GENETIC DIVERSITY, SPECIES NICHE MODELLING AND ETHNOBOTANICAL USES OF *STRYCHNOS HENNINGSII* FOR SUSTAINABLE CONSERVATION AND UTILIZATION

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(PLANT SCIENCE)

JOMO KENYATTA UNIVERSITY OF

AGRICULTURE AND TECHNOLOGY

2018

Genetic diversity, Species Niche Modelling and Ethnobotanical uses of *Strychnos henningsii* for Sustainable Conservation and Utilization

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A thesis submitted in partial fulfillment for the degree of Doctor of philosophy in Plant Science in the Jomo Kenyatta University of Agriculture and Technology

2018

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

This work is dedicated to the Almighty God who has strengthened me all through my research study. I also dedicate this work to my family for their Prayers, patience, moral and financial support. God bless you all.

ACKNOWLEGMENT

Firstly I would like to gratefully acknowledge JKUAT-Research, Production and Extension institution for providing me with research funds to pursue my degree and making this research project possible. I would like to thanks the Director KEFRI through the chief scientist Dr. David W. Odee for allowing me to undertake my research work at KEFRI Biotechnology Laboratory and utilize the instituon's facilities. Thanks to all my supervisors Prof Victoria Wambui Ngumi, Dr Peter K. Njenga, Prof Laura Nyawira Wangai and Prof Esther Magiri for being patient, encouraging and for their guidance during the entire course.

My tributes are also to the chief technologist at KEFRI Biotechnology Laboratory, Mr John Gicheru for his dedication to this research work especially in the collection of samples and molecular characterization work in the laboratory. It was quite an experience working under his guidance and have learnt a lot from him. I am indebted to Mr Stephen Kiama and Ms Cynthia Wangechi Kanyingi at KEFRI GIS Department for helping me entangle my ideas on ecological characterization of *Strychnos henningsii* species into being clear and concise study area of species niche modeling. To you I appreciate the moving discussions that took place in every meeting. Thanks to Mr Sayah Abiud, biometrician at KEFRI for data analysis and interpretation on seed germination experiments.

My gratitude also goes to my parents and the entire family for their moral and financial support. Thanks also to all my friends for their encouragement during my study. Words may not express my gratitude but may God bless you all. Above all I thank and praise God for everything for he has strengthened me during the entire course of my research.

TABLE OF CONTENT

DECLARATION II

DEDICATION III

ACKNOWLEGMENT IV

TABLE OF CONTENT V

LISTS OF TABLES X

LIST OF FIGURES XIII

LIST OF PLATES XV

LIST OF APPENDICES XVI

ABBREVIATIONS AND ACRONYMS XVII

ABSTRACT XX

CHAPTER ONE 1

INTRODUCTION 1

1.1 Background Information 1

1.1.1 Description of Strychnos henningsii 1

1.1.2 Propagation and Management 2

1.1.3 Economic Importance 3

1.2 Statement of the problem 4

1.3 Justification 6

1.4 Hypotheses 7

1.5 Broad Objective 7

1.6 Specific Objectives..7

1.7 Scope of the Study- 9

CHAPTER TWO 10

LITERATURE REVIEW..10

2.1 Introduction 10

2.2 Population genetic diversity 10

2.2.1 Morphological markers 11

2.2.2 Molecular markers 12

2.3 Species Distribution modeling in conservation strategies 18

2.4 Ethnobotanical studies of medicinal plants...24

CHAPTER THREE 29

MATERIALS AND METHODS 29

3.1 Introduction 29

3.2 Genetic diversity studies of S. henningsii 29

3.2.1 Study areas 29

3.2.3 Collection of Plant Materials 30

3.2.4 DNA Extraction 31

3.2.5 DNA Quantification and Quality Evaluation 33

3.2.6 PCR-RAPD Assay 34

3.2.7 PCR-ISSR Assay 34

3.2.8 DNA analysis 36

3.3 Species Distribution Modeling of S. henningsii 36

3.3.1 Environmental variables 36

3.3.2 Model development and evaluation 37

3.4 Ethnobotanical Studies of S. henningsii 39

3.4.1 The Study Areas 39

3.4.2 Research Design 39

3.4.3 Target Population, Sampling, sample size and measurements 40

3.4.4 Methods of statistical data Analysis 40

CHAPTER FOUR 41

RESULTS 41

4.1 Introduction 41

4.2 Genetic diversity of S. henningsii 41

4.2.1 RAPD Analysis 43

4.2.2 ISSR analysis 51

4.3 Species Distribution Modeling of S. henningsii 57

4.3.1 Predicted potential suitable habitats for S. henningsii 58

4.3.2 Model Evaluation 58

4.3.3 Analysis of variable importance...61

4.4 Ethnobotanical studies of S. henningsii 66

4.4.1 Location, gender and Age category 66

4.4.2 Economic Activities 69

4.4.3 Land size and use 69

4.4.4 Ethnobotany of Strychnos henningsii 72

4.4.5 Trading on S. henningsii 75

4.4.6 Uses of S. henningsii...76

4.4.7 Extraction/ processing of S. henningsii..79

4.5 Synopsis of the major findings of the study...83

CHAPTER FIVE...84

DISCUSSION, CONCLUSION AND RECOMMENDATIONS...............................84

5.1 Introduction 84

5.2 Discussions....84

5.2.1 Genetic diversity of S. henningsii 84

5.2.2 Species Niche Modeling of S. henningsii 90

5.2.3 Ethnobotanical Studies of S. henningsii 94

5.3 Conclusions....98

5.3.1 Genetic diversity of S. henningsii 98

5.3.2 Species niche modeling of S. henningsii....98

5.3.3 Ethnobotanical study of S. henningsii....99

5.4 Recommendations....99

5.4.1 Genetic diversity of S. henningsii 99

5.4.2 Species niche modeling of S. henningsii....100

5.4.3 Ethnobotanical study of S. henningsii....100

REFERENCES....102

APPENDICES 147

LISTS OF TABLES

Table 3.1: List of ten RAPD primers used for molecular characterization of *S. henningsii* 34

Table 3.2: List of nine ISSR primers used for molecular characterization of *S. henningsii* 35

Table 3.3: Codes and details of the bioclimatic variables....................38

Table 4.1: Genetic diversity analysis of nine populations of S. henningsii as revealed using RAPD and ISSR markers in PopGene software....................45

Table 4.2: Genetic diversity analysis of nine populations of *S. henningsii* as revealed by RAPD and ISSR markers in GenAlex software.....................46

Table 4.3: RAPD population's Specific loci....................47

Table 4.4: Nei's (1978) unbiased measures of genetic Identity and Genetic Distance from RAPD markers in PopGene software....................48

Table 4.5: RAPD pairwise population matrix of Nei's unbiased measure of Genetic Identity and Genetic Distance using GenAlex software 49

Table 4.6: Analysis of Molecular Variance of *S. henningsii* as revealed by RAPD markers....................49

Table 4.7: ISSR population's Specific loci....................53

Table 4.8: Nei's (1978) unbiased Measure of Genetic Identity and Genetic Distance fom ISSR markers in PopGene software....................54

Table 4.9: ISSR pairwise matrix of Nei's (1978) Genetic Identity and Genetic Distance using GeneAlex software 54

Table 4.10: Analysis of Molecular Variance for *S. henningsii* as revealed by ISSR markers 55

Table 4.11: Age category of respondents in five study areas/locations 67

Table 4.12: Comparison between the age category and study areas 68

Table 4.13: Comparison between sex of the respondents and age categoty in five study locations 68

Table 4.14: Other types of economic activities apart from farming among the respondents from the study areas 70

Table 4.15: Mean land size in acreage among the study locations 71

Table 4.16: Comparison between study locations and land use 71

Table 4.17: Association and comparison of growth of *S. henningsii* on farmer's land among the study locations 73

Table 4.18: Association and comparison of sources of *S. henningsii* among the study areas 74

Table 4.19: Association and comparison on how *S. henningsii* was obtained among the study areas 75

Table 4.20: Trading on *S. henningsii* among the study areas 76

Table 4.21: Overall uses of *S. henningsii* in all five study areas 76

Table 4.22: Uses of *S. henningsii* among the study locations 77

Table 4.23: Plant parts used among the study locations 79

Table 4.24: Methods used for processing *S. henningsii* plant parts among the study areas 80

Table 4.25: Association and comparison on processing of *S. henningsii* plants parts among the study locations 81

Table 4.26: Mixing of *S. henningsii* plants parts with other substances 81

Table 4.27: Difficulties experienced with *S. henningsii* plant collection.........................82

Table 4.28: Suggested solutions to the identified difficulties by the respondents 82

LIST OF FIGURES

Figure 3.1: Map showing areas for *S. henningsii* genetic diversity study 32

Figure 3.2: Map showing areas for ethnobotanical study of *S. henningsii* in Kenya......39

Figure 4.1: RAPD marker profile of amplified loci of samples from Baringo population using primer KFP-23. Lane (1-30) are samples, M:-Marker DNA 100bp ladder..41

Figure 4.2: RAPD marker profile of amplified loci of samples from Narok population using primer KFP-14. Lane (1-30) are samples, M:-Marker DNA 100bp ladder..42

Figure 4.3: ISSR marker profile of amplified loci of samples from Baringo population using primer 862. Lane (1-30) are samples, M:-Marker DNA 100bp ladder42

Figure 4.4: ISSR marker profile of amplified loci of samples from Narok population using primer 818. Lane (1-30) are samples, M:-Marker DNA 100bp ladder43

Figure 4.5: A three dimensional plot of the Principal oordinate Analysis (PCA) of RAPD data showing the clustering of *S. henningsii* populations.................48

Figure 4.6: Percent of Molecular Variance for RAPD data...50

Figure 4.7: UPGMA cluster analysis of the nine *S. henningsii* populations based on Nei's (1978) unbiased genetic distance 51

Figure 4.8: A three dimensional plot of the Principal Coordinate Analysis (PCA) of ISSR data showing the clustering of *S. henningsii* populations....................53

Figure 4.9: Percentage of Molecular variance of ISSR data..55

Figure 4.10: UPGMA clustering analysis of nine *S. henningsii* populations based on Nei's (1978) unbiased genetic distance 56

Figure 4.11: Receiver Operating Characteristic with Area under the Curve (AUC) measure..60

Figure 4.12: Omission Vs Predicted area for *S. henningsii* 61

Figure 4.13: Jackknife test of regularized training gain for *S. henningsii* 62

Figure 4.14: Jackknife test of regularized test gain for *S. henningsii* 63

Figure 4.15: Jackknife test of regularized AUC for *S. henningsii* 64

Figure 4.16: (a-d): Relationship between the strongest variables and *S. henningsii* probabilities of presence...65

Figure 4.17: Percentage frequency distribution of respondents per study area. 66

Figure 4.18: Other activities engaged apart from farming...69

Figure 4.19: Land use across the study areas...70

Figure 4.20: Percentage frequency of *S. henningsii* growing on farmer's land 72

Figure 4.21: Frequency on how *S. henningsii* was obtained..74

Figure 4.22: Individual use of *S. henningsii* among the study locations 78

Figure 4.23: Parts of *S. henningsii* used among the study locations..............................79

LIST OF PLATES

Plate 1.1: a, b and c are *S. henningsii* Shrub, fruits and seed 2

Plate 1.2: a, b, c and d: Destruction of *S. henningsii* in Nyeri, Taita taveta and Ngong forest. 6

Plate 4.1: Model output showing the map of the predicted potential areas of *S. henningsii* in Kenya. 58

LIST OF APPENDICES

Appendix 1: Common thresholds and corresponding omission rates for the threshold dependent binomial test of omission 147

Appendix 2: Estimation of relative contributions of the enviromental variables to the MaxEnt model 148

Appendix 3: Questionnaire for ethnobotanical study 149

Appendix 4: Georeference points of individuals of *S. henningsii* selected from nine populations in Kenya 151

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
Arc GIS	Aeronautical Reconnaissance Coverage Geographic Information System
ASCII	American Standard Code for Information Interchange
AUC	Area under the Curve
BIOCLIM	BioClimatic variable
BRT	Boosted Regression Tree
CAPs	Cleaved Amplified Polymorphic Sequence
CIFOR	Center for International forest Research
CSV	Comma-Separated Values
CTAB	Cetyltrimethylammonium Bromide
DBH	Diameter at Breast Height
DNA	De-oxyribonucleic Acid
dNTPs	deoxyribonucleotides triphosphates
DSM	Decimal Minutes Seconds
EDTA	Ethylenediaminetetraacetic Acid
EPSG4326	European Petroleum Survey Group 4326
ESRI	Environmental Systems Research Institute

FAO	Food and Agriculture Organizaion
GARP	Genetic Algorithim for Rule-set Production
GIS	Geographical Information System
GLMs	Generalized Linearized Models
GTIFF	GeoTag Image File format
ICRAF	World Agroforesrty Center
ID	Identity
IPGRI	International Plant Genetic Resource Institute
ISSRs	Inter Simple Sequence Repeats
IUCN	International Union for Conservation of Nature
IUFRO	International Union of Forest Research Organisation
KEFRI	Kenya Forestry Research Institute
LSD	Least Significance Difference
MARs	Multivariate Adaptive Regression Splines
MaxEnt	Maximum Entropy
NCEAS	National Center for Ecological Analysis and Synthesis
NMK	National Museum of Kenya
PCR	Polymerase Chain Reaction

Ppm parts per milliom

PVP polyvinylpyrrolidone

QGIS Quantum Geographical Information System

RAMs Random Amplified Microsatellites

RAPD Random Amplified Polymorphic DNA

RFLPs Restriction Fragments Length Polymorphism

ROC Receiver Operating Curve

Rpm Revolution per Minute

RT Room Temperature

SDMs Species Distribution Models

SDS Sodium dedocyl Sulfate

SPSS Statistical Package for the Social Sciences

SSCP Single Stranded Conformation Polymorphism

SSRs Simple Sequence Repeats

TE Tri-EDTA

UV Ultra violet radiation

WGS84 World Geodetic System of 1984

Wrap Warwick Research Archive Portal

ABSTRACT

Strychnos henningsii (Gilg.) belongs to the family Loganiaceae. It is a narrow endemic and endangered medicinal plant species largely confined to the dry land parts of Kenya. It is highly over exploited for medicinal purposes, which has resulted in its decline in the natural habitats. A research study was therefore undertaken to determine the genetic diversity, species niche modeling and ethnobotany of *Strychnos henningsii* in order to come up with strategies for its conservation, cultivation and sustainable utilization. Genetic diversity study of two hundred and seventy individuals sampled from nine populations of *Strychnos henningsii* in Kenya was carried out using RAPD and ISSR markers.This was undertaken to determine the genetic variation within and among its populations and recommend those that revealed sufficient genetic variation as the best sources of the planting materials. Ten RAPD and nine ISSR primers were selected from forty RAPD and ISSR markers screened for the study. The mean percentage polymorphism detected was 38.97 % for RAPD and 43.40 % for ISSR markers. The mean values of Shannon information index (I) and genetic diversity (H) showed by these primers were 0.3310, 0.2005 (RAPD) and 0.4473, 0.2889 (ISSR) indicating low genetic variability among *.S. henningsii* genotypes. The similarity coefficient based on the Nei's unbiased genetic distance revealed low level of divergence among the different populations of *S. henningsii*. The values ranged from 0.8422 to 0.9796 (RAPD) and 0.7239 to 0.8803 (ISSR) AMOVA analysis revealed high genetic variation among populations than within populations ie 54 % and 46 % ($p>0.001$) respectively for RAPD and 58 % and 41 % ($p>0.001$) for ISSR analysis. Low genetic diversity could be attributed to the self pollination mechanism in *S. henningsii.* Therefore, conservation efforts should aim to preserve all the extant populations of this threatened species. The potential suitable habitats for growth and conservation of this species in Kenya were determined using maxEnt modeling method. MaxEnt performance in delineating the potential distribution areas of *S. henningsii* in Kenya was good and reliable. The area under the ROC curve (AUC) indicated the values for training and test data were 0.986 and 0.983 respectively. These values were close to 1 thereby showing the accuracy of the model in prediction. The predicted areas were Taita hills, shimba hills, Kilifi (Arabuko sokoke forest), Mt Kilimanjaro game reserve), Marsabit, Huri hills, Marigat, Baragoi, Malaral (dryland areas in Kenya), Ngong and Karura forests. These aera could be targeted for conservation of *S. henningsii*. Ethnobotanical study was conducted on *Strychnos henningsii* to determine the traditional knowledge of this species by the local people using semi structured questionnaires to collect the data. The study showed clearly that *Strychnos henningsii* a wide range of uses in all the areas of study and that majority of the respondents (50 %) obtained the plant species with lot of difficulty. The key difficulties identified were scarcity (29 %) and long distance walking (35 %) indicating the decline of this species in its natural habitats. Of all the persons interviewed sixty eight percent did not grow *Strychnos henningsii* in their farms while ninety eight percent collected their plant materials from the forests. The results also indicated that the roots (43 %) and stem (47 %) were the most frequently used plant parts for medicinal purposes and often crude methods which are destructive to this species are employed to

collect the plant materials. The respondents suggested that training them on proper harvesting methods (32 %), establishment of nurseries (23 %) and planting this species locally (33 %) would be some of the ways to overcome the difficulties experienced while dealing with not only this species but also many other medicinal plant species.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

1.1.1 Description of *Strychnos henningsii*

Strychnos henningsii (Gilg.) belongs to the family Loganiaceae. The genus Strychnos has about 190 species mainly found in the tropics. The species epithet honors Professor Paul Christopher Henning, a mycologist at the Loyal Botanical Gardens, Berlin-Dahlem. The Synonyms include *S. albersii* (Gilg.), *S. barbata* (Chiov.) and *S. elliotii* (Gilg.) among others (Maundu & Tengäs, 2005). The common names are Red bitter berry (English) (Gachathi, 2007), Henning's Strychnos (Maundu & Tengäs, 2005). The local names include Muteta (Kikuyu & Kamba), Maset (Kipsigis), Entuyesi (Maasai), Mutambi (Mbeere), Muchimbi (Meru), Kapkamkam (Pokot), Nchipilikwa (Samburu), Hadesa (Somali), Turukukwa (Tugen) and Yapoliss (Turkana) (Maundu & Tengäs, 2005).

It varies in size from a shrub or small erect tree, much-branched tree of about 2 to 15 m tall with green-reddish stem (Plate 1.1a). The bark is pale grey and smooth in young trees but becomes dark brown and somewhat flaky in specimens. The twigs have pale ashy or straw-colored and waxy skin splitting lengthwise. Lenticels are few and inconspicuous. Leaves are opposite, sub-sessile or ovate, 2.5 to 6.5 cm long and 0.8 to 4.5 cm wide. They have entire margin and acuminate leaf tips. The leaves are strongly three to five nerved from base cuneate or rarely sub-cordate at base; a characteristic feature in Strychnos species (Van wyk *et al.*, 1997). Floral cymes is borne on flat clusters in the leaf axils, 2 to 2.5 mm long and 4 mm wide when open, scented, yellowish-green in color turning orange with age. The ovary is globose with a short style. The fruit is up to 1.9 cm long and 6 to 11 cm wide (Plate 1.1b), oblong or roundish

with one to two seeds (coffee-like) red, brown or orange when ripe (Plate 1.1c) (Beentje 1994; Gachathi 2007; Maundu & Tengäs 2005).

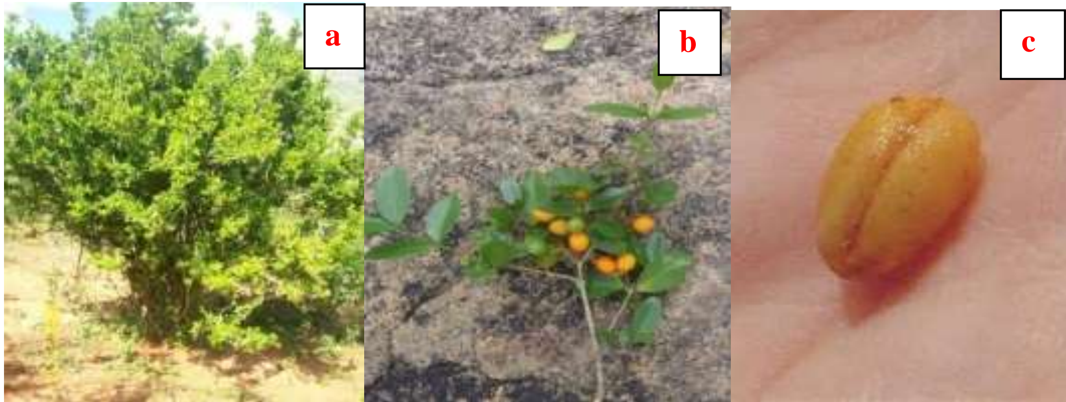


Plate 1.1: a, b and c are *S. henningsii* Shrub, fruits and seed

S. henningsii commonly occurs in the dry and moist forests, wooded hillsides, and thickets, on rocky hills, coastal forests and stream banks. It is native in Angola, Mozambique, South Africa, Swaziland, Tanzania and Uganda. In Kenya it is widely distributed in Nairobi, Kakamega, and in the Central province. It is often associated with dry Podocarpus and Olea forests, hillsides, thickets and *Combretum* bushland (Maundu & Tengäs 2005).

1.1.2 Propagation and Management

It is raised from seedlings or wildings. The species also suckers well. The pulp is removed before sowing the seeds. The seeds exhibit orthodox storage behavior. It is managed through pruning and coppicing (Maundu & Tengäs 2005).

1.1.3 Economic Importance

In the African traditional medicine, it is used for the treatment of various ailments including rheumatism, gastrointestinal complications, abdominal pains, syphilis, and possibly of value in dysmenorrhoea (Hutchings, 1989; Watt & Breyer, 1962; Pujol, 1993; Hutchings 1996; Oyedemi *et al.,* 2009). Roots bark and green fruits of Strychnos species are used as a remedy for snakebites (Tits *et al*., 1991; Van Wyk *et al*., 1997) and hookworm infections in Tanzania (Oyedemi *et al.,* 2009). The bark decoction is employed as a remedy for rheumatism and arthritis (Palgrave, 1988; Beentje, 1994). A decoction of the plant has been used in traditional Kenyan medicine for the treatment of rheumatism, gynecological complaints, chest pain, internal injuries and malaria (Kareru *et al.*, 2007). The ground bark is a mouth antiseptic and applied onto the wounds in cattle and horses to hasten healing (Gachathi, 2007).

In South Africa, the decoction or infusions from the stem bark is widely used for the management of Diabetes mellitus (Oyedemi *et al.,* 2009). The aqueous bark extract is also used in South Africa for the treatment of stomache, colic, dizziness and as a purgative agent (Oyedemi *et al.,* 2013). About five compounds have been isolated including indolinic alkaloids, strychnine, brucine, curanine, and bitter glycosides with significant values (Penelle *et al.,* 2000; Oyedemi *et al*., 2010a). Other compounds that have been isolated from the stem and root bark of *S. henningsii* are holstiine, diaboline, strychnochromine and guianensine (Angenot & Tits 1981). Strong antioxidant and free radical scavenging activity have been observed and attributed to the presence of phenolic compounds in *S. henningsii* extracts indicating the ethnotherapeutic usage of this plant for the management of oxidative stress induced diseases (Oyedemi *et al.,* 2011).

S. henningsii's **medicinal value as an appetite stimulant has long been recognized by the indigenous people of Kenya (Ogeto, 1983). In East Africa,** ***S. henningsii*** **is used in the preparation fatty-meat and milk soups (Chapman** ***et al*****., 1997). Roots, stem and bark are boiled in soup for fitness, painful joints and the general body**

pains among the Kikuyu, Maasai and Kamba communities (Palgrave, 1988; Beentje, 1994; Maundu *et al*., 1999; Gachathi, 2007). The soup is claimed to be an aphrodisiac and is used as a remedy for colic, to relieve nausea and treat syphilis (Palgrave, 1988).

The fruits and the bark contain a poisonous bitter alkaloids; used in traditional medicine as a purgative (Palgrave, 1988; Noad & Birnie, 1989). Some alkaloids have been used in anesthesiology due to their muscle relaxing effects (Bruneton 1995). Mbeere people use the fruits to flavor their beer (Maundu *et al*., 1999; Gachathi, 2007) (Njoroge *et al.,* 2010). *S. henningsii* has a potential in the development of new antinociceptive and antispasmodic drugs due to the presence of retuline-like alkaloids (Penelle *et al.,* 2000), (Tits *et al*., 1991). *In Vitro* anti-diabetic effects of *S. henningsii* stem bark extract have been demonstrated on the fasting blood glucose and glucose tolerance in diabetic rat (Oyedemi *et al.,* 2011).

It is valued timber is dark gray, heavy, hard, durable and termite resistant. Wood is used for fencing and making hut poles and tool handles by Maasai community (Maundu & Tengäs, 2005; Gachathi, 2007). The species is important in protecting soils from water erosion in highland areas. Its physical attributes, shiny foliage, pleasant shade and fragrant flowers make it a suitable choice for gardening (ICRAF, 1992).

1.2 Statement of the problem

The substantial contribution to human health and well-being made by medicinal plants is now well accepted. Indeed, there is an increased demand for these species both in developing and developed countries. For instance, *S. henningsii* was reported as a useful plant species in the control of malaria and Kenya being one of the tropical countries, the fight against malaria is never ending. It is a plant species with a wide scale use for medicinal purposes. This has resulted in higher number of harvesters targeting scarce resources. It suffers from over harvesting of its bark, stem, roots and green fruits for

medicinal use as shown in plate 1.4 below. The methods used to obtain the plant materials are crude and destructive to the plant. *S. henningsii* is also characterized by having restricted geographical distribution in the areas of occurrence, slow grwth rrate and poor seed germination. These factors and over exploitation exceed the natural regeneration of this plant species nd hence calls for efforts for its conservation.

Plate 1.2: a, b, c and d: Destruction of *S. henningsii* in Nyeri, Taita taveta and Ngong forest.

1.3 Justification

The dwindling of the *S. henningsii's* abundance in the wild habitats due to over-exploitation calls for its conservation. Therefore the study on genetic diversity of *S. henningsii* will assist in identifying the populations with sufficient genetic variation within and among the populations and recommend them as sources of planting material. Genetic diversity is a primordial component of biodiversity and is related to the geographical distribution of a species. It is also an important consideration in species conservation because it influences a population ability to adapt and survive to a changing environment. Due to the wide scale medicinal use of *S. henningsii,* understanding the genetic variation within and among the natural populations of *S. henningsii* is a pre-requisite to formulate appropriate strategies for conservation, utilisation and genetic improvement. A reduction in genetic variation is thought to affect the ability of a population to adapt to the changing environment and survive or can result to unsustainable production due to inbreeding depression

Species distribution modeling will assist in determining the potential suitable distribution areas where the required environmental conditions exists for this species. Such areas can be useful in conservation of identified populations of *S. henningsii.* The pattern of genetic variability revele may be associated to the environmental conditions characterizing the geographical areas of this species and thus reflecting its adaptability and survival in those regions

Ethnobotanical study serves as a bridge between conservation and local community and also has considerable information about the use of many plants or plant parts. Ethnobotany also helps in understanding how local communities relate to environment

and pave way for their active involvement in natural resource conservation. The pattern of genetic variation revealed may also be associated to the varied ethnobotanical uses of this species in different geographical areas. This is because genetic variation implies that the expression of genes for enzymes that help in the biosynthesis of secondary metabolites (responsible for the medicinal properties and help plants to cope up with environmental stresses) varies depending with the environmental areas where the plant grows. From the literature survey, information on genetic diversity, niche modeling and ethnobotany of *S. henningsii* is lacking. Therefore the finding of this research study aims to provide useful information towards conservation of *S. henningsii.*

1.4 Hypotheses

1 There is no genetic variation within and among populations of *S. henningsii* in Kenya

2 There is no potential distribution areas existing for growth and restoration of *S. henningsii*

3 There are no differences in the traditional knowledge of *S. henningsii* from different communities in the areas where it grows in Kenya.

1.5 Broad Objective

To undertake a study on genetic diversity, species niche modeling and ethnobotany of *Strychnos henningsii* in Kenya

1.6 Specific Objectives

1. To assess the extent of genetic variation in the natural populations of *S. henningsii* using RAPD and ISSR markers.
2. To determine the potential distribution areas for reintroduction and growth of *S. henningsii* in Kenya
3. To evaluate the ethnobotanical knowledge of *S. henningsii* from the local communities in the areas where it grows.

1.7 Scope of the Study-

This study focuses on providing useful insights for conservation and sustainable utilization of *S. henningsii.* This was achieved by assessing the genetic variation within and among nine populations of this species in Kenya as stipulated in the materials and method section. The study also determined the potential suitable areas that exist in Kenya for conservation and reintroduction of *S. henningsii.* This was carried out using the environmental variables in the areas of *S. henningsii* occurrence and were projected to the areas of study in Kenya to highlight the areas that meet the requirements for growth of this species. These areas were then modeled using MaxEnt software and the results presented in form of a map. The study also evaluated the ethnobotanical study of *S. henningsii* in five selected counties as in the materials and methods section.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter discusses other research studies that have previously been done by other researchers in this area of research study. More over this chapter also reveal the current techniques that have been used in conducting research in the areas of study and present the techniques that have been used in achieving the specific objectives

2.2 Population genetic diversity

The conservation of threatened and endangered medicinal plants species is indispensable for the future humankind. The number of threatened species is increasing annually as a result of adverse environmental conditions and anthropogenic activities (Khan *et al*., 2012). Over exploitation including habitat destruction, expansion of urban activities, overgrazing, selective species removal and mutilation is leading to genetic erosion (Gaafar *et al.*, 2014). Therefore, scientific approaches to the conservation and utilization of these genetic resources require detailed knowledge of their genetic diversity (Dawson *et al.*, 1993). Genetic diversity refers to any variation in nucleotides, genes, chromosomes, or whole genomes of organisms. Genetic diversity can be assessed at different levels within a species or among species (Wang *et al*., 2009). Phylogenetic relationships reflect the relatedness of a group of species based on a calculated genetic distance (sequence conservation or diversification) in their evolutionary history (Wang *et al*., 2009).

The determinants of the level and structure of genetic variation within plant species include mating systems, evolutionary history, life history characteristics, population density and mechanisms of gene flow (Schaal, 1980; Loveless & Hamrick, 1984; Hamrick *et al*., 1992; Hamrick & Godt, 1996; Loveless, 1992). In addition to the within

species issues, potentially complex interactions among species must be considered (Rieseberg & Brusfeld, 1992; Riesberg & Wendell, 1993).

Genetic diversity within populations is of great concern to the conservation biologists because paucity of genetic variation is thought to reduce the ability of populations to adapt to the changing environments and to survive thereby causing their extinction (Beardmore, 1983; Yang *et al*., 2012). Therefore, an understanding of the patterns of genetic variation within and among populations of medicinal plants is essential for devising optimum genetic resource management strategies for their conservation, sustainable utilization and genetic improvements (Panda *et al*., 2015; Souza *et al*., 2012; Yang *et al*., 2012; Yeh, 1989; Hamrick, 1993).

Natural populations of medicinal plants species are extensively exploited due to their heavy demands. In such cases, long term survival of many medicinal plants depends on the maintenance of sufficient genetic variability within and among populations to accommodate new selection pressures exerted by continuous environmental changes (Barrett & Kohn, 1991). There are various approaches to assess genetic diversity in organisms including:-

2.2.1 Morphological markers

Traditionally, morphological character and various reproduction traits have been used to characterize levels and pattern of diversity. These traits alone represent only a small proportion of the genome. They are also influenced by the environmental factors thereby limiting their utility in describing the potentially complex genetic structure, which may exist within and among the taxa (Avise, 1994). To overcome these constraints, biochemical and molecular approaches have been devised for detection of genetic variability.

2.2.2 Molecular markers

2.2.2.1 Isozymes

The use of isozymes has been applied more than any other molecular technique to characterize patterns of genetic diversity and differentiation in plant species (Dawson & Chamberlain, 1996; Second, 1982; Tanksley & Orton, 1983). This is because of the simplicity of the approach, which allows data to be collected quickly from a large number of samples, low cost compared to the other molecular techniques, early recognition and availability in the history of marker development (Avise, 1994). This technique is limited by the relatively low level of polymorphism detectable (Dawson & Chamberlain, 1996). The weakness of isozyme markers relies on the expression of analyzed proteins which depend on tissue/organ, development stage, and also environment (Kumar *et al.,* 2009).

2.2.2.2 Restriction Fragments Length Polymorphism (RFLPs)

This technique overcomes the problems of isozymes. Its utility relies on the ability of the restriction endonucleases enzyme to cut the DNA at specific recognition sites within the genome of an organism (Bernatsky & Tanksley 1989). If individuals differ in their distribution of recognition sites, so that the fragments of DNA of different lengths are created after digestion, the polymorphism can be resolved as the mobility differences during the gel electrophoresis. Detection of variation at individual loci requires DNA fragments resolved by electrophoresis to be blotted on the nylon membranes and probed by a radioactively labeled DNA sequence complementary to the locus in question (Dawson & Chamberlain 1996). The probe binds specifically to the corresponding nucleotide sequences on the membrane, allowing that locus to be detected by autoradiography.

The RFLPs are co-dominant markers and could potentially detect all alleles from a given locus which may be highly desirable for detecting recessive traits (Kesawat & Kumar,

2009). RFLP analysis is therefore a very powerful tool in genetic, taxonomic, and evolutionary studies of plants. However, this technique is time-consuming and labor intensive due to blotting and hybridization with radioactively labeled probes and requires large amount of pure DNA (Kumar *et al*., 2009). RFLP analysis has been use to study genetic diversity in various plant genera including Lycopersicon (Miller & Tanksley, 1990), Leucaena (Hughes & Harris, 1994), and Vicia (Ven Van Den *et al*., 1993). The use of RFLP analysis in tropical trees has been reviewed by Neale *et al*., 1992). Arie F B *et a*l., (2018) reported on studies of genetic diversity of bioactive Plants of family Verbenaceae and Lamiaceae using RFLP and AFLP markers.

2.2.2.3 Polymerase Chain Reaction- based markers

The development of the Polymerase Chain Reaction (PCR) (Bachmann, 1994) has caused revolution in the analysis of plant and animal genetic variation. The technique allows the selective *in vitro* amplification of DNA. It involves the binding of short DNA sequences (oligonucleotides) to complementary sequences in the DNA of the organism. This is followed by the synthesis of new DNA primed by these oligonucleotides and catalyzed by DNA polymerase, using the supplied deoxyribonucleotides triphosphates (dNTPs) as substrates.

DNA amplification is then brought about by repeated temperature-controlled cycling through three basic steps namely; denaturation of the double stranded DNA to produce two complementary single strands, annealing of the oligonucleotides (primers) to the target sequences using the single stranded DNA and extension of the primers using the target DNA as the template, thereby producing new double stranded DNA copies of the original DNA (Brown, 1995). The amplification is automated by employing thermal cycling machines and an extremely thermostable *Taq* polymerase (Saiki *et al*., 1988) capable of surviving at 95 °C, the temperature required to denature the double stranded DNA. Based on PCR techniques, a wide range of approaches has become available for the detection of genetic variation in plant and animals (Bachmann, 1994). These

methods differ in the type of primers employed for analysis, which can be targeted to specific regions, or chosen at random to amplify unspecified regions in the genome.

Targeted approaches include Cleaved Amplified Products (CAP) analysis in which the detection relies on restriction endonucleases digestion of PCR products in a manner similar to RFLP analysis, but avoiding the requirement of the large initial quantities of DNA or radioactive detection for example genetic variation study in *Crassostrea virginica* (Karl & Avise, 1992). Single Stranded Conformation Polymorphism (SSCP) analysis, in which differences are detected as changes in secondary structure between single stranded DNA on non-denaturing gels, is an example of genetic variation study in *Homo sapiens* (Orita *et al*., 1989) and Simple Sequence Repeat (SSR) analysis in which the length variation is assessed across short iterative repeats in the genome (e.g. in *Dioscorea tokoro* (Terauchi & Konoma, 1994).

2.2.2.3.1 Random Amplified Polymorphic DNA (RAPD)

Random Amplified Polymorphic DNA (RAPD) analysis is based on unspecified targets. It relies on primers or arbitrary sequences to detect polymorphism as the presence or absence of amplified products between individuals. In this case, the basis of polymorphism apparently reflects differences in complementarity at potential primer binding sites, either allowing or preventing binding and amplification (Williams *et al*., 1990).

The use of RAPD markers does not need either large amount of DNA, prior knowledge of the DNA sequences, less labor intensive and does not require the use of radioisotopes (Cardoso *et al*., 1998; Ganie *et al*., 2015). It requires short oligonucleotides (primers) of arbitrary sequence use to achieve random amplification of DNA. These primers detect polymorphisms in the absence of specific nucleotide sequence information and polymorphism acts as genetic markers (Williams *et al*., 1990). However RAPD markers have got reproducibility problems (Moulin *et al*., 2012; Safari *et al.,* 2013; Sarwat *et al.,* 2016) but the method will probably be important as long as other DNA-based techniques

remain unavailable in terms of cost, time and labor (Lashermes *et al*., 1996). It is also a dominant marker which does not allows the differentiation between some heterozygous and homozygous individuals (Priyono & Putranto, 2014).

RAPD provide an accurate measure of the distribution of genetic variation over populations and geographical areas (Russell *et al*., 1993). RAPD analysis has been applied widely to assess genetic variation in plants, including *Eucalyptus globulus* (Nesbitt *et al.,* 1995) and *Populus* (Liu & Furnier, 1993), *Breonadia salicina* (Gaafar *et al*., 2014), *Rheum officinale* (Wang *et al*., 2012). Its utilization in studies of genetic diversity of medicinal and aromatic plant species is extensive including *Lepidium sativa* (Bansal *et al*., 2012), Shankhpushpi (Ganie *et al*., 2015), *Dendrobium nobile* (Bhattachryya & Kumaria, 2015), *Catharanthus roseus* (Shaw *et al*., 2009), *Achilee fragrantissims* (Rawashdeh *et al*., 2011), *Withania somnifera* (Khanna *et al*., 2014), *Brassica* species (Prajarat *et al*., 2015), *Morinda citrifolia*, (Bordallo *et al.,* 2017), *Coptis omeiesis* (Zhang *et al*., 2010), *Cassia tora* (Tilwari *et al*., 2016) and *Carum carvi* (Labiri *et al*., 2011).

2.2.2.3.2 Amplified Fragment Length Polymophism (AFLPs)

The Amplified Fragment Length Polymorphism (AFLPs) technique was developed by Vos *et al*., 1995). It utilizes fragments of DNA amplified using direct primers from restriction digested genomic DNA (Vos *et al*., 1995). The AFLP marker technique is robust, safe, and highly reproducible, and it allows the detection of high levels of polymorphism. It does not also require prior knowledge of the of an organism's genome (Kladmook *et al.,* 2010). This method has bee used widely in assessing the genetic variation in many medicinal plant species. They include *Adhatoda vasica* and *Andrographis paniculata* (Varma & Neeta 2018)*, Croton antisyphiliticus* Mart. (Oliveira *et al.,* 2016), *Zingiber cassumunar* Roxb (Kladmook *et al*., 2010) among others.

2.2.2.3.3 Simple Sequence Repeat (SSRs)

A major advancement in molecular markers was the discovery that up to thirty to ninety percent of the genome in most eukaryotes and to a lesser extent in prokaryotic species comprises of polymorphic repetitive DNA (Jarne & Lagoda 1999; Vaughan & Lloyd 2003). These regions contain loci composed of several hundred allelic forms differing in length, sequence or both (Wang *et al.*, 2009). They are interspersed in tandem arrays ubiquitously. They are also the locations of numerous genomic mutations. Therefore, repetitive DNA and mutations together form the basis of a number of markers that are useful for various applications in plant genetic studies (Wang *et al*., 2009).

SSRs are also referred to as microsatellites and minisatellites (Jeffrey's *et al.*, 1985). Minisatellites are tandem repeats with monomer repeat length of about 11 to 60 base pair while microsatellites consists of 1 to 6 base pair long repeated monomer sequence (Jeffrey's *et al*., 1985). These loci contain variable number of tandem repeats units between genotypes. Microsatellites are targeted by designing specifying primers of about 18 to 24 base pairs, which bind on either side of the repeated elements and amplified using PCR analysis .The difference in repeat length among individuals within a population can be assessed by separating the respective PCR products using electrophoresis followed by gel imaging (Wang *et al*., 2009). Currently automated sequencer systems are accessible to most researchers to detect microsatellites alleles (Gyllenstrand *et al.* 2002; Exeler *et al*. 2008; Kim *et al*. 2008a; Pol *et al*. 2008).

SSRs markers have great potential in genetic and breeding studies (Shoba *et al*., 2010). They have advantageous features over many other markers as they are highly polymorphic, robust, can be automated; only very small DNA is required, highly abundant, analytically simple, multi-allelic in nature, chromosome specific, readily transferable and have a co-dominant inheritance (Matsuoka *et al.*, 2002; Rakshit *et al*., 2012a; Wang *et al.*, 2014). SSRs have widely been used for assessment of genetic diversity in several crop species including Sorghum (Rakshit *et al*., 2012a), Groundnuts (Shoba *et al*., 2010), Chinese spring soybean (Wang *et al*., 2008), Cassava (Montero-

Rojas *et al.,* 2011), cultivated and wild sorghum (Mutegi *et al*., 2011), *Vitis vinifera* (Doulati-Baneh *et al*., 2013), Mulberry (Wangari *et al*., 2013), *Shorea platyclados* (Javed *et al*., 2014).

2.2.2.3.4 Inter Simple Sequence Repeats (ISSRs)

ISSRs markers were developed by Zietkiewicz *et al.,* (1994) and are known as Random amplified microsatellites (RAMs). ISSRs are segments in the genomic DNA that are flanked on both ends by microsatellites segments. Using arbitrary primers that contain repetitive sequences complementary to the microsatellites regions in the genome, random DNA fragments in the genome can be PCR amplified and used as a dominant multi-locus marker for genetic variation/diversity studies (Warner *et al*., 2015; Ng WL & Tan SG, 2015). ISSRs markers are highly variable and ubiquitously distributed across the genome achieving higher reproducibility compared to RAPDs and less costly in time and money compared to AFLPs. They also do not require prior knowledge of the genomic DNA sequences (Coral *et al*., 2016; Feitosa- Alcantara *et al*., 2017). These markers have been used successfully for various studies in genetic variation/diversity such as *Rheuma palmatuma and R. tanguticum* (Wang *et al*., 2012); *Tomistoma schlogelii* (Shafiei-Astani *et al*., 2015), *Satureja bachtiarica* (Khadivi *et al.,* 2015), *Varronia curassarica* (Brito *et al*. 2016), *Hyptis pectinata*, (Feitosa-Alcantara *et al*., 2017), *Capparis spinosa L*., (Liu *et al*., 2015), Rheum species, (Tabin *et al*., 2016), *Withania somnifera* (Khan and Shah 2016), *Peganum harmala* L. (Zerbarjadi *et al.,* 2016) and *Croton tetradenius* (Almeida-Pereira *et al.,* 2017) among others. RAPD and ISSR Markers have also been widely utilized in combination in the studies of genetic diversity of medicinal plant species including *Costus Pictus*, (Naik *et al*., 2017), *Canthium parviflorum* (Kala *et al*., 2017), *Penthorum chinense* (Mei *et al*., 2017), *Hedychium coronarium* (Parida *et al*., 2017), *Stevia rebaudiana* (Sharma *et al*., 2016) and Ocium species (Sarwat *et al*., 2016).

2.3 Species Distribution modeling in conservation strategies

Human beings have a profound impact on the geographical distribution of plant populations and environmental degradation has become a serious problem (Miller & Knouft 2006). Methods for establishing biodiversity conservation priorities are urgently required as the number of species and habitats that are threatened increases relative to the material resources available for their conservation (Balram *et al.,* 2004). The identification of priority areas demands the integration of biophysical data on ecosystems together with social data on human pressures and planning opportunities (Balram *et al.,* 2004). However, comprehensive and reliable data are rarely available to demarcate where the need for action is most urgent and where the benefits of conservation strategies can be maximized (Balram *et al.,* 2004).

Predictive modeling of species geographical distribution based on environmental conditions of sites of known occurrence is an importance technique applied in biological fields such as ecology, systematic, evolution conservation among others (Peterson, 2006; Peterson & Shaw, 2003; Scott *et al*., 2002; Welk *et al*., 2002). Predicting and mapping of potential suitable habitat for threatened and endangered species is critical for monitoring and restoration of their declining native populations in the natural habitats, artificial introductions or selecting conservation sites and conservation and management of native habitats (Gaston, 1996). However, distribution data of threatened and endangered species are often sparse and clustered making it difficult to model their suitable distribution using commonly used modeling approaches (Kumar & Stohlgren, 2009).

Most biodiversity data exist in a difficult, fragmented system, sampling only document presence but rarely absence; sampling is rarely systematically planned so as to permit detailed statistical analysis and institution hold specimens in different countries and regions (Peterson *et al.,* 1999). Inferential procedures that provide robust and reliable prediction of species' geographical distributions thus become critical to biodiversity

analyses (Peterson, 2001) and therefore ecological niche models have been developed to solve this problem.

Species distribution models (SDMs) are also known as ecological niche models. They are algorithmic tools that relate known occurrences of species to environmental conditions that meet a species ecological requirement and predict the relative suitability of habitat (Elith & Leathwick, 2009; Peterson, 2006; Warren & Seifert, 2011). Distribution Models are broadly classified into two groups: "correlative models" and "process-based or mechanistic models" (Dormann *et al.,* 2012).

Correlative models associate species occurrence data with spatial environmental layers of the area of study and produce maps of probability presence or relative suitability of a species (Kumar *et al*., 2014). The process based or mechanistic model uses species' functional traits and physiological tolerances for model fitting (Kearney *et al*., 2010). This approach requires detailed experimental data that may not be available for the target species (Dormann *et al.,* 2012). Both methods have been used in quantifying and mapping the potential distribution of species in areas outside their current distributional range (Lozier & Mill, 2011; Ni *et al.,* 2012). These approaches have found extensive application in ecology, systematic and conservation (Elith *et al*., 2006; Peterson *et al*., 2003; Yang *et al*., 2013) and have been used for prioritizing field surveys (Graham *et al*., 2008). SDMs are often used to estimate the relative suitability of a habitat in geographical area not known to be occupied by the species.

SDMs in its simplest manifestations provide a framework by which one can interpolate between known populations of a species and to anticipate existence of other unknown populations. Some species are sufficiently poorly unknown or endangered that finding new populations can make a difference in understanding their distributions and planning for their conservation. SDMs can also be used to predict the geographical distributions of unknown species closely related to known species. Examples of such studies have been reported by (Bourg *et al.,* 2005; Raxworthy *et al*., 2003).

SDMs approaches have been used to estimate the relative suitability of a habitat in geographical area known to be occupied by the species. However, elements of biodiversity are so poorly known that the first key step is basically to understand the ecological requirements relevant to a species geographical distribution. But for most species only a few geographical occurrences are known without detailed information about their natural history, ecology, behavior etc. Some information can be inferred from SDMs; such studies have been published by Austin and Meyers 1996; Costa *et al*., 2002; Luoto *et al.*, 2006; Ron 2005; Peterson *et al*., 2004a. Identification of areas where appropriate environmental conditions exists to sustain a species is vital to biogeographical and conservation studies (Chefaoui *et al*., 2005). It allows identification of environmentally suitable regions that are still not colonized or where species has become extinct; then the contribution of unique historical or geographical factors to the shaping of the current distribution can be judged (Chefaoui *et al*., 2005).

For conservation purposes, identification of potential distribution area can help locate sites suitable for translocation and reintroduction programs. SDMs provides a framework within which areas may be evaluated for their potential suitability for establishment of populations of species under intensive conservation management examples (Danks & Klein, 2002; Peterson *et al.,* 2006a). Species niche modeling of potential distribution has been used to evaluate the different ecological and evolutionary aspects such as competition between phylogenetically related species (Anderson *et al*., 2002) or variation in species niche requirements through evolutionary times (Peterson *et al*., 1999; Peterson & Holt, 2003).

SDMs also have considerable potential in identifying geographical phenomena that limit species distributional potential. Therefore, SDMs provide a tool by which the biogeography of a species can be evaluated giving information about the species distribution that was basically unavailable Such SDM application include (Anderson *et al.,* 2002; Graham *et al*., 2004; Robertson *et al.,* 2004). Other applications of SDMs are to predict effects of habitat loss, potential for species invasion and climate change effects (Peterson *et al.,* 2006a).

There are many methods that have been used in species distribution modeling including BIOCLIM, DOMAIN, CLIMEX, GAM, GLM, GARP and Maximum entropy (MaxEnt) (Kriticos & Randall 2001; Phillips *et al*., 2004; Guisan & Thuiller 2005; Elith *et al.,* 2006; Sun & Liu, 2010). MaxEnt unravels the best predictive capacity and was the most precise model (Moffett *et al.,* 2007; Wang *et al.,* 2007; Giovanelli *et al.,* 2008; Saatchi *et al*., 2008; Wu & Li, 2009; Kumar *et al*., 2014) giving the most accurate distribution function based on best entropy (Kumar *et al.,* 2014). Several studies comparing up to sixteen of modeling approaches indicated that maxent performed well or better than other approaches (Elith *et al*., 2006; Hernandez *et al.,* 2006; Phillips *et al*., 2006). The use of these models/algorithms is guided by the ultimate objective of the study (Jaryan *et al*., 2013).

BIOCLIM is a model that identifies locations where the climatic indices fall within the range that has been determined based on ground observations. The model treats each climatic axis independently, thus leading to unsound predictions (Carpenter *et al*., 1993). The model does not perform well with respect to precipitation. DOMAIN is a multivariate distance-based model. Though it uses presence only data, performance of the model is limited and sometimes additional information on absence location is required (Graham *et al*., 2008). GLM is a linear regression model that considers response variables that have other than normal distribution (Guisan *et al*., 2002). Compared to traditional models, such as DOMAIN, GLM performs well (Elith *et al*., 2006).

MARS provides an alternative regression-based method for fitting nonlinear responses using piece-wise linear fits (Elith *et al*., 2006). It is faster than GLM, but highly sensitive to sample size (Wisz *et al*., 2008). BRT constructs a combination of trees and is quite effective. It is difficult to identify the significant predictor variable in BRT and it is relatively time-consuming (Graham *et al*., 2008). Maxent is a comparatively popular model for accurately predicting species distribution and therefore it has been recommended (Wisz *et al*., 2008; Meyer *et al*., 2006; Berry *et al*., 2002; Hijmans *et al*., 1999).

MaxEnt is a maximum entropy-based method in which relative entropy is minimized between the two probabilities of presence data and area of study (Elith *et al*., 2011). It focuses on relating the environmental conditions of the area where a species is present to the environmental conditions across the area of study (Phillips *et al*., 2006; Phillips & Dudik 2008). Thus maxEnt estimates a target probability distribution by finding the probability distribution of maximum entropy i.e. most spread out or closest to uniform, subject to a set of constrains that represent our incomplete information about a species distribution (Phillips *et al*., 2004; 2006; Grendár & Grendár 2001).). It focuses on relating the environmental conditions of the area where a species is present to the environmental conditions across the area of study (Phillips *et a*l., 2006; Phillips & Dudik 2008). Therefore the resultant output represents how much better the model fits a location data than would a uniform distribution (Phillips *et al.*, 2004; 2006).

MaxEnt has been found to perform best among many different modeling methods (Elith *et al*., 2006; Ortega-Huera & Peterson 2008) and remain effective despite small sample sizes (Hernandez *et al*., 2006; Peterson *et al*.,2007; Papes & Gaubert, 2007; Wisz *et al*.,2008; Benito *et al*., 2009). MaxEnt offer many advantages over the other methods that use presence data only together with environmental information for the whole study areas. It can utilize both continuous and categorical data (Baldwin, 2009), insensitive to a sample size (Edith *et al.,* 2006; Phillip *et al*., 2006), in some cases only five locations were used to develop a model (Hernandez *et al.,* 2007; Pearson *et al*., 2007) although greater than 30 locations are recommended as more locations are likely to result in a more precise and accurate model (Wisz *et al*., 2008). The reason for MaxEnt's relative insensitivity to sample size appears to be driven by its regularization procedure which compensates for over fitting when using only a few locations. The relatively small number of locations required for accurate model construction is a very beneficial aspect of the MaxEnt approach as there is often a lack of reliable locations available for mapping the distribution of many species (Badwin, 2009). However such models should be interpreted cautiously as the accuracy of the model can be strongly influenced by the

biasness of the sampling effort (Hernandez *et al.,* 2007; Pearson *et al*., 2007; Wisz *et al.*, 2008; Phillips *et al.,* 2009).

The resulting model may not accurately define all areas that this species uses. Nevertheless Phillips *et al.* (2009) pointed out that targeting background locations from areas where locations were sampled, even if location sampling was biased toward a specific area, could counter this sample-selection bias. When developing models with a small number of locations, the resultant distribution should be defined as areas that have similar environmental conditions where the species is known to occur rather than to define the species environmental requisites throughout their range (Pearson *et al.*, 2007). Models constructed from very few location sites may be utilized in pointing out areas where future survey efforts should be focused e.g. conservation of a species (Wisz *et al.*, 2008).

MaxEnt also has concise mathematical definitions hence amenable to statistical analysis (Phillips *et al*., 2006). Some statistical analyses are carried out to evaluate the model performance or accuracy in prediction and in estimating the importance of each variable to the model performance. MaxEnt is prone to over fitting resulting to predictions that are clustered around location points. Therefore a relaxation components called regularization has been added to maxent to constrain the estimated distribution thus allowing the average values of each sampled variable to approximate its empirical average but not equal to it. This regularization component can be adjusted for each sampling area. However, recent simulations have indicated that default settings perform as well as adjusted settings (Phillips & Dudík, 2008). This method has been used in modeling potential distribution areas for many species among them *Canacomyrica monticola* in New Calendonia (Kumar & Stohlgren, 2009), *Bradypus variegatus* and *Microryzomys minutus* (Philips *et al*., 2006), baobab trees (Sanchez *et al.,* 2010), dipterocarps species (Amaludin, 2012), *Sapium sebiferum* (Jaryan *et al.,* 2013), *Basella alba* L (Reddy *et al*., 2015), Iberian *Copris* species (Chefaoui *et al*., 2005) and also in wildlife research (Baldwin, 2009).

Characterization of plant genetic resources according to environmental conditions of the geographical regions through GIS helps in understanding and assessing the genetic variability of large germplasm collections. Normally, those environmental conditions are associated with the different patterns of genetic variability, reflecting processes of adaptation of plant species to the environmental conditions (Burley *et al.,* 2007). In addition, this type of characterization provides information to users that may help to define strategies to rationalize and intensify the proceedings of germplasm evaluation to specific purposes (Burley *et al.,* 2007). Therefore, species niche modeling of *S. henningsii* was carried out in Kenya using maxent modeling method to identify the potential distribution areas for conservation and reintroduction of this species. These areas can then be used to develop *S. henningsii* demo plots, or orchards. People living within these niches could be encouraged to adopt the plant for agro-forestry in order to conserve the species.

2.4 Ethnobotanical studies of medicinal plants

The historical dependency of human beings on forests is still intact either directly or indirectly for fulfilling their various needs such as food, fodder, fiber, medicine and cultural epistemic (Kala, 2011). The age-old traditional interactions of people living in forests and forest fringes with their surroundings natural resources, ecosystems and environments have developed some specific knowledge on the use of forest and forests resources (Gadgil *et al.,* 1993; Berkes *et al*., 2000; Kala, 2005). Most of these forest dwellers are tribal communities who collect various forest products for their own consumption as well as income generation (Kala, 2011). Despite the influence of modernization, cultural diffusion and market forces, most of the traditional practices are adopted by these traditional communities are still endowed with certain beliefs, values, norms and institutional mechanisms (Kala, 2005; 2009).

In promotion of conservation agenda, it is important to understand how local community use and manage natural resources. Traditional healers provide considerable information about the use of many plants or plant parts (Survase & Raut, 2011). Plant traditional

knowledge is an important component in improving the livelihoods (Reyes-Garcia *et al*., 2008), management of natural resources (Mackinson & Nottestad, 1998; Berkes *et al.*, 2000; Huntington, 2000) and practices related to protection of ecosystem and species (Shackeroff & Campbell, 2007). Studies in ethnobiology and traditional ecological knowledge are known to serve as a significant bridge between conservation and local communities (Njoroge *et al.,* 2010). These studies help in understanding how local communities relate to their environment and pave a way for their active involvement in natural resource conservation (Duchelle, 2007). Empowerment of local communities to conserve and sustainably use biodiversity is increasingly becoming an important policy shift as most of the local people in rural areas depend on natural resources for their livelihoods (Bagine, 2006).

Ethnobotanical studies reveal how human beings have utilized plants for a wide diversity of primary survival and aesthetic purposes. It is a science that covers historical as well as present usage of plants (Nyazema, 1996). Ethnobotanical studies are often significant in revealing the importance plants species especially for the discovery of crude drugs (Cox & Blicks, 1996; Flaster, 1996). Such survey also reveal the process of domestication which is a major evolutionary force bringing about different forms of plants through human solution (Casas *et al*., 1996). Ethnobotanical studies are also a source of suitable information regarding useful plants that can be targeted for domestication. Domestication of important medicinal plants in East Africa has been seen as a way of increasing income and availability of the products to the healers and other resource users (Derry & Otsyina, 2000).

Over the last century ethnobotany has evolved into a specific discipline that looks at the people-plant relationship in a multi-disciplinary manner such as ecology, economic botany, pharmacology and public health (Balick, 1996). The need for ethnobotanical research to document important medicinal plants cannot be over-emphasized (Grace *et al.*, 2008). Most of the information is scattered in literatures, hence the need to identify them within communities in order to avoid loss of knowledge on these plants which has remained poorly recorded (Van Wyk *et al.,* 2008). There is a need to identify indigenous

medicinal plants, document information on them as well as the ingredients that bring relief and possible toxic implications of these plants (Wintola & Afolayan, 2010). Studies have been undertaken to try to document and describe the traditional herbal products used by traditional societies and also to validate their use (Light *et al*., 2005).

The use and commercialization of traditional medicinal plant products has been found to be an important livelihood strategy in developing countries where rural people are economically vulnerable (Shackleton *et al*., 2009) hence improving income and living standards (Sunderland & Ndoye, 2004). Traditional medicines form a central component in health care systems in developing countries where 80 % of the population has been reported to depend on traditional medical systems (http:www.who.int/; Gupta *et al*., 2010; Survase and Raut 2011). The use of herbal medicines is on the increase even in developed countries because of their natural origin (Jacobson *et al*., 2009). Herbal medicine are assumed to be of great importance in the primary health care needs of individuals (Sheldon *et al*., 1997) and communities in many developing countries as the herbal medicines are comparatively safer than synthetic drugs (Survase & Raut, 2011). Plant based traditional knowledge has become a recognized tool in search for new sources of drugs and neutraceuticals (Sharma & Mujundar, 2003).

Global demand for herbal medicines is not only large, but also steadily growing (Marles, 1996; Srivastava, 2000; Light *et al*., 2005). This, combined with continued habitat loss and erosion of plant-based traditional knowledge, is endangering many important medicinal plant species and thus creating an urgent need for improved methods of conservation and sustainable use of these vital plant resources (Leaman *et al*., 1999). Unmonitored trade of medicinal plant resources, destructive harvesting techniques, over-exploitation, habitat loss, and habitat change are the primary threats to medicinal plant resources in most developing countries (IUCN, 2001 2002).

Most of the plants used in traditional medicine are collected from the wild, and only a few have been domesticated. There is, therefore, a real danger of genetic erosion, which in turn calls for the need for collection and conservation, research on propagation and

cultivation, and investigation into possible modifications of the active ingredients due to changes in the growing environment. For conservation of rare plant species, cultivation is often considered an alternative to wild collection (IUCN, 2001).

In Kenya, 90% of the population has used medicinal plants at least once for the various health conditions (Chirchir, 2006). In Central Kenya, the rural poor prefer traditional plant based remedies to solve medical problems as the prices of biomedicine have escalated to unaffordable levels. In some cases, the medical facilities are inaccessible with only one or two government hospital per county. This has caused and is still causing some valuable indigenous plants species to become threatened or even extinct (Williams *et al*., 2000; Shingu, 2005). *S. henningsii* offers a recent example of such species being over exploited and in fact it has been reported as disappearing in Mwingi areas in Kenya (Musila *et al*., 2004; Schmeltzer, 2008). *S. henningsii* has been reported among the most traded species but in short supply in some urban areas of the central Kenya (Njoroge, 2012).

As demand for medicinal plants continue to accelerate, species preservation is perceived to depend on sustainable methods and cultivation (Njoroge *et al*., 2010). Cultivation of medicinal plants species may be the only solution for their rapid conservation (Lange 1998). In Asia, more and more medicinal plants are being depleted some becoming endangered hence cultivation is being viewed as a viable alternative source of these resources despite the challenges in *ex-situ* management strategies (Bisht *et al*., 2006; Sher *et al*., 2010).

In view of the rapid loss of natural habitats, traditional community life, cultural diversity and knowledge of medicinal plants, documentation of African medicinal plants is an urgent matter (Wyk *et al*., 2002). The documentation of medicinal plants prioritized by the local people as well as their understanding of possible diversity loss and strategies of conservation are some aspects in ethnobotanical studies (Bisht *et al*., 2006). Further the extent to which important medicinal plants are cultivated is often unclear, even in regions where large amounts of medicinal plants are being commercialized (Bussmann

et al., 2008). This study explored the traditional knowledge of *S. henningsii* among the local people in five natural habitats in Kenya. This study will provide information necessary for sustainable use, conservation methods as well as domestication processes for this important plant species.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter describes the materials and methodologies adopted in carrying out the research study. The research aimed at carrying out the genetic diversity, species niche modeling and ethnobotanical studies of *S. henningsii* in Kenya. This chapter also explains the research design and sampling techniques followed, the study areas, collection of plant materials, data collection and analysis. The chapter has been organized according to the specific objectives.

3.2 Genetic diversity studies of S. henningsii

3.2.1 Study areas

A reconnaissance survey was carried out to identify areas where *S. henningsii* grows naturally. Nine populations were selected from areas identified from the following places: Taita-Taveta (Mwache forest), Kilifi (Arabuko Sokoke forest), Narok (Tipilikwani forest in Talek near Maasai Mara game reserve), Baringo (Tugen hills), Kitui (Ndumooni hills), Marsabit (Marsabit forest reserve), Nyeri (Kabiruini forest), Kiambu (Karura forest) and Kajiado (Ngong forest) (Fig 3.1). At least thirty individual plants were selected for the genetic diversity study from each population (Appendix 4). The first plant was randomly selected then the other plants in each population systematically selected at a distance of fifty to two hundred meters apart to avoid repeated sampling from the same population.

3.2.2 Sampling method

The effectiveness of a sampling method depends on the avoidance of the redundant genotyping of clones. One way to avoid this is to select samples sufficiently apart in the sampling design used (Khanlou *et al.,* 2011). Inappropriate sampling strategy bias genetic diversity parameters whereas excessive sampling inflates costs (Suzuki *et al.,* 2004). The main aim is to infer a minimal sample size required to accurately assess genetic variation within and among *S. henningsii* based on widely used parameters. The grid-based approach has been applied successfully in investigating the genetic diversity of many species (Gustine and Elwinger 2003; Khanlou *et al.,* 2011). It ensures that there is adequate inter-sample interval to avoid collection of related individuals and maximize the precision of the information obtained. In several genetic diversity studies a sample size of 20-30 individuals have been recommended with at least 40 to 50 m apart (Pruett *et al*., 2008; Lu-Na and De-Xing 2004; Zhao *et al*., 2006; Khanlou *et al.,* 2011). In this study the first plant was randomly selected and the others were systematically selected by using a distance of at least 50 m apart and. The plant locations were marked using a GPS handset tools

3.2.3 Collection of Plant Materials

Samples of leaves failed to yield DNA for molecular analysis.Therefore, small discs of cambium tissues were collected directly from the tree trunk using a hollow metal and a hammer. The hollow metal was hammered into the bark until reaching the wood of the plant which generally offered resistance to the metal entry. A thin layer of tissues (cambium tissues) just beneath the bark were removed with a scalpel blade and preserved in absorbent envelopes containing silica gel until they arrived in the laboratory where they were dried in an oven at 30 °c and the stored at room temperature until extraction (Esau, 1977).

3.2.4 DNA Extraction

About 0.1 to 0.2 g of dried and powdered cambium tissues was used for DNA extraction. DNA extraction was carried out using CTAB (Doyle & Doyle, 1990) protocol with a few modifications. It contained high levels of CTAB and salts for suppressing co-precipitation of polysaccharides and DNA. The equipments and materials required were: (1) shredding machine, (2) 2.0 mls microfuge tubes, (3) gliding metal balls, (4) vortex, (5) water bath (65 °c) and (6) centrifuge 13000 rpm.

The reagents and solutions required for DNA extraction were:-

Sorbitol buffer extraction containing: 10 % polyethylene glycol (MW 6000), 0.35 M sorbitol, 0.5 % Tris-HCl (pH 7.5) and 0.5 % β- mercaptolethanol. CTAB Buffer containing: 10 % CTAB, 0.05M Tris-HCl, 1.4M NaCl, 5 % PVP, 0.5 M EDTA,; Chloroform: Isoamyl (CIA) 24:1, 0.5 % β- mercaptolethanol, ice-cold Isoporopanol, 70 % ethanol and Tris EDTA buffer consisting of 10 mM Tris-HCl and 1m M EDTA (pH 8.0).

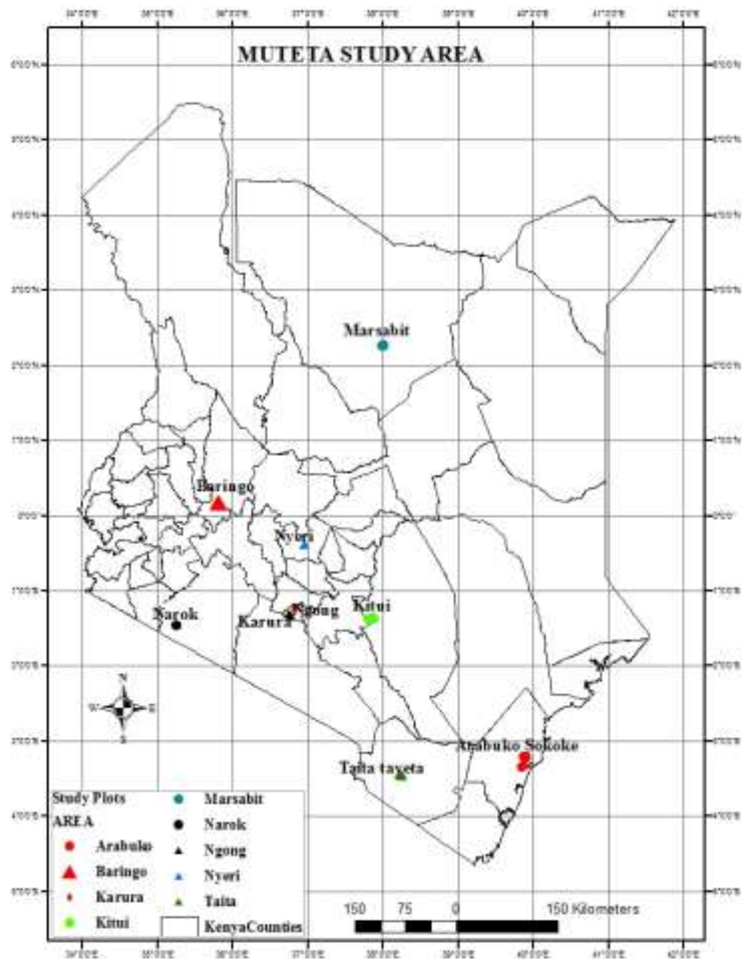


Figure 3.1: Map showing areas for *S. henningsii* genetic diversity study

3.2.4.1 DNA extraction protocol

DNA was extracted using CTAB method by Doyle and Doyle (1990) method with modifications. A sorbitol buffer was initially utilized for removing mucilaginous polysaccharides, followed by tissue extraction with a CTAB buffer containing high salt concentration for suppressing co-precipitation of polysaccharides and DNA.

To the powdered 0.1 to 0.2 g of cambium tissues, 500 µls of sorbitol buffer were added and mixed by vortex then centrifuged for 10 min at 14000 rpm at 4 °c. The supernatant solution was removed and discarded, then 750 µls sorbitol buffer were added again and remixed by vortexing. The sorbitol buffer cleaning was repeated until no visible mucilage layer was present in the sample after centrifugation (usually 3 or 4 times). The supernatant solution was removed and 750 µls CTAB buffer solution added and mixed well. The samples were incubated at 65°c in a water bath for 30-60 min and at RT with gentle inversions to mix. There after equal volume of (750 µls) CIA was added and mixed by inversion for 10-20 min and then centrifuged at 14000 rpm at RT for 10-20 min. The upper aqueous solution was transferred into a new 2.0 ml microfuge tube and equal volume (750 µls) of CIA added and mix by inversion for 10-20 min then centrifuged at 14000 rpm at RT for 10-20 min. The upper aqueous solution was transferred into a new microfuge tube and 1/10 volume of 3M Ammonium acetate or Sodium acetate was added. Equal volume of isopropanol was added and the solution mixed by inversion then stored at -20°c overnight. The samples were centrifuged at 14000 rpm at 4 °C for 5 min and 750 µl of 70 % ethanol was added to wash the DNA and this was repeated 2 to 3 times then centrifuged at 14000 rpm at 4 °c for 5 min. The supernatant was discarded and the pellet dried and 100 µls TE added to dissolve the DNA.

3.2.5 DNA Quantification and Quality Evaluation

DNA quantification was performed by spectrophotometry using an aliquot of 1 µl total genomic DNA with the spectrophotometer (Biospec Model-Nano (Shimadzu- Biotech). The concentration of DNA was obtained by absorbance at 260 nm. The ratio of nucleic acids to proteins in the sample was evaluated by the ratio of absorbance at 260 nm and 280 nm (A260/280) and the ratio for most samples was above the optimal limit of 1.8(Sambrook & ussell, 2001). The presence and quality of DNA obtained was also evaluated by electrophoresis on a 1 % agarose gel stained with SYBER-SAFE dye and viewed under UV light and photographed using gel documentation system.

3.2.6 PCR-RAPD Assay

Forty RAPD (Invitrogen custom from F & S Scientific LTD) primers were screened and ten that gave clear and scorable bands were selected for their ability to amplify the genomic (Table 1) DNA. PCR assay was performed in a reaction mixture of 12.5 µls containing 12.5 ng/µl DNA template, 1 Unit Taq polymerase, 10 µM primers, 10 mM of each dNTPs, 25 mM $MgCl_2$, 10 × PCR buffer, 25 % PVP, and 6.6 µls PCR grade water. The amplification was carried out in an Applied Biosystems® thermocycler with an initial 5 min denaturation step at 95 °C, followed by 35 cycles of 30 s denaturation step at 95 °C, 45 s annealing step at 37 °C, and 1 min extension step at 72 °C and a final extension step for 5 min at 72 °C.

Table 3.1: List of ten RAPD primers used for molecular characterization of *S. henningsii*

No. RAPD	Primer Code	Sequence (5' -3')
1	KFP-3	GTT AGC GGC G
2	KFP-8	ACG CGC TGG T
3	KFP-10	ACG GTG CGC C
4	KFP-12	GAC CCC GGC A
5	KFP-14	GGG TAA CGC C
6	KFP-16	GCATGG AGC T
7	KFP-17	CCG AAG CCC T
8	KFP-23	GCT CGT CAA C
9	KFP-27	TCC TCG CGG C
10	KFP-28	AAT CGG CGT G

3.2.7 PCR-ISSR Assay

Forty ISSR (Invitrogen by Thermo Fisher Scientific) primers were screened and nine that gave clear and reproducible bands were selected for their ability to amplify the

genomic DNA of *S. henningsii* (Table 2). The reaction mixture consisted of a total volume of 12.5 µls which contained.12.5 ng/µl DNA template, 1 Unit Taq polymerase, 10 × PCR buffer, 10 mM dNTPS, 25 mM $MgCl_2$, 10 µM primers, 25 % PVP and PCR grade water. The amplification was carried out in a 96 Veriti Applied Biosystems® thermacycler. This consisted of an initial 5 min denaturation step at 95 °C followed by 35 cycles of 30 s denaturation step at 95 °C, 45 s annealing step at 47°C, 2 min extension step at 72 °C and a final extension step for 5 min at 72 °C. The amplified DNA fragments for both RAPD and ISSR markers were separated by electrophoresis on a 2% agarose gel stained with 1 % SYBER SAFE dye in 1× TBE buffer at 100V for 3-4 h. DNA molecular marker (1517-bp ladder) was also included in the gel to estimate the molecular weight of the DNA fragments obtained. After the run, the gels were photographed under UV light using gel documentation system and then scored for the presence/absence of a band for each primer .The data was recorded in MS excel for analysis**.**

Table 3.2: List of nine ISSR primers used for molecular characterization of *S. henningsii*

NO. ISSR.	Primer	Primer Sequence (5' -3')
1	UBC-807	AGA GAG AGA GAG AGA GT
2	UBC-809	AGA GAG AGA GAG AGA GG
3	UBC-811	GAG AGA GAG AGA GAG AC
4	UBC-818	CAC ACA CAC ACA CAC AG
5	UBC-820	GTG TGT GTG TGT GTG TC
6	UBC-825	ACA CAC ACA CAC ACA CT
7	UBC-830	TGT GTG TGT GTG TGT GG
8	UBC-861	ACC ACC ACC ACC ACC ACC
9	UBC-862	AGC AGC AGC AGC AGC AGC

3.2.8 DNA analysis

The binary data matrix in MS excel was subjected to POPGENE and GENEALEX software to determine the genetic diversity parameter for each population and for each marker. The percentage of polymorphic bands (PPB), total number of bands produced per primer, Nei's (1973) genetic diversity (h), Shannon information index (I) (Shannon & Weaver, 1949), Nei's unbiased genetic distance and Nei's genetic differentiation index (Gst) among population were estimated using POPGENE version 32 software (Yeh *et al*., 2000). Cluster analysis based on Nei's (1978) genetic distance was carried out using an unweighted pair-wise group method with arithmetic averaging (UPGMA) by Sneath and Sokal (1973) using TFPGA Software (Miller 1997) and a dendrogram was constructed using MEGA Ver. 7 (Kumar *et al*., 2016).

By assuming that there was a similar random mating pattern in all populations, analysis of molecular variance (AMOVA) was carried out as described by Excofier *et al.* (1992) using hierachial levels; individuals, populations and their groups to partition genotypic variance within and among *Strychnos henningsii* in Kenya. Analysis of molecular variance (AMOVA) and Principal Coordinate Analysis (PCA) were performed using GenAlEx 6.1 software (Peakall & Smouse, 2006). The Principal Coordinate Analysis was carried out based on the genetic distance matrix using GenAlex software to visualize the genetic relationship among genotypes to confirm the clustering analysis. The multivariate approach was chosen to complement the cluster analysis information because cluster analysis is more sensitive to closely related individuals whereas PCA is more informative regarding distances among major groups (Hauser & Crovello, 1982).

3.3 Species Distribution Modeling of S. henningsii

3.3.1 Environmental variables

These variables were obtained from WorldClim dataset (Hijmans *et al*., 2005), available at http://www.worldclim.org). Four set of variables comprising of Maximum

temperature, Minimum Temperature, Precipitation (for the twelve months of the year) and Altitude in ZIP files were obtained from this website. The environmental layers option in maxEnt can only runs 19 variables at a time. The altitude ASCII file was added as one of the layers. The remaining The 18 layers were obtained by selecting the maximum temperature, minimum temperature and precipitation for the months of January, March, May, July, October, and December. All variables were re-sampled to 1 KM spatial resolution. Therefore, nineteen environmental variables were used as predictors of *S. henningsii* potential suitable habitats in Kenya (Table 3.3). The files format containing the environmental grid layers were converted to ASCII (American Standard Code for Information Interchange using ArcGIS (Aeronautical Reconnaissance Coverage Geographic Information System) 9.3 version in order to be compatible with MaxEnt software

3.3.2 Model development and evaluation

MaxEnt (version 3.3.3k) downloaded from the portal (www.cs.princeton.edu) (Phillips *et al*., 2006) was used in this study. The occurrence data was portioned into two parts; 75 % of the data was randomly selected for model training and the remaining 25 % of the data for testing the model. The model was then run using the default settings of maxent and 75 % of the occurrence points to predict the potential suitable habitats of *S. henningsii* in Kenya. The model produced prediction values ranging from 0 to 1 representing cumulative probabilities of occurrences. The predictions were mapped in ArcGIS 9.3 version. A jackknife procedure was used to measure the variables importance in model development. Receiver Operating Characteristic curve analysis was used to assess the model quality (Fielding & Bell, 2007). The ROC curve is a plot between sensitivity (true positive fraction), i.e. absence of omission error, and the proportion of incorrectly predicted observed absences (1-specificity) or false positive fraction, i.e. commission error for all available probability thresholds (Manel *et al*., 2001). The area under the curve (AUC) value is the single measure of the area under the ROC ranging from 0.5 to 1.

Table 3.3: Codes and details of the bioclimatic variables

Codes	Parameters
prec_1_res	Precipitation for the month of January
pre_10_res	Precipitation for the month of October
prec_7_res	Precipitation for the month July
tmax_10_res	Maximum temperature for the month of October
tmax_1_res	Maximum temperature for the month of January
alt_res	Altitude of the geographical locations
prec_12_res	Precipitation for the month December
prec_5_res	Precipitation for the month May
tmin_12_res	Minimum temperature for the month of December
tmin_5_res	Minimum temperature for the month of May
prec_3_res	Precipitation for the month March
tmin_1_res	Minimum temperature for the month of January
tmax_7_res	Maximum temperature for the month of July
tmin_10_res	Minimum temperature for the month of October
tmax_12_res	Maximum temperature for the month of December
tmax_5_res	Maximum temperature for the month of May
tmax_3_res	Maximum temperature for the month of March
tmin_7_res	Minimum temperature for the month of July
tmin_3_res	Minimum temperature for the month of March

3.4 Ethnobotanical Studies of S. henningsii

3.4.1 The Study Areas

Figure 3.2: Map showing areas for ethnobotanical study of *S. henningsii* **in Kenya**

3.4.2 Research Design

Survey and Naturalistic design was used because of their descriptive nature that aid in learning respondent's perception, attitude, beliefs, values, behavior, opinions, likes and dislikes, habits and desires on *S. henningsii*. In particular, the baseline survey was appropriate because of variables of assessment such as awareness and availability of *S. henningsii*, sources, uses, parts of plant used, extraction and processing of .*S. henningsii* among different communities in Kenya.

3.4.3 Target Population, Sampling, sample size and measurements

The target population for the study was household providers. Purposive and convenience sampling was used in selection of respondents from the study sites (Bernard 2006; Patton 2002). This was so as to only use respondents who interact with *S. henningsii* as the herbal medicine users, plant materials collectors and traders. The data was collected from five counties that were purposefully selected for this study based on the presence of *S. henningsii* plant and also users and traders. The counties for study included, Narok, Nyeri, Kajiado, Meru and Kiambu (Fig 3.2).

A semi structured questionnaire was used in data collection (Appendix 3). The variables assessed were household characteristics such gender, location, age, marital status, economic status, land size and land use management. The other variables assessed included awareness and availability of *S. henningsii,* sources and availability of the species, trading in *S. henningsii,* uses of the plant, extraction of the species and difficulties experienced in accessing of *S. henningsii* plant materials.

3.4.4 Methods of statistical data Analysis

Descriptive statistics such as frequencies, percentages, cross tabulation and exploration were undertaken to identify pattern of data for inferential analysis. Chi-square test was used to check association among variables such as location and gender, location and age, gender and age, location and uses of *S. henningsii,* among others. Kruskal-Wallis test was used to compare qualitative independent variables such as location and land use, location and species use, gender and parts of the plant used, location and extraction methods among others. One way analysis of variance was used to compare land sizes among locations. Least significant difference (LSD) was used for mean comparisons of land sizes among locations. Statistical significant differences were declared at 5 % confidence level. Data was coded and entered into statistical package for social scientists (SPSS V18) which was used in data analysis. The results were presented in tables, bar graphs and pie charts.

CHAPTER FOUR

RESULTS

4.1 Introduction

This chapter presents and interprets the data obtained from the study. The findings of the analyzed data have been presented on the basis of the specific objectives. The results have been presented using tables, graphs, figures, plates and charts where appropriate.

4.2 Genetic diversity of *S. henningsii*

Two hundred and seventy individuals randomly selected from nine populations (Fig 3.1) of *S. henningsii* in Kenya were used for the genetic diversity study. A total of forty arbitrary RAPD primers and forty ISSR primers were initially screened for their ability to produce clear and scorable bands. Out of these, only ten and nine primers for RAPD and ISSR respectively (Table 1 & 2) were selected which gave reproducible and distinct polymorphic amplified products (Plates 4.1 & 4.2; 4.3 & 4.4) respectively. All populations revealed clear polymorphism over the primers used in this study as shown in Table 4.

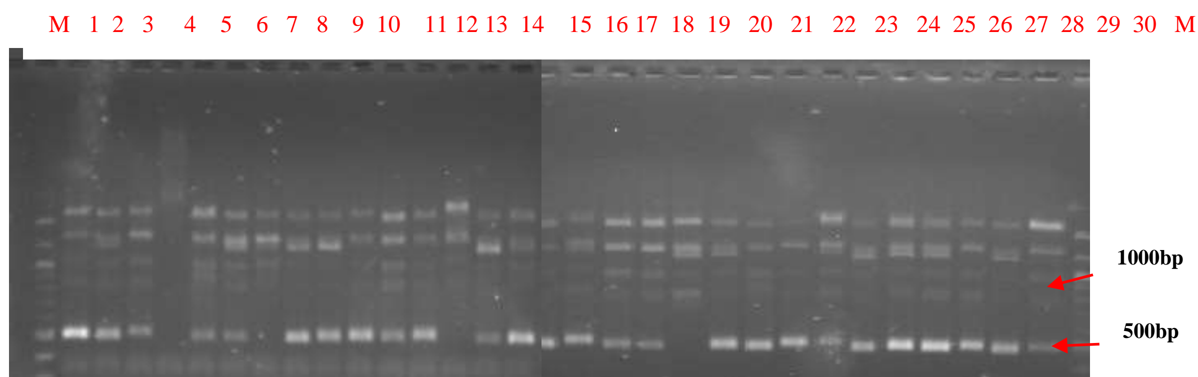


Figure 4.1: RAPD marker profile of amplified loci of samples from Baringo population using primer KFP-23. Lane (1-30) are samples, M:-Marker DNA 100bp ladder

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 2526 27 28 29 30M

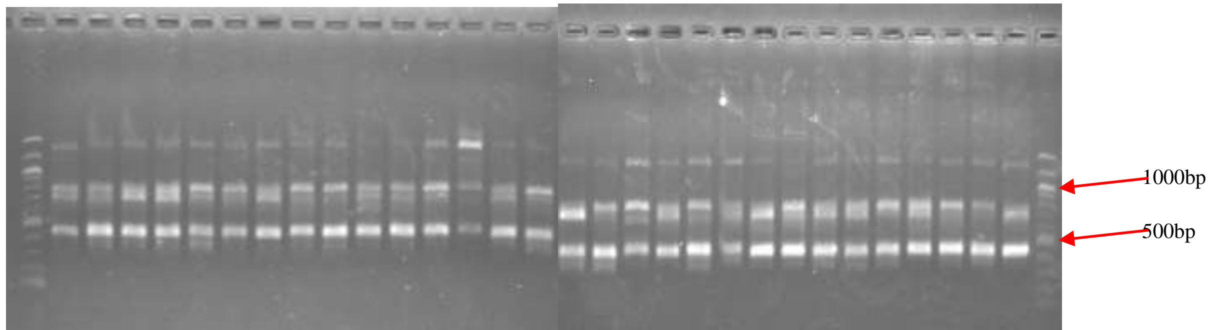


Figure 4.2: RAPD marker profile of amplified loci of samples from Narok population using primer KFP-14. Lane (1-30) are samples, M:-Marker DNA 100bp ladder

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 M

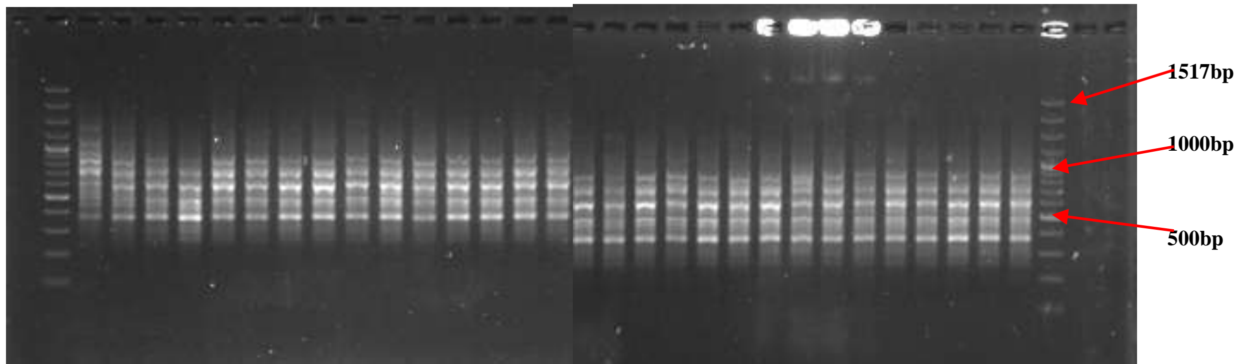


Figure 4.3: ISSR marker profile of amplified loci of samples from Baringo population using primer 862. Lane (1-30) are samples, M:-Marker DNA 100bp ladder

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 M

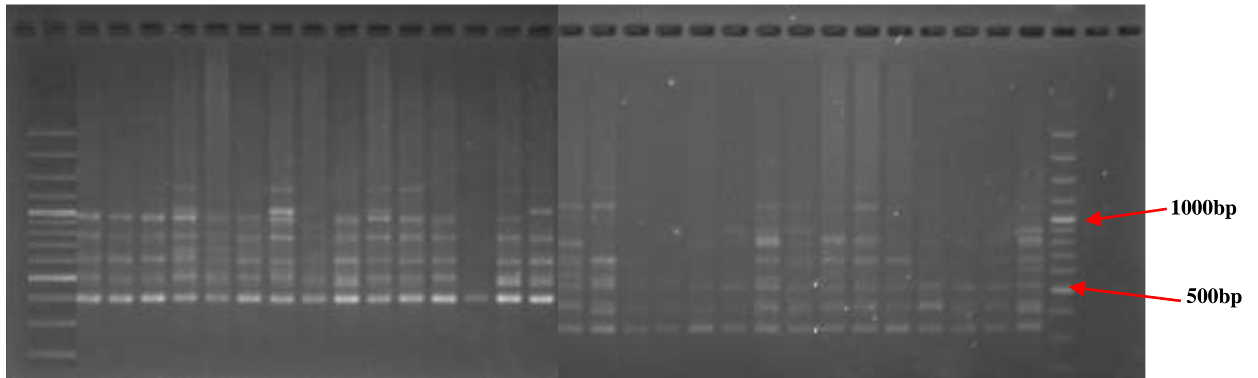


Figure 4.4: ISSR marker profile of amplified loci of samples from Narok population using primer 818. Lane (1-30) are samples, M:-Marker DNA 100bp ladder

4.2.1 RAPD Analysis

This analysis revealed that Kitui population was the most polymorphic with 75 polymorphic loci detected (55.15 %) and Baringo population was the least polymorphic with 35 polymorphic loci detected (25.74 %) (Table 4.1). The mean percentage polymorphism detected was 38.97 % (Table 5). The ten primers revealed a total of 25 population specific loci as follows: Kitui (5), Marsabit (4), Baringo (1), Nyeri (3), Karura (4), Ngong (5), Jilore (2), Taveta (1) and Narok (0) (Table 6). The overall genetic diversity observed among the studied plant materials revealed a range of diversity indices as shown in Tables 4 and 5 for example Na, Ne, H, I, % P and He indices are higher for Kitui population than in all other populations.

The polymorphism and expected heterozygosity revealed in this study were comparable to the results of the Principal Coordinate Analysis (PCA) where sample dispersion was highest in the more genetically diverse and the genetically similar populations. The first two axes accounted for 49.83% in RAPD.analysis (Fig.4.5). According to Nei's (1978) unbiased measure of genetic identity and distance matrix (Tables 4.4), the genetic

similarity coefficient ranged from 0.8422 (Jilore and Nyeri) to 0.9796 (Jilore and Baringo populations) whereas the genetic distance ranged from 0.0206 (Jilore and Baringo populations) to 0.1717 (Jilore and Nyeri populations). Thus the most genetically distant populations were Jilore and Nyeri whereas Jilore and Baringo were the most genetically close populations (Table 4.4). Pairwise Population Matrix of Nei's (1978) unbiased measure of genetic identity and distance matrix revealed a genetic identity coefficient of 0.836 (Ngong and Baringo populations) to 0.955 (Taveta and Kitui populations) whereas the genetic distance ranged from 0.046 (Taveta and Kitui populations) to 0.179 (Ngong and Baringo population). This showed that Taveta and Kitui were the most genetically close populations while Ngong and Baringo were the most genetically diverse populations (Table 4.5). Analysis of molecular variance (AMOVA) revealed a higher genetic variation (54 %, $p < 0.001$) among than within (46 %, $p < 0.001$) the *Strychnos henningsii* populations (Table 4.6; Fig 4.6).

Table 4.1: Genetic diversity analysis of nine populations of S. henningsii as revealed using RAPD and ISSR markers in PopGene software

Population	N	PPL	%P	Na*	Ne*	H*	I*
RAPD Markers							
Kitui	30	75	55.15	1.5515	1.2325	0.1483	0.2337
Marsabit	30	66	48.53	1.4853	1.2461	0.1458	0.2221
Baringo	30	35	25.74	1.2574	1.1538	0.0867	0.1289
Nyeri	30	66	48.53	1.483	1.2410	0.1431	0.2189
Narok	30	41	30.15	1.3015	1.1712	0.1015	0.1525
Karura	30	50	36.76	1.3676	1.2003	0.1179	0.1781
Ngong	30	54	39.71	1.3971	1.1764	0.1067	0.1653
Jilore	30	36	26.47	1.2647	1.1260	0.0769	0.1185
Taveta	30	54	39.71	1.3971	1.1531	0.0969	0.1548
Overall	270	136	100	2.0000	1.2979	0.2005	0.3310
ISSR Markers							
Kitui	30	42	43.75	1.4375	1.3067	0.1720	0.2514
Marsabit	30	40	41.67	1.4167	1.2548	0.1469	0.2189
Baringo	30	28	29.17	1.2917	1.1594	0.0955	0.1448
Nyeri	30	38	39.58	1.3958	1.2823	0.1590	0.2317
Narok	30	41	42.71	1.4271	1.2715	0.1558	0.2303
Karura	30	49	51.04	1.5104	1.3764	0.2071	0.2994
Ngong	30	51	53.12	1.5312	1.3148	0.1828	0.2728
Jilore	30	36	37.5	1.3750	1.2346	1.1366	0.2030
Taveta	30	50	52.08	1.5208	1.2977	0.1773	0.2673
Overall	270	96	100	2.0000	1.4683	0.2889	0.4473

Table 4.2: Genetic diversity analysis of nine populations of *S. henningsii* as revealed by RAPD and ISSR markers in GenAlex software.

Population	%P	N	Na	Ne	I	He	UHe	PSL
RAPD Markers								
Kitui	55.15%	30.000	1.103	1.233	0.234	0.148	0.151	5.000
Marsabit	48.53%	30.000	0.978	1.246	0.222	0.146	0.148	4.000
Baringo	25.74%	30.000	0.603	1.154	0.129	0.087	0.088	1.000
Nyeri	48.53%	30.000	0.978	1.241	0.219	0.143	0.146	3.000
Narok	30.15%	30.000	0.654	1.171	0.153	0.101	0.103	0.000
Karura	36.76%	30.000	0.735	1.200	0.178	0.118	0.120	4.000
Ngong	39.71%	30.000	0.809	1.176	0.165	0.107	0.109	5.000
Jilore	26.47%	30.000	0.581	1.126	0.118	0.077	0.078	2.000
Taveta	39.71%	30.000	0.794	1.153	0.155	0.097	0.099	1.000
Mean	38.97%	30.00	0.804	1.189	0.175	0.114	0.116	
ISSR Markers								
Kitui	43.75%	30.000	0.917	1.307	0.251	0.172	0.175	5.000
Marsabit	41.67%	30.000	0.865	1.255	0.219	0.147	0.149	1.000
Baringo	29.17%	30.000	0.688	1.159	0.145	0.096	0.097	0.000
Nyeri	39.58%	30.000	0.802	1.282	0.232	0.159	0.162	0.000
Narok	42.71%	30.000	0.885	1.271	0.230	0.156	0.158	2.000
Karura	51.04%	30.000	1.063	1.376	0.299	0.207	0.211	2.000
Ngong	53.13%	30.000	1.115	1.315	0.273	0.183	0.186	2.000
Jilore	37.50%	30.000	0.781	1.235	0.203	0.137	0.139	0.000
Taveta	52.08%	30.000	1.052	1.298	0.267	0.177	0.180	2.000
Mean	43.40%	30.000	0.907	1.278	0.236	0.159	0.162	

N= population size, PPL= population polymorphic loci, %P= percentage polymorphism, Na= Number of observed alleles, Ne= number of effective alleles, H= Nei's genetic diversity, I= Shannon information indices, He= expected Heterozygosity, UHe=unbiased expected Heterozygosity, PSL=population specific loci.

Table 4.3: RAPD population's Specific loci

population	NPL	primers	sequences	Band size (bp)
Kitui	5	3	GTT AGC GGC G	2000
		8	ACG CGC TGG T	500
		12	GAC CCC GGC A	1100
		12	GAC CCC GGC A	1200
		14	GGG TAA CGC C	1517
Marsabit	4	3	GTT AGC GGC G	1200
		3	GTT AGC GGC G	900
		8	ACG CGC TGG T	2000
		23	GCT CGT CAA C	1200
Karura	4	16	GCATGG AGC T	1517
		12	GAC CCC GGC A	500
		28	AAT CGG CGT G	700
Ngong	5	8	ACG CGC TGG T	1000
		8	ACG CGC TGG T	400
		8	ACG CGC TGG T	300
		10	ACG GTG CGC C	1517
		17	CCG AAG CCC T	1200
Nyeri	3	3	GTT AGC GGC G	1100
		3	GTT AGC GGC G	1360
		3	GTT AGC GGC G	750
Jilore	2	3	GTT AGC GGC G	500
		17	CCG AAG CCC T	600
Baringo	1	17	CCG AAG CCC T	600
Narok	0	0		0

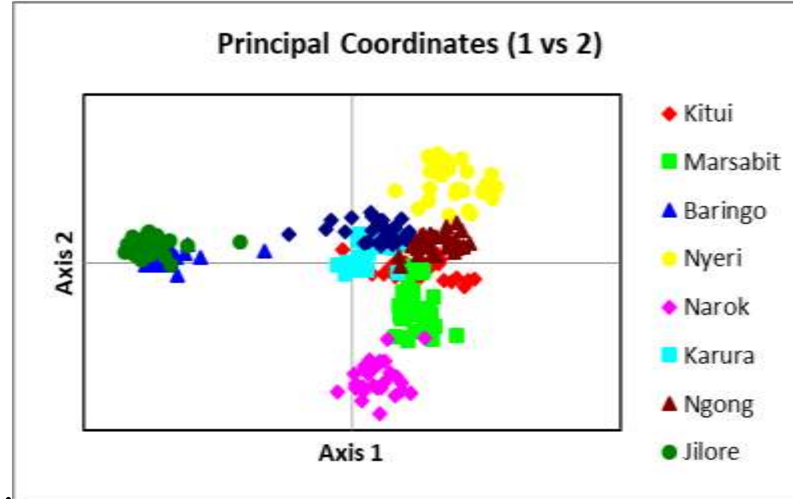


Figure 4. 5: A three dimensional plot of the Principal oordinate Analysis (PCA) of RAPD data showing the clustering of *S. henningsii* populations.

*The first and second principal coordinates account for 30.73 and 19.10% of total variation, respectively

Table 4.4: Nei's (1978) unbiased measures of genetic Identity and Genetic Distance from RAPD markers in PopGene software

pop ID	Kitui	Marsabit	Baringo	Nyeri	Narok	Karura	Ngong	Jilore	Taveta
Kitui	****	0.9331	0.8915	0.9274	0.8985	0.9447	0.9209	0.8730	0.9545
Marsabit	0.0693	****	0.8785	0.9104	0.8981	0.9334	0.8963	0.8667	0.9253
Baringo	0.1148	0.1295	****	0.8551	0.8644	0.8968	0.8586	0.9796	0.8979
Nyeri	0.0753	0.0939	0.1565	****	0.8598	0.9087	0.9095	0.8422	0.9261
Narok	0.1070	0.1075	0.1457	0.1511	****	0.8788	0.8877	0.8491	0.8921
Karura	0.0569	0.0689	0.1090	0.0958	0.1292	****	0.9246	0.8836	0.9456
Ngong	0.0824	0.1095	0.1524	0.0949	0.1191	0.0784	****	0.8481	0.9317
Jilore	0.1358	0.1431	0.0206	0.1717	0.1636	0.1238	0.1648	****	0.8845
Taveta	0.0466	0.0776	0.1077	0.0768	0.1141	0.0559	0.0707	0.1228	****

*Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 4.5: RAPD pairwise population matrix of Nei's unbiased measure of Genetic Identity and Genetic Distance using GenAlex software

Pop ID	Kitui	Marsabit	Baringo	Nyeri	Narok	Karura	Ngong	Jilore	Taveta
Kitui	****	0.934	0.854	0.928	0.899	0.945	0.922	0.874	0.955
Marsabit	0.069	****	0.837	0.911	0.899	0.934	0.897	0.868	0.926
Baringo	0.158	0.178	****	0.844	0.848	0.848	0.836	0.891	0.889
Nyeri	0.075	0.093	0.170	****	0.861	0.909	0.910	0.844	0.927
Narok	0.106	0.107	0.165	0.150	****	0.880	0.889	0.850	0.893
Karura	0.056	0.068	0.165	0.095	0.128	****	0.925	0.885	0.946
Ngong	0.082	0.109	0.179	0.094	0.118	0.078	****	0.849	0.932
Jilore	0.135	0.142	0.115	0.170	0.162	0.123	0.163	****	0.885
Taveta	0.046	0.077	0.117	0.076	0.113	0.055	0.070	0.122	****

*Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 4.6: Analysis of Molecular Variance of *S. henningsii* as revealed by RAPD markers

Source	df	SS	MS	Est. Var.	%	P>0.05
Among Regions	2	628.360	314.180	0.000	0%	0.630
Among Pops	6	1896.662	316.110	10.249	54%	0.010
Within Pops	261	2258.567	8.654	8.654	46%	0.010
Total	269	4783.589		18.902	100%	

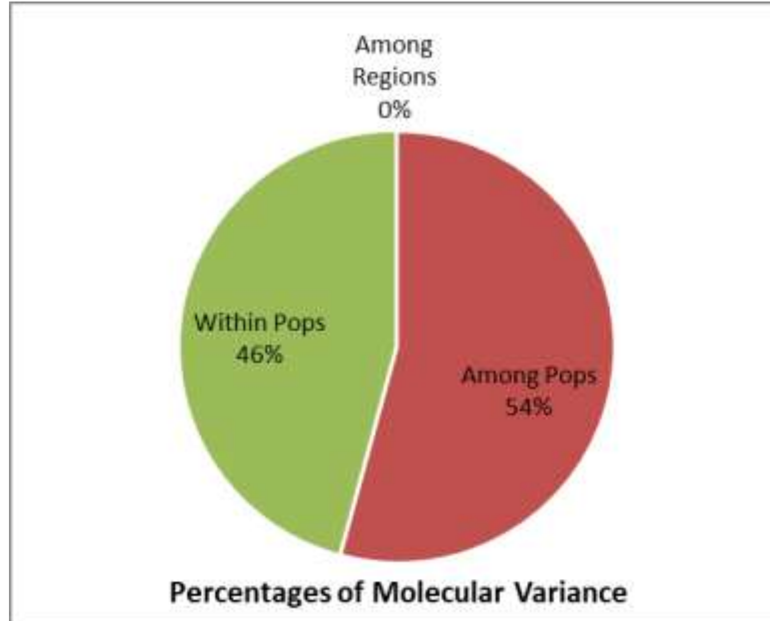


Figure 4.6: Percent of Molecular Variance for RAPD data

4.2.1.1 RAPD-based clustering analysis

The genetic similarity coefficient revealed ranged between 0.8422 (Jilore and Nyeri) to 0.9796 (Jilore and Baringo) (Table 4.4). The UPGMA cluster analysis based on RAPD data grouped nine populations into two clusters. Cluster I consisted of 7 populations (Kitui, Taveta, Karura, Marsabit, Ngong, Nyeri and Narok) while cluster II consisted of two populations (Baringo and Jilore) based on their unbiased genetic distance (Fig. 4.7). The clustering analysis did not completely match to the geographical areas of plant collection.

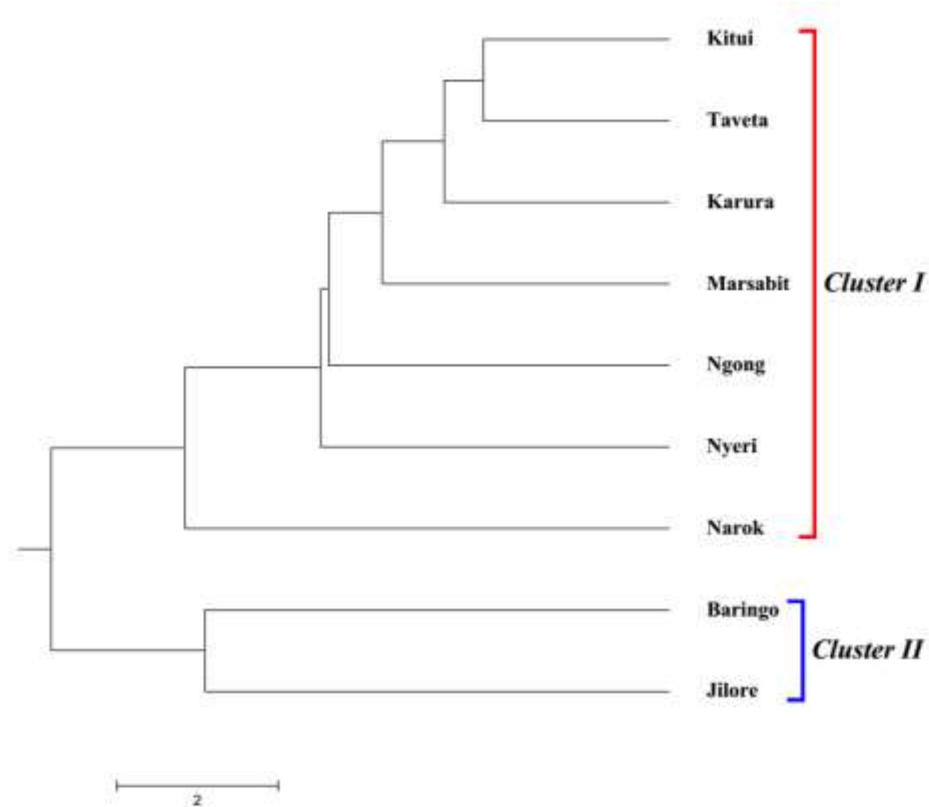


Figure 4.7: UPGMA cluster analysis of the nine *S. henningsii* populations based on Nei's (1978) unbiased genetic distance

4.2.2 ISSR analysis

The results of analysis slightly differed from RAPD where Ngong population was the most polymorphic population with 51 (53.13 %) polymorphic loci detected and Baringo population was the least polymorphic population with 28 (29.17 %) polymorphic loci detected (Table 5). The mean percentage polymorphism detected was 43.40 % (Table 6). The nine primers revealed a total of 13 specific loci as follows: Kitui (5), Marsabit (1), Narok (2), Karura (2), Ngong (2), Taveta (2), Nyeri (0), Jilore (0) and Baringo (0) (Table 4.7).

The polymorphism and expected heterozygosity revealed in this study were comparable to the results of the Principal Coordinate Analysis (PCA) where sample dispersion was highest in the more genetically distant and the genetically similar populations. The first two axes accounted for 44.91 % in ISSR analysis (Fig 4.4) of the genetic diversity found in the entire study materials. According to Nei's (1978) unbiased measure of genetic identity and distance matrix (Tables 4.8), ISSR analysis revealed the.genetic identity coefficient range between 0.7239 (Jilore and Baringo populations) and 0.8803 (Taveta and Marsabit populations) while the genetic distance ranged between 0.1275 (Taveta and Marsabit populations) and 0.3231 (Jilore and Baringo populations). Hence the most genetically distant populations indicated from this analysis were Jilore and Baringo while Taveta and Marsabit were the most genetically close populations (Table 4.8).

Pairwise Population Matrix of Nei's (1978) Unbiased measure of genetic identity and distance matrix revealed a genetic identity coefficient of 0.724 (Jilore and Baringo populations) and 0.885 (Ngong and Nyeri populations) whereas the genetic distance ranged between 0.122 (Ngong and Nyeri populations) and 0.323 (Jilore and Baringo populations) (Table 4.9). This results indicated that Jilore and Baringo the most genetically distant populations while Ngong and Nyeri were the most genetically close populations. Analysis of molecular variance (AMOVA) revealed a higher genetic variation (58 %, $p < 0.001$) among than within (41 %, $p < 0.001$) the *Strychnos henningsii* provenances (Table 4.10; Fig 4.9).

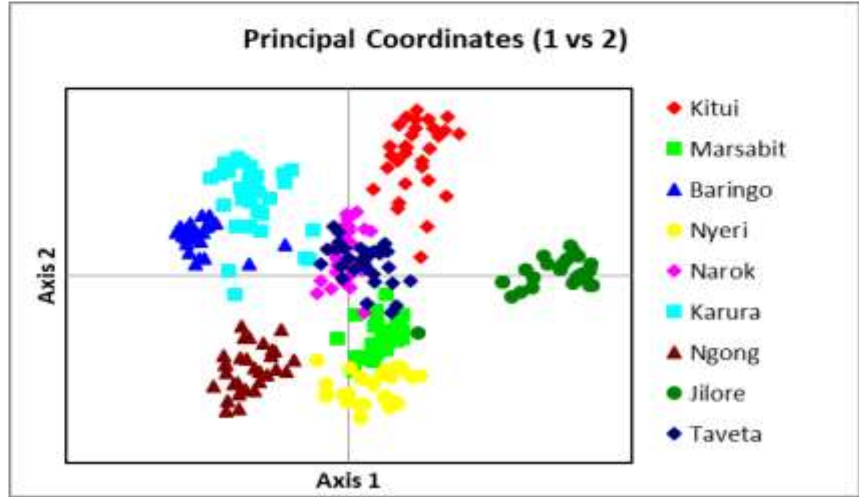


Figure 4.8: A three dimensional plot of the Principal Coordinate Analysis (PCA) of ISSR data showing the clustering of *S. henningsii* populations

*The first and second principal coordinates account for 25.13 and 19.78% of total variation, respectively

Table 4.7: ISSR population's Specific loci

population	NPL	primers	sequences	Band size (bp)
Kitui	5	807	AGA GAG AGA GAG AGA GT	400
		861	ACC ACC ACC ACC ACC ACC	900
		862	AGC AGC AGC AGC AGC AGC	500
		862	AGC AGC AGC AGC AGC AGC	400
		818	CAC ACA CAC ACA CAC AG	500
Marsabit	3	811	GAG AGA GAG AGA GAG AC	1000
		811	GAG AGA GAG AGA GAG AC	500
		862	AGC AGC AGC AGC AGC AGC	800
Karura	2	830	TGT GTG TGT GTG TGT GG	500
		861	ACC ACC ACC ACC ACC ACC	400
Ngong	2	825	ACA CAC ACA CAC ACA CT	300
		861	ACC ACC ACC ACC ACC ACC	500
Nyeri	0			
Jilore	0			
Baringo	0			
Narok	2	807	AGA GAG AGA GAG AGA GT	400
		809	AGA GAG AGA GAG AGA GG	400

Table 4.8: Nei's (1978) unbiased Measure of Genetic Identity and Genetic Distance fom ISSR markers in PopGene software

pop ID	Kitui	Marsabit	Baringo	Nyeri	Narok	Karura	Ngong	Jilore	Taveta
Kitui	****	0.8556	0.7992	0.8141	0.8471	0.8422	0.8494	0.8428	0.8366
Marsabit	0.1560	****	0.8203	0.8755	0.8481	0.8417	0.8298	0.8337	0.8803
Baringo	0.2241	0.1981	****	0.8019	0.8361	0.8250	0.7675	0.7239	0.8004
Nyeri	0.2057	0.1330	0.2208	****	0.8606	0.8236	0.8064	0.8436	0.8639
Narok	0.1659	0.1648	0.1791	0.1502	****	0.8269	0.8146	0.8201	0.8705
Karura	0.1718	0.1723	0.1924	0.1941	0.1900	****	0.8074	0.7790	0.8713
Ngong	0.1632	0.1866	0.2647	0.2152	0.2050	0.2139	****	0.8363	0.8438
Jilore	0.1710	0.1819	0.3231	0.1700	0.1983	0.2497	0.1788	****	0.8314
Taveta	0.1784	0.1275	0.2226	0.1463	0.1387	0.1377	0.1698	0.1847	****

*Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 4.9: ISSR pairwise matrix of Nei's (1978) Genetic Identity and Genetic Distance using GeneAlex software

Pop ID	Kitui	Marsabit	Baringo	Nyeri	Narok	Karura	Ngong	Jilore	Taveta
Kitui	****	0.856	0.799	0.814	0.847	0.842	0.806	0.843	0.837
Marsabit	0.156	****	0.820	0.876	0.848	0.842	0.867	0.834	0.880
Baringo	0.224	0.198	****	0.802	0.836	0.825	0.818	0.724	0.800
Nyeri	0.206	0.133	0.221	***	0.861	0.824	0.885	0.844	0.864
Narok	0.166	0.165	0.179	0.150	****	0.827	0.831	0.820	0.870
Karura	0.172	0.172	0.192	0.194	0.190	****	0.873	0.779	0.871
Ngong	0.215	0.143	0.201	0.122	0.185	0.135	****	0.766	0.837
Jilore	0.171	0.182	0.323	0.170	0.198	0.250	0.266	****	0.831
Taveta	0.178	0.128	0.223	0.146	0.139	0.138	0.178	0.185	****

*Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Table 4.10: Analysis of Molecular Variance for *S. henningsii* as revealed by ISSR markers

Source	df	SS	MS	Est. Var.	%	p>0.05
Among Regions	2	669.272	334.636	0.269	2%	0.001
Among Pops	6	1868.083	311.347	10.139	58%	0.001
Within Pops	261	1876.100	7.188	7.188	41%	0.001
Total	269	4413.456		17.595	100%	

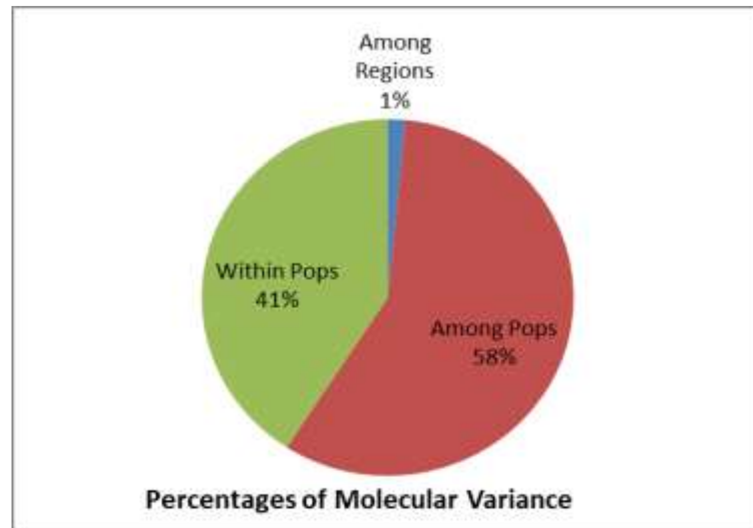


Figure 4.9: Percentage of Molecular variance of ISSR data

4.2.2.1 ISSR-based cluster analysis

The genetic similarity coefficient revealed ranged between 0.7239 (Jilore and Baringo) to 0.8803 (Taveta and Marsabit) (Table 4.9). Cluster analysis of ISSR data based on the unbiased genetic distance generated a dendrogram with three major groups. Cluster I consisted of three popupations namely Kitui, Ngong and Jilore. Cluster II consisted of five populations (Marsabit, Taveta, Nyeri, Narok and Karura) and cluster III consisted of Baringo population (Fig. 4.10). The clustering groups did not correlate to the

geographical areas of plant collection. The Principal Coordinate Analysis confirmed the clustering analysis (Fig 4.8).

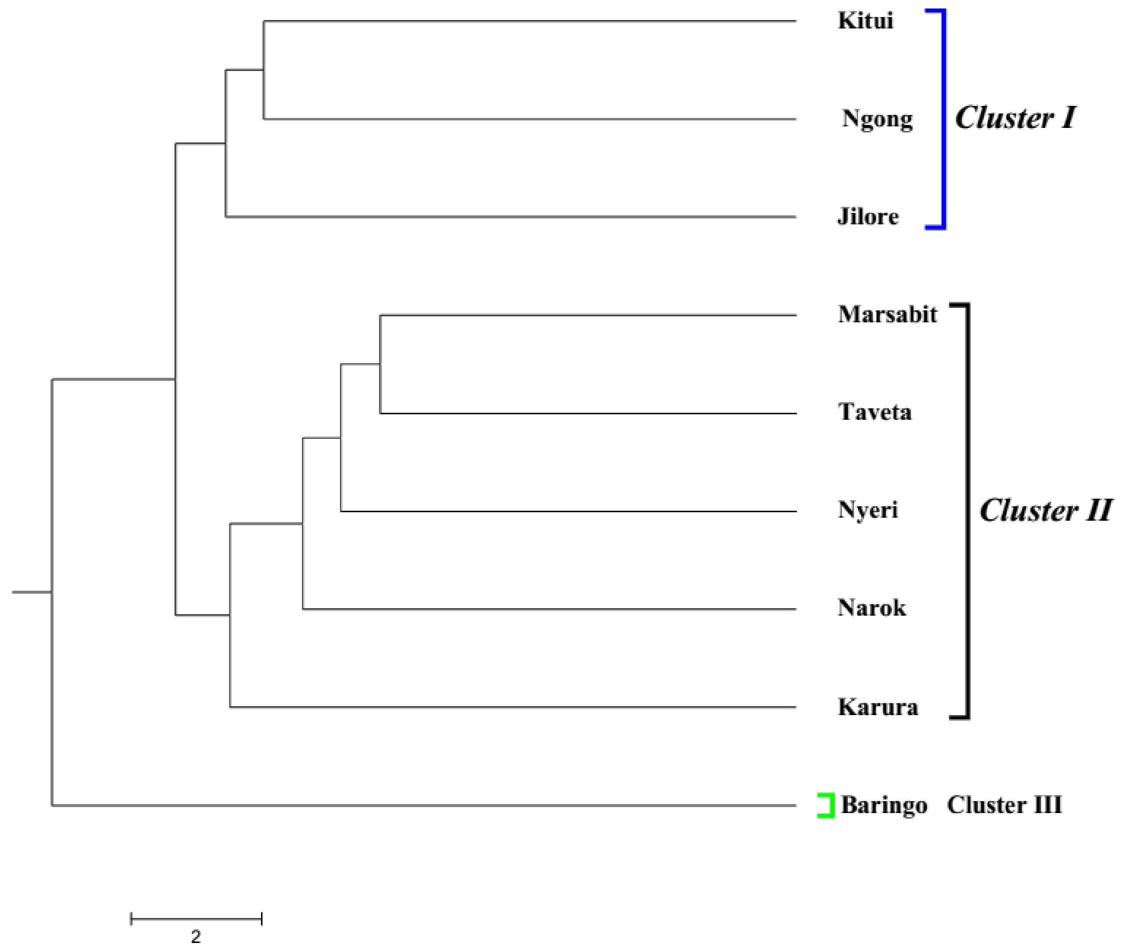


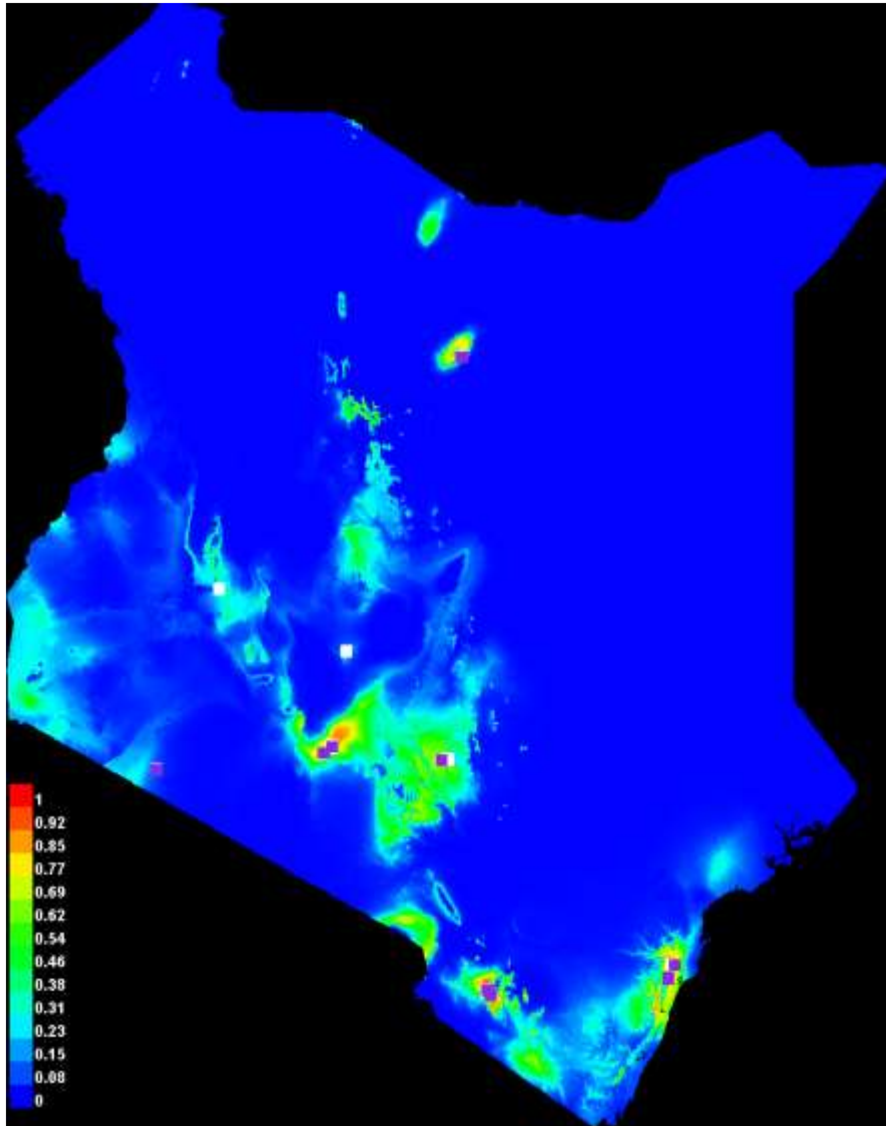
Figure 4.10: UPGMA clustering analysis of nine *S. henningsii* populations based on Nei's (1978) unbiased genetic distance

4.3 Species Distribution Modeling of *S. henningsii*

The results in this section show the model output designed by maxEnt software highlighting the predicted potential areas suitable for *S. henningsii* growth and conservation. It also indicates the statistical analysis methods used for model evaluation to determine its validity and reliability.

In this study, environmental conditions of the occurrence locations of *S. henningsii* were utilized to predict the probability of suitable conditions existing for the same species in other locations in Kenya using maxent model. In (Plate.4.1), the image uses colors to indicate predicted probability that conditions are suitable, with red indicating high probability of suitable conditions for *S. henningsii*, green indicating conditions typical of those areas where the species is found, lighter green shades indicating low predicted probability of suitable conditions and the blue color representing areas of unsuitable conditions.

4.3.1 Predicted potential suitable habitats for *S. henningsii*



Key

0-1: Probability

0-< 0.23: No Prob. (Blue)

0.24-0.38: Lowest Prob. (Light green)

0.39-0.69: moderate prob. (Green)

> 0.70 High Prob. (Red)

Plate 4.1: Model output showing the map of the predicted potential areas of *S. henningsii* in Kenya.

4.3.2 Model Evaluation

The evaluation of model accuracy is an essential step as it indicates the level of accuracy of the estimations. The concept of model validation (Bair 1994; Oreskes 1998) is

generally accepted and interpreted in terms of suitability for a particular purpose (Rykiel Jr. 1996; Sargent, 2001). Several methodologies have been used for model accuracy assessment in species distribution modeling. The receiver operating characteristic (ROC) and defined thresholds are important methodologies used for the evaluation of MaxEnt model quality (Reddy *et al*., 2015). Apendix 1 describes the common thresholds and the corresponding omission rates for the threshold-dependent binomial tests of omission.

4.3.2.1 Receiver Operating Characteristics (ROC) analysis

ROC is a graph obtained by plotting sensitivity on the y-axis and 1- specificity on the X-axis for all possible thresholds. Sensitivity refers to the fraction of all pixels that fall in the areas suitable for *S. henningsii* i.e. absence of omission error (true positive rate) while 1- specificity is the fraction of the pixels that falls in the areas unsuitable for *S. henningsii* but predicted as suitable or (false positive fraction) i.e. commission error across all possible threshold between 0 and 1. A model will be considered to perform better than chance if the curve lies above the diagonal of no discrimination that is if the AUC is higher than 0.5.

To develop ROC a certain proportion of the location data is selected for training data while the remaining portions is used as test data. For *S. henningsii* 75 % of the presence data was used for training and 25 % as test data. A good model is defined by a curve that maximizes sensitivity for low values of false positive fraction. The significance of this curve is measured by the area under curve (AUC) and has values that typically range from 0.5–1.0. For *S. henningsii,* the area under the curve (AUC) for the Receiver Operating Characteristic analysis measured at all possible thresholds was 0.986 and 0.983 for training and test data respectively. These values were close to 1 thereby showing the accuracy of the model in prediction (Fig. 4.11).

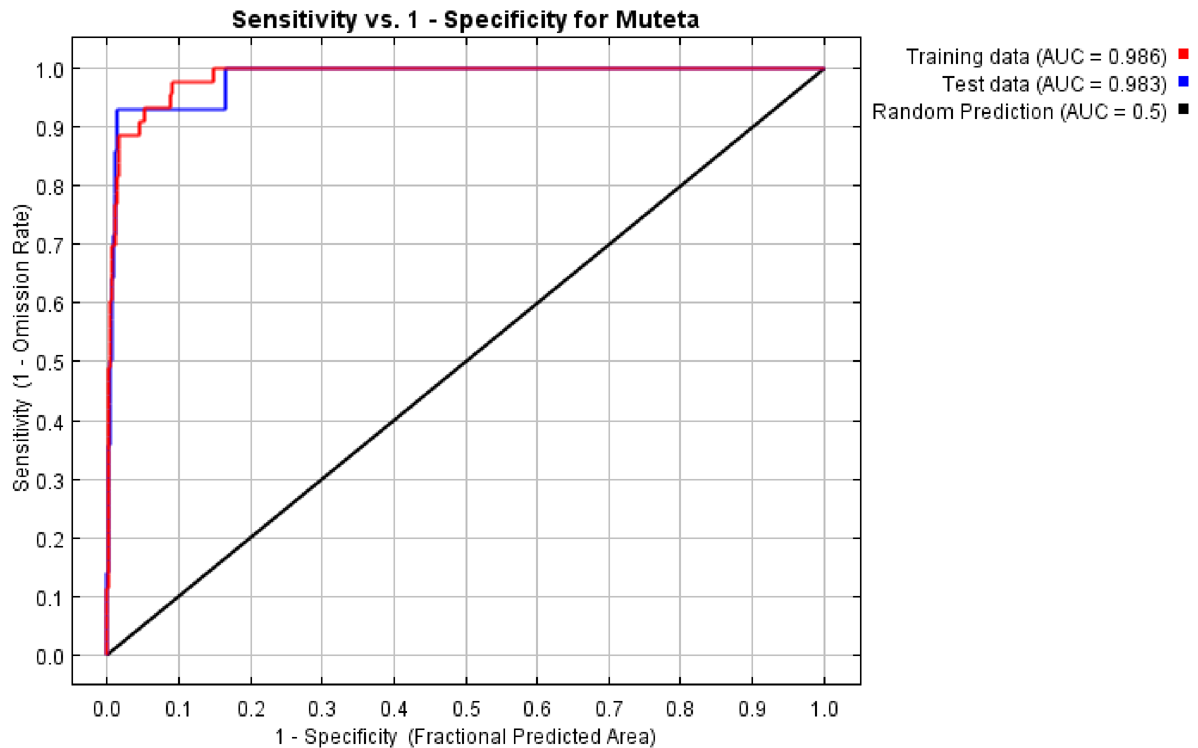


Figure 4.11: Receiver Operating Characteristic with Area under the Curve (AUC) measure.

4.3.2.2 Omission and commission rate analysis

Another approach to test and evaluate the model is through the analysis of omission and commission rate, which is a threshold dependent binomial test. It is based on omission and predictive areas (Fig.4.8). Omission is the fraction of the test localities that falls on the pixels not predicted as suitable for the species. Predicted areas are fraction of pixels that are predicted as suitable for the species. The red line indicates fraction of background predicted, black line indicates predicted omission, blue line indicates omission on training samples and the light blue line indicates omission on test samples. The omission rate is calculated both on the training presence records and on the test records (Fig. 4.12)

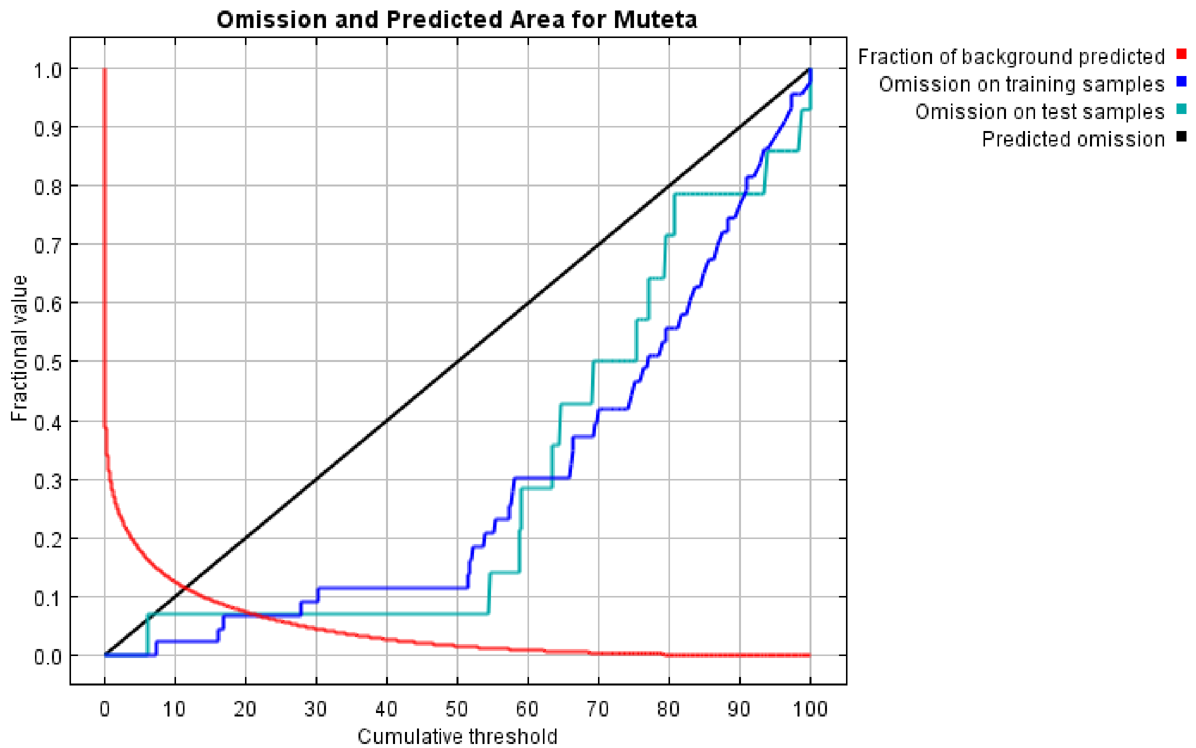


Figure 4.12: Omission Vs Predicted area for *S. henningsii*

4.3.3 Analysis of variable importance

This was conducted to determine the influence of each variable on the distribution of the species being modeled and subsequently illustrate which variable has the greatest influnce on the model prediction. MaxEnt does this in two ways; first by providing the percent contribution of each variable to the final model (Apendix 2) or by using a jack-knife test approach (Fig. 4.13, 4.14 & 4.15). MaxEnt can also constructed the response curves for selected variables to illustrate their effects on the probability of a species occurence in the study area (Fig. 4. 16a-d).

MaxEnt also performed the jack-knife test to evaluate the relative influence of different environmental variables in the model prediction of *S. henningsii* distribution. Therefore, the environmental variables with highest gain (provide much information) when used in isolation are tmax_1_res (Maximum temperature for January) in Fig 4.13 tmax_10_res

(Maximum temperature for October) in Fig.4.14 and 4.15 which therefore appears to have the most useful information by themselves. The environmental variable that decreases the regularized gain the most when it is omitted is pre_10_res, Fig 4.14, pre_12_res and pre_3_res (Fig. 4.14) which therefore appears to have the most information that is not present in the other variables. Therefore, *S. henningsii* seems to have high affinity for high temperature and moderate precipitation conditions. The following are jack-knife plots for training, test and AUC for *S. henningsii* respectively.

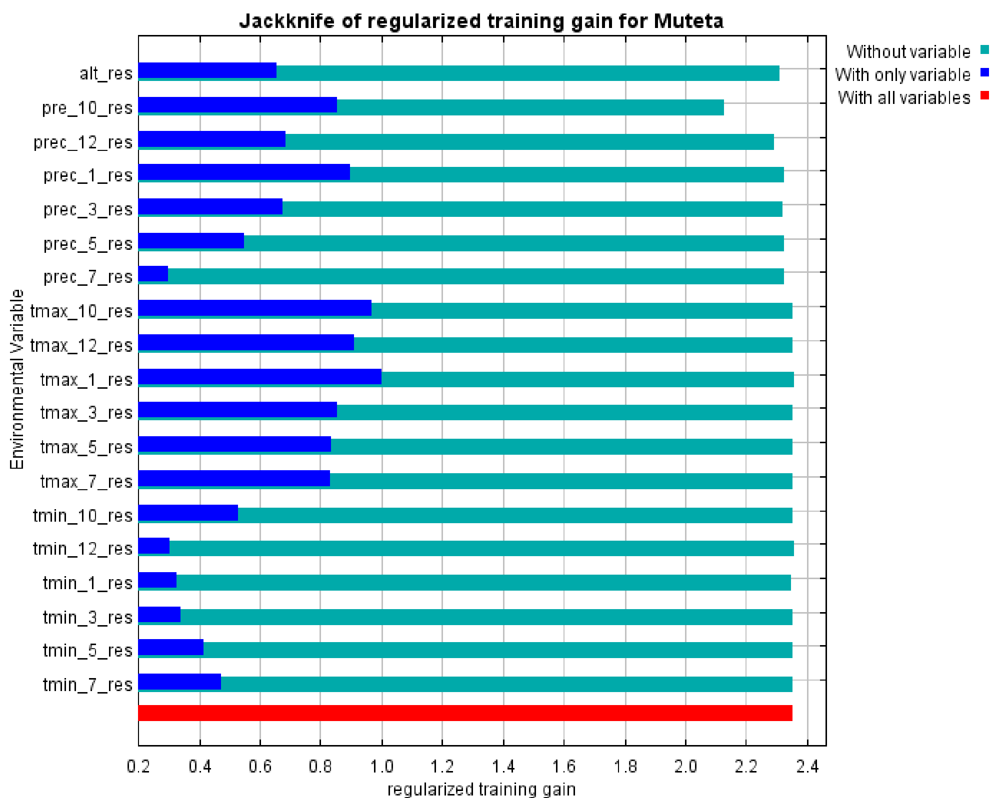


Figure 4.13: Jackknife test of regularized training gain for *S. henningsii*

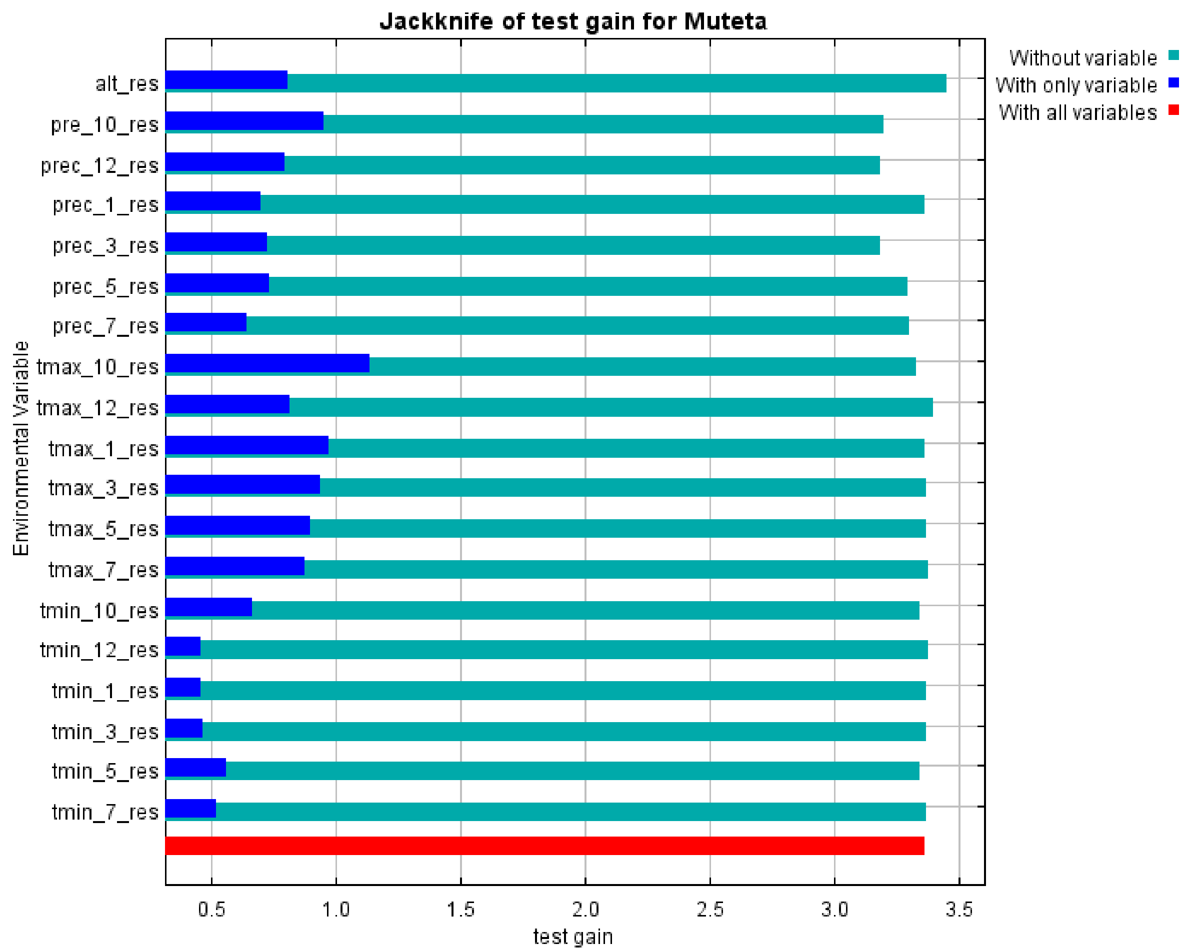


Figure 4.14: Jackknife test of regularized test gain for *S. henningsii*

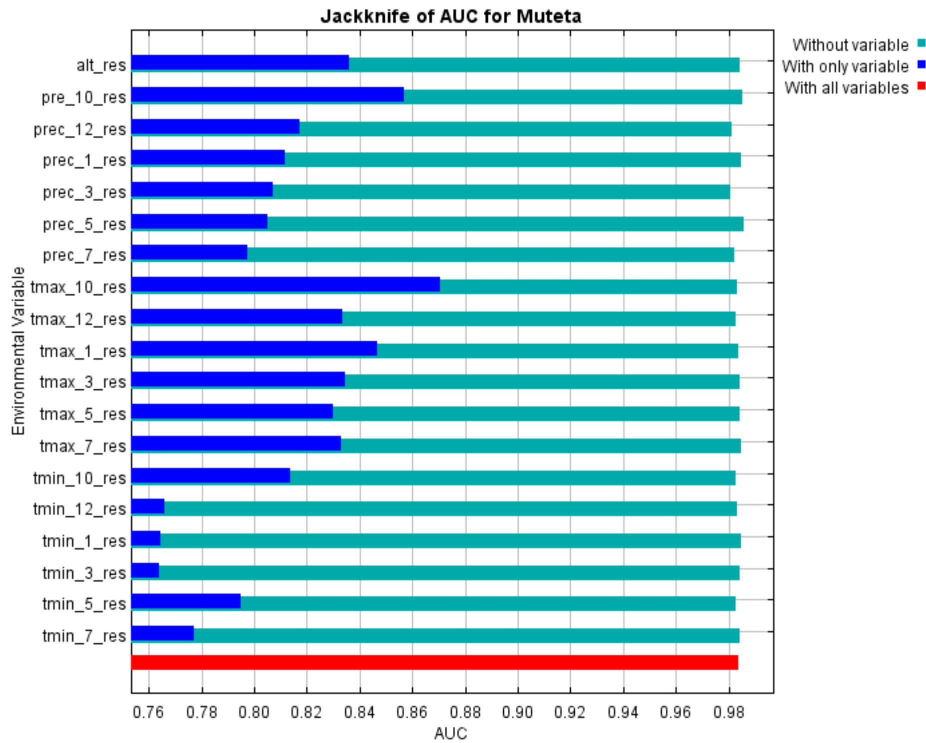
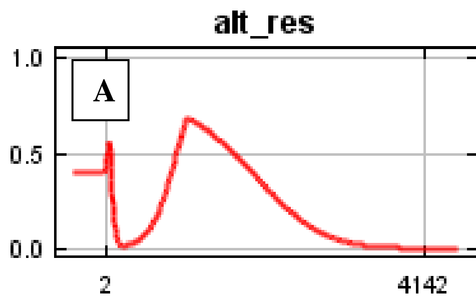
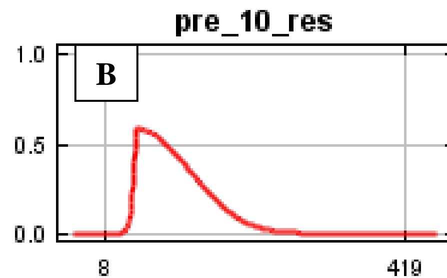


Figure 4.15: Jackknife test of regularized AUC for *S. henningsii*

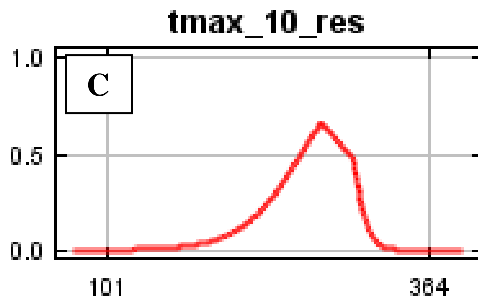
Fig. 4.13, 4.14 & 4.15: Relative importance of environmental variables based on Jackknife test (a) training gain (b) Testing gain and (c) AUC. As a measure of model's predictive ability

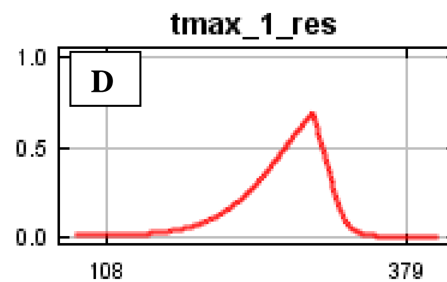
MaxEnt generated species' response curves that showed relationships between predicted probabilities of presence for *S. henningsii* and the different variables (Fig. 4.12).





Altitude of different ecological zones **precipitation for the month of October**





Maximum temperature for the month of October

Maximum temperature for the month of January

Figure 4.16: (a-d): Relationship between the strongest variables and *S. henningsii* probabilities of presence.

(A): Altitude of different ecological zones, (B): Precipitation for the month of October, (C): Maximum temperature for the month of October. (D): Maximum temperature for the month of January. Each of these curves is based on different maxent models created using only the corresponding variable

4.4 Ethnobotanical studies of *S. henningsii*

This section provides the findings in relation to the defined variables as prescribed in materials and methods:-

4.4.1 Location, gender and Age category

Kajiado had the highest number of respondents followed by Nyeri/ Narok with the least number in Kiambu (Fig. 4.17). Of all the respondents, 81 % and 19 % are male and female respectively. This was not significantly associated (χ^2 =5.763; d.f. =4; p= 0.218) with location, implying that both male and female respondents were proportionately represented in the study areas /location. The respondents aged 36-45 were most interviewed followed by 46-55 years (Table 4.11).

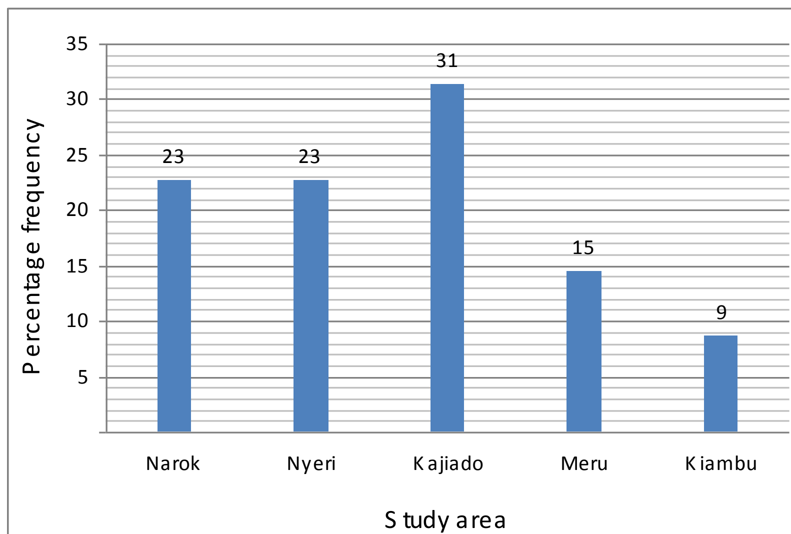


Figure 4.17: Percentage frequency distribution of respondents per study area.

Table 4.11: Age category of respondents in five study areas/locations

Age Category	Frequency (n)	Percentage frequency
26-35years	30	14
36-45 years	94*	43
46-55yesrs	79*	36
>55 years	16	7
Total	219	100.0

There are significant associations ($p<0.05$) and differences between study area and age category (Table 4.12) where Narok had the highest (57 %) proportion of respondents in the age category of 36-45 whereas Kiambu had (53 %).in the age category 46-55 years. In contrast, Meru had the least of respondents (6 %) in the category of 26- 35 years (Table 4.12). It is clear from the study that the age between 36 -55 years were the active group and hence the respondents were readily available for the interview. Consequently, there were significant associations ($p< 0.05$) between the age category of the respondents and sex/gender of the respondents (Table 4.13).

Table 4.12: Comparison between the age category and study areas

	Percentage frequency and mean rank on age category in years and study area/location					
Study area	26-35	36-45	46-55	>55	Total (n)	Mean rank
Narok	20	57*	22	0	49	83.76
Nyeri	22	12	22	5	50	114.6
Kajiado	7	44	37	12	6	119.80
Meru	6	50	34	9	32	115.2
Kiambu	10	37	53*	0	19	115.79
Chi square test	χ^2=26.919;d.f.=12;p =0.008					
Kruskal Wallis test	χ^2 =12.343;d.f. =4; p =0.015					

Key* significance difference

Table 4.13: Comparison between sex of the respondents and age categoty in five study locations

Percentage frequency and mean rank on the age category in years and the study areas						
Study areas	26-35	36-45	46-55	>55	Total (n)	Mean rank
Male	16	37	38	9	174	109.83
Female	5	65*	30	0	40	97.35
Chi square test	χ^2 =12.739;d.f=3; p=0.005					
Mann Whitney U test	χ^2 =30.74; p=0.218					

4.4.2 Economic Activities

Majority (70 %) of the respondents were self-employed (own their small business) as compared to one percent who were informal employment (Fig.4 18). This significantly varied (p<0.05) among the study location where there was more informal employment in Meru and Nyeri whereas more of self-employment in Narok, Kajiado and Kiambu (Table 4.14).

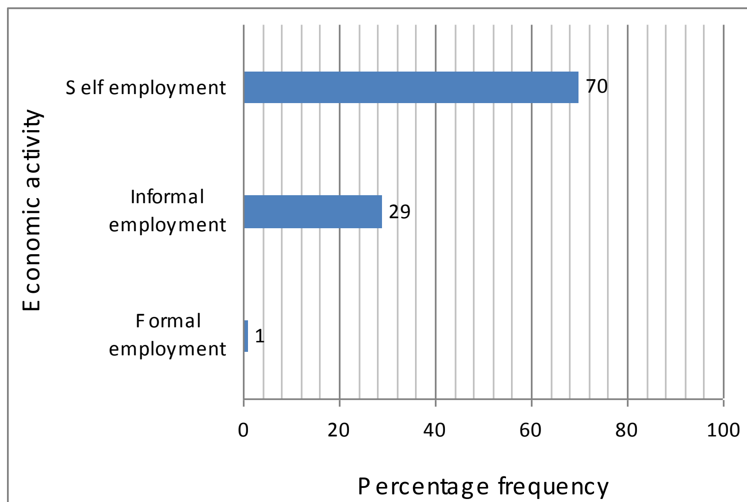


Figure 4.18: Other activities engaged apart from farming

4.4.3 Land size and use

There was a significant difference (P< 0.01) on land size among the study locations where Narok had the highest land size with the least size in Kiambu (Table 4.15). Majority of the respondents were using their land for growing crops and keeping livestock (Fig 4.19). This significantly varied (p<0.05) varied among the study areas whereby majority of farmers in Nyeri, Meru and Kiambu grew crops while in Narok and Kajiado kept livestock (Table 4.16).

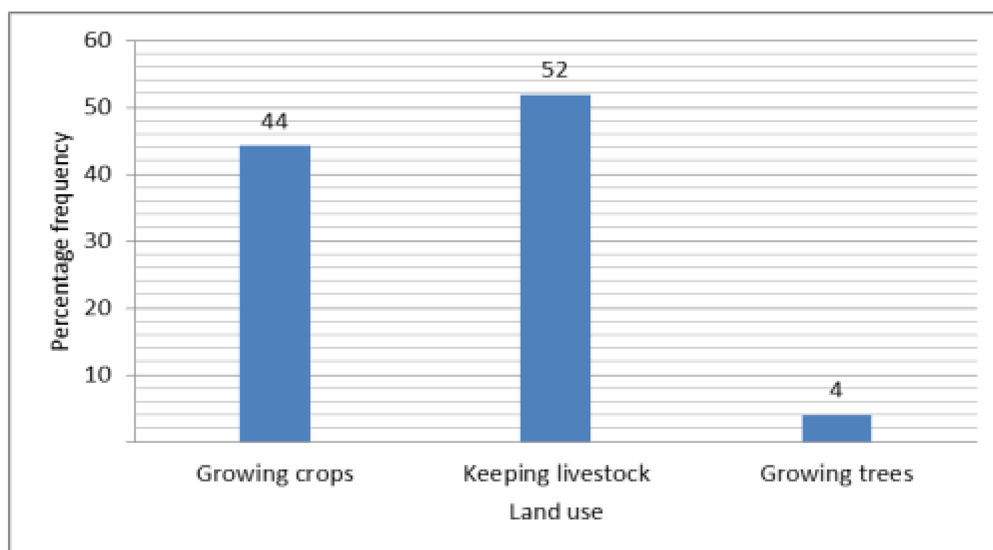


Figure 4.19: Land use across the study areas

Table 4.14: Other types of economic activities apart from farming among the respondents from the study areas

	Percentage frequency and mean rank on economic activities among study locations				
Study area	Formal employment	Informal employment	Self-employment	Total(n)	Mean rank
Narok	0	4	96*	49	137.63
Nyeri	2	64*	34	50	70.72
Kajiado	0	10	90*	68	130.99
Meru	3	68*	29	31	65
Kiambu	5	5	90*	19	129.0
Chi square test		χ^2=9.418;d.f=8;p<0.01			
Kruskal Wallis test				χ^2 =84.976;d.f=4; p<0.01	

Table 4.15: Mean land size in acreage among the study locations

					95% confidence Interval for Mean			
Study locations	n	Mean	Std. deviation	Std. error	Lower Bound	Upper Bound	Minimum	Maximum
Narok	43	7.2*	1.42	0.217	6.8	7.6	5.0	10.0
Nyeri	37	3.8	1.91	0.314	3.2	4.5	1.0	8.0
Kajiado	47	6.2*	2.86	0.417	5.3	7.0	1.0	13.0
Meru	28	4.2	2.29	0.434	3.3	5.1	1.0	10.0
Kiambu	18	1.7*	0.91	0.214	1.2	2.1	1.0	4.0
Total	173	5.1	2.73	0.209	4.7	5.5	1.0	13.0

Table 4.16: Comparison between study locations and land use

Percentage frequency on land use and mean rank				
Study areas			Total (n)	Mean rank
	Crops	Livestock		
Narok	50	50	48	109.25
Nyeri	79*	21	43	81.49
Kajiado	33	67*	52	125.78
Meru	100*	0	31	61.50
Kiambu	94*	6	17	67.12
Chi square test		χ^2=54.490;d.f=4; p<0.01		
Kruskal- Wallis Tests		χ^2=54.205;d.f=4; p<0.01		

4.4.4 Ethnobotany of *Strychnos henningsii*

Ethnobotany of *S. henningsii* focused on the awareness of the *S. henningsii*, place of growth, sources of the plant, uses, parts of the plant used, quantity used , processing of the plant products for use, accessibility of the plant and difficulties experienced when dealing with the plant.

4.4.4.1 Availability and awareness of *S. henningsii*

One hundred percent of the respondents knew *S. henningsii* plant and its various uses in the study locations. However majority of the respondents did not grow the tree on their lands (Fig 4.20. This was significantly ($p < 0.05$) associated and varied among study locations (Table 20). Majority of the farmers in Kajiado (82 %) and Kiambu (100 %) did not grow *S. henningsii* on their land as compared to 59 % in Narok who grew *S. henningsii* on their farms (Table 4.17).

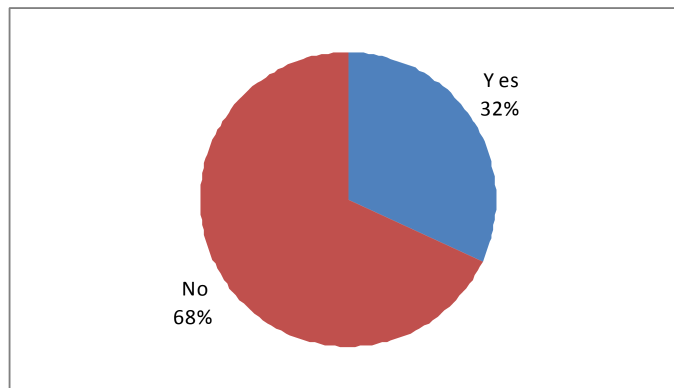


Figure 4.20: Percentage frequency of *S. henningsii* growing on farmer's land

Table 4.17: Association and comparison of growth of *S. henningsii* on farmer's land among the study locations

	Percentage frequency on growth of *S. henningsii* and mean rank			
Study Areas	Yes	No	Total (n)	Mean rank
Narok	59*	41	42	71.37
Nyeri	41	59*	46	89.3
Kajiado	18	82*	0	11.94
Meru	23	77*	31	107. 76
Kiambu	0	100*	18	130.0
Chi square test	χ^2=31.592;d.f=4; $p<0.01$			
Kruskal Wallis test	χ^2 =31.431; d.f=4; $p<0.01$			

4.4.4.2 Sources and availability of *S. henningsii*

The results showed that 98 % of the respondents across all study locations obtained *S. henningsii* from forests as compared to two percent from suppliers. This was significantly ($p<0.05$) associated and varied among the study locations (Table 4.18). Generally high proportion (50 %) of respondents obtained the plant with lots of difficulties with many citing an increase in the gathering effort (Fig. 4.21). This was significantly ($p< 0.05$) associated and varied among the study locations. For instance, majority (69 %) of respondents from Nyeri obtained the plant easily as compared to 77 % and 88 % of respondents from Meru and Kiambu who obtained the plant with difficulty respectively (Table 4.19).

Table 4.18: Association and comparison of sources of *S. henningsii* among the study areas

	Percentage frequency on sources of *S. henningsii* and mean rank			
Study Areas	Forests	Suppliers	Total (n)	Mean rank
Narok	100	0	49	94.5
Nyeri	98	2	42	96.8
Kajiado	98	2	53	96.3
Meru	100	0	32	94.5
Kiambu	88	12	18	106.5
Chi square test	χ^2=10.262;d.f=4;p=0.036			
Kruskal Wallis test	χ^2 =10.209;d.f=3; p=0.037			

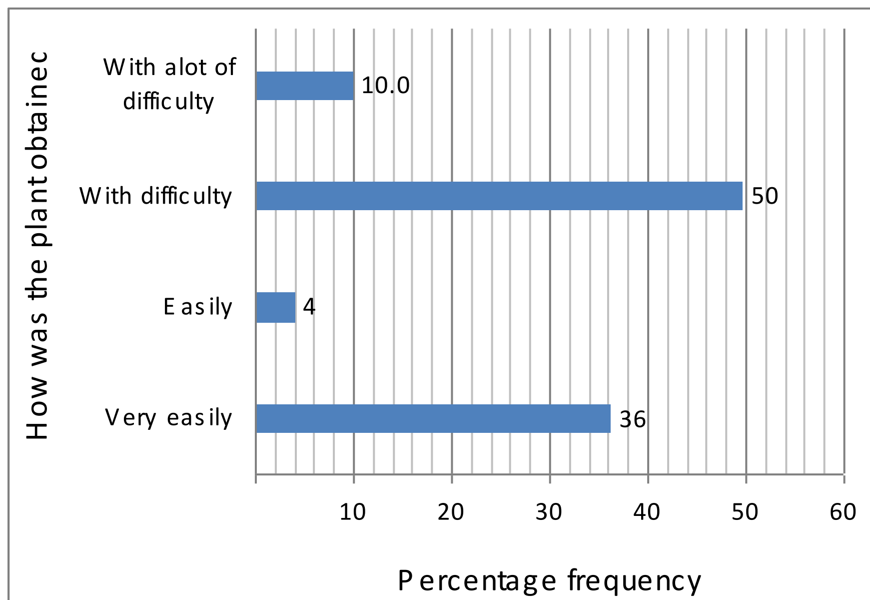


Figure 4.21: Frequency on how *S. henningsii* was obtained

Table 4.19: Association and comparison on how *S. henningsii* was obtained among the study areas

	Percentage frequency on how *S. henningsii* was obtained and mean rank					
Study Areas	Very easily	Easily	With difficulty	With a lot of difficulty	Total (n)	Mean rank
Narok	52*	4	22	22	46	92.11
Nyeri	69*	0	21	4	45	68.58
Kajiado	21	8	64*	7	61	109.74
Meru	17	0	77*	7	30	118.47
Kiambu	0	0	88*	12	17	137.00
Chi square test	χ^2=69.062;d.f=12; p<0.01					
Kruskal Wallis test	χ^2=31.587;d.f=4; p<0.01					

4.4.5 Trading on *S. henningsii*

Majority (64 %) of the respondents across the study locations were not trading on *S .henningsii* as compared to 36 % who did so. This was significantly ($p<0.05$) associated and varied among the study locations (Table 4.20). For instance 100 % of respondents in Nyeri and Meru were not selling the species as compared to 42 % and 58 % who were selling in Narok and Kajiado (Table 4.20). Of those who were selling, they traded in roots and bark at Kshs. 20 per piece or teaspoon.

Table 4.20: Trading on *S. henningsii* among the study areas

	Percentage frequency trading on *S. henningsii* and mean rank			
Study Areas	Yes	No	Total (n)	Mean rank
Narok	42*	58	12	30.67
Nyeri	0	100	13	44
Kajiado	58*	42	31	25.42
Meru	0	100	8	44
Kiambu	-	-	-	-
Chi square test	χ^2=18.544;d.f=3; p<0.01			
Kruskal Wallis test	χ^2=31.431;d.f=4;p<0.01			

4.4.6 Uses of *S. henningsii*

Ninety percent of the respondents across all the study location knew the uses of *S. henningsii* as compared to two percent who did not know. This was consistent with study locations where 100 % of the respondents from Meru, Narok and Kajiado knew the uses of the plant as compared to 93 % and 95 % from Nyeri and Kiambu respectively. They identified the following uses of the plant (Table 4.21).

Table 4.21: Overall uses of *S. henningsii* in all five study areas

Uses of S. henningsii	Frequency (n)	Percentage frequency
Treating stomach	43	13.1
Treating headache	53	16.2
Treating common cold	56	17.1
Source of energy	7	2.1
soup	35	10.7
Malaria treatment	56	17.1
Blood cleanser	2	0.6
Joint pain treatment	51	15.5
Deworming children	22	6.7
Appetizer	1	0.3
Chest pain	2	0.6
Total	328	100.0

Consequently, comparing the uses of the plant among the study locations, there were significant differences ($p < 0.05$) on the uses of the plant. For instance in Narok, the plant was mainly used for treating malaria whereas in Nyeri for making soup, Kajiado for treating, Meru for de-worming children and in Kiambu for treating stomach (Table 4.22).

Table 4.22: Uses of *S. henningsii* among the study locations

	Study locations and % frequency on uses of plant					
Uses of S .henningsii	Narok	Nyeri	Kajiado	Meru	Kiambu	Total (n)
Treating stomach	11	14	29*	10	26*	37
Treating headache	2	0	11	3	16	12
Treating common cold	18	5	5	3	21	18
Source of energy	2	2	0	0	0	2
Making soup	13	33*	16	7	0	32
Malaria treatment	42*	5	22	7	16	40
Blood cleanser	2	2	0	0	0	2
Joint pain treatment	7	35	18	16	16	37
Deworming children	2	0	0	55*	5	19
Mean rank	95.89	109.6	81.90	148.81	78.95	
Kruskall-Wallis test	χ^2=32.601; d.f=4; p<0.01					

On the other hand, cross tabulating between age bracket and use of *S. henningsii*, there was no significant difference ($p=0.910$) implying that the use of the plant was not in any way influenced by the age bracket of the respondents in all study locations**.**

4.4.6.1 Individual use of *S. henningsii*

The results showed that 99 % used *S. henningsii* as compared to one percent who did not. The common use was making soup (Fig. 4.22).

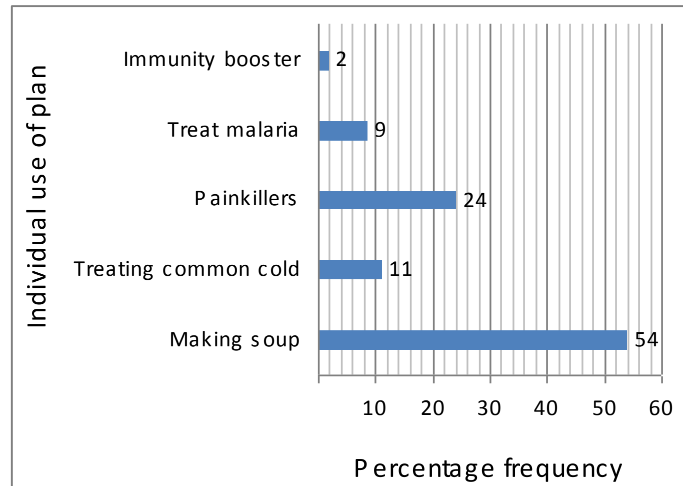


Figure 4.22: Individual use of *S. henningsii* among the study locations

4.4.6.2 Parts of *S. henningsii* used in herbal medicines

In this study, the plant parts of S. *henningsii* commonly used were stems and roots or their bark ground into powder. The leaves were not commonly used (Fig. 4.23). These varied significantly ($p<0.05$) among the study locations where roots were mainly used in Narok, Kajiado and Kiambu and stem in Nyeri and Meru (Table 4.23). Of these plant parts used, 98 % used mature ones as compared to two percent who used any. Consequently, 100 % used small portion to taste.

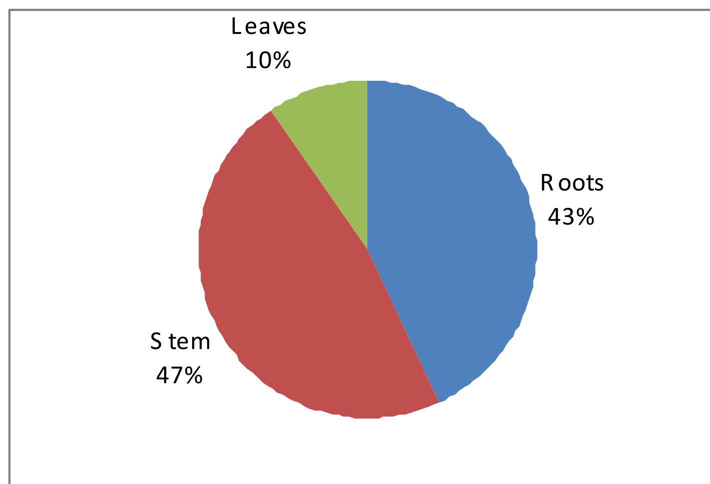


Figure 4.23: Parts of *S. henningsii* used among the study locations

Table 4.23: Plant parts used among the study locations

	Percentage use of plant parts among the study locations				
Study location	Roots	stem	Leaves	Total (n)	Mean rank
Narok	90*	6	4	48	80.56
Nyeri	18	82*	0	49	153.71
Kajiado	88*	12	0	65	80.92
Meru	25	72*	3	32	147.97
Kiambu	95*	5	0	19	73,53
Chi-square test	χ^2=110.608;d.f=8;p<0.01				
Kruskal-Wallis test	χ^2=94.418; d.f=4; p<0.01				

4.4.7 Extraction/ processing of *S. henningsii*

The study revealed that majority (72 %) of the respondents processed their plant parts through boiling in water (Table 4.24). Comparing the processing methods of the plant extracts, there was a significant differences (p<0.05) among the study locations (Table

4.25). Nyeri, Kajiado, Meru and Kiambu mainly boiled the plant parts in water whereas Narok graded the bark and then boiled in water

Table 4.24: Methods used for processing *S. henningsii* plant parts among the study areas

Methods of extraction	Frequency (n)	Percentage frequency
Boiling in water	138*	72
Grade the bark then boil in water	47	25
Put the scrapings in water and let stand for 5minutes	5	2.5
Dry the leaves, crush them and boil	1	0.5
Total	191	100.0

4.4.7.1 Mixing of *S. henningsii* plant parts with other things

The results showed that 67% mixed *S. henningsii* plant with other plants or substances such as honey and milk things as compared to 33 % who did not. They mainly mixed with honey, soup tea and other plant (Table 4.25). The key difficulties identified in relation to *S. henningsii* were scarcity and long distance walking (Table 4.26). The key suggestions were training of locals on tree conservation strategies and sustainable use, establishment of tree nurseries to enhance planting locally (Table 4.27). This would provide an alternative source of the plant and thus reduce the pressure exerted on the few individuals remaining in the wild habitat.

Table 4.25: Association and comparison on processing of *S. henningsii* plants parts among the study locations

	Percentage frequency on processing methods and mean rank					
Study Areas	Boiling in water	Grade the bark then boil in water	Put the scrapings in water and let stand for 5 minutes	Dry the leaves, crush them and boil	Total (n)	Mean rank
Narok	31	56*	11	2	45	136.76
Nyeri	96*	4	0	0	47	73.44
Kajiado	73*	27	0	0	51	94.89
Meru	86*	14	0	0	29	82.26
Kiambu	89*	11	0	0	19	79.24
Chi square test	χ^2=63.664;d.f=12;p<0.01					
Kruskal Wallis test	χ^2=59.968;d.f=4;p<0.01					

Table 4.26: Mixing of *S. henningsii* plants parts with other substances

Mixed with	Frequency (n)	Percentage frequency
Tea	3	15.0
Soup	7	35.0
Honey	9	45.0
Milk	1	5.0
Total	20	100.0

Table 4.27: Difficulties experienced with *S. henningsii* plant collection

Difficulties experienced	Frequency (n)	Percentage frequency
Scarcity	72*	29
Long distance	86*	35
Bad weather	9	4
Insecurity	21	9
Ignorance	4	2
Deforestation	13	5
Expensive buying	30	12
Confusing it with other trees	7	3
Identifying	5	2
Total	247	100.0

Table 4.28: Suggested solutions to the identified difficulties by the respondents

Suggested solutions	Frequency (n)	Percentage frequency
Establish tree nurseries	56	23
Train locals on tree conservations and use	79	32
Design better methods of harvesting	1	0.4
Plant it locally	81	33
Processing the medicine from the plant and sell it in shops	1	0.4
Afforestation	12	5
Protect and safeguard	5	2.4
Proper harvesting	12	5
Total	247	100.0

4.5 Synopsis of the major findings of the study

Genetic diversity study indicated that low genetic diversity exists in *S. henningsii* populations as revealed by the various parameters of genetic diversity. For instance the mean percentage polymorphism revealed were 38.97% and 43.40 % for RAPD and ISSR markers respectively while Nei's genetic diversity (H) and Shannon Informtion index (I) ranged between 0.0867-0.1483; 0.1289-0.2337 respectively for RAPD markers and 0.0955-0.1828; 0.1448-0.2728 respectively for ISSR arkers. Analysis of Molecular Variance (AMOVA) indicated a higher genetic variation 54 % and 58 % for RAPD and ISSR markers respectively among populations than within 46 % and 42 % populations.

MaxEnt modeling was used to delineate the potential suitable areas for *S. henningsii* in Kenya. The results revealed that this model performed better than a random distribution in prediction as showed by the Receiver Operating Characteristic (ROC) analysis. The Area under the Curve (AUC) values achieved for the training and tests data were 0.986 and 0.983 respectively. These values showed the accuracy of the model in prediction as they are closer to one. Therefore the areas highlighted in the model meet the requirements for growth of *S. henningsii* and could be used for its conservation. The jackknife tests analysis indicated that the variable of importance were maximum temperature, precipitation and altitude and hence contributed more information in explaining the distribution of *S. henningsii*.

Ethnobotanical study showed that *S. henningsii* is threatened in all the areas of study. The key difficulties experienced by the respondents in this study were scarcity and long distance walking. This showed that that *S. henningsii* population size had declined locally and that they had to walk for long distance to collect the plant material for medicinal use from the natural forests. The roots and the stems were the main plant parts harvested. More often poor and destructive methods are used to collect the plant materials and thus threatening the survival of many medicinal plant species including *S. henningsii*.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

This chapter focuses on the discussions of the major findings of the research study and has been organized according to the specific objectives. It has provided the interpretations of the results obtained by comparing them with findings from previous studies under taken in the same areas of research study.

5.2 Discussions

5.2.1 Genetic diversity of *S. henningsii*

5.2.1.1 Extraction of DNA

DNA extraction is an essential step for obtaining good results in a molecular analysis. However isolation of DNA from plants high in secondary metabolites such as tannins, phenolics and polysaccharides can be problematic (Tibbits *et al*., 2006); the yield of DNA isolated is often low and the quality is poor (Li *et al*., 2001). Contamination of extracted DNA by these cellular components inhibits downstream molecular analysis and therefore their removal is critical to the success of most molecular based research.

Typically, molecular studies involving plant species have used leaves as their main sources of DNA. Different problems arise while dealing with the leaves as the only source of DNA. For instance, sampling of leaves from tall trees may be a laborious task; deciduous and semi-deciduous trees may have all their leaves fallen or senescence during dry seasons and leaves are also hampered with high quantities of secondary metabolites (Novaes *et al*., 2009). Plant extracts of *S. henningsii* have been found to contain different types of secondary metabolites (Angenot and Tits 1981; Oyedemi *et al*., 2010a; Oyedemi *et al*., 2013; Penelle *et al*., 2000). DNA isolation from dried young

mature leaves of *S. henningsii* was not achieved using both SDS and CTAB methods even after modifying the protocols. This was probably due to the reason that leaves of this plant species contained high quantities secondary metabolites.

Methods published by Doyle and Doyle 1987; Murray and Thompson 1980; Wagner *et al*., 1987 suggests the use of CTAB and is frequently used for tree species. Some researchers (Devey *et al*., 1996; Kim *et al*., 1997; Steward and Via 1993) have modified them also with PVP-40. Others methods based on SDS (Jobes *et al*., 1995; Lin and Kuo 1998) have also been used but less frequently used for tree species. In most cases, after the initial extraction and precipitation, many cleaning steps are required to separate the DNA from the contaminants (Tibbits *et al.*, 2006). Several protocols have been developed for DNA isolation from cambium tissues (Colpaert *et al.*, 2005; Rachmayanti *et al*., 2006; Tibbits *et al*., 2006; Maxine *et al.*, 2015; Cabral *et al*., 2017; Ebert *et al*., 2017) among others.

In *S. henningsii* DNA isolation was achieved by extraction of DNA from cambium tissue using increased concentration of CTAB (2 %) and NaCl (1.4 M) in the extraction buffer. This prevented co-precipitation of polysaccharides with DNA. However the DNA pellets obtained were brown in color indicating its contamination by phenolic compounds. The best DNA quality was achieved when an additional cleansing step with sorbitol buffer was used. The powdered cambium tissues were initially washed with sorbitol buffer several times until the mucilaginous polysaccharides were not visible in the samples. DNA was the extracted using modified CTAB method resulting into white DNA pellets. Sorbitol has been used by other researchers in DNA isolation (Souza *et al.,* 2012; Li *et al*., 2001). This protocol could be useful not only for DNA isolation of *S. henningsii* but also other species that have high levels of secondary metabolites.

5.2.1.2 RAPD and ISSR Analysis

Genetic diversity assessment of medicinal plant species is an essential component in germplasm characterization, evolution, breeding and conservation (Wu *et al.,* 1999).

However, from literature survey information on genetic diversity of *S. henningsii* is lacking. Therefore efforts have been made in the present study to characterize 270 individuals from nine populations of *S. henningsii* in Kenya using RAPD and ISSR markers in order to provide insight useful for its conservation and sustainable utilization. Many studies on genetic diversity of medicinal plant species have been reported using the same markers for example, *Withania somifera* (Khan and Shah 2016), *Plumbago zeylanica* (Panda *et al*., 2015) *Canthium Parviflorum* (Kala *et al*., 2017), *Angelica sinensis* (Mei *et al*., 2015) among others.

In the present study, the results revealed that all the loci detected by both RAPD and ISSR markers in *S. henningsii* genotypes were polymorphic. A higher percentage polymorphism was revealed by ISSR markers than RAPD markers. In other studies on the genetic diversity of *Costus pictus* similar results were obtained where a higher percentage polymorphism was revealed by ISSR markers (42.47 %) than RAPD (37.57 %) (Naik *et al*., 2017). Moreover, low levels of polymorphism in ISSR and RAPD markers have also been reported in previous studies for example 26.43 % RAPD; 24.36 % ISSR in *Bruguirra gymnorrhiza* and 14.4 % RAPD;12.73 % ISSR in *Heritiera fomes* (Dasgupta *et al.,* 2015). Yet in other studies a high polymorphism (92.26 %) RAPD; 82.76 % ISSR was reported in *Chlorophytum borivilianum* (Samantary and Ngangkham 2017), *Ziziphus spina-christi* (L.) (Alansi *et al*., 2016) reported 93.4 % percentage polymorphism, (Liu *et al*., 2013) reported 76.1 % in *Thuja sutchuenensis* and 71.8 % RAPD; 80.9 % ISSR percentage polymorphism in *Aloe vera* (L) (Chandra *et al*., 2014).

Several studies on natural populations have indicated the percentage polymorphism as a measure of genetic diversity (Naik *et al*., 2017). However, despite being the most commonly used indicator of genetic diversity, variation in this value is observed (Soares *et al*., 2016). Nei (1987) reported that percentage polymorphism is not a significant measure of genetic variation and that the parameter of genetic diversity (H) is more appropriate. In this study, the mean values for genetic diversity (H) and Shannon index (I) attained using these markers wee low. However these values were highest in Kitui using RAPD and Ngong population using ISSR markers.

These values indicate low genetic (allelic) diversity for *S. henningsii* genotypes. The results obtained could be attributed to the pollination, propagation and seed dispersal mechanisms in *S. henningsii*. This plant species has cleistogamous reproduction (self-pollinating) (Adowale 2015; Bruce and Lewis 1960) and bears small and brightly colored flowers which indicate a high possibility of entomophilous pollination. Insects transfer pollen for short distance mainly on flowers in a single tree resulting in the production of inbred seeds with poor germination (Adowale 2015; Bryndum and Hedgegart 1969; Mathew *et al*., 1987; Indira and Mohandas 2002; Tangmitcharoen *et al.,* 2009). It is propagated through seeds, wildings as well as suckers (Maundu and Tengas 2005). Seed dispersal is mainly by mammals especially birds and apes (Adowale 2015). It is also known to occupy restricted geographical zones in areas where it is naturally found. All these above factors have resulted in the narrow and common gene pool in *S. henningsii* populations. Other studies that reported low level of genetic divergence in the medicinal plant species using the same markers in their study include *Thuja sutchuenensis* (Liu *et al*., 2013), *Croton tetradenius* (Pereira –Almeida *et al*., 2017), *Costus pictus* (Naik *et al*., 2017) and *Peganum harmals* L (Zebarjadi *et al.,* 2016). The differences in the results obtained from the two markers may be due to the fact that they target different regions in the genomic DNA.

Analysis of molecular variance (AMOVA) revealed a high genetic variation among populations than within populations using ISSR and RAPD markers. This may be due to the reproductive system in *S. henningsii*. Additionally, genetic drift may have also contributed to the higher among population diversity through the loss of some alleles with successive generations in some population. In *Withania somnifera* genetic diversity study, a high variation among populations than within populations was reported using RAPD and ISSR primers (Khan and Shah 2016). This was attributed to the self pollinating nature of the species. Panda *et al*., (2015) also reported a higher genetic variation among *Plumbago zeylanica* populations revealed by RAPD and ISSR markers due to habitat fragmentation. Reduction and fragmentation in wild medicinal plants due to over-exploitation in the forest cover could be one of the main causes that led to an

increase in genetic differentiation and reduced gene flow between populations (Panda *et al.,* 2015).

In this study, Kitui, Taita–Taveta and Nyeri populations were highly over exploited and thus revealed a high genetic variation due to the reduction in gene pool within these populations. *S. henningsii* has been reported as disappearing due to over exploitation (Musila *et al.*, 2004). A decline in population size lowers the genetic diversity and leads to inbreeding depression (Liu *et al*., 2013). This is a highly possible scenario in the analyzed populations of *S. henningsii* which was a common species in the past but has rapidly declined due to destructive harvesting methods and over-exploitation. This can be one of the reasons that led to genetic drift. In the long run, a reduction in genetic variation could lower the ability of a population to adapt and survive under the changing environmental conditions (Liu *et al*., 2013). Adaptability and evolution of any species are based on the level of genetic diversity present in populations, which reflect the richness of diverse germplasm in a specific environment or location (Li and Chen 2004). Genetic drift may have also contributed to the higher among population diversity through the loss of some alleles with successive generations in some population.

Clustering analysis grouped the populations two groups based on the Nei's (1978) unbiased genetic distances using RAPD markers. Cluster I consisted of seven populations (Kitui, Taveta, Karura, Marsabit, Ngong, Nyeri and Narok), while Cluster II consisted of two populations Baringo and Jilore. ISSR markers grouped the populations into three clusters. Cluster I consisted of three populations namely Kitui, Ngong and Jilore, Cluster II consisted of five populations (Marsabit, Taveta, Nyeri, Narok and Karura) while cluster III had only one population, Baringo. The Principal Coordinate Analysis confirmed the results of the Cluster analysis where the dispersion of individuals based on their genetic distance placed those that are genetically close together. However, both UPGMA and the PCA analysis did not indicate a clear pattern of clustering and the geographical trend among the populations. The genetic divergence did not match to the geographical places of plant collection. Similar results have also been reported in studies on other medicinal plants species such as (Zebarjadi *et al*.,

2016) in *Peganum harmala* L., (Liu *et al*., 2013) in *Thuja sutchuenensis* and (Pereira-Almeida *et al.,* 2017) in *Croton tetradenius* using RAPD and ISSR markers, (Varma and Shrivasava 2018) in *Andrographis paniculata* used AFLP markers and (Gaafar *et al*., 2014) in *Breonandia salicina* using ISSR markers. Geographical isolation is one of the major factors influencing genetic differentiation by limiting the amount of gene flow through seeds and pollen (Pfeifer and Jetschke (2006). The lack of significant correlation between genetic distance and geographical locations indicate that genetic drift has played an important role in influencing the genetic structure and increasing the genetic variation among populations (Fischer *et al*., 2000).

In conclusion, the results of this study showed that low genetic variation exist at species and population level as assessed by RAPD and ISSR markers. However, high genetic variation among populations exists in *S. henningsii*. Therefore efforts should be made to preserve all the existing populations of this plant species and their habitat. Due to its wide scale medicinal use, it would be sustainable if plantation of new populations can be established to meet its demand and also widen the genetic base by collecting the planting materials from different populations. In this way we can alleviate the over exploitation of the natural resources of *S. henningsii.*

The genetic variation pattern revealed in this study can be associated with the ethnobotanical uses of *S. henningsii* which revealed that this species is used for treatment of different diseases in different areas and that different part of this plant are used as a source of medicine in different areas as well. A study by Bailie *et al.,* (2016) on phytogeographic and genetic varation of a medicinal plant Sorbus Spp reported that the plants increased the production of secondary metabolites in areas where the plants were exposed to high environmental stress. The expression of the genes responsible for the biosynthesis of the secondary metabolites was high in areas of stressful environmental factors. Their results conformed to the local traditional knowledge that harvesting of medicinal plants should be carried out in areas where plants showed efficient traditional medicinal use i.e. along the coastal and the northern latitude. Plants growing in Northern areas are faced with greater environmental stress due to the greater

range of temperature while coastal plants have to cope with greater amounts of salt in their soils. Consequently; these plants cope with these stressors by producing more secondary metabolites, which in turn have medicinal value (Bailie *et al*., 2016). Fraser *et al.*, (2007) reported that plant species also exhibit differences in antioxidant capacity according to locality and tissue type. Furthermore, differences in phenolic compounds and activities observed according to tissue type were supported by traditional medicinal practitioners, which use decoction of specific parts of the plant for different symptoms (Fraser *et al*., 2007).

Secondary metabolites are known to aid plants in dealing with environmental stresses (McCune and Johns 2007; Theis and Lerdau, 2003; Figueiredo *et al.,* 2008) and are also responsible for the medicinal properties in plants. In this view, the expression of the genes for the production of the secondary metabolites and their accumulation in the plant tissues in *S henningsii* varies depending with the environmental factors in their locality. This could explain why *S. henningsii* is used for treating various ailments in different areas and different plant parts used in different geographical areas as a source of medicine.

5.2.2 Species Niche Modeling of *S. henningsii*

5.2.2.1 **MaxEnt Output**

The potential habitats distribution for *S. henningsii* predicted by maxEnt showed that the areas suitable for growth and reintroduction of this species area along the coastal regions (Taita hills, Shimba hills, Kilifi (Arabuko sokoke forest), Mt Kilimanjaro game reserve), Marsabit, Huri hills Marigat, Baragoi, Malaral (dryland areas in Kenya), Ngong and Karura forests (Plate 4.5). The predicted probability of suitable condition was generated on a scale from 0 to 1. Lowest suitability areas are represented by 0 while 1 represents the areas with highest suitability. The probability less than 0.23 are areas of unsuitability represented by the blue shade. The probabilities between 0.23 to 0.38 are low suitable areas represented by lighter green color, 0.38 to 0.69 are moderate or typical of those

areas where *S. henningsii* is growing represented by green color while areas of higher suitability i.e. greater than 0.77 are represented by the red color in the prediction map

Receiver operating characteristic (ROC) is a threshold-independent approach analyzed for both training and test data. In ROC, the performance is measured on the basis of area under curve (AUC) (Hanley and McNeil, 1982). ROC curve is a plot between sensitivity (true positive fraction), i.e. absence of omission error and the proportion of incorrectly predicted observed absences (1-specificity) or false positive fraction, i.e. commission error. The specificity is defined using predicted area, rather than true commission. The area below the ROC curve, i.e. the value of the area under the curve (AUC) indicates the predictive accuracy of the model.

ROC analysis was carried out both on the training data and test data records for *S. henningsii.* The AUC values achieved on the training and the test data were 0.986 and 0.983 respectively. AUC has values that range from 0.5 –1.0. Values close to 0.5 indicate that the model is close to random and is a poor indicator, whereas a value of 1 indicates best run (Engler *et al*., 2004; Swets 1988). AUC is a ranked approach for assessing model fit that determine the probability that a presence location will be ranked higher than a random background location i.e. AUC > 0.9 = very good; AUC 0.7 -0.9 = good and AUC < 0.7 = uninformative (Swets 1988; Phillips *et al*., 2006). In general, the higher the AUC value, the more accurate the prediction of the constructed model (Elith, 2002) and when the AUC values are more than 0.75, the constructed model is applicable. Therefore maxEnt model for *S. henningsii* performance was very good and applicable in predicting the potential suitable areas for this species in Kenya since the values for AUC were greater than 0.9.

AUC's are developed from ROC plots for assessing differences in species suitability for developed models compared to a random distribution. A binomial test of omission (known areas of presence/ predicted absence) can then be used to test whether or not this difference is significant (Philips *et al*., 2006). This test is a threshold dependent method

based on omission and predicted area to test the suitability of the model in prediction (Phillips *et al*., 2002; Phillips and Dudik 2008).

The '25' entered for the random test percentage commanded the program to randomly set aside 25% of the sample presence data for testing. This allows the program to do some statistical analysis and much of the analysis uses threshold to make a binary prediction with suitable conditions predicted above the threshold and the unsuitable below. Fig 4.8 shows the omission and predicted areas as a function of the cumulative threshold. The omission is calculated on both training (75%) and test (25%) records respectively. The omission rate should be close to the predictive omission because of the definition of the cumulative threshold. Fig 4.8 shows how training and test omission and predicted area vary with the choice of cumulative threshold.

The omission rate on test records (sky blue line) is a very good match to the predicted omission rate (black line) and the omission rate for test records is drawn from maxEnt distribution itself. The predicted omission rate is a straight line (black line) by definition of the cumulative output format. In some case, the test omission rate lies well below the predicted omission line while in other situations the test line lies well above the predicted omission line. A common reason being that the test and the training records are not independent e.g. if they are derived from the same spatially auto-correlated presence data as was the case for *S. henningsii*. This indicates that maxEnt model was significantly better than random in the binomial test of omission and predicted area curve.

The other approach used for evaluation of maxEnt model in this study was the defined threshold. This approach involves selecting thresholds to establish the sites that are considered suitable or unsuitable for the species of interest. Once a threshold has been identified the locations can be classified as suitable or unsuitable for the species of interest. These thresholds are established to maximize sensitivity while minimizing specificity (Fielding and Bell, 1997; Phillips *et al*., 2006). Threshold values differ for each model and are selected to provide a desired balance between omission and

commission (Fielding and Bell, 1997; Hernandez *et al.,* 2006). Where this threshold is applied is selected at the discretion of the modeler, for example, when dealing with endangered species the modeler may decide to maintain zero omission error while identifying the minimum predicted areas. However, if he is interested in identifying any possible area that a species may utilize, then he may want to minimize commission error (Pearson *et al*., 2007).

5.2.2.2 Variables Importance

It is important to determine how each variable influences the presence of the modeled species and subsequently which variables have the greatest influence and in what way these variables influences the species distribution. MaxEnt determines the importance of variables in two ways: - First by providing the percent contribution of each variable to the final model (Badwin and Bender 2008; Phillips 2009). The contribution values are determined by the increase in gain in the model provided by each variable (Phillips 2009). However caution must be used when employing this method as strong co linearity can influence results by indicating more importance for one of two or more highly correlated variables. In this study, the relative contribution of the environmental variables as generated by maxEnt is shown in Apendix 2. The variables that contributed most to the model development are t_max_10 res, pres_1 res and t_ max_1 res.

Alternatively a jackknife test approach can be used for assessing variable importance (Phillips 2009; Yost *et al*., 2008). Jackknife test approach is a method that excludes one variable at a time when running the model. This provide information on the performance of each variable in the model in terms of how each variable is importance in explaining the species distribution and how much unique information each variable provides (Phillips 2009; Yost *et al*., 2008). In the present study the results revealed that maximum temperature, precipitation and altitude contributed a lot in designing the model for the three jackknife tests as compared to the minimum temperature. This indicated that *S. henningsii* prefers areas with high temperature and moist conditions. This also conforms with the potential areas predicted on the map by maxent model. These areas are found in

the dryland areas of Kenya with high temperature and also experience short term rainy seasons Majority of the potential suitability areas for *S. henningsii* are found in low altitude areas and the probability of presence decline with increase in the altitude as indicated by the respose curve.

MaxEnt also generated the response curves to explain in which way each variable influence the species distributions ie giving us an indication of areas where the species is likely to be found. MaxEnt is an exponential model, the probability assigned to a location is proportional to the exponential of the selected combination of variables, thus allowing construction of response curves to illustrate the effect of selected variables on probability of occurrences (Baldwin and Bender 2008; Phillips *et al*., 2006; Yost *et al.,* 2008; Hoenes and Bender 2010). These response curves consist of a chart with specified metrics for the variable in question represented on the x-axis and the predicted probability of suitable conditions along the y-axis (Phillips 2009). Upward trends for variables indicate a positive association, downward movements represent a negative relationship and the magnitude of these movements indicates the strength of these relationships. Therefore, maxEnt response curves showed that the variables that strongly contributed to the model prediction were maximum temperature, altitude and precipitation. as in the jackknife tests.

5.2.3 Ethnobotanical Studies of *S. henningsii*

Local people have interacted with plants from time immemorial and they have a vast knowledge on the uses of plants, geographical distribution, growth conditions and conservation status. In order to develop strategies for the conservation of medicinal plants, it is necessary to collect traditional knowledge of the plants, their distributions and traditional conservation practices (Qureshi *et al.,* 2006). Herbal medicine plays an important role in rural areas and various locally produced drugs are still being used as household remedies for different ailments (Qureshi and Ghufran 2005) and thus an ethnobotanical study of *S. henningsii* was carried out to collect its traditional knowledge useful in guiding its sustainable use and conservation.

Generally more male respondent were interviewed in this study compared to the female, although both sexes were proportionately represented in the study areas. This is probably because men go frequently to the forests than females to collect wild food and medicine and even grazing their livestocks. Men are also more involved in herbal medicine trade (Liu *et al.,* 2017). In this study, age category 36-45 represented the highest number of informants. However, among the study areas Narok had the highest representation followed by Kiambu in the age categories 36-45 and 46-55 years respectively thus the interviewees ranged between 36- 55 years old and the older people were more knowledgeable to plants than the young people. Liu *et al.,* (2017) reported that interviewees over the age of fifty years tended to have much more traditional knowledge.

The age categories represented in this study were the most active groups of people encountered in the study areas. The specific areas targeted for the interviews were the butcheries, slaughter houses, hotels and market places where there was a likelihood of finding people dealing with herbal medicine practices such as soup making, herbal tea and trading on medicinal plants species. In other studies (Simbo, 2010; Teklehaymanot & Giday 2007) reported that majority of their informants were men in the age between 28 -70 years. Qureshi *et al*., 2006 reported that males above the age of fifty years were more knowledgeable than their female counterparts due to their involvement in trading activities. Most of the people under thirty years old have left to find jobs in urban areas or for college study while others remain in the village. There seems to be an erosion of ethnobotanical knowledge among the young generation. This has been reported in many parts of the world such as Guangxi village in China (Liu *et al*., 2017), Brazil and Benin (Sogbohossou *et al.*, 2015; Voeks and Leony 2014); Tropical regions (Acharya & Acharya, 2009; 2010). Thus there is a need for systematic documentation of indigenous knowledge and biological resources (Liu *et al*., 2017).

The results of this study indicated that majority of the informants were self-employed. In Narok and Kajiado counties majority respondents were involved with trading in herbal medicines (women) and in soup making (men). In other areas the respondents were

mainly gathered from slaughtering houses, hotels or butcheries and hence they were either self-employed or in informal employment. Across the study areas the respondents from Narok, Kajiado and Kiambu were self-employed while in Meru and Nyeri they were informally employed. These respondents were also engaged in other economic activities such as growing crops and trees and livestock keeping. Majority were using their land for livestock keeping particularly those from Narok and Kajiado while the farmers from Nyeri, Meru and Kiambu grew crops. Their land size varied among the study location with the highest acreage in Narok and the least in Kiambu.

All respondents interviewed in the study knew about *S. henningsii* although they did not grow it on their farms but obtained the plant materials from forest. In Narok County, the Maasai community grazed their livestock in their farms which are also the forested areas in that region. In Kajiado, Meru, Nyeri and Kiambu counties *S. henningsii* is found in the natural forests where they obtained it. The plant could also be obtained from suppliers who travelled to as far as Tanzania to collect the plant materials and sell to the local people

Majority of the respondents obtained the plant species with difficulty and they reported an increase in gathering effort.. This confirmed the decline of the plant species that the herbal practitioners used to collect within the vicinity of their homesteads only few years ago but now move for long distances to collect the same kind of plants. The results conformed to the study on Conservation Status and Use of Medicinal Plants by Traditional Medical Practitioners in Mwingi District, Kenya (Musila *et al*., 2004; Njoroge *et al*., 2010). In Meru county *S. henningsii* occurs in the natural forest (Ngaya forest in Maua) which is very far while in Kiambu County it is found in the protected natural forest which is Karura forest. In Nyeri County, the species is easily obtained from Kabiru-ini forest which is unprotected natural forest although currently there are restrictions to the entry into the forest due to over exploitation of this species. In Narok County the plant materials are obtained easily since the forested areas are the grazing farm lands for the Maasai community.

The higher percentages of respondents interviewed in Kajiado and Narok counties were mainly from the Maasai community who are deeply rooted to their traditional herbal medicinal practices. They engaged in buying and selling of different types of medicinal plant species. In Nyeri, Meru and Kiambu counties hospitals and others health facilities are readily available and hence majority do not use or trade on herbal medicine. The most used and traded plant parts were the roots and the bark/stem.

Different uses of *S. henningsii* were identified in the study which varied among the respondents from different study locations and the most common use was soup making. Different types of preparations are made from medicinally important plants include decoction, juice, powder and oils (Survase and Raut 2011). The roots and the stems are the most frequently used plant parts by the traditional healers (Survase and Raut 2011). In this study the plant parts of *S. henningsii* that were frequently used were also the roots and the stems or their bark grounded into powder. This varied in different study areas where in Narok, Kajiado and Kiambu counties used the roots whereas in Meru and Nyeri counties used the stems. In others studies (Simbo 2010; Wintola and Afolayan 2010) the leaves were the most used plant parts yet in other studies (Okello *et al*., 2010; Njoroge and Bussmann 2006) the roots were the most frequently used plant parts.

The study revealed that majority of the respondents processed their plant parts through boiling in water however, the processing methods of the plant extracts differed among the study locations. In Nyeri, Kajiado, Meru and Kiambu Counties they boiled the plant parts in water whereas Narok they graded the bark and then boiled in water. Oral consumption was recommended against diseases like cold, cough, rheumatism, joint pains, fever diarrhea etc. Drugs were prescribed in small doses to taste either from parts of *S. henningsii* only or in combination with plant parts from other plant species. These combinations are more effective in curing diseases or enhancing the immunity of the patient (Survase and Raut 2011; Musila *et al*., 2004; Schmeltzer, 2008). The study showed that *S. henningsii* was also mixed with other substances such as milk, honey, soup or tea while being administered.

The key difficulties identified in relation to *S. henningsii* were scarcity and long distance walking. These were much related because with scarcity of the plant it would be expected that individuals searching for it have to walk long distances. The key suggestions were training of locals on tree conservation strategies and sustainable use, establishment of tree nurseries to enhance planting locally. Proper training and involvement of the local communities in conservation issues practices has also been recommended in other studies (Aumeeruddy 1996). This would provide an alternative source of the plant and thus reduce the pressure exerted on the few individuals remaining in the wild habitats.

5.3 Conclusions

Since the ultimate goal of this study was to conserve *S. henningsii* the conclusions drawn from this research study were as follows:

5.3.1 Genetic diversity of *S. henningsii*

There was low level of genetic variation revealed within *S. henningsii* populations coupled with relatively high among population genetic diversity hence the need for an urgent conservation of *S. henningsii* before its extinction. This suggests the hypothesis that there has been a common gene pool existing and exchanged within populations for very long time. Therefore conservation efforts should aim at preserving all the existing extant of this plant species

5.3.2 Species niche modeling of *S. henningsii*

MaxEnt modeling for *S. henningsii* performance was good and applicable in predicting the potential suitable areas for this species in Kenya since the values for AUC were greater than 0.9 i.e. 0.986 and 0.983 for training and test data respectively. The potential distribution areas in Kenya for *S. henningsii* exist along the coastal regions (Taita hills, Shimba hills, Kilifi (arabuko sokoke forest), Mt Kilimanjaro game reserve), Marsabit, Huri hills Marigat, Baragoi, Malaral (dryland areas in Kenya), Ngong and Karura

forests. These areas could be targeted for reintroduction and conservation of this species. People living in those areas could also be encouraged to include *S. henningsii* in their agroforestry. The variables that strongly contributed to the model prediction and in explaining the distribution of *S. henningsii* were maximum temperature, altitude and precipitation as shown by maxEnt response curves and the Jackknife tests.

5.3.3 Ethnobotanical study of *S. henningsii*

The older people were more knowledgeable to plants than the young people, that is thirty years and above and respondents above fifty years had more information. There was no in farm cultivation of *S. henningsii* but it is obtained from the natural forests. The roots and the stems were the main plant parts collected for medicinal uses and boiling them in water was the preparation process of the decoctions. These decoctions were orally administered for treatment. Scarcity and long distance walking were the difficulties experienced in this study and thus showed clearly that *S. henningsii* is heavily exploited in all the areas of study due to its high demand for medicinal properties. Poor and destructive havesting methods are often used for the collection of plant materials threatening their survival.

5.4 Recommendations

Drawing from this study results the following recommendations have been put forward:-

5.4.1 Genetic diversity of *S. henningsii*

This research study provides the first report on the molecular characterization of *S. henningsii*. A possible management strategy for *S. henningsii* is also suggested concerning a study on the mating systems in *S. henningsii* to verify the suggested self pollination mechanism in this study. The outcome of such a study would have important implications for *S. henningsii* conservation and management as the knowledge of mating system is a factor in determining the genetic variation levels at both species and

populations. A study on the possibility of induced /forced cross pollination in this plant species could also be carried out to to widen the genetic base of the plant populations.

5.4.2 Species niche modeling of *S. henningsii*

Further study on niche modeling can be carried out using other modeling techniques to determine the potential suitable areas for *S. henningsii*. Another important consideration in restoration of endangered and threatened plant species is population size. Establishment of on-site protection zones for *S. henningsii* to reduce the impacts of human activities would allow the habitats to increase in size through natural regeneration to reach effective population size. Establishment of *in-situ* conservation areas to preserve the extant populations would also maintain the existing genetic variability for this species. Plants can be introduced from different populations via propagation and seedling management to increase the chances of gene exchange and recombination and thus improve the level of genetic diversity over time. *Ex-situ* conservation programs can be established to capture most of detected genetic variation and increase the genetic diversity by crossing different populations. Collection of seeds and germplasm in botanical gardens and home gardens and other institutions could be of significant practical value for conservation of genetic diversity. Establishment of regeneration system for this endangered plant species through tissue culture techniques as this would guarantee its *ex-situ* and *in-situ* conservation and sustainable survival.

5.4.3 Ethnobotanical study of *S. henningsii*

It was indicated that medicinal plants were being indiscriminately collected from the wild habitats both for domestic and commercial use without any strategies reported to conserve them. This lack of efforts to sustain plant resources may results in their depletion from their natural habitats. There is a great need therefore to create awareness among the indigenous communities about the endangering of medicinal plants due to over exploitation to meet the market demand. Therefore education and involvement of the local communities in natural resources management and conservation is important.

This can be done through workshops and seminars as such forum will not only educate but also help the scientists to identify research areas that are important and relevant to the local communities. Finally, similar research study could be carried out on ethnobotany, genetic diversity and niche modeling of economically important plant species in the effort for their conservation for the present and future generations.

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APPENDICES

Appendix 1: Common thresholds and corresponding omission rates for the threshold dependent binomial test of omission

Cumulative threshold	Logistic threshold	Description	Fractional predicted area	Training omission rate	Test omission rate	P-value
1.000	0.016	Fixed cumulative value 1	0.286	0.000	0.000	2.51E-8
5.000	0.058	Fixed cumulative value 5	0.180	0.000	0.000	3.618E-11
10.000	0.109	Fixed cumulative value 10	0.125	0.023	0.071	2.363E-11
7.408	0.081	Minimum training presence	0.149	0.000	0.071	2.147E-10
30.210	0.299	10 percentile training presence	0.046	0.093	0.071	4.954E-17
21.451	0.222	Equal training sensitivity and specificity	0.070	0.070	0.071	1.224E-14
16.143	0.178	Maximum training sensitivity plus specificity	0.090	0.023	0.071	3.225E-13
20.975	0.219	Equal test sensitivity and specificity	0.071	0.070	0.071	1.638E-14
54.476	0.575	Maximum test sensitivity plus specificity	0.013	0.209	0.071	5.335E-24
2.722	0.037	Balance training omission, predicted area and threshold value	0.224	0.000	0.000	8.037E-10
15.059	0.165	Equate entropy of thresholded and original distributions	0.095	0.023	0.071	6.554E-13

Appendix 2: Estimation of relative contributions of the enviromental variables to the MaxEnt model

Variable	Percent contribution	Permutation importance
prec_1_res	20.9	2.8
pre_10_res	17.5	33.2
prec_7_res	13.4	1.5
tmax_10_res	12.3	9
tmax_1_res	11.2	0
alt_res	7	9.8
prec_12_res	4.2	17.4
prec_5_res	3	3.1
tmin_12_res	2.5	0
tmin_5_res	2.2	3.6
prec_3_res	2.2	4
tmin_1_res	2	2.7
tmax_7_res	1.3	1.9
tmin_10_res	0.2	4.8
tmax_12_res	0.2	5.7
tmax_5_res	0	0.5
tmax_3_res	0	0
tmin_7_res	0	0
tmin_3_res	0	0

Appendix 3: Questionnaire for ethnobotanical study

The purpose of this Questionnaire is to collect data on conservation status and use of *Strychnos henningsii* by communities. The information you will provide will be used as data for purposes of Mary Wahu Kuria PhD thesis development and will not be disclosed to anyone else.

Questionnaire for ethnobotanical study

General information

1. Name of the respondent:
2. Gender: male/ female
3. Age ()
4. What is your marital status
5. Number of Children
6. Respondents level o their education : 1) lower primary 2) Upper primary 3) Lower Secondary 4) Upper secondary 5) University 6) Non formal education
7. Who is the head of your household? 1) Myself 2) Husband (3 Wife (4) Mother (5) Father 6) sister
8. Do you own any land? Yes/ No. If so what is your land size?
9. How do you use your land?
10. What are the economic activities carried out in your land?

Ethnobotanical information

11. Do you know/ are aware of *S. henningsii*? Yes / No
12. If so how do you know it?
13. Where does *S. henningsii* grow?
14. Do you or have you ever used it? Yes / No
15. If yes, how have you ever used it?
16. Where do you get the plant material?

17. Is the plant locally available i) Yes ii) No
18. What is effort used in the gathering the plant? Easily/ with difficulty
19. What part of the plant do you use?
20. Do you use different parts of the plant for the same herbal preparation? Yes/No
21. What method (s) do you use to get the plant materials?
22. Which parts of *S. henningsii* are popular in herbal medicines?
23. How do you prepare your medicine from this plant?
24. How do you administer your medicine?
25. Do you mix *S. henningsii* with other plants or substances such as honey or milk?
26. . Do you trade in herbal plants? Yes / no
27. If so what plants?
28. Which parts of these plants are popular in trade?

Appendix 4: Georeference points of individuals of S. henningsii selected from nine populations in Kenya

KILIFI POPULATION (Arabuko Sokoke forest)				
	Georeference points			
S. No.	Southings	Eastings	Altitude MASL	Height
1	03° 12' 54.4"	039° 55' 21.9"	81	8
2	03° 12' 56.3"	039° 55' 21.0"	81	7
3	03° 13' 00.8"	039° 55' 20.6"	83	5
4	03° 12' 53.6"	039° 55' 22.6"	84	8
5	03° 12' 51.8"	039° 55' 17.1"	85	10
6	03° 12' 55.2"	039° 55' 23.8"	85	15
7	03° 12' 20.9"	039° 54' 49.5"	54	7
8	03° 12' 23.7"	039° 54' 47.2"	53	8
9	03° 12' 25.3"	039° 54' 45.0"	59	9
10	03° 12' 23.4"	039° 54' 42.8"	61	5
11	03° 12' 20.2"	039° 54' 39.3"	64	6
12	03° 12' 17.2"	039° 54' 35.6"	65	7
13	03° 12' 15.5"	039° 54' 32.8"	69	8
14	03° 12' 13.5"	039° 54' 30.0"	67	6
15	03° 12' 12.0"	039° 54' 28.0"	63	7
16	03° 12' 11.1"	039° 54' 21.8"	53	6
17	03° 12' 12.8"	039° 54' 14.0"	63	8
18	03° 12' 14.2"	039° 54' 09.2"	64	7
19	03° 12' 13.9"	039° 54' 06.7"	64	8
20	03° 12' 15.4"	039° 54' 00.0"	63	7
21	03° 12' 16.4"	039° 53' 54.6"	64	7
22	03° 12' 17.8"	039° 53' 48.3"	62	7
23	03° 12' 18.9"	039° 53' 42.2"	60	6
24	03° 12' 21.2"	039° 53' 35.8"	59	7
25	03° 12' 17.0"	039° 53' 36.1"	51	7
26	03° 12' 27.3"	039° 53' 28.2"	54	8
27	03° 12' 35.4"	039° 53' 19.0"	54	8
28	03° 20' 38.6"	039° 51' 55.0"	104	9
29	03° 19' 52.8"	039° 52' 10.1"	100	7.5
30	03° 19' 45.1"	039° 52' 10.2"	98	8
TAITA TAVETA POPULATION (Mwache forest in Mwatate sub county)				
S. No	Southings	Eastings	Altitude MASL(M)	Height
1	03° 28' 56.5"	038° 15' 58.1"	1001	4
2	03° 28' 56.5"	038° 15' 53.8"	996	5
3	03° 28' 48.7"	038° 15' 51.1"	1006	5.5

4	03° 28' 15.4"	038° 15' 41.3"	1015	6
5	03° 27' 34.6"	038° 15' 45.7"	1042	7
6	03° 27' 34.2"	038° 15' 28.3"	1030	8
7	03° 28' 04.7"	038° 14' 47.8"	1037	7.5
8	03° 27' 41.8"	038° 14' 05.5"	1084	7
9	03° 26' 46.6"	038° 14' 01.7"	1105	7.5
10	03° 26' 44.8"	038° 14' 06.0"	1107	6.5
11	03° 26' 42.4"	038° 14' 04.3"	1109	8
12	03° 26' 42.7"	038° 14' 08.7"	1104	6.5
13	03° 26' 44.1"	038° 14' 11.9"	1102	6.58
14	03° 26' 45.0"	038° 14' 16.1"	1093	6
15	03° 26' 52.7"	038° 13' 54.2"	1105	6
16	03° 26' 27.2"	038° 14' 04.9"	1095	6.5
17	03° 26' 17.1"	03°8 14' 12.1"	1104	8
18	03° 26' 00.1"	038° 14' 20.9"	1124	5.5
19	03° 25' 57.3"	038° 14' 41.2"	1114	6
20	03° 26' 12.3"	038° 15' 33.0"	1109	6.5
21	03° 26' 09.1"	038° 15' 33.2"	1113	7
22	03° 26' 01.6"	038° 15' 29.1"	1118	6
23	03° 27' 29.8"	038° 15' 55.7"	1057	7.5
24	03° 27' 01.0"	038° 15' 28.5"	1065	5
25	03° 27' 00.6"	038° 15' 24.1"	1071	5
26	03° 27' 03.0"	038° 15' 20.8"	1070	5
27	03° 26' 59.9"	038° 15' 18.9"	1075	6.5
28	03° 26' 35.4"	038° 15' 17.2"	1084	5
29	03° 26' 34.4"	038° 15' 13.6"	1078	7.5
30	03° 26' 58.8"	038° 14' 04.7"	1070	7

KITUI POPULATION (Nduumoni Hills)

S. NO.	Southings	Eastings	Altitude MASL(M)	Height(CM)
1	01° 22' 13.2"	037° 53' 01.6"	1119	7
2	01° 22' 15.1"	037° 53' 00.3"	1115	7
3	01° 22' 17.8"	037° 53' 00.9"	1117	7.5
4	01° 22' 20.8"	037° 53' 01.7"	1115	9
5	01° 22' 23.8"	037° 53' 04.2"	1119	7
6	01° 22' 23.3"	037°53' 10.1"	1154	10
7	01° 22' 26.6"	037° 53' 12.1"	1146	10
8	01° 22' 29.0"	037° 53' 12.1"	1132	8
9	01° 22' 30.5"	037° 53' 13.6"	1133	5
10	01° 22' 33.8"	037° 53' 15.7"	1133	6
11	01° 22' 36.3"	037° 53' 21.1"	1138	7
12	01° 22' 27.9"	037° 53' 18.8"	1146	6

13	01° 22' 18.4"	037° 53' 10.4"	1177	10
14	01° 22' 08.5"	037° 52' 53.6"	1102	5
15	01° 22' 11.7"	037° 52' 57.5"	1114	8
16	01° 22' 07.4"	037° 52' 58.1"	1109	8.5
17	01° 22' 04.5"	037° 52' 52.4"	1110	6
18	01° 22' 04.1"	037° 52' 49.7"	1104	2
19	01° 22' 06.8"	037° 52' 51.2"	1097	8
20	01° 22' 09.5"	037° 52' 48.7"	1076	6
21	01° 22' 00.2"	037° 52' 47.0"	1107	7
22	01° 21' 26.4"	037° 52' 55.1"	1110	7
23	01° 21' 25.1"	037° 52' 53.0"	1114	7
24	01° 21' 25.6"	037° 52' 49.1"	1122	8
25	01° 21' 24.4"	037° 52' 47.2"	1114	7
26	01° 21' 22.2"	037° 52' 48.2"	1124	7.5
27	01° 22' 21.8"	037° 49' 31.5"	1199	4
28	01° 22' 23.2"	037° 49' 27.8"	1198	3.5
29	01° 22' 21.6"	037° 49' 26.1"	1206	4
30	01° 22' 22.7"	037° 49' 24.2"	1195	4
MARSABIT POPULATION (Marsabit forest)				
S. No.	**Northings**	**Eastings**	**Altitude MASL (M)**	**HEIGHT (CM)**
1	02° 15' 51.0"	038° 00' 56.2"	1018	8.5
2	02° 15' 49.6"	038° 00' 54.0"	1036	7
3	02° 15' 48.4"	038° 00' 49.9"	1045	9
4	02° 15' 52.4"	038° 00' 48.7"	1053	8
5	02° 15' 55.5"	038° 00' 46.2"	1061	11
6	02° 15' 57.6"	038° 00' 44.3"	1069	18
7	02° 16' 01.7"	038° 00' 43.5"	1073	20
8	02° 15' 44.2"	038° 00' 48.8"	1048	12
9	02° 15' 40.9"	038° 00' 49.6"	1042	16
10	02° 15' 37.5"	038° 00' 52.6"	1041	6
11	02° 15' 33.4"	038° 00' 54.9"	1039	5
12	02° 15' 29.5"	038° 00' 53.4"	1040	12
13	02° 15' 26.4"	038° 00' 56.0"	1038	16
14	02° 15' 23.9"	038° 00' 59.5"	1035	20
15	02° 15' 19.8"	038° 01' 01.1"	1035	22
16	02° 15' 15.6"	038° 01' 02.4"	1031	7
17	02° 15' 11.1"	038° 01' 03.8"	1026	11
18	02° 15' 09.2"	038° 01' 00.2"	1020	20
19	02° 15' 13.2"	038° 00' 40.6"	1025	18
20	02° 15' 20.4"	038° 00' 39.0"	1031	10

21	02° 15' 23.5"	038° 00' 37.7"	1036	11
22	02° 15' 26.7"	038° 00' 31.8"	1034	12
23	02° 15' 25.4"	038° 00' 25.8"	1025	10
24	02° 15' 21.9"	038° 00' 26.8"	1028	18
25	02° 15' 18.4"	038° 00' 29.8"	1034	16
26	02° 15' 14.7"	038° 00' 31.6"	1033	12
27	02° 15' 10.0"	038° 00' 32.7"	1027	12
28	02° 15' 05.9"	038° 00' 30.1"	1029	12
29	02° 15' 02.5"	038° 00' 26.7"	1031	8
30	02° 15' 09.9"	038° 00' 02.3"	1063	9
BARINGO POPULATION (Tugen Hills)				
S. NO.	**Northings**	**Eastings**	**MASL (M)**	**Height(CM)**
1	00° 09'42.1	035° 49'40.0"	1595	1
2	00° 09 42' 43.3"	035° 49' 41.5"	1604	25
3	00° 09' 44.5"	035° 49 ' 42.5"	1613	15
4	00° 09' 47.4"	035° 49' 43.0"	1630	15
5	00° 09' 47.7"	035° 49' 42.5"	1637	11
6	00° 09' 49.1"	035° 49' 40.7"	1638	7, 6, 8, 7
7	00° 09' 50.6"	035° 49' 41.9"	1648	3, 6, 2
8	00° 09' 52.5"	035° 49' 41.0"	1653	15,12, 11, 10
9	00° 09' 54.8"	035° 49' 41.3"	1664	7, 12, 10
10	00° 09' 57.4"	035° 49' 41.3"	1667	15, 2
11	00° 10' 00. 3"	035° 49' 41.3"	1668	12, 10, 12, 11
12	00° 10' 01. 3"	035° 49' 43.0"	1669	12, 6, 7
13	00° 10' 01. 7"	035° 49' 45.7"	1669	21
14	00° 10' 04.0"	035° 49' 46.6"	1652	16
15	00° 10' 05.9"	035° 49' 44.5"	1632	12
16	00° 10' 04.5"	035° 49' 43.2"	1638	11
17	00° 10' 03.2"	035° 49' 41.7"	1636	15, 14
18	00° 10' 02.5"	035° 49' 38.6"	1630	11, 16
19	00° 10' 00.0"	035° 49' 37.1"	1629	6, 11
20	00° 09' 57.3"	035° 49' 37.6"	1624	5, 6
21	00° 09' 54.4"	035° 49' 37.4"	1613	2, 3
22	00° 09' 51.9"	035° 49' 37.1"	1603	18
23	00° 09' 49.0"	035° 49' 35.9"	1596	15
24	00° 09' 46.8"	035° 49' 38.0"	1592	10, 12, 6
25	00° 09' 45.0"	035° 49' 39.6"	1591	5, 4, 5, 3
26	00° 09' 52.8"	035° 49' 31.2"	1550	5, 6, 5
27	00° 09' 55.3"	035° 49' 30.6"	1549	12
28	00° 09' 59.9"	035° 49' 30.1"	1551	10
29	00° 10' 03.0"	035° 49' 30.2"	1555	20

30	00° 10' 05.9"	035° 49' 30.4"	1550	18
NAROK POPULATION (Tipilikwani forest In Talek)				
S. NO.	**Southings**	**Eastings**	**MASL (M)**	**Height(CM)**
1	01° 27' 20.3"	035° 15' 54.3"	1625	8
2	01° 27' 19.9"	035° 15' 53.1"	1623	5
3	01° 27' 21.9"	035° 15' 52.3"	1612	7
4	01° 27' 22.8"	035° 15' 53.6"	1620	8
5	01° 27' 22.2"	035° 15' 55.0"	1625	9
6	01° 27' 23.5"	035° 15' 56.3"	1626	8.5
7	01° 27' 24.6"	035° 15' 57.8"	1620	7
8	01° 27' 25.5"	035° 16' 00.2"	`1612	8
9	01° 27' 23.8"	035° 16' 00.4"	1619	8
10	01° 27' 22.3"	035° 16' 00.8"	1623	6
11	01° 27' 20.9"	035° 15' 59.7"	1626	6
12	01° 27' 20.0"	035° 15' 58.1"	1627	8
13	01° 27' 18.0"	035° 15' 57.8"	1625	6
14	01° 27' 17.8"	035° 16' 00.0"	1623	8
15	01° 27' 19.4"	035° 16' 02.0"	1621	7
16	01° 27' 21.0"	035° 16' 03.3"	1613	8
17	01° 27' 22.4"	035° 16' 04.5"	1610	6
18	01° 27' 25.1"	035° 16' 06.0"	1606	8
19	01° 27' 27.2"	035° 16' 08.2"	1605	8
20	01° 27' 29.7"	035° 16' 08.6"	1603	5.5
21	01° 27' 31.3"	035° 16' 09.7"	1595	6
22	01° 27' 31.1"	035° 16' 12.6"	1591	5
23	01° 27' 29.3"	035° 16' 14.8"	1595	5
24	01° 27' 27.4"	035° 16' 14.0"	1602	6
25	01° 27' 25.8"	035° 16' 12.9"	1602	8
26	01° 27' 23.9"	035° 16' 11.0"	1603	7
27	01° 27' 22.4"	035° 16' 08.5"	1606	8
28	01° 27' 20.3"	035° 16' 07.0"	1609	6
29	01° 27' 18.1"	035° 16' 03.8"	1617	5
30	01° 27' 16.6"	035° 15' 59.3"	1620	5
NYERI POPULATION (Kabiruini forest)				
S. NO.	**Southings**	**Eastings**	**MASL (M)**	**Height(CM)**
1	00° 23' 24.1"	036° 58' 09. 1"	1786	8
2	00° 23' 25.9"	036° 58' 08.2"	1782	9
3	00° 23' 27.1"	036° 58' 11.9"	1779	11
4	00° 23' 30.1"	036° 58' 14.6"	1775	13
5	00° 23' 31.6"	036° 58' 14.6"	1772	11
6	00° 23' 30.8"	036° 58' 16.5"	1772	13

7	00° 23' 28.6"	036° 58' 17.2"	1772	10
8	00° 23' 28.7"	036° 58' 19.1"	1769	11
9	00° 23' 35.5"	036° 58' 18.7"	1748	5
10	00° 23' 36.1"	036° 58' 19.6"	1747	14
11	00° 23' 36.2"	036° 58' 08.3"	1767	12
12	00° 23' 32.4"	036° 58' 10.5"	1770	16
13	00° 23' 31.1"	036° 58' 09.3"	1774	6
14	00° 23' 31.8"	036° 58' 20.0"	1712	10
15	00° 23' 30.3"	036° 58' 21.3"	1784	13
16	00° 23' 28.6"	036° 58' 21.8"	1784	18
17	00° 23' 28.2"	036° 58' 24.0"	1778	18
18	00° 23' 27.7"	036° 58' 26.5"	1773	21
19	00° 23' 25.0"	036° 58' 27.5"	1773	14
20	00° 23' 26.2"	036° 58' 24.7"	1770	21
21	00° 23' 28.5"	036° 58' 22.8"	1770	25
22	00° 23' 26.8"	036° 58' 19.5"	1772	11
23	00° 23' 24.7"	036° 58' 17.0"	1773	21
24	00° 23' 21.3"	036° 58' 16.5"	1767	18
25	00° 23' 19.7"	036° 58' 16.3"	1765	15
26	00° 23' 20.5"	036° 58' 10.1"	1776	17
27	00° 23' 22.0"	036° 58' 11.4"	1774	25
28	00° 23' 20.5"	036° 58' 11.3"	1774	20
29	00° 23' 20.2"	036° 58' 12.7"	1777	6
30	00° 23' 17.7"	036° 58' 21.5"	1729	11
KIAMBU POPULATION(Karura forest)				
S. NO.	**Southings**	**Eastings**	**Altitude MASL (M)**	**Height(CM)**
1	01°14' 58.9"	036° 50' 24.8"	1683	10
2	01°15' 58.9"	036° 50' 24.8"	1679	7
3	01°15' 00.6"	036° 50' 23.3"	1670	15
4	01°14' 58.6"	036° 50' 22.1"	1670	9
5	01°14' 54.4"	036° 50' 20.2"	1673	11
6	01°14' 59.3"	036° 50' 21.3"	1676	8
7	01°15' 00.4"	036° 50' 21.8"	1673	7.5
8	01°14' 56.4"	036° 50' 25.3"	1684	9
9	01°14' 36.4"	036° 50' 20.4"	1685	12
10	01°14' 32.1"	036° 50' 22.6"	1691	8
11	01°14' 30.8"	036° 50' 23.6"	1688	12
12	01°14' 29.1"	036° 50' 25.6"	1678	20
13	01°14' 28.2"	036° 50' 27.0"	1669	10
14	01°14' 27.7"	036° 50' 28.6"	1656	6

15	01°14' 26.6"	036° 50' 26.6"	1657	7
16	01°14' 26.0"	036° 50' 24.9"	1662	12
17	01°14' 24.2"	036° 50' 21.7"	1665	15
18	01°14' 24.3"	036° 50' 19.6"	1668	12
19	01°14' 23.9"	036° 50' 20.0"	1695	13
20	01°14' 24.6"	036° 50' 16.8"	1664	12
21	01°14' 22.4"	036° 50' 11.9"	1666	8
22	01°14' 22.9"	036° 50' 10.9"	1676	14
23	01°14' 26.0"	036° 50' 09.0"	1687	14
24	01°14' 25.0"	036° 50' 11.9"	1677	10
25	01°14' 27.0"	036° 50' 13.8"	1679	9
26	01°14' 29.1"	036° 50' 13.8"	1675	7
27	01°14' 53.5"	036° 50' 24.8"	1632	12
28	01°14' 51.5"	036° 50' 26.1"	1641	6
29	01°14' 44.3"	036° 50' 13.5"	1641	8
30	01°14' 35.2"	036° 50’13.08.0"	1646	14
KAJIADO POPULATION (Ngong forest)				
S. NO.	**Southings**	**Eastings**	**Altitude MASL (M)**	**Height(CM)**
1	01°18' 43.7"	036° 46' 06.5"	1787	15
2	01°18' 41.0"	036° 46' 03.7"	1790	7
3	01°18' 38.6"	036° 45' 57.0"	1799	15
4	01°18' 42.1"	036° 45' 57.6"	1804	11
5	01°18' 43.6"	036° 45' 58.0"	1804	8
6	01°18' 45.8"	036° 45' 59.3"	1801	7
7	01°18' 43.8"	036° 46' 03.9"	1798	8
8	01°18' 48.3"	036° 46' 01.7"	1817	10
9	01°18' 47.9"	036° 46' 06.8"	1805	15
10	01°18' 48.5"	036° 46' 07.3"	1805	17
11	01°18' 50.0"	036° 46' 08.0"	1803	7
12	01°18' 47.0"	036° 46' 08.2"	1802	12
13	01°18' 46.5"	036° 46' 08.8"	1798	7
14	01°18' 45.6"	036° 46' 10.9"	1798	15
15	01°18' 47.8"	036° 46' 08.8"	1799	10
16	01°18' 48.1"	036° 46' 11.3"	1794	15
17	01°18' 41.4"	036° 45' 52.4"	1799	11
18	01°18' 43.4"	036° 45' 50.4"	1805	7
19	01°18' 44.7"	036° 45' 47.6"	1807	9
20	01°18' 47.3"	036° 45' 48.2"	1808	5
21	01°18' 46.2"	036° 45' 50.4"	1812	6
22	01°18' 46.6"	036° 45' 55.0"	1808	4

23	01°18' 47.3"	036° 45' 56.7"	1808	12
24	01°18' 45.6"	036° 45' 57.2"	1807	11
25	01°18' 41.5"	036° 45' 55.2"	1806	12
26	01°18' 40.5"	036° 45' 55.4"	1802	11
27	01°18' 38.1"	036° 46' 03.4"	1790	8
28	01°18' 39.5"	036° 46' 03.7"	1793	13
29	01°18' 38.3"	036° 46' 07.4"	1791	4
30	01°18' 40.9"	036° 46' 15.4"	1786	12