Tracking route". GPX file was created by the GPS and was uploaded on ArcMap GIS software which coverted the gpx file to "shapefile" which was used to create distribution maps for Juniper and Manyenjere Forests (Figures 10 and 11).

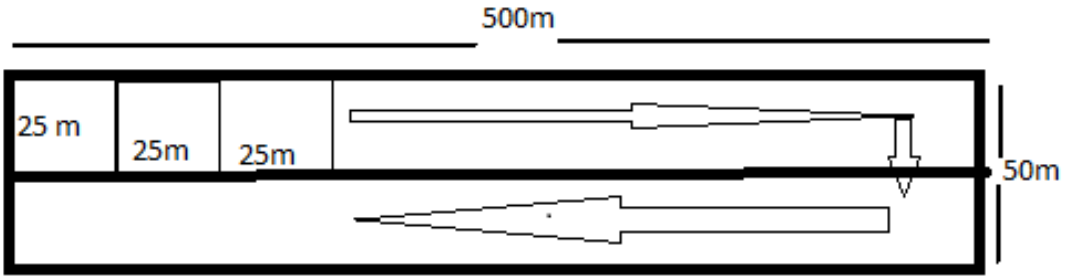


Figure 3:Belt transect showing data collection plan

Asrat and Tesfaye, (2013) reported that for sampling plants, especially forest trees with a height over 3m, rectangular or square plots with a sampling area greater than or equal to 100m^2 have been commended to yield better results hence the adoption of the 25m x 25m plots. The plot method was used because it is one of the basic and commonly accepted procedures for sampling many types of sessile organisms like plants.

A total of 2 belts with 40 plots were sampled in each stratum (Riparian or Non-riparian) representing 160 plots in both the Juniper and Manyenjere Forests. Ten (10) hectares of the area were sampled.

3.3.2 Soil physiochemical analysis

Soil samples were collected from the study area at the beginning of the study. Three (3) soil samples per plot containing *P.africana* were collected at a depth range of 0 to 15cm after removing the top organic matter under each identified study plot (Provin, *et al.*, 2002). Collected soil samples from both Juniper and Manyenjere Forests plots were taken to FRIM for laboratory analyses.

3.3.2.1 Soil physical analysis

Collected soil samples were air-dried at room temperature (25-30) degrees Celsius. Particle-size analysis was done using a combination of sieving and hydrometer analysis. Sieved soil

was pre-treated with hydrogen peroxide as described by Gee and Bauder (1986). The hydrometer method was used to determine the clay, silt and sand fractions, (fine, medium and coarse). Calculations were made from each reading to determine the percentage of sand, silt and clay. The results were then referred to the Soil triangle where the textural class for each soil sample was classified.

3.3.2.2 Chemical properties of soil.

The air-dried soil samples were sieved using a 2 mm sieve. Soil pH was determined using the $CaCl_2$ method (Okalebo, *et al.,* 2002). Organic carbon was determined using the Walkley - Black method (Anderson and Ingram, 1993). Total nitrogen was then determined using the Kjeldal digestion method where it went through a distillation process and finally colorimetric titration (Okalebo, *et al*. 2002). Exchangeable bases Calcium and Magnesium were determined using the titration method with (Ethylene diamine tetraacetic acid) EDTA. Phosphorus was extracted using the Mehlich lll method and determined using a UV- VIS spectrophotometer.

3.3.3 Phytochemical analysis

Phytochemical analysis was carried out on samples from eight trees (8) four from each site which were picked at random and during selection, no factors were considered as the primary purpose was to profile all phytochemicals found in the study sites.

3.3.3.1 Sample collection, preparation and extraction

Prunus africana bark samples were obtained from Juniper and Manyenjere forests. Samples from eight (8) trees picked at random (four from each site) were cut into small pieces and air-dried under a shade. After drying, samples were milled into a fine powder.

A hundred grams(100g) of each powdered bark sample from the two sites was sequentially soaked with 600 ml hexane, dichloromethane, and ethylacetate. After soaking for 48 hrs, each

extract was filtered using Whatmann filter no. 1. Further, the filtrate was concentrated using a vacuum at reduced pressure with the temperature set at 40°C using a Heldolph Laborata 4000-efficient rotary evaporator.

3.3.3.2 Gas Chromatography- Mass spectrometry analysis and sample preparations

One milligram of each sample was weighed into 1.5 ml Eppendorf tubes. Each sample was dissolved with one milliliter of hexane, dichloromethane, ethylacetate, and methanol and each mixture vortexed for 30 seconds. Each sample was then sonicated for five minutes and centrifuged for five minutes at 13000 rpm at room temperature. Each sample was then transferred to a 2 ml auto sampler vial and analysed using GC-MS.

3.3.3.3 Gas Chromatography- Mass spectrometry conditions

The oven temperature (35^{o}c), the flow rate of the gas used and the electron gun for the GC-MS were programmed before sample analysis. Analysis was carried out using a GC-MS (7683 Agilent Technologies, Inc., Beijing, China) comprising a gas-chromatograph interfaced to a 5975C inert XL EI/CI mass spectrometer equipped with an HP-5 MS (5% phenyl methyl siloxane) low bleed capillary column of 0.25 mm diameter, 30 m length and 0.25 µm film thickness. Detection was done using an electron ionization system with ionization energy of 70Ev. Helium (99.99%) was used as the carrier gas at a constant flow rate of 1.25 ml/min. Further, the injector and mass transfer line temperature were set at 250°C and 200°C respectively. An injection volume of 1 µl (splitless mode) was used. The oven temperature was set at 35°C for 5 minutes, with an increase of 10°C/min to 280°C for 10.5 minutes, then 50°C/min to 285°C for 29.9 minutes with a run time of 70 minutes. MS parameters were as follows: ionization energy, 70eV; ion source temperature, 230°C, solvent cut time, 3.3 min, relative detector gain mode, scan speed 1666 µ/sec; scan range 40-550 m/z, the interface temperature was 250°C. The total running time of GC-MS was 70 min. In this study,

hexadecenoic acid was used as an external standard to construct a calibration curve for the quantification of compounds.

3.3.3.4 Liquid chromatography Mass Spectrometry

Samples (1g) were each dissolved in 1mL (90:10 MeOH, ddH2O), vortexed for 10s, sonicated for 30 min, centrifuged at 14000 rpm for 10 min at 4 °C and supernatant analysed using UPLC-MS/MS (0.1µL). Chromatographic separation was performed on a ACQUITY UPLC I-class system (Waters Corp., Milford, MA) fitted with an ACQUITY UPLC BEH C18 column (2.1 mm X 150 mm, 1.7 µm particle size; Waters Corp., Wexford, Ireland, oven temp 45 °C). The autosampler tray was cooled to 5°C. The mobile phase comprised of (A) water and (B) methanol (solvent B) both acidified with 0.01% formic acid. The gradient system used was 0–2 min, 5% B, 2–4 min, 40% B, 4–7 min, 40% B, 7–8.5 min 60% B, 8.5–10 min 60% B, 10–15 min, 80% B, 15–19 80% B, 19–20.5 min, 100% B, 20.5–23 min, 100% B, 23– 24 min 95% B, 24–26 min, 95% B. The flow rate was held constant at 0.2 ml/min. The UPLC was interfaced with electrospray ionization (ESI) Waters Xevo TQ-S operated in full scan MS in positive ionization mode. Data were acquired over. The m/z range is 40–2,000 with a capillary voltage of 0.5 kV, sampling cone voltage of 30 V, source temperature of 150 °C desolvation temperature of 120 °C. The nitrogen desolvation flow rate was 800 L/h. Data was acquired using MassLynx version 4.1 SCN 712 (Waters). Potential assignments of compounds were determined after the generation of the mass spectrum for each peak, establishing the molecular ion peaks using adducts, common fragments, literature online databases (METLIN, ChemSpider), and where available, confirmed with authentic samples through co-injections.

3.4. Data Collection

Spatial data on *Prunus africana* distribution was collected using GPS Garmin etrex 35. All coordinates, where *Prunus africana* was sighted, were recorded.

Data on the number of *P. africana* encountered (n), dbh (cm), Height (m), and Canopy cover (%) were collected using ecological data sheets. Soil major elements (Ca, pH, N, C, Mg, and P) were detected through laboratory analysis and quantified. Phytochemical compounds were profiled and quantified using GC-MS (µg/mg) and LC-MS (ng/g) units.

3.5. Data Analysis

All data in 3.4 above were analysed using GenStat version 12.1, Minitab version 16.1, past4, PC-ORD version 6.08 and Arc GIS ArcMap 10.8.1 software. The spatial data was processed into distribution map using Arc GIS ArcMap 10.8.1(Figures 10 and 11). To determine the significant difference in the parameters, ANOVA test, *Kruskal-Wallis* tests, T-test, and *Mann–Whitney U* test were performed at a 5% critical level. Before testing, normality tests in GenStat and Past4 were performed on all parameters to determine if they satisfy ANOVA & T-tests assumptions (Quinn and Keough, 2002). Data on density, and development stages were log-transformed before analysis to permit the use of parametric statistics. In data sets that contained zeros, one was added to every observation before transformation (i.e. X 1 = log (X + 1) to eliminate the zeros. Data on percentages (canopy cover) were arcsine transformed to remove the imposed limits before analysis. ANOVA test was applied to all data comparing parameters amongst vegetation types (dbh, heights, density) and population structure (seedlings, saplings & mature trees) in the study sites. T-test was conducted on heights, dbh, crown cover, densities, soils (Ca, PH, N, C, Mg, and P), and phytochemical amounts which were meant to compare the parameters between Nyika Malawi and Nyika Zambia whilst *Mann–Whitney U* test was performed on phytochemical profile frequency between the two study sites which were not

normally distributed. Cluster analysis, Shannon's Diversity index analysis was applied to derive species association levels and diversity using PC-ORD version 6.08 software. Density was calculated by the number of individual species divided by area (ha). Abundance was found by summing up individual counts of *P.africana* in all plots and sites.

CHAPTER 4: RESULTS

4.1 Abundance of *Prunus africana* study sites

The abundance of *P. africana* trees in the study sites are presented in Table 4. A total of 47 individuals of *P. africana* were found in 16.25% of sampled plots. A total of 160 plots were sampled across the sites. Results show that the abundance of trees was highly significant ($p<0.05$) between study sites. Juniper Forest recorded higher tree abundance (38) as compared to the Manyenjere Forest (9). In terms of vegetation type, the riparian forest type (36) was highly significant ($p<0.05$) than the non-riparian forest type (11) in the abundance of *P. africana*.

4.1.1 Stocking density of Prunus africana in study sites

The mean stocking density was 5 trees/ha in the study sites. Tree stocking density was highly significant ($P<0.05$) between sites. Juniper Forest had the highest, while Manyenjere Forest had the lowest tree density. The Juniper riparian habitat type (14 trees/ha) had the highest stocking densities followed by Manyenjere non-riparian forest (4 trees/ha). Based on the results, the least density was the Manyenjere riparian vegetation (0 trees/ha) (Table 4).

Table 4: Abundance, Density, Mean DBH, Height and Canopy cover (%) in relation to vegetation in the study sites

Name of forest and forest type	Abundance	Density (Trees/ha)	Mean dbh (cm)	Mean Height (m)	Canopy Cover (%)
Juniper riparian Forest	36	14.4	30.5±18.1	15.4±7.3	55±10
Juniper non-riparian	2	0.8	24.6±3.9	12.7±2.7	48±19
Manyenjere riparian	0	0.0	0.0	0.0	92.25±8
Manyenjere non-riparian	9	3.6	28.4±18.1	21.1±11.2	87±7

Table 5 below depicts One –way ANOVA results for tree Density, dbh, Height and Crown cover in *Prunus africana* study sites. Statistical test shows that density, dbh and canopy cover varied significantly whilst tree height did not vary between forest types (Table 5).

Table 5: One-way ANOVA results for Prunus africana in the study sites

Variable	**F-value**	**df**	**P- Value**
Density	6.97	3	0.001
dbh	145.90	3	0.001
Tree height	1.64	2	0.235
Canopy cover	148	3	0.001

4.1.2 Tree Diameters at Breast Height (dbh) in study sites

Juniper Forest recorded a (29.9 cm) mean dbh whilst Manyenjere (28.4 cm). Juniper Forest recorded a mean height of 14.9 m while Manyenjere recorded 21.1m as a mean height. The results of the study show no significant difference in relation to both dbh and height between Juniper Forest and Manyenjere Forest (Table 5). However, results comparing forest types, show highly significant differences ($p<0.05$) amongst the four vegetation types (Table 5). Tree diameters ranged from 5.5 - 66.3 cm in Juniper Forest, and 7.9- 53.5cm in Manyenjere Forest (Table 6).

Table 6: Diameter at Breast Height(cm) and heights(m) ranges in the study sites.

JUNIPER FOREST			MANYENJERE FOREST		
Plot	**dbh(cm)**	**Height(m)**	**Plot**	**dbh(cm)**	**Height(m)**
1	5.5	5.3	1	7.95	7.2
2	8.6	5.8	2	12.1	11.2
3	10	6.2	3	17.2	13.6
4	10.6	6.3	4	23.2	18.7
5	10.8	7.7	5	35	29.3
6	18.6	10.8	6	49.95	33.5
7	21.8	10.8	7	53.5	34.3
8	26.7	14.6	**Total**	**198.9**	**147.8**
9	27.3	14.6	**Mean**	**28.4**	**21.1**
10	27.3	15.5			
11	28.9	15.6			
12	30.5	16.1			
13	42	17			
14	43.1	19.3			
15	43.5	19.6			
16	45.5	22.3			
17	45.7	23			
18	54.8	25.3			
19	66.3	26.7			
Total	**567.5**	**282.5**			
Mean	**29.9**	**14.9**			

4.1.3 dbh size-class distribution in study sites

Figure 4 shows frequencies of tree diameter classes found in the two study sites. There were no significant differences (F=0.5131, df=2.713, p=0.7605) among dbh size class distribution (Figure 4). However, a greater number of individuals was recorded in size-class 21-30.9 cm (6 individuals); followed by<10.9 cm and 41-50.9 cm class (5 individuals each), respectively. More individuals were found in Juniper Forest than in Manyenjere Forest (Figure 4).

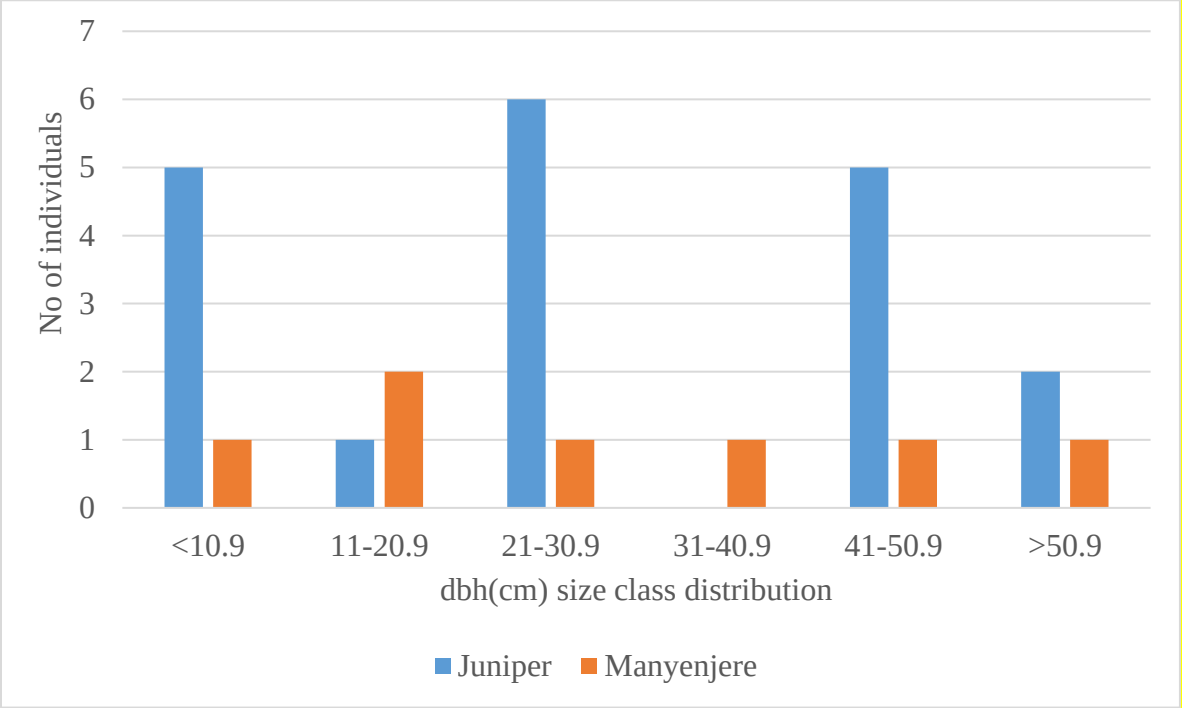


Figure 4: Tree size class distribution of Prunus africana in the two study sites

4.1.4 Tree heights (m) in the study sites

Juniper Forest recorded a *P. africana* tree mean height of 15.4±7.3m whilst Manyenjere Forest recorded a *P. africana* tree mean height of 21.11±11.17m. The Juniper Forest heights ranged from 5.3m to 26.7m while Manyenjere Forest ranged from 7.2m to 34.3m. However, there were no statistically significant differences in mean height (Two Sample t-tests, t = -1.64; DF = 23; p =0.114) between study sites.

4.1.5 Forest canopy cover% in the occurrence sites

Tree canopy cover in the two study sites ranged from 7 to 95%. The canopy covers for Juniper Forest ranged from 7 to 75% while Manyenjere Forest ranged from 75% to 95%. Comparatively, Juniper Forest recorded a lower mean canopy cover (52 ±14 %) whereas Manyenjere Forest had the highest (94% ±5.9) average canopy cover ($p<0.001$, Table 5).

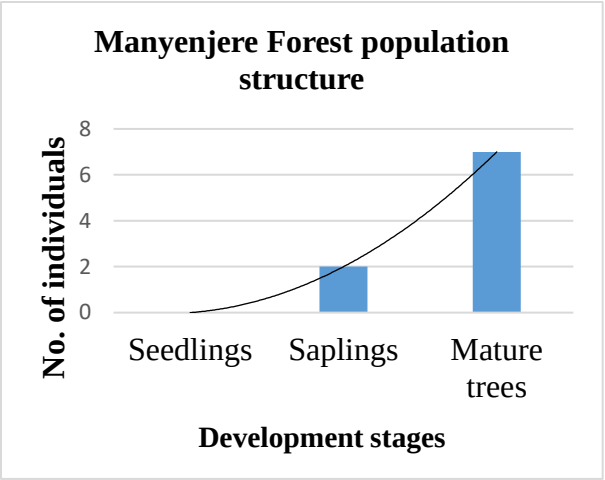
4.1.6 Tree canopy cover in habitat types

Canopy cover percentage varied significantly ($P<0.05$) among the studied habitat types in Manyenjere and Juniper Forests. Manyenjere Riverine (riparian) Forest had 94% followed by Manyenjere non-riparian Forest (87.7%). Furthermore, there were significant variations between Juniper riverine (Riparian) and Manyenjere riparian forests ($p <0.05$) with a mean canopy (%) of 55±10 and 92.25±8, respectively.

In contrast, the non-riparian habitats recorded a significant difference ($P<0.05$) in mean canopy cover of 48±19 and 87±7 % between Juniper and Manyenjere Forests, respectively.

4.1.7 *Prunus africana* population structure in the study site

The main three development stages (seedlings, saplings and mature trees) of *P. africana* in both forests are presented in Figure 5. Results show significant differences ($P<0.05$) in terms of individual counts of the three development stages ($F= 5$; DF 2; $P=0.007$) between sites. Comparatively, Juniper Forest recorded higher seedlings, saplings and mature trees than Manyenjere Forest (Figure 5). The observed population structure, reveals nonexponential curves, but typical J-shaped curves of the three development stages of *Prunus africana* (Figure 5).



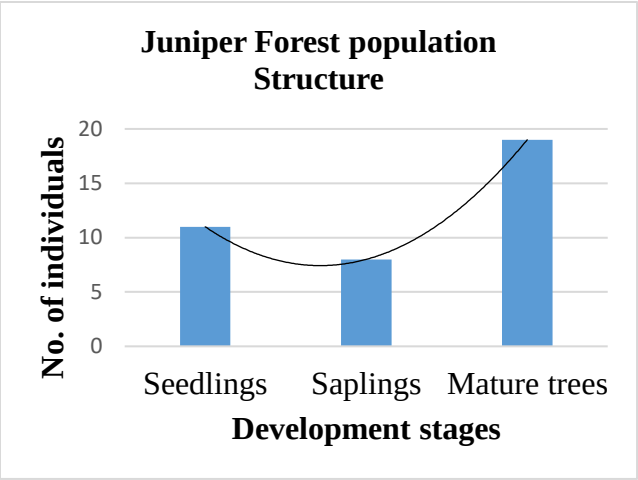


Figure 5: Prunus africana development stages in the study areas

4.1.8 Prunus africana plant associated species.

Plant association was analysed in terms of species richness and diversity as in Nguta (2012).

4.1.8.1 Species richness

A total of sixty-seven (67) associated tree species were found belonging to 43 Genera in the study sites (Appendix 3). Kruskal-Wallis ranking test revealed no significant variations (H=1.62, DF=1, P=0.109) between study sites in terms of species richness. Manyenjere Forest recorded a median of 13 while Juniper Forest had 11 species (Figure 6). This result may indicate similarity in associated tree species.

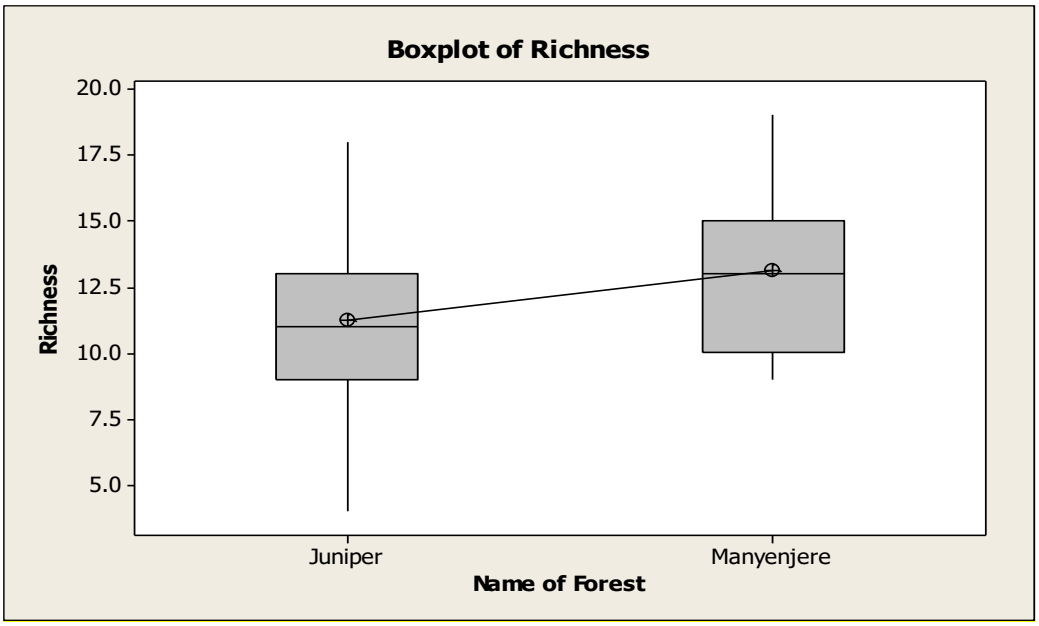


Figure 6: Boxplot of species richness in the two study sites

4.1.8.2 Species diversity

Shannon's index of diversity for the associated tree species found in the two *Prunus africana* occurrence sites revealed no significant variation (Kruskal-Wallis analysis H= 2.01, DF=1, P= 0.157) in the diversity of tree species. Juniper Forest recorded a median of 2.381 while Manyenjere Forest had a median of 2.561. The most common species found were *Diospyros whyteana, Teclea nobilis, Psychotria zombamontana, Parinari excelsa, Bersama abyssinica, Clausena anisata, Garcinia kingaensis, Ilex mitis, Maytenus acuminata, Rapanea melanophloeos, Agauria salicifolia, Juniperus procera, Macaraga capensis, Podocarpus milanjianus, Hagenia abyssinica and Tecomaria capensis*.

4.1.8.3 Relative abundances

The relative abundance values showed that *P. africana* tree communities were considerably dominated by *Diospyros whyteana, Teclea nobilis, Psychotria zombamontana, Parinari excelsa, Bersama abyssinica, Clausena anisata, Garcinia kingaensis, Ilex mitis, Maytenus*

acuminata, Rapanea melanophloeos, Agauria salicifolia, Juniperus procera, Macaraga capensis, Podocarpus milanjianus, Hagenia abyssinica and Tecomaria capensis (Figures 7, 8 and 9).

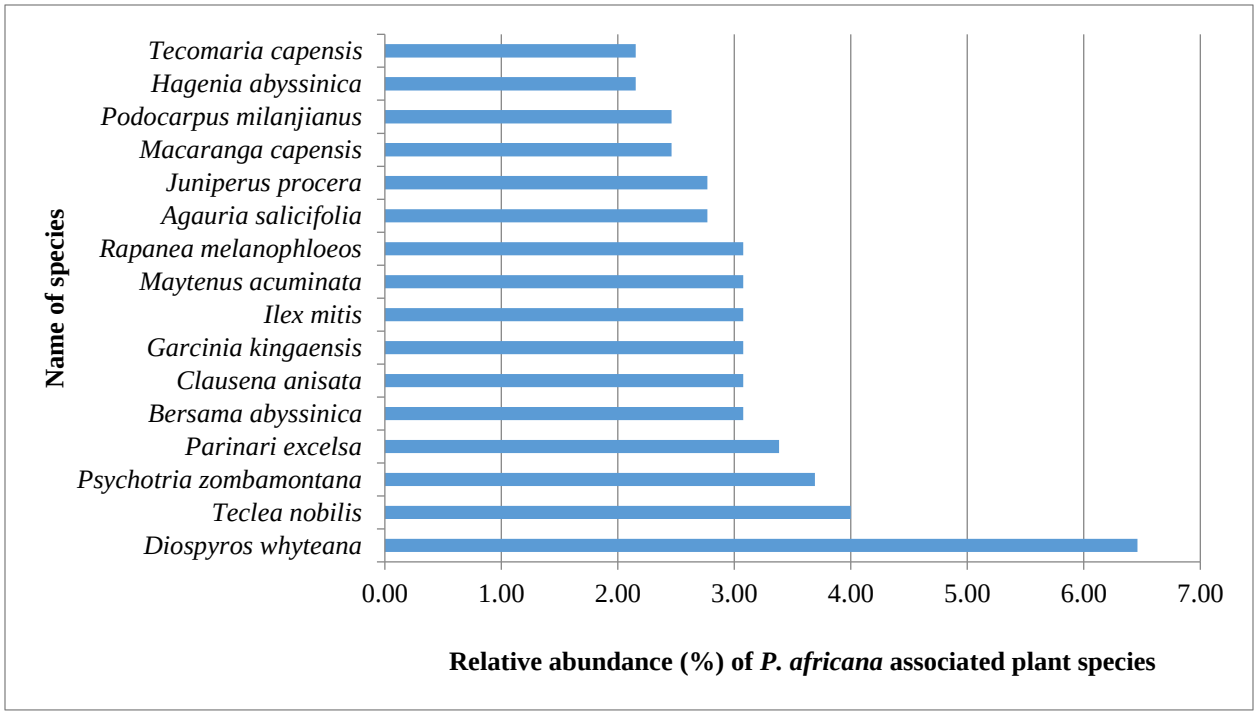


Figure 7: Relative abundance of Prunus africana associated plant species in the study sites

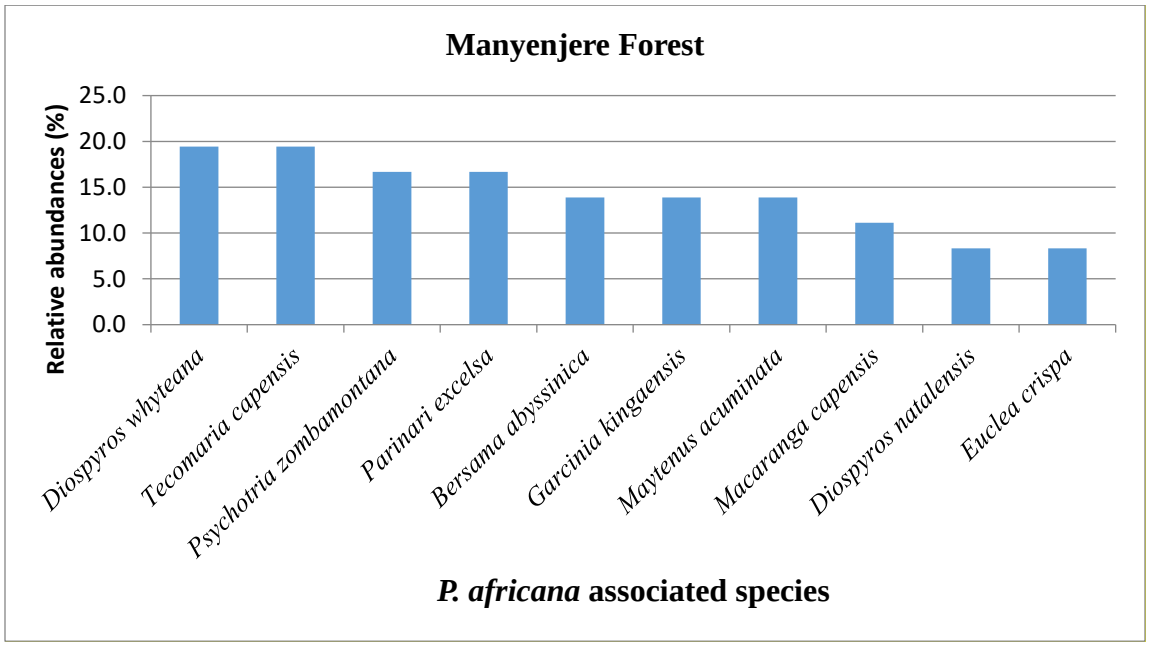


Figure 8: Common associated plant species of Prunus africana in Manyenjere Forest

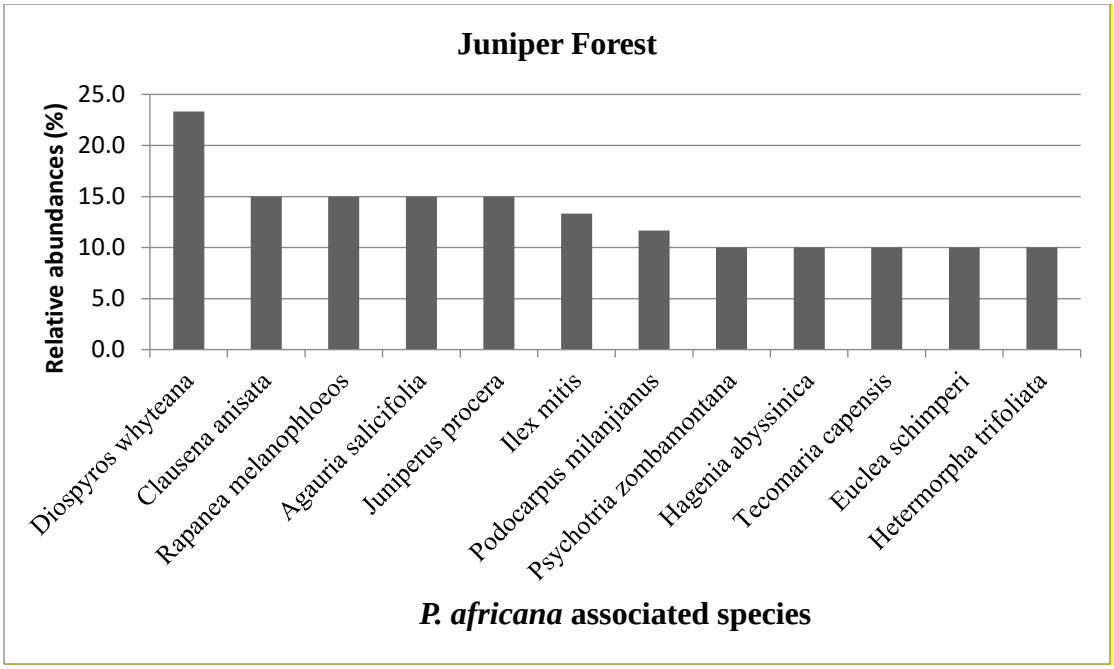


Figure 9: Common plant species associated with Prunus africana in Juniper Forest

4.2 Soil physicochemical properties

Soil analysis involved soil texture, soil particle size distribution, and physicochemical composition.

4.2.1 Soil texture

Table 7 shows soil structure, indicating the relative content of particles of various sizes (sand, silt, and clay) in the soil. In the Juniper Forest plots, (84%) of them were loamy sand with most of the plots revealing high sand content (above 80%). The other 16% of the plots had sand content which was below 70%. This was the same with Manyenjere plots where 84 % of the plots had above 70% sand content while the remaining plots were sandy loam (Table 7) content.

Table 7: Particle size distribution of plots under different soil cover

Occurrence Sites	**Particle size distribution**			**Soil texture class**
	Sand%	Clay%	Silt%	
Juniper plot -01	80	6	14	Loamy sand
Juniper River Bank- 02	66	12	22	Sandy loam
Juniper plot -05	82	2	16	Loamy sand
Juniper plot -06	80	2	18	Loamy sand
Juniper plot -07	80	4	16	Loamy sand
Juniper plot -08	68	4	28	Sandy loam
Juniper plot- 09	84	2	14	Loamy sand
Juniper plot -10	80	2	18	Loamy sand
Juniper plot -11	80	2	18	Loamy sand
Juniper plot -12	76	4	20	Loamy sand
Juniper plot -14	80	2	18	Loamy sand
Juniper plot -16	84	2	14	Loamy sand
Juniper plot -17	78	2	20	Loamy sand
Juniper plot -18	70	8	22	Loamy sand
Manyenjere plot -01	78	4	18	Loamy sand
Manyenjere plot -02	76	2	22	Loamy sand
Manyenjere plot -03	70	2	28	Loamy sand
Manyenjere plot -05	66	4	30	Sandy loam
Manyenjere plot -06	74	4	22	Loamy sand
Manyenjere plot -07	72	6	22	Loamy sand

4.2.1 Soil chemical properties in Prunus africana plots in the study sites

Table 8 presents soil physicochemical properties in the two study sites. Nitrogen (N), Calcium (Ca), Magnesium (Mg), pH, and Carbon(C) were the same in the study area. T-test showed no significant differences ($p>0.05$) in mean values between Juniper and Manyenjere Forests soil samples (Table 8). Phosphorus (P) varied significantly ($p<0.05$) between the two study sites. Mean values (Mean ± SD) and probability values (p-value) for all the major soil elements have been tabulated (Table 8).

Table 8: Chemical properties of soil in Prunus africana occurrence sites.

SITES	**N (%)**	**P(mg/kg)**	**Ca (%)**	**Mg (%)**	**pH**	**C (%)**
Juniper	0.49±0.19	9.01±4.7	6.35±3.29	4.02±2.29	5.25±0.41	3.20±0.46
Manyenjere	0.51±0.14	3.83±3.4	5.66±2.0	3.29±2.38	5.15±0.37	3.27±0.63
P-value	0.83	0.028*	0.643	0.524	0. 589	0.787

Values are expressed as Mean ± SD (n=3). (*) Values less than 0.05 are significantly different.

4.3 Phytochemical analysis

Phytochemical analysis of *Prunus africana* extracts from Juniper and Manyenjere Forests were analysed at Jomo Kenyatta University in Kenya Biochemistry Laboratory. GC-MS **(*ethyl acetate, dicloromethane {DCM}* & hexane**) analysis and LC-MS analysis were conducted to detect possible compounds in *Prunus africana.* The results of GC-MS and LC-MS are presented in the subsequent sections.

4.3.1 Gas Chromatography Mass spectrometry

GC-MS analysis detected a total number of 163 phytochemical volatile compounds in *P. africana* ethyl acetate, dichloromethane (DCM), and hexane extracts from both Juniper Forest and Manyenjere Forest. Juniper Forest recorded 92 phytochemicals whereas Manyenjere Forest 111 phytochemicals. Methyl linoleate and Squalene had the highest frequency

occurrences (20) seconded by Methyl octadecanoate and Octadecanoic acid with (19) frequency occurrences (Appendix 5). The variation in the number of compounds detected in the study sites was statistically different (Mann-Whitney *U*: 11530; *Z*: 2.1726; p= 0.0295). There were no significant differences (F=2.18, DF=2, p=0.115) in the number of phytochemicals from all the three extracts (Ethyl acetate, DCM and hexane). Table 9 shows volatile compounds in *P. aficana* ethyl acetate extracts from the two study sites.

The 6-Octadecanoic acid and Octadecanoic acid were the major volatile compounds in *P. africana* stem bark from the two study sites. Other compounds included γ-Sitosterol, stigmastanol, and oleic acid (Table 9). There were no significant differences ($p > 0.05$) in the amount of most volatile compounds between the two sites. However, *P. africana* extract from Manyenjere Forest contained more oleic acid than the one from Juniper Forest. Phenol, 2,4-bis(1,1-dimethylethyl)- and Stigmastanol were detected in *P. africana* extract from Juniper Forest only while n-Hexadecanoic acid was detected in Manyenjere Forest only. A comprehensive list of phytochemical compounds detected under ethyl acetate extract method has been recorded (Appendices 7-13).

Table 9: Concentrations of phytochemical compounds in the ethyl acetate extracts in the study sites**(**µg/mg)

Chemical compound	**Juniper Forest**	**Manyenjere Forest**
Oleic acid	1.00±1.37[a]	19.48±34.46[b]
9-Octadecenoic acid, methyl ester, (E)-	10.99±8.36[a]	19.75±14.9[a]
γ-Sitosterol	9.94±18.77[a]	25.01±23.34[a]
Phenol, 2-4-bis(1,1-dimethylethyl)-	5.56±11.11[a]	0.00[a]
6-Octadecanoic acid	109.25±73.5[a]	91.37±46.16[a]
Stigmastanol	20.73±23.89[a]	0.00[b]
Octadecanoic acid	57.13±71.77[a]	30.12±7.02[a]
n-Hexadecanoic acid	0.00[a]	4.49±8.98[a]
Methyl, linoleate	2.69±1.88[a]	9.63±9.77[a]
Stigmast-7-en-3-ol(3.beta,5.alpha,24S)-	0.79±1.57[a]	1.2±0.5[a]
Methyloctadecanoate	4.54±4.23[a]	5.85±4.89[a]
Squalene	6.57±4.4[a]	8.19±4.93[a]

Values are expressed as Mean ± SD (n=4). Values followed by the same super script along rows are not significantly different (p>0.05).

Table 10 shows phytochemicals in *P. africana* DCM extract from the two study sites. All phytochemicals did not differ significantly between the sites (P>0.05) except γ-Sitosterol, 6-Octadecanoic acid, Stigmastanol and Stigmast-7-en-3-ol(3.beta,5.alpha,24S)-which varied significantly between the sites (P<0.05). Phenol, 2-4-bis (1,1-dimethylethyl)- and D:A-Friedooleanan-2-one were detected in Juniper *P.africana* extracts only. The most bioactive compound in *P. africana* extracts from both study sites was 6-Octadecanoic acid (Table 10). Full list of all compounds detected in *P. africana* DCM extracts is presented in ppendices 14-19).

Table 10: Amount of volatile compounds in DCM extracts from the two populations **(**µg/mg)

Chemical compound	**Juniper Forest**	**Manyenjere Forest**
Oleic acid	28.9±47.54[a]	27.23±51.1[a]
9-Octadecenoic acid, methyl ester, (E)-	13.86±9.38[a]	5.68±0.32[a]
γ-Sitosterol	39.2±17.31[a]	0.06±0.13[b]
Phenol, 2-4-bis(1,1-dimethylethyl)-	4.85±9.7[a]	0[a]
6-Octadecanoic acid	154.37±111.36[a]	69.47±138.94[a]
Stigmastanol	2.62±3.03[a]	15.23±24.05[b]
Octadecanoic acid	29.35±21.33[a]	19.06±13.95[a]
n-Hexadecanoic acid	5.1±10.19[a]	2.86±5.73[a]
Methyl, linoleate	3.98±0.52[a]	10.42±9.86[a]
D:A-Friedooleanan-2-one	0.03±0.06[a]	0[a]
Stigmast-7-en-3-ol(3.beta,5.alpha,24S)-	10.92±17.48[a]	0.39±0.79[b]
Methyloctadecanoate	4.03±1.6[a]	4.83±5.58[a]
Squalene	5.91±4.22[a]	10.25±7.06[a]

Values are expressed as Mean ± SD (n=4). Values followed by the same super script along rows are not significantly different (p>0.05).

Table 11 shows volatile phytochemicals in hexane extracts. The results showed variations in the amount of compounds. For example, 9-Octadecenoic acid methyl ester, γ-Sitosterol, 6-Octadecenoic acid, Methyl linoleate, and Stigmast-7-en-3-ol(3.beta,5.alpha,24S)- varied significantly (P<0.05) between the two sites. The amount of volatile compounds in *P. africana* hexane extract from Manyenjere Forest were more than those from Juniper Forest. The other five (5), Oleic acid, Octadecanoic acid, n-Hexadecanoic acid, Methyloctadecanoate, and Squalene did not vary (P>0.05) between the sites. The 9-Octadecenoic acid, methyl ester, (E)-, and 6-Octadecanoic acid were not detected in *P.africana* extracts from Juniper Forest. The

major compounds in *P. africana* extracts from both study sites were 6-Octadecanoic acid, Oleic acid, and Octadecanoic acid (Table 11). A full list of phytochemicals detected in hexane extracts has been presented in appendices 20-27.

Table 11: Concentrations of phytochemical compounds in hexane extracts in the study sites(µg/mg)

Chemical compound	Juniper Forest	Manyenjere Forest
Oleic acid	51.28±102.56[a]	104.96±181.79[a]
9-Octadecenoic acid, methyl ester, (E)-	0.00[a]	10.89±16.8[b]
γ-Sitosterol	5.12±5.02[a]	25.9±44.37[b]
6-Octadecanoic acid	0.00[a]	273.02±201.22[b]
Octadecanoic acid	44.91±69.15[a]	94.22±70.08[a]
n-Hexadecanoic acid	32.55±42.85[a]	30.2±51.51[a]
Methyl linoleate	0.86±1.33[a]	16.5±13.58[b]
Stigmast-7-en-3-ol(3.beta,5.alpha,24S)-	0.46±0.92[a]	36.27±62.83[b]
Methyloctadecanoate	0.54±0.94[a]	4.32±5.19[a]
Squalene	8.38±11.08[a]	14.18±12.76[a]

Values are expressed as Mean ± SD (n=4). Values followed by the same super script along rows are not significantly different (p>0.05).

4.3.2 Liquid Chromatography Mass Spectrometry

The results of liquid chromatography-mass spectrometry (LC-MS) analysis of *P. africana* from Manyenjere and Juniper Forests, are presented in Table 12. Results showed that Oleanolic acid, β-sitostenone, Luteolin, Rutin, Apigenin 6-c—glucoside differed significantly ($p<0.05$) between the two sites. The amount of other compounds were almost the same ($p>0.05$) in both sites (Table 12). Chlorogenic acid, Oleic acid and Quercetin were the major compounds in *P. africana* from Manyenjere Forest. In contrast, Apigenin 6-c—glucoside, Quercetin, and

Chlorogenic acid, were the major compounds in Juniper Forest. Oleic acid was detected by both GC-MS and LC-MS. Details of the phytochemical profile and their quantities have been presented in appendices 28 - 29.

Table 12: Concentrations of non-volatile phytochemicals in P. africana extracts from Manyenjere and Juniper Forests (ng/g).

Chemical Compound	**Manyenjere**	**Juniper**
Chlorogenic acid	118.11±36.33[a]	111.04±36.49[a]
Oleanolic acid	53.78±34.02[a]	2.96±2.09[b]
β-sitostenone	48.48±33.29[a]	8.98±7.76[b]
Ellagic acid	55.41±18.70[a]	46.74±33.86[a]
Oleic acid	73.21±21.34[a]	50.32±8.79[a]
Quercetin	65.58±24.09[a]	116.62±30.11[a]
Luteolin	41.53±27.57[a]	4.41±5.23[b]
Prunasin	39.71±21.15[a]	30.57±19.68[a]
P-coumaric	43.93±45.39[a]	28.32±24.34[a]
Rutin	35.68±19.96[a]	4.92±2.54[b]
Ursolic acid	5.46±1.11[a]	1.51±1.15[a]
Apigenin 6-c--glucoside	33.66±23.77[a]	147.99±27.04[b]
Campesterol	16.93±10.00[a]	7.82±4.87[a]
Zeatin	20.60±11.42[a]	5.43±5.37[a]
Kaempterol	6.06±5.52[a]	4.63±6.25[a]
Cynanidin-0-galactoside	8.03±4.85[a]	11.61±19.88[a]
β-sitesterol	42.62±29.11[a]	1.98±2.15[b]

Values are expressed as Mean ± SD (n=4). Values followed by the same super script along rows are not significantly different ($p>0.05$).

CHAPTER 5: DISCUSSION

5.1 *Prunus africana* abundance and density in the study sites

5.1.1 Abundance and stocking density of Prunus africana in Juniper and Manyenjere Forests

The study showed that the Juniper Forest had a higher stocking density of *Prunus africana* than the Manyenjere Forest (Table 4). This could be attributed to forest canopy cover which showed significant differences (Table 5). *Prunus africana* is a light-demanding species and regeneration and establishment is difficult in areas where the canopy cover is thick (Nkeng *et al.,* 2010; Nguta, 2012; Ingabire *et al.,* 2019). The other possible reason could be the variability in soil phosphorus in the two study sites (Table 8) which varied significantly. Phosphorus is a major element in plant growth. According to The University of Nebraska-Lincoln (2015), healthy levels of P in soil ranges from 25 to 50 ppm (25 to 50mg/kg). When phosphorus concentration in plants is sufficient, it allows plants to operate at optimum rates and the growth and development of plants proceed at a normal pace. The deficiency of P in plants, is manifested in terms of stunted growth, reduced yield, and delayed maturity (Ahemad *et al*., 2009). Coupled with the dense forest canopy cover in Manyenjere, *Prunus africana* might not compete favourably with other species hence the disparities. The other possibility for the variation is the deposition of organic matter by the river which makes the river bank very fertile and promote quick growth and development of *Prunus africana* seedlings to saplings and trees. A study of soils in Michigan demonstrated potential crop-yield increases of about 12% for every 1% increase in organic matter (Magdoff. and van Es, 2021).

5.1.2 Prunus africana habitat preference in the study sites

Prunus africana was mostly distributed along Uyaghaya River (Riparian vegetation) in Juniper

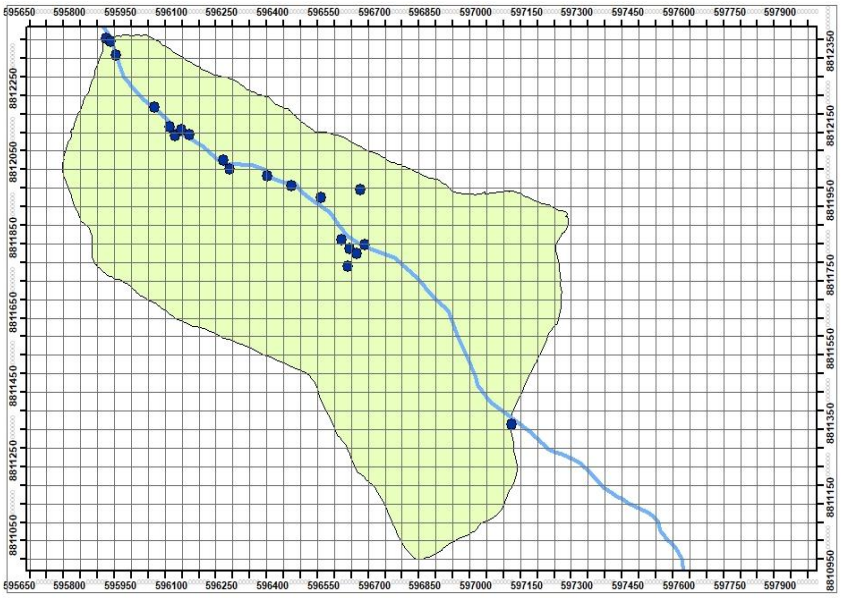


Figure 10:Distribution pattern of Prunus africana along Uyaghaya River in Juniper Forest

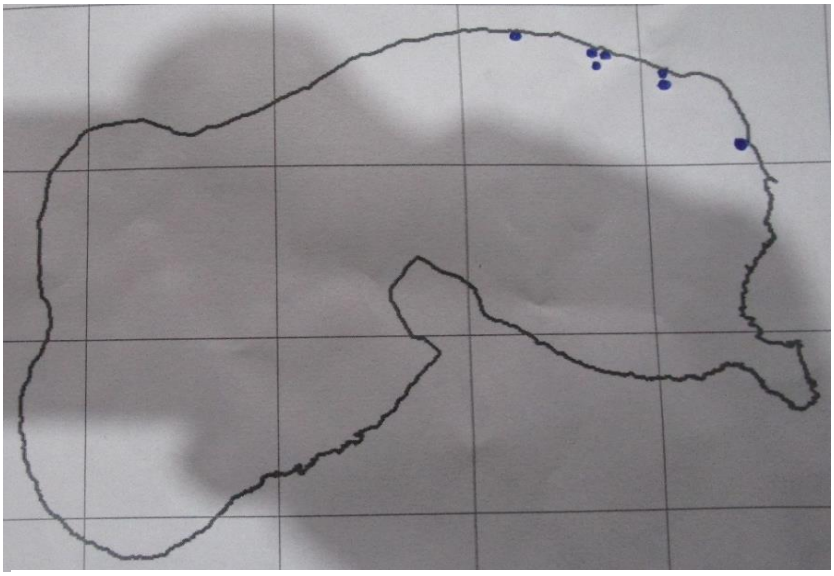


Figure 11: Distribution pattern of Prunus africana in Manyenjere Forest

Forest (Figure 10) whilst in Manyenjere the species grows along the forest edge (Non-riparian) (Figure 11). The results in Manyenjere agree with (Nguta, 2012; Nyamai *et al*. 2015; Onyango *et al.,* 2018) who also reported that *Prunus africana* in most occurrence sites prefer growing along the forest edges because it's not shade-tolerant species. Manyenjere has a thick forest

and dense canopy cover that forces *Prunus africana* to grow along the forest edge in search of light energy while in Juniper, the forest is relatively open which allows it to grow well along the forest edge and inside the the forest. The open canopy in Juniper was influenced by a number of factors which include herbivory. Elephants were seen pushing down a number of big trees in the forest. The African elephant is a keystone species that can radically change an ecosystem through its destructive feeding behaviour (Van de Koppel and Prins, 1998). No evidence of herbivory was noted in Manyenjere Forest (Zambia Nyika) and the forest was thick. However, the density along the forest edge might have been also affected by uncontrolled fires which are seen destroying part of the forest. In Juniper, the growing pattern can also be well explained by the influence of flush floods. Water is one of the major agents of seed disposal (Howe & Smallwood, 1982). The river might have dispersed the seeds on its banks which might have led to the linear distribution pattern of the species along the Uyaghaya River.

5.1.3 Age class distribution of Prunus africana in the Juniper and Manyenjere Forests.

The results of the study revealed that both sites are dominated by mature trees (Figure 5). In Manyenjere Forest, no seedling was observed and the Juniper Forest had few seedlings (10). There is an indication that no recruitment is taking place in Manyenjere Forest. *Prunus africana* requires light to develop from seedlings to saplings (Navarro-Cerrillo *et al*., 2008; Ingabire *et al.*,2019). With an average canopy cover of 94% for Manyenjere Forest (Table 4) recruitment of the *P. africana* might be difficult. Juniper Forest recorded some seedlings and saplings with more mature trees. This is possibly due to the low canopy cover. In both scenarios, a typical J-shaped curve pattern was exhibited indicating an unstable population (Hall *et al*., 2000; Nguta, 2012;). Stable populations exhibit an inverse J-shaped curve i.e. more seedlings, fewer saplings, and relatively low mature trees (Hall *et al*., 2000; Ingabire *et*

al.,2019). The other reason that might have affected the population structure in the study area could be fire prevalence. Fires may impede the growth of seedlings and saplings into trees (Hoffmann 1996, Hoffmann and Moreira, 2002), and eventually may shape the population structure and spatial distribution of woody species (Silva *et al*., 2009). In this study, evidence of destructive fire was observed in Juniper Forest (Figure 12).

Figure 12: Part of the Juniper Forest destroyed by wildfires

Fires are known to remove mainly the seedlings and saplings and increase the median height of the population (Hoffmann and Solbrig, 2003). High-severity fires change biomass and size structure of the plant community (Higgins *et al.,* 2007). The low-severity fires boost plant species abundance and richness (Pourreza *et al.,* 2014). This could be the reason for the presence of more big trees than seedlings and saplings. In a normal functioning forest system, it is expected that the seedlings will be highest in proportion followed by saplings then finally mature trees (Nowak and Crane, 2002). Besides over-exploitation, *Prunus africana* faces, diseases and pests attack among others (Hall *et al*., 2000; Fashing, 2004; Nkeng *et al*., 2010; Nguta, 2012; Ingabire *et al*., 2019). Evidence of pests was observed especially in fresh fruits. Manyenjere fruits had more pests than those of the Juniper. This is the area that needs more

investigation. Hall *et al*., (2000) reported that disease and pests are other variables that affect the crown health of *Prunus africana*, particularly coleopterous borers which often cause wood degradation. Pests found in these fruits might also contribute to the poor regeneration in the study area especially Manyenjere Forest which recorded no seedlings at all (Figure 13). Seed-feeders affect seedling recruitment and vegetation dynamics in different ways. Seed predators consume seeds and thereby reduce plant reproductive efficiency (Schowalter, 2011).

Tree mean heights exhibited in this study were far below those reported by (Hall *et al*, 2000; Nguta, 2012) of 30m to 40m. This could be attributed to altitude because intensity of light competition declines with altitude (Coomes and Allen, 2007). The study done by Hall *et al*., (2000) trees found at lower altitude were significantly taller than those found at higher altitude of more than 2100m above sea level. Juniper Forest and Manyenjere are found at 2000m above sea level (GoM, 2003).

Figure 13: Pest found inside *Prunus africana* seed in Manyenjere Forest

5.1.4 Prunus africana species diversity, richness and association in the study sites

The study revealed similarities in species associations in the study sites. This could be because both sites are at almost the same altitude (GoM, 2003; Burrow & Willis, 2005). Soil chemistry could also be a possible contributing factor. All soil major elements were the same in the study except phosphorus (P) and this could be a reason behind the similarity in species richness and

diversity. Healthy levels of P in soil range from 25 to 50 ppm (The University of Nebraska-Lincoln, 2015) and the Juniper (9 ppm) and Manyenjere (3ppm) was still in the lower side and did not have any impact on the plant diversity and richness in the study area.

Prunus africana was seen to be in very close association with several species (Figure 7). This study concurs with (Hall *et al*., 2000; Nguta, 2012;) who also reported some species like *Ilex mitis, Juniperus procera, and Podocarpus milanjianus* mostly associating with *P. africana* in most occurrence sites in Kenya and Cameroon. Chapman and White (1970) described *Ilex mitis, Juniperus procera, and Podocarpus milanjianus* as Afromontane species found in the evergreen forest patches on Nyika Plateau.

5.2 Soil texture in the Juniper and Manyenjere Forests

The results (Table 6) showed that *Prunus africana* can thrive best in loamy sandy soils as well as in sandy loamy particle sizes. It is considered to be ideal because of its ability to release nutrients freely to plants, retain water to feed plants and allow excess water to flow away quickly and easily (Gatiboni, 2022). These results conform to the findings by (Hall *et al*., 2000; Nguta, 2012) who also reported similar results in Cameroon and Kenya respectively. Therefore, the similarity observed in particle size distribution between the two sites could be a consequence of vegetation structure as well as vegetation types of the forests. According to Dodd *et al.* (2002), the dominance of woody plants is usually associated with course texture soils. Similarly, the ecotones between the woody and herbaceous plant functional types are associated with soil textural changes. In this study, the observed similarity in soil structure is one factor that could have influenced vegetation structure/species composition in *Prunus africana* occurrence sites. According to FAO (2011), the occurrence of a tree species on a certain soil type depends to some extent on the composition of the surrounding forest cover and on the size of the soil area. In this case, *P. africana* was observed to occur on the edge of

grasslands in Juniper and Manyenjere Forests. The species could have modified the soil type to its preference in an event to prevent other competing species. Furthermore, the differences observed in available phosphorus may also be used as a proxy in growth rates of the species within and between the sites.

5.2 Soil chemical properties in *Prunus africana* occurrences in Juniper and Manyenjere Forests.

5.2.1 Soil pH in the study sites

Soil pH is a measure of the acidity or alkalinity of the soil. A pH value is a measure of hydrogen ion concentration (Queensland Government, 2013). Soil pH affects the amount of nutrients and chemicals that are soluble in soil water, and therefore the amount of nutrients available to plants. Some nutrients are more available under acidic conditions while others are more available under alkaline conditions (Wilson, 2013). This study found that the soil pH in both study sites are acidic with a mean pH value of 5.25±0.41 for Juniper Forest and 5.15±0.37 for Manyenjere Forest (Table 8). Sparks *et al*., (2003) highlighted three conditions plants acidify soils. Plant root respiration with soil organic matter decomposition by microorganisms can release CO_2, increasing the carbonic acid (H_2CO_3) concentration. The other way is plants take up nutrients in the form of ions such as NO_3^-, NH_4^+, Ca_2^+, and $H_2PO_4^-$, but often take up more cations than anions. This makes plant roots release H^+ ions to compensate for the extra positive charge to maintain a neutral charge in the roots. Turner and Lambert (1988) reported that the effect of different tree species on soil pH is most significant in the first 10 cm of the topsoil. The results also support the findings by Hall *et al*., (2000) who reported similar acidic soil conditions in *P.africana* habitat sites in Cameroon.

5.2.2 Soil phosphorus

Phosphorus is a macronutrient that plays several important roles in plants which include, plant reproduction, and biological energy transfer processes that are vital for life and growth (Beegle and Durst, 2002). Phosphorus is commonly a limiting nutrient in crop production. While most soils have adequate P, the amount of available P is low as mineralization of this nutrient is slow. Phosphorus moves very little in the soil and does not leach even with large amounts of precipitation (Syngenta, 2018). Healthy levels of P in soil range from 25 to 50 ppm (25 mg/kg to 50 mg/kg) (The University of Nebraska-Lincoln, 2015). However, the results of this study show that the recommended amount of P were far much lower than the recommended one. Juniper Forest (9.01 ± 4.7 mg/kg) and Manyenjere Forest (3.83 ± 3.4 mg/kg) were below the recommended levels. However, the research has found that phosphorus is always very low in soil and plants adapt to this by developing extensive root systems to reach out to where phosphorus is located (Beegle and Durst, 2002; Griffith, 2011). According to Jensen, (2010) phosphorus (P) availability in the soil is largely controlled by two primary factors: soil pH (low pH), and the amount of organic matter. If soils are too acidic, phosphorus reacts with iron and aluminium and makes it unavailable to plants. Likewise, if soils are too alkaline, phosphorus reacts with calcium and also becomes inaccessible. Variations in the phosphorus amount observed between Juniper Forest and Manyenjere Forest could be attributed to river deposits. Each year, the Uyaghaya River carries a lot of animal waste and other debris depositing them along the river banks and changing the soil chemistry in the process (Beegle and Durst, 2002). Soil erosion and crop removal are the significant ways soil phosphorus is lost (Griffith, 2011). This study agrees with the finding by Kanzunguze (2019) who reported a mean of 9 mg/kg for soil phosphorus on the Nyika Plateau.

5.2.3 Total carbon(C)

Carbon (C) is stored in soil organic matter (Lemus and Lal, 2005). This carbon enters the soil through the decomposition of plant and material residues and other dead micro-organisms (Ontl, and Schulte, 2012). Results of the comparison have shown that, while there were no significant differences ($p>0.05$) in the level of total carbon in the Juniper and Manyenjere plots, there were slight differences in terms of proportional % as Manyenjere plots recorded slightly higher concentrations of carbon (3.27±0.63) as compared to Juniper plots (3.20±0.46). This could be a result of Manyenjere plots having a highly the dense canopy. Thus, the higher litter fall could have contributed to more accumulation of organic matter. This could positively affect the availability of carbon in the soil. The Juniper plots had more canopy spaces hence; less litter fall and less availability of carbon percentage. In addition, Juniper Forests plots were along the perennial stream. According to FAO (2005) climatic conditions like temperature, moisture and rainfall also affect the rate of organic matter decomposition whereby, the decomposition is faster in areas that are warm and humid and slower in cool dry areas

5.2.4 Total nitrogen (N)

Soil total nitrogen (TN) is the major determinant and indicator of soil fertility and quality in an agricultural ecosystem and is closely related to soil productivity (Al-Kaisi *et al*., 2005). Soil type is a major source of variation in soil total nitrogen (Wang *et al*., 2009). The similarity in total nitrogen observed in this study could be attributed to an increase in organic matter found in Juniper and Manyenjere Forests which influences the availability of carbon thus; more nitrogen percentage. This agrees with (Angus *et al*. 2006) who found that litter fall affect positively the availability of total nitrogen in the soils. Li *et al*., (2022) reported that soil total carbon changes with soil profile where the first 0 to 20cm of top soil is richer in TN than 20 to 40 cm and 40 to 60 cm top soil respectively.

5.2.5 Calcium

Calcium (Ca) is a secondary plant macronutrient and is vital for healthy plants (Wade, 2019). It is required for the formation of new cells so is needed for roots, stems and leaves to grow. It is also used by plants when they respond to pest and disease attacks (Wade, 2019).

As other results show that there were no significant differences between the plots of Juniper and Manyenjere plots, this was the same case with calcium percentage available in these plots. There were also no significant differences (P>0.05) in their levels. The overall proportional percentage of soil calcium (Ca) levels in the Juniper and Manyenjere plots were slightly low, ranging from (1.2 to 12.2). These results could be due to the high percentage (above 80%) of sand level content (Table 7) as compared to clay content which was below 10%. In this case, soil texture may be a factor influencing the concentration of Ca levels in the soil. Most sandy soils have low calcium concentrations while, clay soils usually have high calcium concentrations levels ((Bonomelli *et al*., 2019). Under normal circumstances, the higher the calcium level in the soil, the greater the soil clay content (Bonomelli *et al*., 2019). The texture results found in this study support Loide (2004) who found out that a rise in sand content concentration leads to a decrease in calcium levels in the soils and vice versa.

5.2.6 Magnesium

Within each plot, there were no significant differences (P>0.05) in levels of Magnesium between the sites. Nevertheless, comparatively, Juniper soils recorded a high proportional percentage (4.02±2.29) of Magnesium level as compared to Manyenjere soils (3.29±2.38). In addition, Magnesium deficiencies are most encountered on light sand soils as was the case with this study's results where both plots recorded a high percentage of sand soils. This agrees with (Chan *et al.* 1979) who reported that magnesium deficiency in soils of heavier texture appears to be increasing, particularly in strongly weathered and leached soils.

5.3 Phytochemical analysis of *Prunus africana* in the study sites

Prunus africana stem and root bark aqueous extracts have been used to suppress lower urinary tract symptoms by decreasing inflammation, reducing bladder reactivity and prostate size in patients with Benign prostatic Hyperplasia (Andro & Riffaud, 1995; Ishani *et al*; 2000; Nyamai *et al.,* 2016). The extracts are believed to treat or prevent BPH through inhibition of 5-α-reductase, anti-inflammatory activity, inhibition of prolactin levels, and inhibition of prostatic fibroblast proliferation in response to growth factors (Capasso *et al.,* 2003; Nyamai *et al.,* 2015; Komakech *et al.,* 2017). In this study GC-MS and LC-MS were conducted to derive possible phytochemicals from Juniper and Manyenjere Forests stem bark extracts.

5.3.1 GC-MS analysis in Juniper and Manyenjere bark extracts.

GC-MS analysis assists in detecting volatile phytochemicals found in plant extracts (Harborne, 1973; Nyamai *et al.,* 2015). These naturally occurring biologically active compounds belong to different chemical groups such as phenols, flavonoids, anthocyanins, diterpenes, isoflavones etc (Saxena *et al*., 2013; Adegbaju *et al*., 2020). This study detected 163 volatile phytochemical compounds from *P. africana* stem bark extracts from the two study sites. Methyl linoleate and Squalene had the highest frequency occurrences (20) and seconded by Methyl octadecanoate and Octadecanoic acid with (19) frequency occurrences (Appendix 5). Methyl linoleate, Methyl octadecenoate, and Octadecanoic acid are fatty acids while Squalene is a triterpene (Moreda *et al.,* 2001). Fatty acids and sterols are believed to reduce prostate size by blocking the conversion of testosterone to dihydrotestosterone. The mechanism of action of these compounds is by inhibition of the 5-α-reductase enzyme thus preventing the formation of dihydrotestosterone, the modulator of prostate growth (Edeoga 2005; Bent *et al.,* 2006; Nyamai *et al.,* 2015). Most of the phytochemicals known to treat and manage BPH were detected (Tables, 9, 10, and 11). The absence of some phytochemicals in both Juniper Forest and

Manyenjere Forest could be due to environmental conditions. According to Nyamai *et al.*, (2015), environmental conditions (e.g. soil type, closeness to water source, and temperature) and genetic differences were singled out as major sources of variations in phytochemical concentrations in *P. africana*. The temperature differences may cause chilling injury in plants leading to imbalances in metabolism, accumulation of toxic compounds, and increased membrane permeability (Gachie *et al.*, 2012). In this study, *P. africana* in Juniper Forest grows along the river while in Manyenjere Forest, it is distributed along the forest edge (Figure 11 & Figure 12). The two populations are subjected to two different environmental conditions and hence the presence or absence of some phytochemical compounds. For example, Stigmastanol and Phenol, 2-4-bis(1,1-dimethylethyl)- were detected in *P. africana* ethyl acetate extract from Juniper Forest only. In contrast, n-Hexadecanoic acid was detected in *P. africana* ethyl acetate extract from Manyenjere Forest only. This trend was also observed in DCM extracts where Phenol, 2-4-bis (1,1-dimethylethyl)- was present in the Juniper Forest only. The major compound in *P. africana* hexane extract from Juniper Forest was Oleic acid while 6-Octadecanoic acid was the major compound for the Manyenjere Forest. Chemical variations observed in *P. africana* in this study, were also reported by other researchers. Nyamai *et al.* (2015) reported the presence of Cyanidin-3-o-rutinoside in *P. africana* methanolic extract from Karuri but was absent in methanol extract from Muguga and Kabujoi. Chemical variations have also been reported in other plants. For example, *Lipia javanica* essential oil from Nchenachena extension planning area (EPA) in Rumphi district, Malawi, contained perillaldehyde as the major compound while ipsdieonone was reported to be the major volatile compound in *L. javanica* essential oil from Champhira EPA in Mzimba district, Malawi (Kamanula *et al*., 2017). These variations were attributed to factors such as differences in morphological varieties, geographical location, type of soil, genetic make-up, and time of leaf harvesting, among others. The amount of perilladehyde in *L. javanica* leaves harvested in

August was higher (63 %) than in April (1 %) from the same location and same year (2011). Other factors that cause chemical variations in plants include plant parts, extracting solvent and growth phases (Lagnika *et al*., 2016; Adegbaju *et al*., 2020). During this study, most trees in Juniper Forest had fruits and none in Manyenjere Forest. This phenomenon could be another source of variations in volatile components reported in *P. africana* extracts from the two study sites. For example, some studies have found that some plants produce high concentration of flavonoids at the flowering stage which assist the plant in fruit colouration, ultra-violet protection, pigmentation, and aroma in flower production (Saxena *et al*., 2013). The high production of flavonoids during the flowering period of plants is also believed to be a defense mechanism against pests that may attack the flowers (Petrussa *et al.*, 2013) and a means of attracting potential pollinators (Adegbaju *et al*., 2020). Another possible source of variation in this study could be post-harvest (collection) processing (Adegbaju *et al.*, 2020). Two Juniper plots (J003 &J008) did not respond well to hexane solvent and this could be another source of variations though it might be difficult to ascertain specific phytochemicals affected and this could be investigated further.

Some notable phytochemicals such as Stigmastano and γ-Sitosterol detected in this study have been discussed widely in the treatment and management of cancer (Holtz *et al*., 1998; Kadu *et al.*, 2012; Nyamai *et al.*, 2015). A study undertaken by von Holtz *et al*., (1998) found that there was a decrease in cell growth (24%) and induction of apoptosis (fourfold) followed by cell rounding in cancer treatment by sitosterol. The compound has also been reported in soybean extracts (Nyamai *et al.*, 2015; Patel *et al.*, 2017). The high potential of this compound and its analogue in the treatment of various illnesses, classifies this compound as the noteworthy drug of the future, although its role in the treatment of BPH is now approved via clinical trial confirmations (Saeidnia1 *et al*., 2014). According to Nyamai *et al.*, *(*2015), the

pharmacological efficacy of the wild tree bark extracts is thought to be due to the synergistic effect of various compounds some of which are known and others unknown. All phytochemicals detected and presented in (Tables 9, 10, and 11) play a key role in the management and treatment of BPH).

5.3.2 LC-MS analysis of Juniper and ManyenjereForests stem bark extracts.

LC-MS helps to detect non-volatile compounds from plant extracts (Harborne, 1973). In this study LC-MS detected a total of 27 (Appendix 28 and 29) phytochemicals. Oleic acid which was detected by GC-MS (Tables 9, 10, and 11), was also detected by LC-MS. This is because it is mid-polar to polar (Nyamai *et al.*, 2015). The phytochemical profile exhibited in this study is similar to the one reported by Nyamai *et al.*, (2015), where Chlorogenic acid, Ursolic acid and Cyananidin-O-galactoside were present in *P. africana* from Kenya. However, some compounds such as Ellagic acid, Prunasin, p-coumaric, Rutin, and Zeatin reported in this study were different from those reported by Nyamai *et al*. (2015) and other researchers. The difference in chemical variation could be due to geographical location and genetic make-up (Cunningham *et al*., 1997).

Kadu *et al*. (2012) and Komakech *et al*. (2017) reported that Ursolic acid, β-Sitesterol, β-Sitostenone and Apigenin 6-c—glucoside present *P. africana* were the major compounds responsible for the treatment and management of in BPH. However, Nyamai *et al.* (2015) argued that although some compounds may be present in small quantities, they play a major role in the synergistic effect of the plant extract. Previous studies showed that Ursolic acid inhibits the growth of melanoma cells and prostate cancer cells (Nataraju *et al.,* 2007; Nyamai *et al.,* 2015; Komakech *et al.,* 2017), hence an important phytochemical in the treatment of BPH. In another study, *β*-sitosterol and β-sitostenone were reported to exhibit anti-inflammatory effects as they suppress the production of prostaglandins and thus prevent

swelling of the prostate (Shenouda *et al*., 2007; Nyamai *et al.,* 2015; Komakech *et al.,* 2017), meaning that *P. africana* growing in Juniper and Manyenjere Forests has potential for the treatment and management of BPH. Apigenin 6-c—glucoside, which is reported in this study, can inhibit cell proliferation by preventing DNA replication, stimulating DNA damage, and inducing cell cycle arrest (Liu *et al*., 2019).

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The study has shown that the population structure of *P. africana* in both Juniper and Manyenjere Forests is dominated by mature trees which is an indicator of an unstable population. Therefore, the hypothesis that the population structure of *P. africana* is not similar in both study sites is rejected. The results have also shown that the two study sites are mostly comprised of loamy sand textures which are moderately acidic and favours the growth of *Prunus africana*. Hence, the null hypothesis is rejected.

GC-MS and LC-MS analysis showed the presence of Stigmastanol, Oleic acid, Ursolic acid, β-Sitosterol, β-Sitostenone and Apigenin 6-c-glucoside in *P. africana* from Juniper and Manyenjere Forests. These phytochemicals are important in the treatment of BPH and therefore, important to promote domestication and cultivation of the species for medicinal purposes and commercialization of BPH herbal medicines. Based on this study, the null hypothesis that there are no chemical variations in *P. africana* stem bark extracts from the two study sites is accepted and rejected to some extent. This is because some phytochemicals that were present in *P. africana* stem bark from Juniper Forest were also present in *P. africana* stem bark from Manyenjere Forest. However, some compounds were present in *P. africana* stem bark from Juniper or Manyenjere Forest only. Furthermore, the amount of some compounds in *P. africana* stem bark did not differ significantly ($p > 0.05$) between the two study sites.

6.2 Recommendations

- It was, therefore, recommended that pruning in thick forest be promoted to allow light into the forest floor to trigger regeneration (Assisted Natural Regeration). The policy does not allow silvicultural systems in national parks but this study recommends a revision of this policy to promote natural regeneration of *Prunus africana* which is showing difficult recruitment in the study sites.
- There is a need to intensify *ex-situ conservation* initiatives of *P. africana* in both study sites to save *P. africana* from getting locally extinct because the study has found that in both study sites there are more mature trees than seedlings and saplings, which is a sign of unstable population. Further research should be carried out to identify and assess the impacts of the pests on the regeneration of *Prunus africana* in the study area.
- There is a need to establish the relationship between tree sizes in relation to phytochemical profile and quantities in the study area.
- There is also need to study *Prunus africana* soil seed bank in the study area.

6.3. Limitations of the study

- ✓ Phytochemical samples took along time to go to Kenya due to Covid 19 restrictions. This could affect results of laboratory analysis to some degree.
- ✓ Sampling sites were not very far away from each other. Despite, one found in Malawi and the other one in Zambia, the distance between the two sites is 40 km apart. Genetically, the species might not be very different from each other.
- ✓ Some phytochemical samples from Juniper (J003 and J008) did not respond well to Hexane extract which might have implications on phytochemicals found in Juniper Forest.

REFERENCES

Adegbaju, O.D., Otunola, G.A., and Afolayan, A.J. (2020). Effects of growth stage and seasons on the phytochemical content and antioxidant activities of crude extracts of *Celosia argentea* L. *Heliyon* **6** (6): e04086. doi: 10.1016/j.heliyon. 2020.e04086. PMID: 32514483; PMCID: PMC7267717.

Ahemad, M., Khan, M.S., Zaidi, A., and Wani, P.A. (2009). Remediation of herbicides contaminated soil using microbes in M.S. Khan, A. Zaidi, J. Musarrat (Eds.), Microbes in Sustainable Agriculture, Nova Science Publishers, New York, USA.

Al-Kaisi, M. M., Yin, X., and Licht, M. A. T. (2005). Soil Carbon and Nitrogen Changes as Influenced by Tillage and Cropping Systems in Some Iowa Soils. *Agric. Ecosyst. Environ*. **105**, 635–647. doi:10.1016/j.agee.2004.08.002

Angus, J.F. *et al.* (2006). N mineralization in relation to previous crops and pastures. *Soil Research*. **44**: 355-365.

Asrat, Z. and Tesfaye, Y. (2013). Training manual on: Forest Inventory and Management in the Context of SFM and Redd+. Hawassa university. Wondo Genet College of Forestry and Natural Resources. Ethiopia. https://www.forestcarbonpartnership.org/.

Awono, A., Tchindjang, M., Levang, P. (2016). Etat des lieux de la filiere ecorces de *Prunus africana* : Cas des regions du Nord-Quest et Sud-Quest du Cameroun. *Revue Scientifique et Technique Foret et Environment du Bassin du Congo* ***6****: 46-59*

Azadzoi KM, Babayan RK, Kozlowski R, Siroky, M.B. (2003). Chronic ischemia increases prostatic smooth muscle contraction in the rabbit. *J Urol* **170**: 659- 663.

Barlet, A., Albrecht., J., Aubert, A., Fischer, M., Grof F., *et al.* (1990). Efficacy of *Pygeum africanum* extract in the treatment of micturitional disorders due to benign prostatic hyperplasia: evaluation of objective and subjective parameters A placebo-controlled double-blind multicenter study. *Wien Klin Wochenschr* **102**: 667-673.

Barrows, J.E. and Willis, C.K. (eds) (2005). *Plants of the Nyika Plateau: an account of the vegetation of the Nyika National Parks of Malawi and Zambia.* Southern African Botanical Diversity Network Report No. 31. SABONET, Pretoria.

Bauer,, H.W., Bach., D. (1986) Prostaglandin E2 in prostatitis and prostatic adenoma. *Urol Int* **41**: 139-144.

Bent, S., Kane, C., Shinohara, K., Neuhaus, J., Hudes, E.S., et al. (2006). Saw palmetto for benign prostatic hyperplasia. N Engl J Med 354: 557-566.

Berry, S.J., Coffey, D.S, Walsh P.C, and Ewing, L.L. (1984). The development of human benign prostatic hyperplasia with age. *J Urol* **132**: 474-479.

Betti, J.L. (2008). Non-detriment finding report on *Prunus africana* (Rosaceae) in Cameroon.

Betti J.L. and Ambara J. (2013). Mass of *Prunus africana* stem barks on Tchabal mbabo and Tchabal Gang Daba Mountain Forests, Cameroon. *Afr. J of Env. Sci. Technol.* (**7**):204-221

Bombardelli, E. and Morazzoni, P. (1997). *Prunus africana* (Hook.f.) Kalkman. *Fitoterapia* **68**: 205-218.

Bonomelli, C., Gil, P.M., and Schaffer, B. (2019). Effect of soil type on calcium absorption and partitioning in young avocado (*Persea americana* Mill.) Trees. *Agronomy* **9**(12).

Catalano S, Ferretti M, Marsili A, and Morelli, I. (1984). New constituents of *Prunus africana* bark extract. *J Nat Prod* **47**: 910.

Chan, K. Y., Davey, B. G. and Geering, H. R. (1979). Adsorption of Mg and Ca by a soil with variable charge. *Soil Sci. Soc. Am. J.*, **43**, 301-304

Chapman, J.D. and White, F. (1970). *The ever-green forests of Malawi*. Commonwealth Forestry institute, Oxford.

Chatelain, C., Autet., W., and Brackman, F. (1999). Comparison of once and twice daily dosage forms of *Pygeum africanum* extract in patients with benign prostatic hyperplasia: a randomized, double-blind study, with long-term open label extension. *Urology* **54** : 473-478.

Clavert A, Cranz C, Riffaud JP, Marquer C, Lacolle JY, *et al.*, (1986). Effects of an extract of the bark of *Pygeum africanum* (V.1326) on prostatic secretions in the rat and in man. *Ann Urol (Paris)* **20**: 341-343.

Coomes, D. A., and Allen, R. B. (2007). Effects of Size, Competition and Altitude on Tree Growth. *Journal of Ecology*, 95(5), 1084–1097. http://www.jstor.org/stable/4496061.

Cunningham, A B. (2005). CITES Significant Trade Review o*f Prunus africana*. CITES Management Authority, Geneva, Switzerland.

Cunningham, M., Cunningham, A.B., and Schippmann, U. (1997). Trade in *Prunus africana* and the implementation of CITES. German Federal Agency for Nature Conservation, Bonn. Germany.

Cunningham, A.B. and Mbenkum, F.T. (1993) Sustainability of Harvesting *Prunus africana* Bark in Cameroon. A Medicinal Plant in International Trade. People and Plants working paper 2. UNESCO, Paris.

Daw, *et al.*, (2006). Cortical substrates for exploratory decision in humans. *Nature* **441**. 876-9. 10.1038/nature04766.

Dowsett-Lemaire, F. (1985). The forest vegetation of the Nyika Plateau (Malawi-Zambia): ecological and phenological studies. *Bull. Jard. Bot. Nat. Belg. / Bull. Nat. Plantentuin Belg.* **55** (4): 301-392.

Dodd, M., Lauenroth, W., Burke, I., and Chapman, P. (2002). Associations between Vegetation Patterns and Soil Texture in the Shortgrass Steppe. *Plant Ecology*, **158**(2), 127-137. Retrieved February 25, 2021, from http://www.jstor.org/stable/20051192

Edeoga, H.O., Okwu, D.E., Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* **4**: 685-688

FAO. (2005). The importance of soil organic matter - key to drought-resistant soil and sustained food production. *FAO Technical Paper. Rome, Italy.*

FAO. (2011). Assessing forest degradation Towards the development of globally applicable guidelines. Forest Resources Assessment Working Paper 177. Rome. Italy.

Farnsworth, N.R., Akerele, O. and Bingel, A.S. (1985). Medicinal plants in therapy.

*Bulletin of World Health Organizatio*n ***63***: 965-981.

Farwig, R., Kozono, H., and Sohr, H. (2007). Local in time regularity properties of the Navier-Stokes equations. *Indiana Univ. Math. J.* **56:** 2111–2132.

Fashing, P. (2004). Mortality trends in the African cherry (*Prunus africana*) and the implications for Colubus Monkeys (*Colubus guereza*) in Kakamega Forest, Kenya. *Journal of Biol. Conserv.* ***120:*** 449-459.

Fourneau, C., Hocquemillar, R., and Cave, A. (1996) Triterpenes from *Prunus africana* bark. *Phytochemistry* **42:** 1387-1389.

Gacci M, Eardley I, Giuliano F, Hatzichristou D, Kaplan SA, *et al*. (2011). Critical analysis of the relationship between sexual dysfunctions and lower urinary tract symptoms due to benign prostatic hyperplasia. *Eur Urol* **60:** 809-825.

Gachie, P.K., Koech, E.K., Njunge, J.T, Simons, A.J, and Ndalut, P.K. (2012). Variation in yield and composition of crude bark extracts of *P. africana* in different provenances of Kenya. *Forests, Trees and Livelihoods* **21**: 56-62.

Gatiboni, L. (2022). Soils and Plant Nutrients, Chapter 1. In: K.A. Moore, and. L.K. Bradley (eds). *North Carolina Extension Gardener Handbook*, 2nd ed. NC State Extension, Raleigh, NC. https://content.ces.ncsu.edu/extension-gardener-handbook/1-soils-and-plant-nutrients.

Geldenhuys, C. J., and Venter, S. (2002). Plant communities and biodiversity of the Limpopo Province forests: relevance and management options. *Multiple use management of natural forests and Savanna Woodlands*: *Policy refinements and scientific progress*, 23-37.

Gormley G.J., Stoner, E., and Bruskewitz, R.C., *et al.* (1992) The effect of finasteride in men with benign prostatic hyperplasia. The Finasteride Study Group. *N Engl J Med* **327:** 1185-1191.

Hall, J., E. O‟Brien and Sinclair. F. (2000). *Prunus africana*, A monograph. University of Wales Bangor, Mount Cameroon Project, IRCRAF. Sciences, S. o. A. F. Publication No 18, University of Wales Bangor, School of Agricultural and Forest Sciences 104 p.

Higgins, S. I., Bond, W. J., and February, E. C. *et al*., (2007). Effects of four decades of fire manipulation on woody vegetation structure in savanna. *Ecology* **88**, 1119–1125. doi: 10.1890/06-1664

Hoffmann, W.A., (1996). The effects of cover and fire on seedling establishment in a neotropical savanna. *Journal of Ecology,* **84(3)**: 383-393.

Hoffmann, W.A. and Moreira, A.G. (2002). The role of fire in population dynamics of woody plants. *The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna* (eds P.S. Oliveira & R.J. Marquis), pp. 159–177. Columbia University Press, New York.

Holm M, and Meyhoff H.H. (1997) Chronic prostatic pain. A new treatment option with finasteride?. *Scand J Urol Nephrol* **31**: 213-215.

Howe, H.F., and Smallwood, J. (1982). Ecology of Seed Dispersal. *Ann Rev. Ecol. Syst*.**13**: 201-228

Ingabire, V., Sylvie, I., and Grace, M. A. (2019). Assessment of abundance, distribution and threats on *Prunus africana* in Rwanda, Case study: Nyungwe and Gishwati-Mukura National Parks.

Ingram, V. (2007). Problem analysis, assessment of impacts and status of the *Prunus africana* chain. Bamenda, November 22-23, 2007. SNV & CIFOR

Irani, J., Brown, C.T, van der Meulen J. and Emberton, M. (2003). A review of guidelines on benign prostatic hyperplasia and lower urinary tract symptoms: are all guidelines the same? *British Journal of Urology International*, **92:** 937–942.

Irmak, S., Jonnala, R.S., and MacRitchie, F. (2008). Effect of genetic variation on phenolic acid and policosanol contents of Pegaso wheat lines. *Journal Cereal Science*, **48**: 20–26.

Ishani, A., MacDonald, R., Nelson, D., Rutkis, I and Wilt, T.J. (2000). *Pygeum africanum* for the treatment of patients with benign prostatic hyperplasia: A systematic review and quantitative meta-analysis. *American Journal of Medicine* **109:** 564–664.

Jaenicke, H., Munjuga, M., Were, J., Tchoundjeu, Z. and Dawson, I. (2000). *Prunus africana* - propagation techniques for the conservation of an endangered medicinal tree in Africa. In: Darwin plant conservation manual for the tropics (edited by C. Hankamer). Royal Botanic Gardens, Kew.

Jacob, J., Tiwari, K., and Correa-Betanzo, J. *et al.,* (2012). Biochemical basis for functional ingredient design from fruits. *Annual Review of Food Science & Technology*, **3:** 79-104.

Jansen, B., Tonneijck, F., and Hooghiemstra, *et al.*, (2010). How an advanced combination of soil science, biogeochemistry, and paleo-ecology helps Ecuadorian cloud forest management. 19th World Congress of Soil Science, Soil Solutions for a Changing World 1 – 6 August 2010, Brisbane, Australia.

Jenkin, R.N., Howard, W.J., Thomas, P., Abell, P.M.B. & Deane, G.C. (1977). Forestry development prospects in the Imatong Central Forest Reserve, southern Sudan. Volume 2. Main report. *Land Resource Study,* 28: 1-217.

Jimu, L. (2011). Threats and Conservation strategies for the African cherry (*Prunus africana)* in its natural range- A review. *Journal of Ecology and Natural Environment*.3: 118-130.

Kadu, C. A., Parich, A. and Schueler, S., *et al.,* (2012). Bioactive constituents in *Prunus africana:* Geographical variation throughout Africa and associations with environmental and genetic parameters. *Phytochemistry*, **83**: 70-78.

Kadu, C.A.C., Schueler, S., and Konrad, *et al.*, (2011). Phylogeography of the Afromontane Prunus africana reveals a former migration corridor between East and West African highlands. *Molecular Ecology* **20**: 165-178.

Kalkman, C. (1988). The phylogeny of the Rosaceae. Botanical Journal of the Linnean Society, 98: 37–59.

Kamanula, J.F., Belmain, S.R., Hall, D.R., Farman, D.I., Goyder, D.J., Mvumi, B.M., Masumbu, F.F., Stevenson, P.C. (2017). Chemical variation and insecticidal activity of *Lippia javanica* (Burm. f.) Spreng essential oil against *Sitophilus zeamais* Motschulsky. *Industrial Crops and Products* **110:** 75-82.

Kampa, M., Alexaki, I., and Notas, G., *et al.*, (2004). Anti-proliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells: Potential mechanisms of action. *Breast Cancer Research*, **6:** 63–74.

Kanzunguze, A. (2019). Ecology of Bracken Fern invasion in Nyika National Park: Assessment of its spatial temporal distribution and potential threat to plant species diversity. https://nyika-vwaza-trust.org/research/.

Kaplan, S., MacConell, J. & Roehrborn, C. (2006). Combination therapy with doxazosin and finasteride for benign prostate hyperplasia in patients with lower urinary tract symptoms and a baseline total prostate volume of 25 ml or greater. *Journal of Urology*, **175(1):** 217-220.

Kapoor, A. (2012). Benign prostatic hyperplasia (BPH) management in the primary care setting. *Can J* Urol **19:** 10-17.

Kazuki, K., Masaki, Y. and Yukio, H. (2006). Silodosin, a new alpha-1a adrenoreceptor selective antagonist for treating benign prostatic hyperplasia: results of a phase 3 randomized, placebo-controlled double-blind study in Japanese men. *BJU international*, **98**(5): 1019-1024.

Kideghesho, J. and Msuya, T. (2010). Gender and socio-economic factors influencing domestication of indigenous medicinal plants in the West Usambara Mountains, northern Tanzania. *International Journal of Biodiversity Science, Ecosystems Services & Management* **6**. 3-12. 10.1080/17451590.2010.480946.

Komakech, R., Kang, Y., Lee, J., and Omujal, F. (2017). "A Review of the Potential of Phytochemicals from *Prunus africana* (Hook f.) Kalkman Stem Bark for Chemoprevention and Chemotherapy of Prostate Cancer," *Evidence-Based Complementary and Alternative Medicine*, vol. 2017, Article ID 3014019,10pages, 2017. https://doi.org/10.1155/2017/3014019

Koros, *et al.*, (2016). Indigenous Knowledge, Uses and Conservation of *Prunus africana* (Hook. F.) Kalkman in Nandi Forests. *Wuhan University Journal of Natural Sciences* **6.** 58-62.

Lagnika L., Amoussa A.M.O., Adjileye, R.A., Laleye A., Sanni A. (2016). Antimicrobial, antioxidant, toxicity and phytochemical assessment of extracts from A cmella uliginosa, a leafy-vegetable consumed in Bénin, West Africa. *BMC Compl. Altern. Med* **16**(1):34.

Latalski, M., Spruch, T. & Obuchowska, D. (1979). The ultrastructure of the epithelium of bulbourethral glands after administration of the tadenan preparation. Folia Morphology. (Warsz.), 38: 193-201.

Lemus, R. and Lal, R. (2005). Bioenergy Crops and carbon sequestration. *Critical Reviews in Plant Sciences* **24**, 1-21

Li, M., Han, X., Li, L.J. (2022). Total Nitrogen Stock in Soil Profile Affected by Land Use and Soil Type in Three Counties of Mollisols. *Front. Environ. Sci.* **10 :** doi=10.3389/fenvs.2022.945305.

Liu, J., Qu, L., and Meng, L. *et al.*, (2019). Topoisomerase inhibitors promote cancer cell motility via ROS-mediated activation of JAK2-STAT1-CXCL1 pathway. *J Exp Clin Cancer Res* **38,** 370. https://doi.org/10.1186/s13046-019-1353-2.

Loide, V. (2004). About the effect of the contents and ratios of soil's available calcium, potassium and magnesium in liming of acid soils. *Agronomy Research* **2**(1): 71–82

Longo, R. and Tira, S. (1981). Constituents of *Pygeum africanum* bark. *Planta Medica Journal***, 42**: 195-203.

Madersbacher, S. and Alivizatos G. *et al.*, (2004) EAU 2004 guidelines on assessment, therapy and follow-up of men with lower urinary tract symptoms suggestive of benign prostatic obstruction (BPH guidelines). *Eur Urol* **46:** 547-554.

Malawi Government, (2003). Management Plan for Nyika National Park. Unpublished report. DNPW. Lilongwe.

Mangoale, R. M., and Afolayan, A. J. (2020). Comparative Phytochemical Constituents and Antioxidant Activity of Wild and Cultivated *Alepidea amatymbica* Eckl & Zeyh. *BioMed research international*. https://doi.org/10.1155/2020/5808624

Marcoli M, D'angelo L, Del Vecchio A, Caravaggi M, Lecchini S, *et al*., (1986). Anti-inflammatory action of Pygeum africanum extract in the rat. *Farmaci e Terapia* **3:** 135-137.

Matés, J.M., and Sánchez-Jiménez, F.M. (2000). Role of reactive oxygen species in apoptosis: implications for cancer therapy. *Int J Biochem Cell Biol* **32:** 157-170.

McClure, M.W. (2002). An overview of holistic medicine and complementary and alternative medicine for the prevention and treatment of BPH, prostatitis, and prostate cancer. *World J Urol* **20**: 273-284.

Moreda, W., Pérez-Camino, M.C., Cert, A. (2001). Gas and liquid chromatography of hydrocarbons in edible vegetable oils. *Journal of Chromatography A* **936**(1-2): 159-171.

Munjuga, M., Were J., Dawson I., Ruigu S., and Simons, A. (2000). Reproductive biology of the over-exploited, medicinal tree *Prunus africana*: Studies in Central Kenya. *East African Journal of Forestry and Agriculture 28: 31.*

Navarro-Cerrillo, R.M., Clemente, M., Padron, E. Hernandez-Bermejo, E. and Garcia-Ferrer, A., *et al.*, (2008). Forest structure in harvested sites of Afromontane forest of *Prunus africana. African Journal of Ecology **46**:* 620-630.

Ndibi, B.P., Kay, E.J. (1997). The regulatory framework for the exploitation of medicinal plants in Cameroon: the case of *Prunus africana* on Mount Cameroon. *Biodiversity and Conservation* **6**: 1409-1412.

Nguta, E.M. (2012). Distribution and Population structure of *Prunus Africana* in Mount Kenya Forest. *MSc Thesis*. University of Nairobi. Nairobi.

Nkeng, P.F., Ingram, V. and Awono, A. (2010) Assessment of *Prunus africana* bark exploitation methods and sustainable exploitation in the South west, North-West and Adamaoua regions of Cameroon. CIFOR.

Nowak, D.J. and Crane, D.E. (2002). Carbon Storage and Sequestration by Urban Trees in the USA. *Environmental Pollution* **116 (3)**: 381-389.

Nyamai DW, Mawia AM, Wambua FK, Abdirahman YA, and Osano K, *et al.*, (2015). Growth Performance of Domesticated *Prunus africana* Population in Muguga, Kiambu County, Kenya. *Biochem Physiol **4**: 185*. doi: 10.4172/2168- 9652.1000185.

Nyamai D.W, Mawia AM, Wambua F.K, Njoroge A, Matheri F, *et al*., (2015). Phytochemical Profile of *Prunus africana* Stem Bark from Kenya. *J Pharmacogn Nat Prod **1**: 110.* doi:10.4172/jpnp.1000110

Nyamai, D.W., Arika W.M, Rachuonyo, H.O, Wambani J.R., and Ngugi M.P. (2016). Herbal Management of Benign Prostatic Hyperplasia. *J Cancer Sci Ther* **8(5):** 130-134. doi:10.4172/1948-5956.100040.

Ontl, T. A. and Schulte, L. A. (2012). Soil Carbon Storage. *Nature Education Knowledge* **3**(10):35

Onyango, A. E., Hitimana, J., Sirmah, P. K., Matonyei, T. (2018). Population Structure of Prunus africana (Hook. f.) Kalkm. and Olea europaea L. in South Nandi Afromontane Forest, Kenya. *Asian Journal of Research in Agriculture and Forestry* **1**(4): 1-13

Orwa, C., Mutual, A., Kindt, R., Jamnadass, R., and Anthony, S. (2009). Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya.

Petrussa E., Braidot E., Zancani M., Peresson C., Bertolini A., Patui S., Vianello A. (2013). Plant flavonoids—biosynthesis, transport and involvement in stress responses. *Int. J. Mol. Sci.***14** (7):14950–14973.

Pourreza, M., Hosseini, S. M., Sinegani, A. A. S., Matinizadeh, M., and Alavai, S. J. (2014). Herbaceous species diversity in relation to fire severity in Zagros oak forests, *Iran. J. Forestry Res.* **25**, 113–120. doi: 10.1007/s11676-014-0436-3.

Provin, T. and Pitt, J.L. (2002). Testing Your Soil: How to Collect and Send Samples. Texas FARMER Collection.

Quinn, G. and Keough, M. (2002). *Experimental Design and Data Analysis for Biologists.* Cambridge University Press. United Kingdom.

Rassweiler, J., Teber D, Kunt, R., and Hofmann, R. (2006). Complications of transurethral resection of the prostate (TURP): Incidence, management, and prevention. *Eur Urol* **50:** 969-979.

Saxena M., Saxena J., Nema R., Singh D., and Gupta, A. (2013). Phytochemistry of medicinal plants. *J. Pharmacogn. Phytochem.***1** (6):168–182.

Scarpato R, Pistelli L, Bertoli A, Nieri E, and Migliore, L. (1998). In vitro genotoxicity and cytotoxicity of five new chemical compounds of plant origin by means of human lymphocyte micronucleus assay. Toxicol In Vitro 12: 153-161.

Schippmann, U., Leaman, D., and Cunningham, A. (2006). A Comparison of Cultivation and Wild Collection of Medicinal and Aromatic Plants Under Sustainability Aspects. *Medicinal and Aromatic Plants* ***17:*** https://doi.org/ 10.1007/1-4020-5449-1_6.

Shenouda, N., Sakla, M., and Newton, L., *et al*., (2007. "Phytosterol *Pygeum africanum* regulates prostate cancer in vitro and in vivo*," Endocrine* **31**: 72–81.

Silva, I.A., Valenti, M.W. and Silva-Matos, D.M. (2009). Fire effects on the population structure of Zanthoxylum rhoifolium Lam (Rutaceae) in a Brazilian savanna. *Braz. J. Biol.*, **69(3**): 813-818

Sparks, D.L. (2003). *Environmental Soil Chemistry*, 2nd Edition ed. Academic Press, San Diego.

Sun C, Kaplin, B.A., and Kristensen, K., *et al*., (1996). Tree phenology in a tropical montane forest in Rwanda. *Biotropica* **28**: 668-681.

Sunderland, T. and Nkefor, J. (1997). Conservation through cultivation. A case study: the propagation of pygeum - *Prunus africana.* Tropical Agriculture Association Newsletter, December 1997, 5-13.

Thompson, C.B. (1995) Apoptosis in the pathogenesis and treatment of disease. *Science* **267**: 1456-1462.

Thomsom, M.J., Robbins, W.E., and Baker, G.L. (1963). The nonhomogeneity of soybean sterol--"gamma-sitosterol". *ScienceDirect* **2**(5): 505-512. doi.org/10.1016/0039-128X(63)90027-8.

Van de Koppel, J., and Prins, H. H. T. (1998). The importance of herbivore interactions for the dynamics of African savanna woodland: An hypothesis. *Journal of Tropical Ecology* **14**: 565-576.

Wang, C. L., Ruijun, W., Qi, D., Lu, W. (2007). Effects of altitude on plant-species diversity and productivity in an alpine meadow, Qinghai–Tibetan plateau. *Australian Journal of Botany **55*** - 10.1071/BT04070.

Wang, Y., Zhang, X., and Huang, C. (2009) Spatial Variability of Soil Total Nitrogen and Soil Total Phosphorus under Different Land Uses in a Small Watershed on the Loess Plateau, China. Geoderma **150,** 141–149. doi: 10.1016/j.geoderma.2009.01.021.

Wasson, K.M., and Watts, S.A. (1998). Proscar@(Finasteride) inhibits 5a-reductase activity in the ovaries and testes of Lytechinusvariegatus Lamarck (Echinodermata: Echinoidea). Comparative Biochemistry and Physiology Part C: *Pharmacology, Toxicology and Endocrinology* **120**: 425-431.

Wilt, T.J., Ishani, A., Stark, G., MacDonald, R., Lau, J., *et al*., (1998). Saw palmetto extracts for treatment of benign prostatic hyperplasia: a systematic review. *JAMA* 280: 1604-1609.

Yarnell., E. (2002). Botanical medicines for the urinary tract. *World J Urol* **20**: 285-293.

Young, D., Ingram, G. and Swartz, I. (1988). The Persistence of Traditional Medicine in the Modern World. *Cultural Survival Quarterly **12(1):*** 39-41.

APPENDICES

Appendix 1: Ecological data collection form

Belt No. ……..Plot No. …….GPS Northings……...……...……Eastings…….……...…….Altitude…….…...…….Plot canopy cover…….

No	*Prunus africana*	DBH(cm)	Height(m)	Crown length(m)	Phenology	ID	Comment

Prunus africana species association

No	Tree species	DBH(cm)	distance(m)	COMMENT

NB	Canopy cover(%)= less than 30%; between 31-60% and above 60%			

Small Regenerants(1-50cm) height			Medium Regenerants(51-150cm height)			Large regenerants(≥151 to 500cm height)		
Species	Tally	Count	Species	Tally	Count	Species	Tally	Count

Appendix 2: One-Way ANOVA for P.africana population structure in the study sites

SUMMARY							

Groups	*Count*	*Sum*	*Average*	*Variance*			
Seedlings	148	0.69315	0.00468	0.00325			
Saplings	148	3.98898	0.02695	0.02717			
Mature trees	148	14.5561	0.09835	0.0589			
ANOVA							
Source of Variation	*SS*	*df*	*MS*	*F*		*P-value*	*F crit*
Between Groups	0.7088	2	0.3544	11.9043		9.22021E-06	3.01617
Within Groups	13.1289	441	0.02977				
Total	13.8377	443					

Appendix 3: List of plant species with their frequency, relative abundances and presence/absence occurrence in the study sites

	Species name	**Family name**	**Frequency**	**Relative abundance %)**	**Manyenjere forest**	**Juniper forest**
1	*Diospyros whyteana*	Ebenaceae	21	6.46	+	+
2	*Teclea nobilis*	Rutaceae	13	4.00	+	+
3	*Psychotria zombamontana*	Rubiaceae	12	3.69	+	+
4	*Parinari excelsa*	Chrysobalanaceae	11	3.38	+	+
5	*Bersama abyssinica*	Melianthaceae	10	3.08	+	+
6	*Clausena anisata*	Rutaceae	10	3.08	+	+
7	*Garcinia kingaensis*	Clusiaceae	10	3.08	+	+
8	*Ilex mitis*	Aquifoliaceae	10	3.08	+	+
9	*Maytenus acuminata*	Celastraceae	10	3.08	+	+
10	*Rapanea melanophloeos*	Rizophoraceae	10	3.08	+	+
11	*Agauria salicifolia*	Ericaceae	9	2.77	-	+
12	*Juniperus procera*	Cupressaceae	9	2.77	-	+
13	*Macaranga capensis*	Euphorbiaceae	8	2.46	+	+
14	*Podocarpus milanjianus*	Podocarpaceae	8	2.46	+	+
15	*Hagenia abyssinica*	Rosaceae	7	2.15	+	+
16	*Tecomaria capensis*	Bignoniaceae	7	2.15	+	+

17	*Euclea schimperi*	Ebenaceae	6	1.85	-	+
18	*Hetermorpha trifoliata*	Apiaceae	6	1.85	-	+
19	*Diospyros natalensis*	Ebenaceae	5	1.54	+	+
20	*Dodonaea viscosa*	Sapindaceae	5	1.54	-	+
21	*Halleria lucida*	Stilbaceae	5	1.54	-	+
22	*Maesa lanceolata*	Myrsinaceae	5	1.54	+	+
23	*Mollera pilulifera*	Myricaceae	5	1.54	-	+
24	*Rhamnus prinoides*	Rhamnaceae	5	1.54	-	+
25	*Allophyllus chaunostachys*	Sapindaceae	4	1.23	+	+
26	*Aphloia theiformis*	Flacourtiaceae	4	1.23	+	+
27	*Ekebergia capensis*	Meliaceae	4	1.23	+	+
28	*Entandophragma excelsum*	Meliaceae	4	1.23	+	+
29	*Euclea crispa*	Ebenaceae	4	1.23	+	+
30	*Mystroxylon aethiopica*	Celastraceae	4	1.23	-	+
31	*Syzgium cordatum*	Myrtaceae	4	1.23	+	+
32	*Tricalysia acocatheroides*	Rubiaceae	4	1.23	+	+
33	*Buddleja salviifolia*	Buddlejaceae	3	0.92	-	+
36	*Chrysophyllam bequetiodendron*	Sapotaceae	3	0.92	-	+
34	*Croton megalobotrys*	Euphorbiaceae	3	0.92	+	+
35	*Tricalysia coriacea*	Rubiaceae	3	0.92	+	+

37	*chrysophyllum gorungosanum*	Sapotaceae	2	0.62	+	-
38	*Cussonia spicata*	Araliaceae	2	0.62	-	+
39	*Faurea rochetiana*	Proteaceae	2	0.62	-	+
40	*Khaya anthotheca*	Meliaceae	2	0.62	+	+
41	*Lepidotrichilia volkensii*	Meliaceae	2	0.62	-	+
42	*Ochna holstii*	Ochnaceae	2	0.62	+	+
43	*Olinia rochetiana*	Oliniaceae	2	0.62	-	+
44	*Polycias vulva*	Araliaceae	2	0.62	+	+
45	*Rhus longipes*	Anacardiaceae	2	0.62	-	+
46	*Rhus natalensis*	Anacardiaceae	2	0.62	-	+
47	*Vernonia myriantha*	Asteraceae	2	0.62	+	+
62	*Alocasia coriacea*	Araceae	1	0.31	+	-
66	*Afrocrania vokensii*	Cornaceae	1	0.31	+	-
48	*Allophyllus africanus*	Sapindaceae	1	0.31	-	+
49	*Apodytes dimidiata*	metteniusaceae	1	0.31	-	+
50	*Bequatiodendron magalismotanum*	Sapotaceae	1	0.31	-	+
51	*Carissa edulis*	Apocynaceae	1	0.31	-	+
52	*cassipourea malosana*	Rhizophoraceae	1	0.31	-	+
63	*cassipourea mollis*	Rhizophoraceae	1	0.31	+	-
64	*Crabbea longipes*	Acanthaceae	1	0.31	+	-

53	*Dombeya Rotundifolia*	Malvaceae	1	0.31	-	+
54	*Dovyalis zeyheri*	Salicaceae	1	0.31	-	+
55	*Erica bengualensis*	Ericaceae	1	0.31	-	+
56	*Maytenus senegalensis*	Celastraceae	1	0.31	-	+
65	*Milletia dura*	Papilionoideae	1	0.31	+	-
57	*Milletia lasiantha*	Papilionoideae	1	0.31	-	+
58	*Myrsine africana*	Myrsinaceae	1	0.31	-	+
59	*Olea europea*	Oleaceae	1	0.31	-	+
60	*Osyris lanceolata*	Santalaceae	1	0.31	-	+
61	*Pittosporum viridiflorum*	Pittosporaceae	1	0.31	-	+
67	*Pachystella brevipes*	Sapotaceae	**1**	0.31	+	-

Appendix 4: Forest and plot plant diversity and evenness in the two study sites.

Plot No.	Name of Forest	Mean	Stand.Dev.	Richness	Evenness	H	D`
01	Manyenjere	0.284	0.454	19	1	2.944	0.9474
02	Manyenjere	0.224	0.42	15	1	2.708	0.9333
03	Manyenjere	0.179	0.386	12	1	2.485	0.9167
04	Manyenjere	0.134	0.344	9	1	2.197	0.8889
05	Manyenjere	0.149	0.359	10	1	2.303	0.9
06	Manyenjere	0.194	0.398	13	1	2.565	0.9231
07	Manyenjere	0.209	0.41	14	1	2.639	0.9286
08	Juniper	0.119	0.327	8	1	2.079	0.875
09	Juniper	0.134	0.344	9	1	2.197	0.8889
10	Juniper	0.209	0.445	13	0.99	2.54	0.9184
11	Juniper	0.134	0.344	9	1	2.197	0.8889
12	Juniper	0.149	0.359	10	1	2.303	0.9
13	Juniper	0.149	0.399	9	0.985	2.164	0.88
14	Juniper	0.194	0.435	12	0.989	2.458	0.9112
15	Juniper	0.149	0.359	10	1	2.303	0.9
16	Juniper	0.179	0.49	10	0.96	2.21	0.875
17	Juniper	0.224	0.42	15	1	2.708	0.9333
18	Juniper	0.149	0.359	10	1	2.303	0.9
19	Juniper	0.179	0.386	12	1	2.485	0.9167
20	Juniper	0.179	0.386	12	1	2.485	0.9167
21	Juniper	0.075	0.317	4	0.961	1.332	0.72
22	Juniper	0.284	0.486	18	0.993	2.871	0.9418
23	Juniper	0.239	0.43	16	1	2.773	0.9375
24	Juniper	0.194	0.435	12	0.989	2.458	0.9112
25	Juniper	0.134	0.344	9	1	2.197	0.8889
26	Juniper	0.194	0.398	13	1	2.565	0.9231
27	Juniper	0.209	0.41	14	1	2.639	0.9286
	Averages	**0.1797**	**0.3942**	**12.04**	**0.995**	**2.411**	**0.9034**

Appendix 5: Phytochemical compound frequency in GC-MS analysis in the study sites

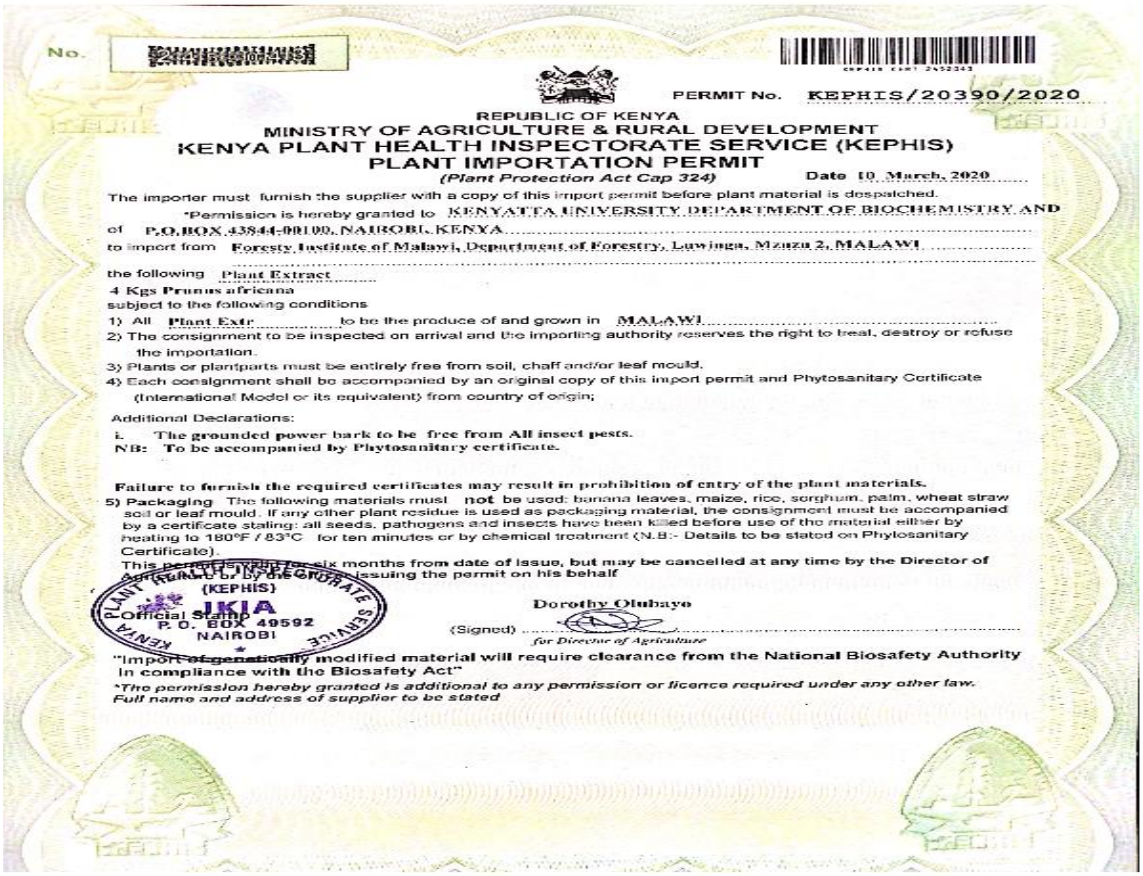
	Juniper	Manyenjere	Total
Compound Name	**Frequency**		
.alpha.-Amyrin	1	0	1
.beta.-Amyrin	1	2	3
11,14-Eicosadienoic acid, methyl ester		1	1
11,14-Octadecadienoic acid, methyl ester		1	1
11-Eicosenoic acid, methyl ester		1	1
11-Hexadecynoic acid, methyl ester	1		1
14-Octadecenoic acid, methyl ester	1		1
1-Heptacosanol	1		1
1-Hexacosanol		1	1
1-Hexadecanol		1	1
22,23-Dihydrospinasterone		1	1
28-Norolean-17-en-3-one	1		1
2-Pentadecanone, 6,10,14-trimethyl-		1	1
2-Propenoic acid, tridecyl ester		1	1
5-(2-Thienyl)pentanoic acid	1		1
5.beta.-Cholest-23-ene, (Z)-		1	1
5-Cholestene-3-ol, 24-methyl-		1	1
6-Octadecenoic acid	7	10	17
79.26 Stigmast-4-en-3-one		1	1
7-Octadecenoic acid, methyl ester	1	1	2
8,11-Eicosadienoic acid, methyl ester	1		1
8-Octadecenoic acid, methyl ester, (E)-	1		1
9,12-Octadecadien-1-ol, (Z,Z)-	1		1
9,12-Octadecadienoic acid (Z,Z)-	2	1	3
9,12-Octadecadienoic acid, ethyl ester		1	1
9-Hexadecenoic acid		1	1
9-Hexadecenoic acid, eicosyl ester, (Z)-	1		1
9-Hexadecenoic acid, hexadecyl ester, (Z)-	1		1
9-Hexadecenoic acid, methyl ester, (Z)-		2	2
9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	1		1
9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	1		1
9-Octadecenoic acid (Z)-, hexadecyl ester	2	3	5
9-Octadecenoic acid (Z)-, methyl ester	2	2	4
9-Octadecenoic acid (Z)-, octadecyl ester	5	4	9
9-Octadecenoic acid (Z)-, tetradecyl ester	3		3
9-Octadecenoic acid, (E)-	2	2	4
9-Octadecenoic acid, methyl ester, (E)-	6	7	13
alpha-Amyrin	1		1
Androstan-17-one, (5.alpha.)-	1		1
beta-Sitosterol acetate		2	2

Betulin	1		1
Butyl 9,12-octadecadienoate	2		2
Butyl 9-tetradecenoate		1	1
Campesterol	1	1	2
Cholest-4-ene-3,6-dione		1	1
Cholest-7-en-3-ol, acetate, (3.beta.)-		1	1
Cholest-8(14)-en-3-ol, acetate, (3.beta.)-	1	1	2
Cholest-8(14)-en-3-ol, acetate, (3-beta.)-		1	1
Cholesta-8,14-dien-3-ol, 4,4-dimethyl-, (3.beta.,5.alpha.)-	1		1
Cholestan-3,26-diol-22-one	1		1
Cholestan-3-one, dimethylhydrazone, (5.alpha.)-	1		1
Cholesterol		1	1
cis-10-Heptadecenoic acid	3	2	5
cis-11-Eicosenoic acid, methyl ester		1	1
cis-13-Eicosenoic acid	1		1
cis-13-Eicosenoic acid, methyl ester		3	3
cis-13-Octadecenoic acid		2	2
cis-13-Octadecenoic acid, methyl ester		2	2
cis-9-Hexadecenoic acid		1	1
Citrost-8(14)-en-3.beta.-ol	1		1
D:A-Friedooleanan-2-one	1		1
Decanedioic acid, bis(2-ethylhexyl) ester	1		1
Decyl oleate	1		1
dl-.alpha.-Tocopherol	1	1	2
Docosanedioic acid, dimethyl ester		1	1
Docosanoic acid, methyl ester	3	5	8
Dodecanoic acid	3		3
Dodecanoic acid, tetradecyl ester	1		1
Dodecyl methacrylate		1	1
E-6-Octadecen-1-ol acetate	1		1
Eicosanoic acid	2	2	4
Eicosanoic acid, methyl ester	5	6	11
Ergost-25-ene-3,5,6-triol, (3.beta.,5.alpha.,6.beta.)-		1	1
Ergosta-4,6,22-trien-3-beta-ol		1	1
Ergosta-4,6,8(14),22-tetraen-3-one		3	3
Ergosta-7,24(28)-dien-3-ol, 4-methyl-, (3-beta,4-alpha-,5-alpha)-	1		1
Ergostanol	2	2	4
gamma-Sitosterol	10	8	18
Heneicosanoic acid, methyl ester		2	2
Heptadecanoic acid		3	3
Heptadecanoic acid, 16-methyl-, methyl ester	1		1
Hexacosanoic acid, methyl ester		1	1
Hexadecanedioic acid, dimethyl ester		1	1
Hexadecanoic acid	1	3	4

Hexadecanoic acid, 2-hydroxy-, methyl ester		1	1
Hexadecanoic acid, dodecyl ester	4	2	6
Hexadecanoic acid, hexadecyl ester		1	1
Hexadecanoic acid, methyl ester		1	1
Hexadecanoic acid, octadecyl ester		1	1
Hexadecanoic acid, tetradecyl ester	6	4	10
Hexadecanol<n->		1	1
Hexadecenoic acid, Z-11-		1	1
i-Propyl 9-octadecenoate		1	1
Isopropyl linoleate		1	1
Isopropyl myristate	1	1	2
Lanost-7-en-3-ol, acetate, (3-beta,9-beta.,13-alpha,14.beta.,17.alpha.)-	1		1
Lanostane-3-beta.,11.beta.-diol, 3-acetate		1	1
Lanosterol		1	1
Limonene		2	2
l-Methionine, n-propargyloxycarbonyl-, ethyl ester		1	1
Methacrylic acid, hexadecyl ester		1	1
Methyl 18-methylnonadecanoate	2	1	3
Methyl 5,9-heptacosadienoate		1	1
Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate		2	2
Methyl benzoate		1	1
Methyl dodecanoate		3	3
Methyl hexadec-9-enoate		1	1
Methyl hexadecanoate	1	1	2
Methyl linoleate	9	11	20
Methyl octadecanoate	9	10	19
Methyl palmitate (Methyl hexadecanoate)		1	1
Methyl stearate	1		1
Methyl tetradecanoate		8	8
Myristyl myristate	1		1
n-Dodecyl methacrylate		3	3
n-Hexadecanoic acid	3	4	7
Nonadecanoic acid, methyl ester		6	6
Nonahexacontanoic acid		1	1
n-Propyl 9,12-octadecadienoate	1		1
Octadec-9-enoic acid		1	1
Octadecanoic acid	8	11	19
Octadecanoic acid, dodecyl ester	5	2	7
Octadecanoic acid, octadecyl ester		1	1
Octadecyl methacrylate		1	1
Octanoic acid, 3-methylbutyl ester	1		1
Oleic Acid	5	6	11
Oleic acid, eicosyl ester	4	5	9
Oleyl alcohol , acetate	1		1

Oleyl oleate	1		1
Oxalic acid, dodecyl isobutyl ester		1	1
Palmitoleic acid		1	1
Pentadecanoic acid, 13-methyl-, methyl ester		1	1
Pentadecanoic acid, 3-methylbutyl ester	1		1
Pentadecanoic acid, methyl ester		4	4
Pentanoic acid, 2-octyl ester	1		1
Phenol, 2,4-bis(1,1-dimethylethyl)-	2		2
Propanoic acid, 3,3'-thiobis-, didodecyl ester	2		2
Squalene	9	11	20
Stigmast-4-en-3-one	8	10	18
Stigmast-7-en-3-ol, (3.beta.,5.alpha.)-	2	4	6
Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	7	8	15
Stigmast-7-en-3-ol, (3-beta,5-alpha.)-	1		1
Stigmast-7-en-3-ol, (3-beta.,5.alpha.,24S)-		1	1
Stigmast-7-en-3-ol, (3-beta.,5-alpha.)-		1	1
Stigmast-7-en-3-ol, (3-beta.,5-alpha.,24S)-		1	1
Stigmast-8(14)-en-3.beta.-ol	4		4
Stigmast-8(14)-en-3-beta.-ol	1		1
Stigmast-8(14)-en-3-beta-ol	1		1
Stigmasta-4,6,22-trien-3.beta.-ol		1	1
Stigmasta-5,24(28)-dien-3-ol, (3-beta,24Z)-	1		1
Stigmasta-7,25-dien-3-ol, (3.beta.,5.alpha.)-		1	1
Stigmastanol	5	3	8
Supraene	1		1
Tetracosanoic acid, methyl ester	4	6	10
Tetradecanoic acid	1	4	5
Tetradecanoic acid, hexadecyl ester	1		1
Tricosanoic acid, methyl ester		4	4
Urs-12-en-28-al		1	1
Urs-12-ene	1		1
Ursane-3,16-diol, (3-beta,16-alpha-,18-alpha.,19-alpha-,20-beta)-	1		1
Vanillin	1	2	3
Vitamin E	2	5	7
Widdrol	1		1
Grand Total	**215**	**280**	**495**

Appendix 6: Phytochemical bark import permit



No.

PERMIT No. KEPHIS/20390/2020

REPUBLIC OF KENYA

MINISTRY OF AGRICULTURE & RURAL DEVELOPMENT

KENYA PLANT HEALTH INSPECTORATE SERVICE (KEPHIS)

PLANT IMPORTATION PERMIT

(Plant Protection Act Cap 324)

Date 10 March, 2020

The importer must furnish the supplier with a copy of this import permit before plant material is despatched.

*Permission is hereby granted to KENYATTA UNIVERSITY DEPARTMENT OF BIOCHEMISTRY AND

of P.O.BOX 43844-00100, NAIROBI, KENYA

to import from Forestry Institute of Malawi, Department of Forestry, Luwinga, Mzuzu 2, MALAWI

the following Plant Extract

4 Kgs Prunus africana

subject to the following conditions

1) All Plant Extr to be the produce of and grown in MALAWI

2) The consignment to be inspected on arrival and the importing authority reserves the right to treat, destroy or refuse the importation.

3) Plants or plantparts must be entirely free from soil, chaff and/or leaf mould.

4) Each consignment shall be accompanied by an original copy of this import permit and Phytosanitary Certificate (International Model or its equivalent) from country of origin;

Additional Declarations:

i. The grounded power bark to be free from All insect pests.

NB: To be accompanied by Phytosanitary certificate.

Failure to furnish the required certificates may result in prohibition of entry of the plant materials.

5) Packaging The following materials must not be used: banana leaves, maize, rice, sorghum, palm, wheat straw soil or leaf mould. If any other plant residue is used as packaging material, the consignment must be accompanied by a certificate stating: all seeds, pathogens and insects have been killed before use of the material either by heating to 180°F / 83°C for ten minutes or by chemical treatment (N.B:- Details to be stated on Phytosanitary Certificate).

This permit is valid for six months from date of issue, but may be cancelled at any time by the Director of Agriculture or by the officer issuing the permit on his behalf

(KEPHIS)

Official Stamp JKIA P. O. BOX 49592 NAIROBI

Dorothy Olubayo

(Signed) .....

for Director of Agriculture

"Import of genetically modified material will require clearance from the National Biosafety Authority In compliance with the Biosafety Act"

**The permission hereby granted is additional to any permission or licence required under any other law.*

Full name and address of supplier to be stated

Scanned with CamScanner

Appendix 7: GC-MS Juniper plot number J003 ethyl acetate extract

RT	Library/ID	µg/mg
19,0515	Phenol, 2,4-bis(1,1-dimethylethyl)-	22,22337
20,4263	Dodecanoic acid	0,636102
21,4208	5-(2-Thienyl)pentanoic acid	0,471234
25,2057	Methyl linoleate	2,927136
25,2525	9-Octadecenoic acid, methyl ester, (E)-	14,69747
25,4689	Methyl octadecanoate	3,36519
25,8024	6-Octadecenoic acid	151,6033
25,896	Octadecanoic acid	30,21036
26,8963	Oleic Acid	2,904549
27,2239	Eicosanoic acid, methyl ester	2,868146
30,4239	Tetracosanoic acid, methyl ester	12,92748
30,7573	Myristyl myristate	9,341673
31,4008	Squalene	8,791598
32,9042	Hexadecanoic acid, tetradecyl ester	5,41104
34,1737	Ursane-3,16-diol, (3-beta,16-alpha-,18-alpha.,19-alpha-,20-beta)-	0,500608
34,9927	9-Octadecenoic acid (Z)-, hexadecyl ester	3,82951
35,1974	9-Octadecenoic acid (Z)-, octadecyl ester	1,936592
35,6479	9-Octadecenoic acid, (E)-	9,557298
36,0106	Octadecanoic acid, dodecyl ester	0,890762
36,7711	9-Octadecenoic acid (Z)-, tetradecyl ester	0,843868
37,0811	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	1,40806
37,6486	Oleic acid, eicosyl ester	4,622882
39,0701	Stigmast-8(14)-en-3-beta-ol	0,744468
39,6961	gamma-Sitosterol	38,08765
39,8774	Stigmastanol	3,261938
40,8953	Stigmast-7-en-3-ol, (3-beta,5-alpha.)-	1,58509
41,2814	1-Heptacosanol	0,875513
42,8375	Stigmast-4-en-3-one	0,888988

Appendix 8: Juniper plot numberJ008 ethyl acetate extract results

RT	Library/ID	µg/mg
20,4263	Dodecanoic acid	0,575298
25,2233	Methyl linoleate	3,482114
25,2759	9-Octadecenoic acid, methyl ester, (E)-	19,57251
25,4865	Methyl octadecanoate	4,609823
25,82	6-Octadecenoic acid	155,6152
25,9194	Octadecanoic acid	36,17406
27,2415	Eicosanoic acid, methyl ester	3,110028
27,9084	Pentadecanoic acid, 3-methylbutyl ester	4,541523
28,2711	Butyl 9,12-octadecadienoate	2,998178
28,8795	Docosanoic acid, methyl ester	5,30914
30,4473	Tetracosanoic acid, methyl ester	13,41968
31,4243	Squalene	8,217755
32,7639	9-Hexadecenoic acid, hexadecyl ester, (Z)-	4,122556
32,9394	Hexadecanoic acid, dodecyl ester	6,847573
34,3258	Hexadecanoic acid, tetradecyl ester	0,721019
35,0278	9-Octadecenoic acid (Z)-, octadecyl ester	3,921739
35,2267	Oleyl oleate	4,767809
36,0574	Octadecanoic acid, dodecyl ester	0,507715
36,4026	9-Octadecenoic acid (Z)-, tetradecyl ester	1,084987
39,1228	Stigmast-8(14)-en-3.beta.-ol	0,44872
39,7897	.gamma.-Sitosterol	0,843931
39,9535	Stigmastanol	48,17946
40,9656	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	3,141426
42,5919	Cholest-8(14)-en-3-ol, acetate, (3.beta.)-	0,075334
42,9136	Stigmast-4-en-3-one	0,174897

Appendix 9: Juniper plot number J010 ethyl acetate extract results

RT	Library/ID	µg/mg
24,8196	cis-10-Heptadecenoic acid	20,25579
25,235	Methyl linoleate	4,343955
25,2876	9-Octadecenoic acid, methyl ester, (E)-	9,694892
25,4982	Methyl octadecanoate	10,18327
26,0247	6-Octadecenoic acid	131,7866
26,1242	Octadecanoic acid	162,1427
27,1596	9,12-Octadecadienoic acid (Z,Z)-	4,750218
27,2591	Eicosanoic acid, methyl ester	3,588644
27,4814	cis-13-Eicosenoic acid	6,94258
27,6744	Eicosanoic acid	13,73168
31,4359	Squalene	9,238442
32,9453	Hexadecanoic acid, dodecyl ester	2,83102
34,9869	Widdrol	0,945282
35,7064	Oleic acid, eicosyl ester	1,234431
36,0106	Ergosta-7,24(28)-dien-3-ol, 4-methyl-, (3-beta,4-alpha-,5-alpha)-	7,911126
36,0691	Octadecanoic acid, dodecyl ester	8,102511
36,4201	Vitamin E	2,043605
36,8121	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	2,146731
37,0578	Cholestan-3,26-diol-22-one	1,773161
37,7188	Oleic Acid	1,117777
38,6665	Lanost-7-en-3-ol, acetate, (3-beta,9-beta.,13-alpha,14.beta.,17.alpha.)-	0,574832
39,1462	Stigmast-8(14)-en-3-beta.-ol	0,646648
39,8248	gamma-Sitosterol	0,826814
39,971	Stigmastanol	1,462225
40,129	Stigmasta-5,24(28)-dien-3-ol, (3-beta,24Z)-	1,094455
40,5034	Urs-12-ene	19,9287
40,9656	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	21,14251
41,5271	.alpha.-Amyrin	2,563495
42,5977	Cholestan-3-one, dimethylhydrazone, (5.alpha.)-	2,797976
42,937	Stigmast-4-en-3-one	1,436808
43,7384	28-Norolean-17-en-3-one	1,028716
47,2894	Betulin	0,465809

Appendix 10: Manyenjere plot number M004 ethyl acetate extract results

RT	Library/ID	µg/mg
24,0824	Hexadecanoic acid	17,96967
24,0884	n-Hexadecanoic acid	19,75028
25,1997	Methyl linoleate	0,809199
25,2465	9-Octadecenoic acid, methyl ester, (E)-	4,768772
25,2467	7-Octadecenoic acid, methyl ester	4,580226
25,4688	Methyl octadecanoate	1,124488
25,469	Methyl tetradecanoate	1,028146
25,6796	Oleic Acid	70,93087
25,7321	6-Octadecenoic acid	86,75642
25,8432	Octadecanoic acid	20,22478
31,389	Squalene	5,295367
32,8867	Hexadecanoic acid, dodecyl ester	1,380366
32,8983	Hexadecanoic acid, tetradecyl ester	2,354822
35,1271	Ergosta-4,6,22-trien-3-beta-ol	0,533288
35,607	9-Octadecenoic acid (Z)-, octadecyl ester	2,408437
37,625	Oleic acid, eicosyl ester	0,947591
39,0407	Stigmast-7-en-3-ol, (3-beta.,5-alpha.,24S)-	0,089934
39,5965	gamma-Sitosterol	18,98879
40,8776	Stigmast-7-en-3-ol, (3-beta.,5-alpha.)-	1,23297
42,8374	Stigmast-4-en-3-one	0,788919

Appendix 11: Manyenjere plot number M005 ethyl acetate extract results

RT	Library/ID	µg/mg
22,0935	Tetradecanoic acid	1,23291
25,2056	Methyl linoleate	1,530121
25,2525	9-Octadecenoic acid, methyl ester, (E)-	9,13325
25,2993	cis-13-Octadecenoic acid, methyl ester	1,075224
25,4689	Methyl octadecanoate	2,274748
25,8199	6-Octadecenoic acid	164,1567
25,9135	Octadecanoic acid	30,32443
31,3949	Squalene	2,75772
32,9042	Hexadecanoic acid, tetradecyl ester	0,91649
34,2848	Hexadecanoic acid, octadecyl ester	0,388555
35,642	Oleic acid, eicosyl ester	5,667686
36,4025	dl-.alpha.-Tocopherol	0,606451
37,6369	9-Octadecenoic acid (Z)-, hexadecyl ester	2,471697
39,6492	gamma.-Sitosterol	23,99076
40,8836	Stigmast-7-en-3-ol, (3.beta.,5.alpha.)-	0,682942
42,8433	Stigmast-4-en-3-one	1,100121

Appendix 12: Manyenjere plot number M006 ethyl acetate extract results

RT	Library/ID	µg/mg
21,5141	Methyl tetradecanoate	3,004754
22,5671	Pentadecanoic acid, methyl ester	0,669233
25,2347	Methyl linoleate	18,17135
25,2932	9-Octadecenoic acid, methyl ester, (E)-	33,20192
25,498	Methyl octadecanoate	11,05681
25,7963	6-Octadecenoic acid	134,7228
25,9016	Octadecanoic acid	33,72212
26,3872	Nonadecanoic acid, methyl ester	3,836636
26,8259	11,14-Octadecadienoic acid, methyl ester	3,884716
27,0482	cis-13-Eicosenoic acid, methyl ester	7,438085
27,2471	Eicosanoic acid, methyl ester	6,68327
28,2826	9,12-Octadecadienoic acid, ethyl ester	5,51199
28,8734	Docosanoic acid, methyl ester	20,02063
29,6339	Tricosanoic acid, methyl ester	24,75996
30,1721	Hexadecanol<n->	8,27283
30,4471	Tetracosanoic acid, methyl ester	21,81007
31,424	Squalene	12,81114
31,7223	Docosanedioic acid, dimethyl ester	3,427091
35,1387	beta-Sitosterol acetate	0,863948
35,6945	Oleic acid, eicosyl ester	12,5694
36,4725	Cholest-8(14)-en-3-ol, acetate, (3-beta.)-	0,553851
39,7836	gamma-Sitosterol	0,49997
39,9474	Ergostanol	0,552632
40,9536	Stigmast-7-en-3-ol, (3-beta.,5.alpha.,24S)-	0,523033
41,1759	Ergosta-4,6,8(14),22-tetraen-3-one	3,874762
42,9309	Stigmast-4-en-3-one	0,738471

Appendix 13: GC-MS Manyenjere plot No. M007 ethyl acetate extract results

RT	Library/ID	µg/mg
17,706	Vanillin	0,484481
25,1999	Methyl linoleate	18,00259
25,2584	9-Octadecenoic acid, methyl ester, (E)-	31,91582
25,4631	Methyl octadecanoate	8,945237
25,8141	6-Octadecenoic acid	195,2826
25,9077	Octadecanoic acid	36,19405
26,3581	Nonadecanoic acid, methyl ester	3,593983
27,0192	1-Hexacosanol	6,69011
27,2122	Eicosanoic acid, methyl ester	5,906383
29,6049	Tricosanoic acid, methyl ester	9,221547
30,4122	Tetracosanoic acid, methyl ester	10,51258
31,3833	Squalene	11,89416
34,2732	Hexadecanoic acid, hexadecyl ester	4,437869
34,9635	9-Octadecenoic acid (Z)-, octadecyl ester	4,518101
35,1565	Octadec-9-enoic acid	5,497564
35,4724	Cholesterol	1,524005
35,6245	Oleic acid, eicosyl ester	11,29152
35,9872	Octadecanoic acid, dodecyl ester	2,834279
37,6135	Oleic Acid	6,992172
39,0233	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	1,006307
39,6961	gamma.-Sitosterol	56,56531
39,8482	Ergostanol	6,378518
40,8602	Stigmast-7-en-3-ol, (3.beta.,5.alpha.)-	4,094377
41,0767	beta-Sitosterol acetate	1,141245
42,8317	Stigmast-4-en-3-one	2,267075
43,6039	Lanostane-3-beta.,11.beta.-diol, 3-acetate	0,037027

Appendix 14: GC-MS Juniper plot No.J003 DCM extract results

RT	Library/ID	µg/mg
20,4321	Dodecanoic acid	0,651175
22,1169	Tetradecanoic acid	0,537729
22,3684	Octanoic acid, 3-methylbutyl ester	0,339957
25,194	Methyl linoleate	4,298399
25,2466	9-Octadecenoic acid, methyl ester, (E)-	16,21522
25,4631	Methyl octadecanoate	3,607015
25,7965	6-Octadecenoic acid	167,0807
25,8901	Octadecanoic acid	31,22241
26,7501	Dodecanoic acid, tetradecyl ester	2,722377
27,2122	Eicosanoic acid, methyl ester	5,611835
27,8499	n-Propyl 9,12-octadecadienoate	2,508058
28,0137	9,12-Octadecadien-1-ol, (Z,Z)-	4,742026
28,7683	Oleyl alcohol , acetate	2,420891
28,8443	Docosanoic acid, methyl ester	4,358562
29,248	Androstan-17-one, (5.alpha.)-	2,75285
30,4122	Tetracosanoic acid, methyl ester	7,202741
31,3833	Squalene	6,898491
32,8925	Hexadecanoic acid, tetradecyl ester	5,291709
35,6303	9-Octadecenoic acid (Z)-, octadecyl ester	11,89608
36,0047	Octadecanoic acid, dodecyl ester	1,911039
36,3616	Vitamin E	1,567539
37,6193	9-Octadecenoic acid (Z)-, hexadecyl ester	5,896604
39,0233	Stigmast-8(14)-en-3.beta.-ol	2,115007
39,6726	gamma.-Sitosterol	47,78359
39,8423	Stigmastanol	5,463378
40,8602	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	3,044062
42,8199	Stigmast-4-en-3-one	0,846483

Appendix 15: GC-MS Juniper plot no.J005 DCM extract results

RT	Library/ID	µg/mg
19,0515	Phenol, 2,4-bis(1,1-dimethylethyl)-	19,39372
19,1744	Methyl stearate	1,351494
22,5439	Isopropyl myristate	0,960254
24,8079	cis-10-Heptadecenoic acid	5,266333
25,2232	Methyl linoleate	4,080995
25,2876	9-Octadecenoic acid, methyl ester, (E)-	19,30555
25,4806	Methyl octadecanoate	5,006892
25,8022	6-Octadecenoic acid	185,2773
25,8024	9,12-Octadecadienoic acid (Z,Z)-	54,51015
26,1709	Octadecanoic acid	35,2814
26,9256	11-Hexadecynoic acid, methyl ester	4,258532
27,025	E-6-Octadecen-1-ol acetate	3,334795
27,1479	8,11-Eicosadienoic acid, methyl ester	2,805622
27,2532	Methyl 18-methylnonadecanoate	3,995246
31,1902	Decanedioic acid, bis(2-ethylhexyl) ester	3,155878
31,4242	Squalene	9,996835
32,7463	9-Hexadecenoic acid, eicosyl ester, (Z)-	3,708895
32,9277	Hexadecanoic acid, dodecyl ester	7,345103
34,3141	Hexadecanoic acid, tetradecyl ester	5,900669
34,5188	6-Octadecenoic acid	3,774577
35,0102	Oleic acid, eicosyl ester	5,674862
36,034	Octadecanoic acid, dodecyl ester	4,21922
36,4201	dl-.alpha.-Tocopherol	1,354902
37,7012	9-Octadecenoic acid (Z)-, octadecyl ester	6,711297
37,0458	Decyl oleate	2,391433
39,0055	Stigmast-8(14)-en-3.beta.-ol	1,204904
39,6607	gamma.-Sitosterol	52,65105
39,807	Ergostanol	7,851096
39,8716	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	37,04025
39,9886	Stigmastanol	4,992509
40,9246	Stigmast-7-en-3-ol, (3.beta.,5.alpha.)-	3,69303
41,5037	.beta.-Amyrin	0,337552
42,8901	Stigmast-4-en-3-one	2,236196
47,7983	D:A-Friedooleanan-2-one	0,114791

Appendix 16: GC-MS Juniper plot no. J008 DCM extract results

RT	Library/ID	µg/mg
24,0706	n-Hexadecanoic acid	20,38144
25,188	Methyl linoleate	3,21686
25,2406	7-Octadecenoic acid, methyl ester	10,96733
25,4629	Methyl octadecanoate	1,974932
25,7145	Oleic Acid	99,2788
27,2062	Eicosanoic acid, methyl ester	2,658215
30,4003	Tetracosanoic acid, methyl ester	4,301297
31,3714	Supraene	2,374967
32,8866	Hexadecanoic acid, tetradecyl ester	1,031083
35,6068	9-Octadecenoic acid, (E)-	1,179404
37,5665	Campesterol	0,412764
39,0056	Stigmast-8(14)-en-3.beta.-ol	0,13962
39,5263	.gamma.-Sitosterol	14,59878
40,8601	Stigmast-7-en-3-ol, (3.beta.,5.alpha.)-	0,128993

Appendix 17: GC-MS Juniper plot no. J010 DCM extract results

RT	Library/ID	µg/mg
18,2968	Vanillin	0,273525
24,1702	Hexadecanoic acid	17,43715
24,8078	cis-10-Heptadecenoic acid	3,221932
25,2232	Methyl linoleate	4,35246
25,2817	9-Octadecenoic acid, methyl ester, (E)-	19,9057
25,3168	9-Octadecenoic acid (Z)-, methyl ester	3,081743
25,4806	Methyl octadecanoate	5,546832
26,0305	6-Octadecenoic acid	265,1031
26,1065	Octadecanoic acid	50,91133
27,1478	Butyl 9,12-octadecadienoate	2,578732
27,2473	Methyl 18-methylnonadecanoate	3,721061
27,686	Eicosanoic acid	10,2544
30,7807	Tetradecanoic acid, hexadecyl ester	10,21624
31,4183	Squalene	6,764486
31,588	8-Octadecenoic acid, methyl ester, (E)-	4,83419
32,9276	Hexadecanoic acid, dodecyl ester	8,172021
34,3082	Hexadecanoic acid, tetradecyl ester	5,37563
35,0219	9-Octadecenoic acid (Z)-, tetradecyl ester	5,058721
35,2091	9-Octadecenoic acid (Z)-, octadecyl ester	5,997676
35,6947	Oleic Acid	16,353
37,6953	Oleic acid, eicosyl ester	7,018631
39,111	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	1,452417
39,8072	gamma-Sitosterol	42,36241
39,9476	Ergostanol	2,954101
40,9479	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	3,608419
41,492	alpha-Amyrin	1,343654
41,6558	Cholesta-8,14-dien-3-ol, 4,4-dimethyl-, (3.beta.,5.alpha.)-	0,285659
42,5801	Citrost-8(14)-en-3.beta.-ol	0,777872
42,8959	Stigmast-4-en-3-one	3,179906

Appendix 18: GC-MS Manyenjere plot no.M004 DCM extract results

RT	Library/ID	µg/mg
22,5437	Isopropyl myristate	0,183805
24,7726	cis-10-Heptadecenoic acid	2,626194
25,1938	Methyl linoleate	3,989532
25,2464	9-Octadecenoic acid, methyl ester, (E)-	22,73482
25,457	Methyl tetradecanoate	5,20535
25,8899	6-Octadecenoic acid	277,8712
25,966	Octadecanoic acid	33,52559
27,1126	Isopropyl linoleate	3,003337
27,1535	Oleic Acid	2,770052
27,212	Eicosanoic acid, methyl ester	3,537483
31,3831	Squalene	16,09348
34,2554	Hexadecanoic acid, tetradecyl ester	2,13184
34,4543	6-Octadecenoic acid	5,661945
34,9515	9-Octadecenoic acid (Z)-, hexadecyl ester	6,86171
35,1504	9-Octadecenoic acid, (E)-	1,094139
37,625	9-Octadecenoic acid (Z)-, octadecyl ester	1,069461
39,7251	gamma.-Sitosterol	0,25947
39,8597	Stigmastanol	0,418959
40,8424	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	9,60591
41,053	Ergosta-4,6,8(14),22-tetraen-3-one	0,531035
41,6088	22,23-Dihydrospinasterone	5,208291
42,8022	79.26 Stigmast-4-en-3-one	0,120011
47,7396	Cholest-4-ene-3,6-dione	0,243456

Appendix 19: GC-MS Manyenjere plot no.M006 DCM extract results

RT	Library/ID	µg/mg
17,8814	Vanillin	0,607224
19,1684	Methyl dodecanoate	0,607241
21,4733	Methyl tetradecanoate	2,36669
23,1757	Hexadecanoic acid, methyl ester	0,998171
23,357	9-Hexadecenoic acid, methyl ester, (Z)-	0,635864
24,1409	n-Hexadecanoic acid	11,45845
24,7903	Oleic Acid	2,291626
25,2232	Methyl linoleate	18,87626
25,2934	9-Octadecenoic acid (Z)-, methyl ester	31,12629
25,4747	Methyl octadecanoate	10,08331
25,931	6-Octadecenoic acid	146,6308
25,9544	i-Propyl 9-octadecenoate	20,11517
26,0363	Octadecanoic acid	22,09346
26,3814	Nonadecanoic acid, methyl ester	3,674869
26,6213	Hexadecanedioic acid, dimethyl ester	7,262825
27,0249	cis-11-Eicosenoic acid, methyl ester	3,618048
27,2297	Eicosanoic acid, methyl ester	10,17219
27,6392	Eicosanoic acid	10,2312
28,8618	Docosanoic acid, methyl ester	13,18384
30,4296	Tetracosanoic acid, methyl ester	14,998
31,3949	Squalene	12,96239
31,6581	5.beta.-Cholest-23-ene, (Z)-	3,817787
34,2789	Hexadecanoic acid, tetradecyl ester	3,474858
34,4076	Methyl 5,9-heptacosadienoate	0,413963
34,624	Oxalic acid, dodecyl isobutyl ester	2,72048
34,9633	9-Octadecenoic acid, (E)-	4,265263
35,1739	9-Octadecenoic acid (Z)-, hexadecyl ester	2,247019
36,186	cis-13-Octadecenoic acid	1,701274
36,4024	Vitamin E	3,667496
37,6543	9-Octadecenoic acid (Z)-, octadecyl ester	8,661947
39,8832	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	72,79137
39,9826	Stigmastanol	9,842169
40,4331	.beta.-Amyrin	0,505805
40,474	Lanosterol	2,751213
40,8952	Stigmast-7-en-3-ol, (3.beta.,5.alpha.)-	5,330046
42,4455	Cholest-8(14)-en-3-ol, acetate, (3.beta.)-	0,610153
42,8491	Stigmast-4-en-3-one	5,472517
47,7397	Cholest-7-en-3-ol, acetate, (3.beta.)-	0,11547

Appendix 20: GC-MS Manyenjere plot no.M007 DCM extract results

RT	Library/ID	µg/mg
21,4967	Methyl tetradecanoate	2,015788
22,5556	Pentadecanoic acid, methyl ester	0,491839
23,3804	Methyl hexadec-9-enoate	0,460074
24,8136	cis-10-Heptadecenoic acid	2,375173
25,2465	Methyl linoleate	18,81646
25,3109	cis-13-Octadecenoic acid, methyl ester	31,05677
25,4981	Methyl octadecanoate	9,223987
25,8198	9,12-Octadecadienoic acid (Z,Z)-	76,07299
25,9661	Oleic Acid	103,8531
26,0539	Octadecanoic acid	20,60518
26,4049	Nonadecanoic acid, methyl ester	3,017419
27,0483	cis-13-Eicosenoic acid, methyl ester	6,208604
27,2472	Eicosanoic acid, methyl ester	5,268992
27,7152	Eicosanoic acid	7,502323
28,8794	Docosanoic acid, methyl ester	9,398498
29,6399	Tricosanoic acid, methyl ester	14,22384
30,4472	Tetracosanoic acid, methyl ester	14,06371
30,7806	Hexadecanoic acid, 2-hydroxy-, methyl ester	9,451699
31,4241	Squalene	11,93254
32,9334	Hexadecanoic acid, dodecyl ester	6,128494
35,7004	Oleic acid, eicosyl ester	0,293581
36,0515	Octadecanoic acid, dodecyl ester	0,482974
38,1808	Octadecanoic acid, octadecyl ester	1,009551
38,6254	Ergost-25-ene-3,5,6-triol, (3.beta.,5.alpha.,6.beta.)-	0,804166
39,9943	Stigmastanol	50,65678
40,4799	.beta.-Amyrin	0,185093
40,9537	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	1,579176
42,9076	Stigmast-4-en-3-one	0,532603

Appendix 21 : GC-MS Juniper plot no. J005 Hexane extract results

RT	Compound	µg/mg
22,18	Pentanoic acid, 2-octyl ester	0,8
23,91	n-Hexadecanoic acid	90,3
25,0302	Methyl linoleate	2,814239
25,077	14-Octadecenoic acid, methyl ester	5,557494
25,2876	Methyl octadecanoate	1,942798
25,4572	Oleic Acid	205,109
25,5976	Octadecanoic acid	145,8511
31,12	Squalene	23,96182
39,5732	gamma-Sitosterol	11,97683
42,1823	Stigmast-4-en-3-one	7,109399
47,225	Propanoic acid, 3,3'-thiobis-, didodecyl ester	3,245702

Appendix 22: GC-MS Juniper plot no. J008 Hexane extract results

RT	Compound	µg/mg
23,6262	Methyl hexadecanoate	2,219304
25,2057	9-Octadecenoic acid (Z)-, methyl ester	4,254077
25,3988	Heptadecanoic acid, 16-methyl-, methyl ester	1,038342
28,657	Docosanoic acid, methyl ester	5,828888
31,114	Squalene	8,606815
39,5672	gamma.-Sitosterol	4,836613
39,7605	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	1,843393
47,2367	Propanoic acid, 3,3'-thiobis-, didodecyl ester	2,793147

Appendix 23: GC-MS Juniper plot no.J010 Hexane extract results

RT	Library/ID	µg/mg
23,8543	n-Hexadecanoic acid	39,88555
25,0067	Methyl linoleate	0,643497
25,2699	Methyl octadecanoate	0,225962
25,6853	Octadecanoic acid	33,8225
31,1258	Squalene	0,95452
39,3099	gamma-Sitosterol	3,671649
42,1529	Stigmast-4-en-3-one	0,545954

Appendix 24: GC-MS Manyenjere plot no.M004 Hexane extract results

RT	Library/ID	µg/mg
21,8945	n-Dodecyl methacrylate	0,899722
21,9939	Tetradecanoic acid	1,613836
23,6846	Palmitoleic acid	188,2908
23,8542	n-Hexadecanoic acid	1,720564
24,7668	Heptadecanoic acid	9,333885
25,0067	Methyl linoleate	3,554165
25,0535	9-Octadecenoic acid, methyl ester, (E)-	3,695589
25,2699	Methyl octadecanoate	2,298175
25,5507	6-Octadecenoic acid	484,5998
25,656	Octadecanoic acid	26,88835
29,4175	Nonahexacontanoic acid	3,084384
31,1199	Squalene	0,799492
35,2968	Vitamin E	0,428687
39,1578	gamma.-Sitosterol	14,8431
40,9771	Stigmasta-7,25-dien-3-ol, (3.beta.,5.alpha.)-	0,5207
42,1178	Stigmast-4-en-3-one	10,78027
42,89	Stigmasta-4,6,22-trien-3.beta.-ol	0,337368

Appendix 25: GC-MS Manyenjere plot no. M005 Hexane extract results

RT	Library/ID	µg/mg
21,9003	n-Dodecyl methacrylate	1,945363
23,6962	Hexadecenoic acid, Z-11-	1,29849
23,8483	n-Hexadecanoic acid	119,0647
25,0066	Methyl linoleate	2,856851
25,0534	9-Octadecenoic acid (Z)-, methyl ester	2,617051
25,2757	Methyl octadecanoate	1,785051
25,5097	Oleic Acid	419,8197
25,6384	Octadecanoic acid	211,4428
26,4983	l-Methionine, n-propargyloxycarbonyl-, ethyl ester	15,22817
27,4285	Methacrylic acid, hexadecyl ester	11,51244
31,1198	Squalene	34,37097
35,2908	Vitamin E	1,317877
39,1109	gamma.-Sitosterol	88,78693
40,9478	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	3,095729
42,1002	Stigmast-4-en-3-one	8,726209

Appendix 26: GC-MS Manyenjere plot no. M006 Hexane extract results

RT	Library/ID	µg/mg
12,1251	Limonene	0,489854
19,028	Methyl dodecanoate	0,470063
20,9585	2-Propenoic acid, tridecyl ester	1,471355
21,2978	Methyl tetradecanoate	0,886313
21,8185	Dodecyl methacrylate	0,9073
21,953	Tetradecanoic acid	0,921212
22,3625	Pentadecanoic acid, methyl ester	0,735653
23,0002	Pentadecanoic acid, 13-methyl-, methyl ester	0,454243
23,3745	Methyl hexadecanoate	28,10894
23,632	9-Hexadecenoic acid	1,751595
23,8894	Hexadecanoic acid	90,07412
24,1351	Butyl 9-tetradecenoate	3,422277
24,7785	Heptadecanoic acid	5,131518
25,0184	Methyl linoleate	33,72417
25,0711	9-Octadecenoic acid, methyl ester, (E)-	39,87367
25,27	Methyl octadecanoate	13,1874
25,6619	6-Octadecenoic acid	292,07
25,738	Octadecanoic acid	61,40714
26,1767	Nonadecanoic acid, methyl ester	7,233972
26,6154	11,14-Eicosadienoic acid, methyl ester	3,594124
26,6622	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate	5,091431
26,826	11-Eicosenoic acid, methyl ester	10,79639
27,0249	Methyl 18-methylnonadecanoate	10,6839
27,8556	Heneicosanoic acid, methyl ester	8,282529
28,6512	Docosanoic acid, methyl ester	12,41659
30,1956	Tetracosanoic acid, methyl ester	13,32044
31,1199	Squalene	7,673288
32,1378	Hexacosanoic acid, methyl ester	3,583675
35,2851	Vitamin E	2,406905
37,4905	Campesterol	0,802127
39,1227	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	145,0938
40,4682	Ergosta-4,6,8(14),22-tetraen-3-one	3,900886
40,7724	Stigmast-7-en-3-ol, (3.beta.,5.alpha.)-	2,182382
42,1237	Stigmast-4-en-3-one	0,836326
46,4527	Urs-12-en-28-al	1,175755

Appendix 27: GC-MS Manyenjere plot no.M006 Hexane extract results

RT	Library/ID	µg/mg
12,1193	Limonene	0,393426
13,4472	Methyl benzoate	2,252651
19,0047	Methyl dodecanoate	0,375394
21,2979	Methyl tetradecanoate	0,53935
21,8185	n-Dodecyl methacrylate	1,233574
21,9531	Tetradecanoic acid	0,82631
21,9999	1-Hexadecanol	0,880413
22,3567	Pentadecanoic acid, methyl ester	0,623725
22,579	2-Pentadecanone, 6,10,14-trimethyl-	0,719533
23,1757	9-Hexadecenoic acid, methyl ester, (Z)-	0,716065
23,3688	Methyl palmitate (Methyl hexadecanoate)	18,48737
23,6203	cis-9-Hexadecenoic acid	1,645223
23,8836	Hexadecanoic acid	87,51114
24,3399	Methyl tetradecanoate	3,53069
24,7786	Heptadecanoic acid	6,028816
25,0126	Methyl linoleate	25,86361
25,27	Methyl octadecanoate	9,386543
25,662	6-Octadecenoic acid	315,4396
25,7322	Octadecanoic acid	77,13006
26,1651	Nonadecanoic acid, methyl ester	12,23211
26,6565	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate	7,560019
26,8203	cis-13-Eicosenoic acid, methyl ester	7,522398
27,025	Eicosanoic acid, methyl ester	12,24706
27,4228	Octadecyl methacrylate	4,011881
27,8557	Heneicosanoic acid, methyl ester	5,743164
28,6455	Docosanoic acid, methyl ester	9,031442
29,4118	Tricosanoic acid, methyl ester	7,186621
30,1899	Tetracosanoic acid, methyl ester	10,97701
31,12	Squalene	14,68963
31,3657	cis-13-Octadecenoic acid	4,127516
35,2852	Vitamin E	3,253827
37,5433	5-Cholestene-3-ol, 24-methyl-	1,243752
39,0701	.gamma.-Sitosterol	102,4575
40,7666	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	0,916041
40,8426	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	0,980018
42,1062	Stigmast-4-en-3-one	3,269022

NB. The hexane extracts for J003 and J008 were not dissolving thus they did not give good results. I have dried them and will try analysing them again

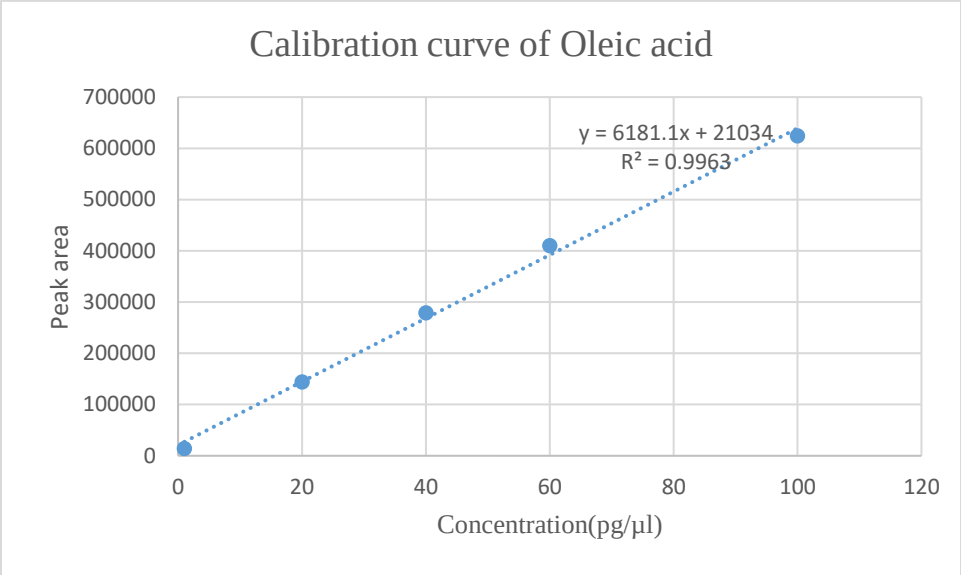
Appendix 28: Results of LC-MS analysis in Manyenjere Forest

LC-MS phytochemical analysis(ng/g)				
	Manyenjere Plot No			
Chemical compound	***M006***	***M005***	***M007***	***M004***
Chlorogenic acid	128.22	138	64.3	141.92
Apigenin	112.5	121.5	158.9	55.5
Oleanolic acid	62.48	77.8	71.2	3.63
Beta-sitostenone	53.58	72	0	68.34
Octadecanoic acid	44.96	65.5	103.2	0
Ellagic acid	44.7	74.8	67.2	34.92
Quercetin 3,3`-demethylether-4`-glucoside	42.68	141.8	96.8	2.34
Oleic acid	41.51	83.7	80.2	87.44
Quercetin	32.22	64.4	79	86.7
Luteolin	27.41	74.5	52.3	11.91
Catechin	12.19	47.1	28	67.27
Prunasin	11.1	53.9	36.5	57.35
Adenosine	7.98	85.2	0	0
Arachidic acid	7.93	43.3	30.1	0
P-coumaric	6.82	110.1	30.9	27.91
Prunetrine	6.79	11.6	10.1	7.15
Rutin	6.59	51.1	39.5	45.51
Ursolic acid	6.3	3.9	6.2	5.44
Apigenin 6-c--glucoside	6.25	64.3	31.9	32.17
Campesterol	6.24	28.3	11.3	21.89
Zeatin	6.06	33.9	22.3	20.14
4-hydroxycinnamic acid	4.78	15.3	15.1	20.62
a,12-octadecadienoic acid (z,z)-	4.28	50.2	0	14.52
Kaempterol	2.17	13.7	6.5	1.85
Cynanidin-0-galactoside	1.54	13.3	8.5	8.79
Beta-sitesterol	0.53	45.7	61.3	62.94
Cholesterol	0	0	0	61.38
Vitexin	0	0	0	6.15

Appendix 29: Results of LC-MS analysis in Juniper Forest

LC-MS Chromatography analysis in Juniper Forest(ng/g)				
	Juniper Plot No			
Chemical compound	***JOO5***	***JO10***	***J008***	***J003a***
Quercetin	135.06	124.32	125	72.1
Chlorogenic acid	131.38	122.97	133.1	56.7
Apigenic 6-c glucoside	124.94	127.11	158.5	181.4
Prunetrin	71.89	1.43	0	1.5
Oleic acid	47.15	2.76	54.5	59.9
Ellagic acid	44.54	26.3	95.2	20.9
P-coumaric	39.3	17.77	0.3	55.9
Prunasin	16.69	24.37	21.5	59.7
Catechin	15.25	9.58	7.1	24.5
Campesterol	12.74	8.53	1.1	8.9
Quercetin 3,3`-demethyl-4`glucoside	11.6	44.16	17	19.7
Apigenin	11.02	103.28	0	103.7
9,12-octadecadienoic acid (z,z)-	10.31	4.82	3.8	9.8
4-hydroxycinnamic acid	5.45	3.53	4.8	12.8
Cyanidin-0-galactoside	5.25	41.2	0	0
beta-sitostenone	5.23	16.9	0	13.8
Zeatin	2.77	0.74	5.2	13
Rutin	2.63	6.45	7.7	2.9
Oleanolic acid	2.18	2.76	1	5.9
Ursolic acid	1.99	1.33	0	2.7
Beta-sitosterol	0.95	4.97	2	0
Luteolin	0.92	4.13	0.7	11.9
Kaempterol	0.05	13.25	5.2	0

Appendix 30: Calibration curve of Oleic acid in LC-MS analysis



Appendix 31: Analysis of variance test for ethyl acetate, DCM & Hexane in detecting compounds

Variate: Amount

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Method	2	36271.	18135.	2.18	0.115
Residual	321	2676163.	8337.		
Total	323	2712434.			

Appendix 32: Anova test for dbh(cm) in the study sites**.**

Sum of sqrs	df	Mean square	F	p (same)
Between groups:	13.6667	5	2.73333	0.5467	0.7379
Within groups:	30	6	5	Permutation p (n=99999)
Total:	43.6667	11	0.7656	

APPENDIX 33: Tests for equal medians comparing the numper of compounds detected in the two study sites

Juniper		Manyenjere	
N:	163	N:	163
Mean rank:	76.368	Mean rank:	87.132

Mann-Whitn U: 11530

Z: 2.1766: *p (same med.)*: 0.029507

Monte Carlo permutation: *p* (*same med.*): 0.0315