BIOPHYSICAL ENVIRONMENTAL FACTORS INFLUENCING THE DISTRIBUTION AND YIELD OF *Osyris lanceolata* (Hochst&Steud): CASE STUDY OF GACHUTHI AND KIBWEZI FOREST, KENYA.

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been submitted or presented for examination in any other institution.

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NM 12/3124/11

Signature_____ Date_____

RECOMMENDATION

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations.

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DEDICATION

To my parents, Mr. and Mrs. Gathara, to my children Patrick, Maryann, Peris, Jacinta and Teresiah, to my sisters and brothers for their support and encouragement throughout my study.

ABSTRACT

East African Sandalwood (*Osyris lanceolata*) is a dioecious shrub growing to 1-7 m tall depending on environmental factors and genetic variation. Sandalwood is widely exploited for extraction of oil, which is used in the fragrance, perfumery and pharmaceutical industries. Efforts are being made to domesticate the species in order to reduce pressure from its natural habitat. However, little is known in regard to its ecological factors, range of oil yields and quality across its distribution in highlands and lowlands areas in Kenya. Therefore, the broad objective of this study was to determine biophysical environmental factors influencing the distribution and oil yield of *Osyris lanceolata* in highland and lowland forests in Kenya. The study employed experimental and ecological survey design where Gachuthi highland forest and Kibwezi lowland dry forest were selected as the study areas. Line transects were laid in areas where *O. lanceolata* was naturally growing and also in areas where it was not found in both forests. Subsequently, nested intensity plots were established along transects where vegetation habit and species data collected, soils and *O. lanceolata* trees sampled. Then, vegetation data was transformed into a species occurrence/ absence matrix for all plots; soils' physical and nutrients properties determined in the soils laboratory; and oil yield and quality in roots, stems and barks determined through chromatography. Indicative species for predicting *O. lanceolata* occurrence and the soil variables where it grows naturally were simultaneously determined through canonical correspondence analysis using CANOCO for windows version 4.15. Variation of soil variables, difference in oil yield and quality among tree components and between sites were analyzed using SPSS version 20.0. Indicative species for *O. lanceolata* occurrence and soil variables influencing its occurrence differed between Gachuthi highland and Kibwezi drylands forests. However, *Rhus natalensis* was a common indicator species in both forests, suggesting its indisputable predictive capacity for *O. lanceolata* occurrence. Soil physical and nutrient variables differed significantly with Kibwezi nutrient levels of nitrogen, phosphorus and potassium higher than Gachuthi forest. Extracted oil yield in roots, stem and bark from both sites showed a significant difference at ($p<0.05$) with highest yield found in Kibwezi roots (2.26%). Quality of oil in reference to concentration of α and β santalol showed that Gachuthi root samples had traces of α santalol, stems were ranging from (0-3.1%) and barks (0-0.06%) when compared to Kibwezi with a range of (10.80-35.21%) in roots, (0.01-5.82%) in stem and (2.30-14.24%) in the bark. In conclusion these findings show that Sandalwood plantations could preferably be established in Kibwezi lowland dry forest.

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LIST OF ABREVIATIONS AND ACRONYMS

µl	- microlitre
ANOVA	- Analysis of Variance
AOAC	- Association of Official Analytical Chemist
CCA	- Canonical Correspondence Analysis
CITES	-Convention on International Trade in Endangered Species
CFS	- Community Forest Service
DBH	- Diameter at breast Height
DSWT	- David Sheldrick Wildlife Trust
FAO	-Food and Agriculture Organization of United Nations
SPSS	- Statistical Package for the Social Sciences
ICIPE	- International Centre of Insect and Plant Physiology
GPS	- Global Positioning System
HCL	- Hydrochloric acid
HQS	- Headquarters
KEFRI	- Kenya Forestry Research Institute
KFS	- Kenya Forest Service
KWS	-Kenya Wildlife Service
M	- Molar
Masl	- Meters above sea level
ML	- milliliter
ng	- nanograms
pH	- Alkalinity or acidity of a media
PPM	- parts per million
QCHD	- Quality Control of Herbal Drugs
GC-MS	- Gas Chromatography Mass Spectra

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Osyris lanceolata (East African Sandalwood) is a dioecious shrub growing to a height of 1-7 m depending on soils, climatic conditions and genetic variation (Subasighe, 2013). The species belongs to the family Santalaceae, and is among the Sandalwood species known for producing fragrant-scented wood from which sandalwood essential oil is extracted (Mbuya *et al.,* 1994). The species is widely distributed in a variety of ecological zones of East Africa that differ in agroclimatic conditions and vegetation (Beentje, 1994, Mwang'ingo *et al.*, 2005). In Kenya, Sandalwood is found in both high rainfall areas and drylands (Beentje, 1994, Maundu and Tengnas, 2005). In the field, Osyris roots are found attached to roots of other trees like *Dodonea viscosa*, *Rhus natalensis* and *Carissa edulis* (Mwang'ingo *et al*., 2005).

Sandalwood is widely harvested in East Africa for extraction of oil, which is used in the fragrance, perfumery and pharmaceutical industries (Teklehaimanot *et al.,* 2012). Traditionally, the species is used for treating infertility, diarrhea, gonorrhea and chronic mucus infections among other diseases (Mbuya *et al.,* 1994, Tshisikhawe *et al.,* 2012). Sandalwood root fibers are used in basketry, whereas the strong red dye from the bark and root is used in skin tanning (Mbuya *et al.,* 1994). Being an evergreen and with long flowering periods, the shrub forms a good animal forage plant (Githae *et al*., 2011).

For years, harvesting of African Sandalwood has been concentrated in Tanzania, particularly in the dry areas, although the species is available in many other countries (Mwang'ingo *et al*., 2010). Illegal sandalwood trade in Kenya has generated hundreds of millions of shillings (Kamondo *et al*., 2007). It has therefore contributed to the economic development of the country. But due to the nature of its trade, not much of the benefits have gone to the local population where the material has been harvested. The cartels involved in trade have been the main beneficiaries. A study by Mwang'ingo *et al.*, (2003) in Tanzania revealed variation of oil yield and quality depending on populations, rainfall, soils, and altitude among other factors.

In Kenya, Sandalwood is heavily overexploited to the extent that the sustainability of the species is threatened. The nature of its exploitation raises concern on its survival in the wild as it involves uprooting the whole tree (Machua *et al.*, 2009). For this reason, Sandalwood was given a five-year Presidential exploitation ban under the Forests Act 2005, (Kenya Gazette notice No. 3176). Nevertheless, smuggling from existing natural stands has continued because of its high value. The protection of the natural populations of the species will be enhanced by putting in place a domestication program where cultivation and harvesting of the species on-farm will ease the pressure on the natural populations.

1.2 Statement of the Problem

Osyris lanceolata is a hemi-parasite plant showing different growth patterns with different host species. Knowledge on its prediction in natural habitats is inadequate in Kenya. Little is known in regard to its oil yield and quality in root, stem and bark from different ecological zones. Also there is inadequate information on ecological factors influencing its distribution and occurrence in different ecological zones. Therefore, it is against this background that this study was conceptualized.

1.3 Objectives

The broad objective of the study was to determine biophysical environmental factors influencing the distribution, oil yield and quality of *Osyris lanceolata*.

1.4 Specific Objectives

1. To determine indicative species for predicting *O. lanceolata* occurrence in natural stands of highland and lowland natural forests.
2. To determine soil physical and chemical parameters where *O. lanceolata* grows naturally in highland and lowland natural forests.
3. To evaluate differences in oil yield among tree components in highland and lowland natural forests.
4. To determine variation of *O. lanceolata* oil quality between highlands and lowlands in naturally growing trees.

1.5 Hypotheses

Ho_1. There is no significant relationship in occurrence of *O. lanceolata* and indicator tree species of highlands and lowlands natural forests.

Ho_2. There is no significant difference on soil physical and chemical parameters where *O. lanceolata* naturally grows in highland and lowland natural forests.

Ho_3. There is no significant difference in oil yield among tree components in selected highland and lowland natural forests.

Ho_4. There is no significant variation in *O. lanceolata* oil quality between highland and lowland natural forests.

1.6 Justification

This study has contributed knowledge for prediction of *O. lanceolata* site suitability using an indicator species and edaphic factors towards domestication and plantation establishment hence ease pressure on existing natural populations. The study has added knowledge to the existing literature on *O. lanceolata* oil yield and quality among different populations in different ecological zones in Kenya. In addition, oil yield and quality data among different tree components of *O. lanceolata* can be used as a guide in the formulation of an appropriate harvesting method. The findings of this study will be disseminated through publications, KEFRI field days and Agricultural Society of Kenya (ASK) exhibitions under research and development category.

1.7 Scope

The study was restricted in Gachuthi forest representing highlands and Kibwezi representing lowlands sites. The choice of the two study sites was based on variation of biotic and abiotic environmental conditions and the common occurrence of Sandalwood populations in both areas. In addition, KEFRI has on-going *O. lanceolata* projects established in the two study areas. Data collection from the field covered a period of two months; May and June, 2013.The biophysical factors mainly focused on indicator species on predicting Sandalwood occurrence and edaphic factors were limited to soil moisture, texture, pH, electro conductivity, nitrogen, potassium and phosphorous content. Oil yield content was determined among populations from both highland and lowland from three tree components namely root,

stem and bark whereas oil quality focused on concentration of α-santalol and β-santalol in the three different plant parts.

The dependent variables of focus were distribution, oil yield and quality. The independent variables consisted of biophysical factors such as indicative species, temperature, humidity, rainfall, altitude, and soil factors in the selected highlands and lowlands environments.

1.8 Limitations

Sampling in Gachuthi forest was slowed by thick under growth of tree climbers that hindered movement within the plots. At Gachuthi forest, *O. lanceolata* trees were sparsely distributed and absent in many plots. This could be attributed to encroachment of human settlements and small land parcels ownership which cannot be enough for woodlot establishment therefore influencing tree poaching for domestic use. Grazing animals in the forest is also a factor contributing to slowed regeneration of the species in the forest. In the same site, *O. lanceolata* trees with an average height of 3.5 m and diameter of 5 cm were absent.

Kibwezi forest is within a national park; therefore, during data collection game wardens from David Sheldrick Wildlife Trust (DSWT) accompanied the researcher in case of an encounter with wild animals. Soil sampling in Kibwezi forest using an auger was difficult because there were huge spreading volcanic rocks that hindered attaining effective depth of 50 cm. Due to past smuggling of *O. lanceolata* from Kibwezi forest before its inclusion in appendix II of CITES, high rate of regeneration and coppicing process from old stamps in situ was observed.

1.9 Definition of Terms

Abundance - is an ecological concept referring to the relative representation of a species in a particular ecosystem and is usually measured as the large number of individuals found per sample plot (Githae *et al.*, 2011).

Biome - are defined as the world's major communities, classified according to the predominant vegetation and characterized by adaptations of organisms to that particular environment (White, 2002).

Biophysical environment – refers to tree density, tree size, soil variables, altitude, temperature and rainfall amount influencing physiological process of oil yield and quality by *O. lanceolata* trees (Hogan, 2012).

Cymes - more or less flat topped cluster of flowers in which the central or terminal flower opens first (White, 2002).

Cytosol - is the intra-cellular fluid that is present inside the cells (Goodsell, 1991).

Dioecious - having the male and female organs in separate and distinct individuals; having separate sexes (Mbuya *et al*., 1994).

Distribution – refers to environment best suited for an organism existence in terms of food, water, air, temperature provision among other needs (Githae *et al*., 2011).

Diversity – refers to the number of different species that are represented in a given community (Tshisikhawe *et al.*, 2012).

Drylands – areas with a moisture index of less than 50%. These are agro climatic zones IV, V, VI and VII in Kenya and mean annual rainfall of less than 1100 mm (Kenya Republic of 1981,Sessional paper No. 4).

Edaphic factors - the physical, chemical, and biological properties of soil that influence the life of organisms (Okalebo *et al*., 2002).

Glaucous - bluish-grey or green appearance of the surface of some plants (White, 2002).

Hemi parasitic – a plant that obtains some nourishment from its host but also photosynthesizes (Irving & Cameron, 2009).

High potential areas - areas with moisture index greater than 50 %. These are agro climatic zones I, II, and III in Kenya with adequate rainfall amount of more than 1100 mm and are productive in terms of mixed farming (Kenya Republic of 1981.Sessional paper No. 4).

Host plant – It supplies water, nutrients and metabolites requirements by connecting its roots with those of other hemi-parasitic plants through a haustorium (Irving & Cameron, 2009).

Indicator plant – refers to a plant that grows in some specific environmental conditions i.e. soils, temperature, rainfall, etc (Tshisikhawe *et al*, 2012).

Lamina – tender shell of unripe nut (White, 2002).

Lanceolate leaf – a leaf that is not divided into parts, narrow and tapering to a point at each end (White, 1983).

Monoecious – with male and female reproductive organs borne in the same flower (Mbuya *et al*., 1994).

Occurrence frequency – refers to number of counts occurring within a specified period or space (Githae *et al*., 2011).

Population Density – refers to number of plant species that a forest hold per unit area (Githae *et al*., 2011)

Sclerophyll – hard leaved forest, heath land and woodland (White, 2002).

Sesquiterpenes - are a group of 15 carbon compounds, they are an important constituent of essential oils in plants and they function as pheromones and juvenile hormones (Rasmann *et al*., 2005).

Tapering off –reduce in amount or narrowing (White, 1983).

Unisexual flowers – a flower that possesses either stamens or carpels but not both (Shobhah, 1990).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Distribution of Sandalwood

Osyris belongs to the Santalaceae family that consists mainly of root parasites (Mwang'ingo *et al.,* 2010). It is represented by eight species namely *Santalum album, Santalum spicatum, Santalum macgregorii, Santalum austroaledonicum, Santalum insulare, Santalum yasi* and *Santalum paniculatum*. *Osyris lanceolata* is the only species commonly found in wide ecological distribution in Eastern and Southern Africa (Mwang'ingo, 2003). In Kenya, it grows at the Coast, Eastern, Rift Valley, Nyanza, Central and Western regions. The species is normally found in both highland and lowland at altitude ranging between 900 and 2550 m above sea level (Maundu & Tengnas, 2005). The tree is known by various local names; Msandali (Swahili) Muthithii (Kikuyu), Mberegesa (Chaga), Olseyeayyesi (Maasai) and Kithawa (Kamba) among others.

2.2 Description and Ecology of *O. lanceolata*

Sandalwood is hemi parasitic plant (Irving & Cameron, 2009). Hemi-parasitic plants obtain part of their water, nutrients and metabolites requirements by connecting their roots with those of other plants through a haustorium and also photosynthesis (Irving & Cameron, 2009). Sandalwood can parasitize over 300 species from grass to other higher plants. It has been found in association with various hosts such as *Dodonea viscosa, Tecomaria capensis, Catha edulis, Apodytes dimidiata, Brachytegia spiciforms, Rhus natalensis* and *Casuarina equisetifolia* (Mwang'ingo *et al.,* 2010). This multi-stemmed, evergreen hemi-parasitic plant has a round to irregular canopy and a grey smooth bark, which later develops into a thick and rough bark with age. Leaves are blue-green, simple, alternate, lanceolate, sometimes egg shaped, slightly glaucous, thick in texture, smooth with a waxy bloom, crowded along the stems; the apex is broadly tapering to round with a fine, sharp tip. The base is broadly tapering; lamina 2.5-7.5 cm, entire and rolled; petiole short, winged up to 0.6 cm, attachment to the stem forming ridges running down the stem. Twigs and leaves point upwards. Flowers are small, unisexual, yellow-green, and red; borne in leaf axils in short panicles or clusters of 2-3 flowers. Fruits are small, edible, 1-seeded drupe, about 1 cm long, fleshy, egg-shaped, and green at first, turning yellow and becoming bright red to purple-black when ripe; crowned with a persistent calyx. Sandalwood is dioecious, flowering from March to August

or even later, September to February with fruits ripening between May and September. In some areas the fruit is available throughout the year (Mwang'ingo *et al*., 2010).

The ecological functions of the species in the ecosystem includes provision of services such as shade and prevention of soil erosion; its trunks and branches support diversity of epiphytic plants such as Orchids, ferns, bryophytes, and lichens. It further provides mechanical support to vines; offers habitat for birds, mammals, reptiles, insects, and arthropods. The flowers provide nectar to insects; birds feed on the flowers, seeds and fruits whereas it is forage to browser animals. *Osyris lanceolata* is a slow growing species and can take 40-50 years to mature and its regeneration potential is low. In Kenya, field observations reveal very few young plants and in their natural habitats, population size range from medium to sparsely spread in a limited area. They are not abundant in most of their natural range habitats (Mukonyi *et al*., 2011).

2.3 Threatened Species

Threatened species are protected under Convention on International Trade in Endangered Species (CITES). These species are endangered because of loss of their habitats or over exploitation. The demand for East African Sandalwood by the perfumery and pharmaceutical industries has increased, as a result of dwindling supply from traditional source countries such as India, Indonesia, Pacific and Australia. The emerging trade on East African Sandalwood and inadequate enforcement of legislations to control cross-border trade for compliance on the Sandalwood trade value chain makes the species more vulnerable. In India and Australia, there exist appropriate legislations for certification on traditional sandalwood species, such as *S. album* and *S. spicatum* whereas no control measures are put in place for the East African Sandalwood. Preliminary results indicate massive uncontrolled wild harvesting from its natural habitats, threatening the species survival in Kenya and Tanzania. Major threats to survival of this species in the wild includes lack of artificial propagation programmes to supplement the wild populations, inadequate data on species population status and ecological functions in the ecosystems, poor regeneration rate, and unsustainable harvesting (Mukonyi *et al*., 2011).

Osyris lanceolata is endangered in Kenya due to over exploitation; it has been smuggled out of gazetted forests, game parks and nature reserves in Kenya to Asian markets where its oil is used as a raw material in production of cosmetics. Being a recent entrant in the

international market and from new sources, there is an urgent need to regulate the international trade of the species to ensure exploitation is not detrimental to its survival. To achieve this, important work has been undertaken with regard to *Osyris lanceolata* under CITES. This work includes, preliminary survey on the exploitation, developing guidelines on sustainable utilization, formation of the National Sandalwood Task force and withdrawal of movement permits (Kamondo *et al.,* 2007).

Currently, research programme on propagation of Sandalwood for domestication and conservation purpose is taking place in Kenya Forestry Research Institute (KEFRI). In undertaking the management plans and conservation actions, the local communities should be involved and empowered to manage the forest through participatory forest management approaches. There is a knowledge gap in appropriate technology on establishment of Sandalwood plantations and sustainable harvesting (Machua *et al.,* 2009). Therefore, objective one in this study has endeavored to address this gap by using *O. lanceolata* indicator species for prediction of site suitability for domestication and establishment of plantations to ease demand on available natural stands in the forest.

2.4 Soil Factors Associated with *O. lanceolata*

In Kenya the species is normally found in various sites including rocky areas and along margins of dry forests, evergreen bushland, grassland, and thickets at altitude of 900 – 2550 m above sea level. A recent study did show the species is more prevalent in nitisols followed by acrisols and more abundant in volcanic than non-volcanic soil habitats (Mukonyi *et al*., 2011). Sandalwood is capable of growing in different soils types including sand, clay red soils, laterite loam, and black-cotton soil provided they are well drained. Even very poor and rocky soils can support Sandalwood growth (Maundu and Tengnas, 2005). Nutrient rich and moist alluvial soils do not support heartwood development in Sandalwood trees and such trees are deficient in oil (Kadamban and Balachandran, 2005). Trees growing in shallow, rocky and sandy soils produce more highly scented wood, giving a better yield and quality oil (Kadamban and Balachandran, 2005). Sandalwood can grow under varying conditions of soil pH ranging from 6.0 to 7.5. In Kenya we do not have sufficient data on how different soil types affect the yield and quality of Sandalwood oil. Therefore, objective two of this study has endeavored to address the gap by comparing soil physical and chemical parameters from

Gachuthi natural forest representing the highlands and Kibwezi natural forest representing lowland dry forest.

2.5 Tree Age in Relation to Heartwood Formation

In India, *Santalum album* and *S. spicatum* mature trees of 30-60 years have a girth of 40-60 cm, with well-developed and demarcated sap and heartwood formation. These trees can yield 19-50 liters of oil (Kadamban and Balachandran, 2005). The value of heartwood varies with age and locality. In Kenya there are no experimental studies that have been undertaken to ascertain the appropriate harvesting age of the East African Sandalwood. Available data from Kenyan smuggled specimens harvested from Taita hills, Marsabit, and Samburu showed age ranging between 15 and 45 years (Mukonyi *et al*., 2011). The mode of harvesting commonly used in East Africa is uprooting the whole tree, hence seriously interfering with the species natural distribution and regeneration (Mbuya *et al*., 1994).

2.6 Essential Oils from Plants

These are liquid components of plant cells which, like lipids, are immiscible with water but which, unlike the fatty compounds, are often volatile at ordinary temperatures and can be distilled at ordinary pressure (Sukhdev *et al*., 2008). Usually, essential oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, hard drinks and insecticides.

Although generally considered immiscible with water, many of the volatile oils are sufficiently soluble in this medium to impart their characteristics odor and taste. The volatile oils are responsible for fragrance of flowers and characteristics aromas of other parts of many plants. Typical examples containing volatile oils are peppermint, spearmint, thyme, eucalyptus, sassafras, cinnamon, cloves and sandalwood among other plants (QCHD, 2001).

2.7 Major component of Sandalwood oil

Pure essential oil from Sandalwood which is 100 percent wholesome is among the most expensive oils, whose global market price ranges from US$10-1000 per kilogram (Scurrah-Ehrhart and Blomley, 2006). The high prices have attracted a number of illegal traders, who have been exporting wood of the species to Europe, the Middle East and Asia, where oil is extracted. The extracted oil is used to manufacture expensive items such as luxurious

cosmetics, perfumes and fragrances (Peters, 2001). The excellent blending and antiseptic properties of Sandalwood oil has also made it valuable as a fixative for other fragrances (Saji *et al.,* 2010). Sandalwood oil, bark and root extracts were used as ancient form of medicine in treatment of inflammatory conditions like genitor-urinary conditions and inflamed skin. At present aromatherapy uses of Sandalwood oil include curing dry skin, acne and general irritation besides helping in cases of dry coughs, sore throat, nausea, diarrhea, laryngitis, catarrh and bronchitis (Hongratanaworakit *et al.*, 2004).

The main part being exploited is the heart wood mainly from the root and the stem for extraction of essential oils as main derivatives though all parts of the plant have uses. The roots are used for extraction of essential oil, the stem for timber and wood carving. In Australia and India, some 15 species occurring in the genus *Santalum*, have valuable aromatic oils within their heartwood (Brand *et al*., 2007). The value of the wood varies between species, due mainly to differences in the mean oil concentration and quality. Oil quality is related to the amounts of α-santalol and β-santalol within the oil, which gives Sandalwood species their unique fragrance (Brand *et al*., 2006). Besides the santalol, Sandalwood oils contain a range of other important components, including trans-farnesol, β-santalene, epi-β-santalene, bergamotol, pinene alpha among others (Brand *et al*., 2007).

2.8 Variation of Oil among Plant Parts

Heartwood of the Sandalwood tree is the most valuable part in oil production. Its formation starts around 10-13 years of age. Ecological determinants for the heart wood formation include factors relating to stress, such as gravelly dry soil and range of elevation. Most of the root portion after a certain age is heartwood; however, in the stem, it is highly variable from place to place. Oil on the stem can range from 90 percent to a negligible amount, or be absent (Ananthpadmanabhe *et al*., 1988).

After harvesting, Sandalwood oils are commonly extracted from the ground wood using methods such as steam distillation or solvent extraction. A non-destructive method of sampling Sandalwood trees entails taking core samples through the centre of the stem. Variation in oil content among tree components has been demonstrated in several research studies. For example in Australia, it was found that within a single *S. spicatum* tree, total extractable oil, and the levels of α-santalol and β-santalol within the oil, all decreased with increasing height in the tree. The level of farnesol within the oil, however, increased higher up in the tree (Brand *et al*., 2007). In India, research on *S. album* trees aged 15 years and

planted in a plantation, revealed that extracted oil from the base, about 15cm, had 64-79% of oil whereas at about 70cm above ground level, produced 57-59% oil. The levels of α-santalol and β-santalol were 46% at the base and 24-27 % (70 cm) above ground of the tree (Howes *et al*., 2004). Another study on 50 years mature natural stands of *S. spicatum* growing in Western Australia arid regions received 282mm annual rainfall and calcareous red loam soils found that within samples taken from 15cm above ground had 3% oil content with 3.1-8.0% and1.3-3.0% α-santalol and β-santalol respectively, compared with samples from 70cm above ground which had 2.25% oil yield and 0.1-2.4% α-santalol and 0-0.6% β-santalol (Brand *et al*., 2006). Therefore, for more accuracy in estimation of the oil content within a tree, core samples should be taken from at least two positions along the tree length, such as near the base and higher up the stem. In addition, the total extractable oil concentration and oil quality (i.e. α-santalol and β-santalol content) should always be expressed together when determining the value of Sandalwood (Howes *et al*., 2004). There is no adequate data on yield and quality of oil from different tree components of Sandalwood in Kenya; therefore to address this gap objective three and four in this study has endeavored to address the gap by sampling *O. lanceolata* trees from Gachuthi and Kibwezi natural forests to extract oil from roots, stem and bark and determine the quality.

. In East Africa, harvesting of Sandalwood has concentrated in Tanzania and Kenya, particularly in the dry areas (Beentje 1994, Mbuya *et al*., 1994). A study by Mwang'ingo *et al*., (2003) revealed a big difference in the yield and quality of oil produced among different natural populations at different altitudes. The study involved four sites, which are Image, Nundu, Sao Hill and Lushoto in Tanzania.

Lushoto area is located within an altitude of about 1250 m with maximum mean temperature of 20.8^{o}C and minimum mean temperature of 13.8^{o}C. It receives a mean annual rainfall of 680 mm. Soils of Lushoto are classified as Dystric nitisols and lithosols with the former being dominant. They are naturally of low fertility (FAO, 1977). The vegetation is Somali-Masai scrub (White, 1983), being characterized by small widely spaced trees over a grass stratum. Sandalwood from Lushoto had oil yield of 6.53% and 11.11% santalol content.

Image area is located within an altitude of 1900 m with maximum and minimum temperature at 26.5^{o}C and 14^{o}C respectively. It is lowland with soils that are predominantly laterised low-humic red earths, being fertile initially, but lose fertility rapidly with frequent

cultivation. The soils support miombo woodland commonly of *Brachystegia* and *Isoberlinia* such as *B. spiciformis, B. utilis* and *B. glaberrima, Isoberlinia* spp., *Combretum* spp. and *Albizia* spp. (Gilchrist, 1952, White, 2002). Sandalwood in Image had 4.8% oil yield and 3.18% santalol content.

Nundu lies within an elevation of 1900 meters above sea level. Maximum temperature is 19.7^{o}C and minimum is 7.9^{o}C. It has soils classified as humic red earths and they support thickets of secondary woodland and scrub of *Agauria* and *Myrica* derived from upland humid evergreen and the dry upland sclerophyll forest (Gilchrist, 1952, White, 2002). Sandalwood oil yield was 8.4% and 3.30% santalol level.

Sao Hill lies within an altitude of 1900 m with maximum and minimum temperature at 21.7^{o}C and 10.8^{o}C respectively. It is a forest with soils which are granitic in origin, being deep and relatively uniform in physical structure, and mostly of sandy clay loam texture, being low in nitrogen, organic carbon, available phosphorus and exchangeable bases (Nshubemuki *et al*., 1996). They are covered with grasses of *Loudetia simplex, Themeda triandra, Hyperrhenia* sp. and *Panicum maximum*. Trees and shrubs are scattered clumps or individuals associated with rocky knolls or gullies with common species being *Searsia natalensis, Parinari curatellifolia, Maytenus senegalensis, Tecomaria capensis* and *Dodonaea viscose* (Mgeni, 1986). Sandalwood oil yield 8.20% and the santalol amount was 2.59%

The findings from this study revealed that Lushoto being lowland at an altitude of 1250 m had the highest amount of santalol in oil compared to the other regions at 1900 masl. The study in Tanzania concluded that there was significant variation in oil yield and quality among population in different ecological regions thus explaining why Sandalwood from different locations in the country is overexploited compared to other regions. In Kenya, data to support oil yield and quality among different population in highland and lowland dry forests is inadequate and for that reason this study has ventured to address the gap by comparing two populations of *O. lanceolata* from Gachuthi and Kibwezi natural forest.

2.9 Conceptual framework

This study was conceptualized based on determination of indicative species for predicting *O. lanceolata* occurrence, soil variables where *O. lanceolata* grows naturally, differences in oil yield and quality among tree components and between populations of highlands and lowlands dry forests. Indicator plant species for predicting *O. lanceolata* occurrence were assessed according to plant habits and their effect on *O. lanceolata* distribution in Gachuthi and Kibwezi natural forest. *O. lanceolata* trees height, diameter at breast height, canopy and stem form were recorded in the field. Recording of seedlings, herbs and grass percent cover was done very close to *O. lanceolata* trees where objective one of indicator plants species was addressed in the field. Soil sampling to determine the physical and chemical properties and their effect on the growth of *O. lanceolata* in both sites was carried out. Four *O. lanceolata* trees were sampled from each site to determine oil yield and quality. Figure 1 illustrates the interaction among dependant, independent and intervening variables of this research. Dependant variables are height, diameter, distribution, oil yield and quality of *O. lanceolata* tree. Independent variables are environmental factors such as humidity, temperature, rainfall, altitude, soil nutrients in the different study sites. Intervening variable of the study is *O. lanceolata* tree growth and density in both study sites.

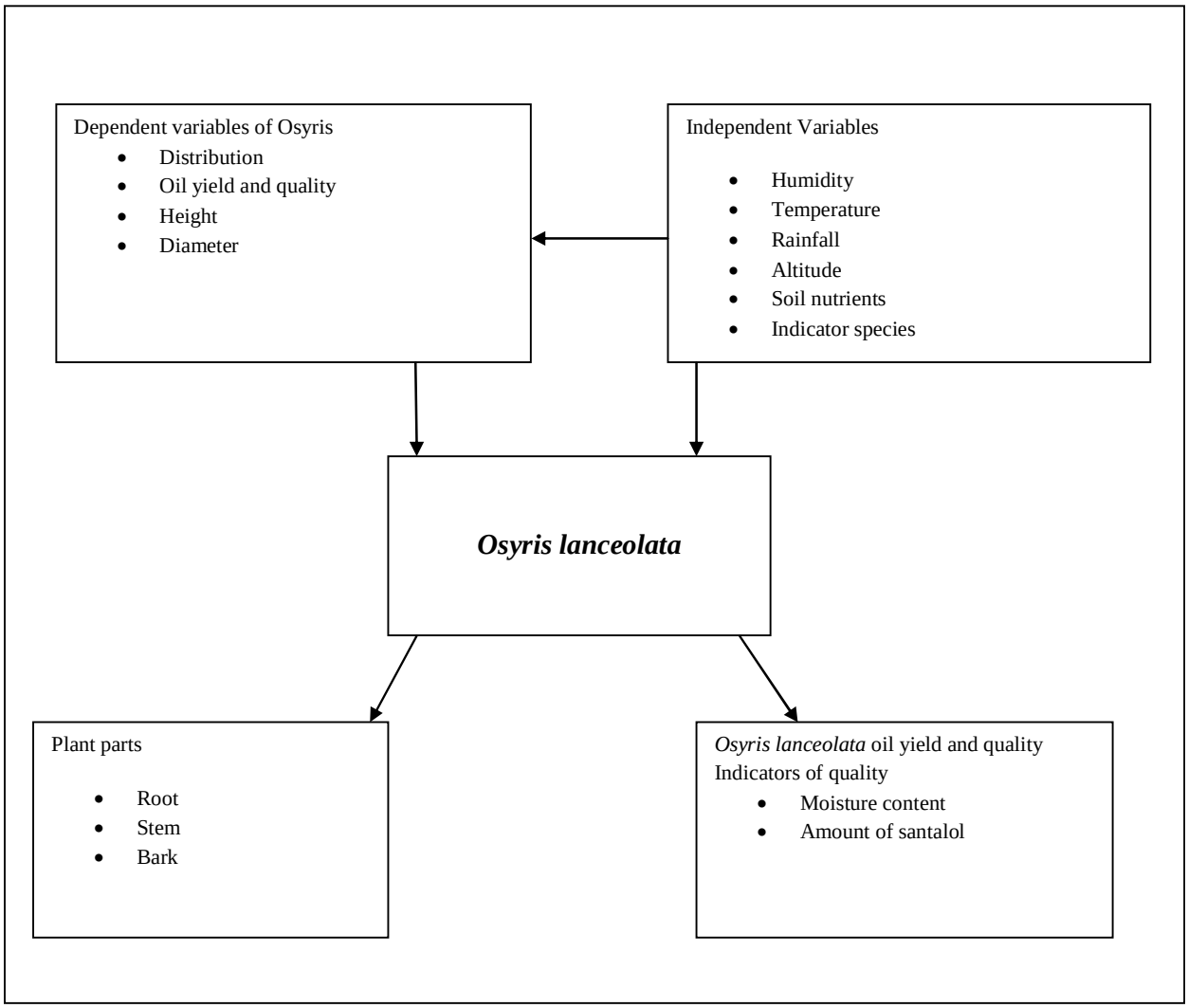


Figure 1: Model on conceptual framework of the study *(Author's survey)*

CHAPTER THREE

3.0 RESEARCH METHODOLOGY

3.1 Research Design

This study employed both experimental and ecological survey design. Primary data was collected from the field as well as from laboratory analysis of samples whereas secondary data was collected through literature review. A comparative study was performed between two sites representing a high rainfall potential land (highlands) and a low rainfall, drylands (lowlands). The highland site was located at Gachuthi natural forest in Muguga and lowland site around Kibwezi natural forest near Chyulu National park (Figure 2 and 3).

3.2 Study Area

The two study sites included Gachuthi forest located in Muguga near KEFRI headquarters and Kibwezi forest close to KEFRI field research station. Muguga is in agroclimatic zone III while Kibwezi is in agroclimatic zone V (Sombroek *et al.,* 1980)

3.2.1 Gachuthi forest

Gachuthi forest in Kerwa Division of Kikuyu District in Kiambu County and lies at about E36^{o}49'49.89'' S-1^{o}10'30.48'' latitude and longitude respectively, 25 km northwest of Nairobi city close to the Nairobi - Naivasha road (Figure 2). The forest external boundaries neighbors densely populated small scale farming communities. The forest is within Muguga estate which is managed by KEFRI mainly for research i.e. (demonstration plots, seed orchards, species and provenance trials, conservation research), and also for commercial production of fuel wood, poles, rails and timber. The forest area consists of open patches, plantations of exotic softwood and hardwood species, and two small portions of indigenous forest vegetation. Muguga estate forest lies within an altitude ranging between 2040 and 2200 meters above sea level with minimum temperature of 12^{o}C and maximum temperature of 25^{o}C. Annual rainfall is between 990-1500mm. Soils in this forest are classified as nitosols that are derived from volcanic rocks. The characteristics of these soils include high clay content (more than 35%), good moisture-storage capacity and aeration; high organic matter content, the cation exchange capacity and the percentage base saturation range from low to high. The soils are acidic (pH < 5.5) due to the leaching of soluble bases (Okalebo, *et al.,* 2002). The natural vegetation of Muguga estate forest is dominated by *Teclea simplicifolia,*

Vangueria madagascariences, Warbugia ugandensis, Ehretia cymosa, Maytenus senegalensis, Zanthoxylum usambarense and *Calodendrum capense.* These tree species are extremely important, as they are the last surviving remnants of the once large dense forest which extended from Ngong hills to Mt. Kenya and around Aberdares and south eastwards along Rift Valley. This forest faces multiple challenges most of which originate from over-exploitation, overgrazing and encroachment by the neighboring communities (KFMP, 1994).

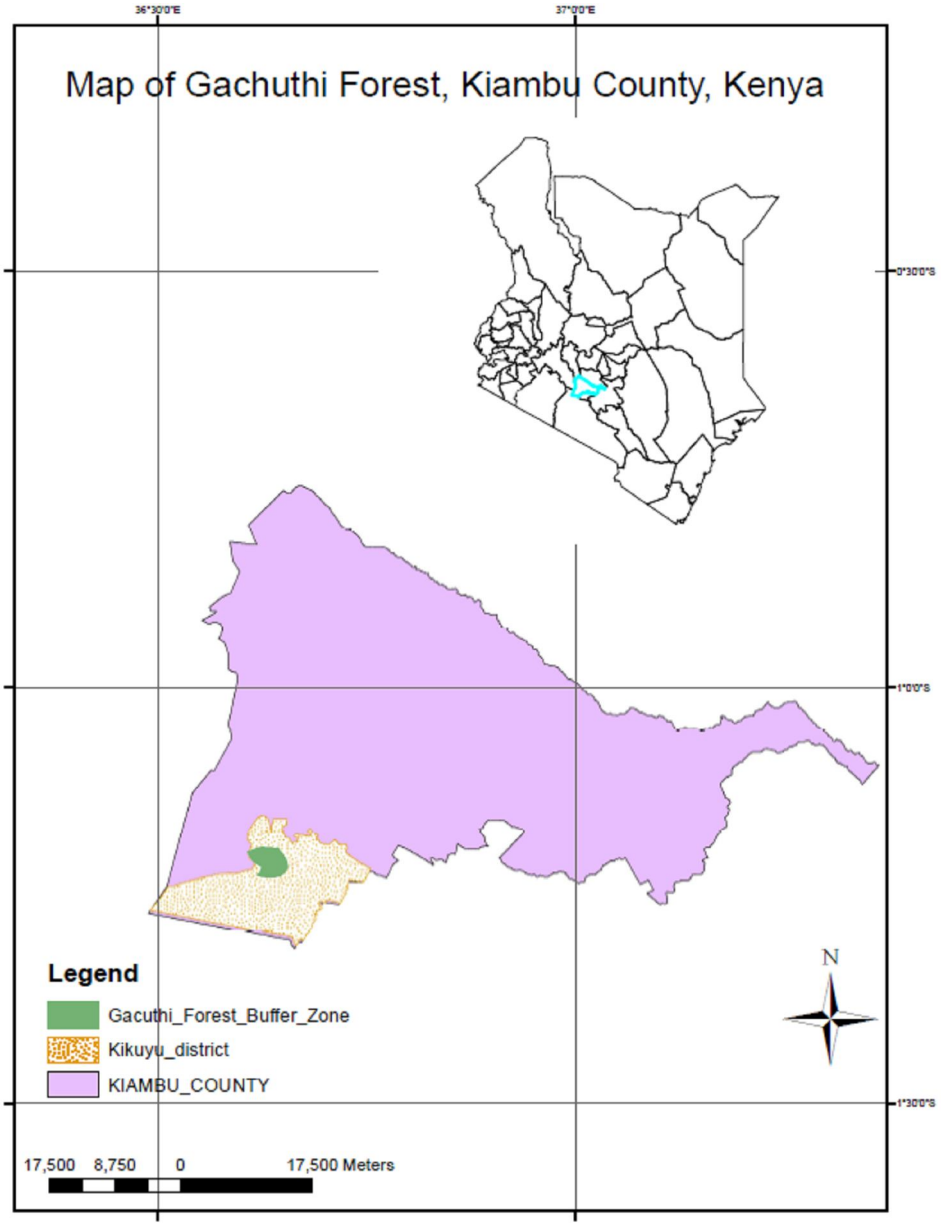


Figure 2: Map of Gachuthi forest ***(Source: GIS office 2014, KEFRI)***

3.2.2 Kibwezi forest

The forest is located near Kibwezi KEFRI field research station, South Eastern part of Makueni County (Figure 3). The latitude and longitude of Kibwezi in Kenya is E37^{o}58'4.25'' S-2^{o}24'37.89'' respectively. The area comprises mainly of ASAL except for very few isolated highland areas such as the Chyulu conservation and catchment areas. The minimum and maximum temperatures of the area are 19^{o}C and 30^{o}C, respectively. The mean annual rainfall ranges between 250mm and 350mm. The soils are generally sandy loams, gravely volcanic and clayey (Okalebo, *et al.,* 2002). Kibwezi forest type of vegetation is Acacia commiphora woodland of varying density. Dominant trees include*: Acacia xanthophloea*, *Acacia tortilis*. *Adansonia digitata, Balanites aegyptica* and *Commiphora* species.

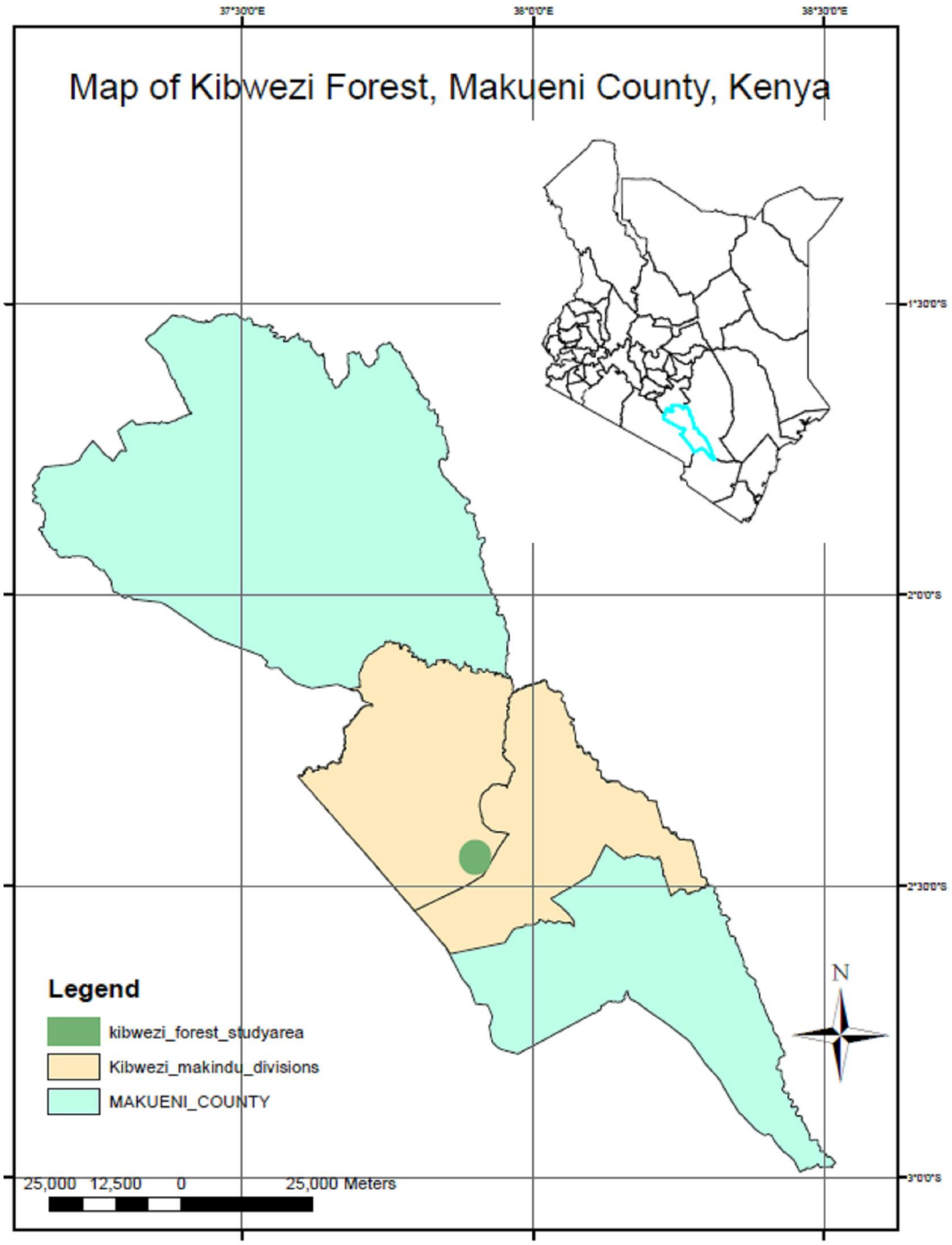


Figure 3: Map of Kibwezi Forest ***(Source: GIS office 2014, KEFRI)***

3.3 Field Activities and Research Design

Due to stiff competition of sunlight and warmth with other large canopy trees, *O. lanceolata* seedlings cannot survive deep inside the forest (Maundu and Tengnas, 2005). In both forests, Osyris trees were abundant at the edges of the forest than deep inside the forest due to effect of crown closure. A reconnaissance visit was undertaken where areas with and with no Osyris trees were identified and a sampling framework determined and data collected. A digitized map of both forests and a GPS was used to mark the plots. Stratification was done 5m from the forest edge on the basis of *Osyris lanceolata* density. A tape measure was used to lay a line transect measuring 600m and sampling plots established on both sides of the transect. To avoid spatial autocorrelation (Tiegs et al., 2005, de Knegt et al., 2010), a distance of ≥50 m was adopted between any two plots. The distance from one sampling point to another was 50m. Figure 4 below shows how sampling was carried out in the field.

Key:

Line Transect

Line marking Stratum

Sampling points

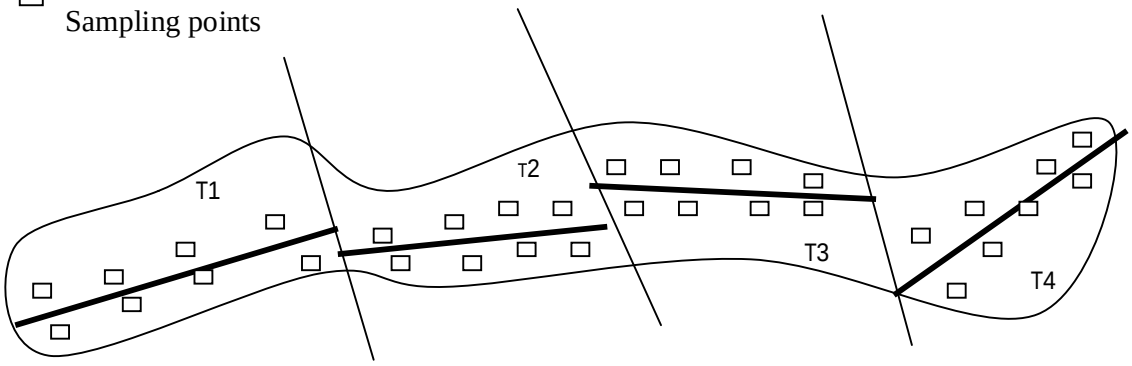


Figure 4: Sampling framework ***(Source: Author's Survey)***

3.4 Sampling and Data Collection

All field data were collected using modified nested-intensity plots (Barnett and Stohlgren, 2003). A nested-intensity plot consists of a main plot measuring (A) 5 by 20m, a middle sub-plot measuring (B) 2 by 5m and four sub-plots (C) 1 by 1m (Figure 5). Normally, the 1 by 1m

sub plots are located near the corners of the main plot but their location was modified in this study to be close to Osyris trees located at the middle of the main plot (Figure 5). Nested intensity plot design was also used for soil sampling from plots with and without *O. lanceolata* tree. In total 24 plots were sampled in each site. In Gachuthi forest, 7 plots randomly fell in plots with *O. lanceolata* and 17 in plots without *O. lanceolata*. In Kibwezi forest, 18 plots were with *O. lanceolata* and 6 plots without *O. lanceolata*. Vegetation data in terms of species and habit were listed as trees, multi-stemmed shrubs and herbaceous weeds and grass.

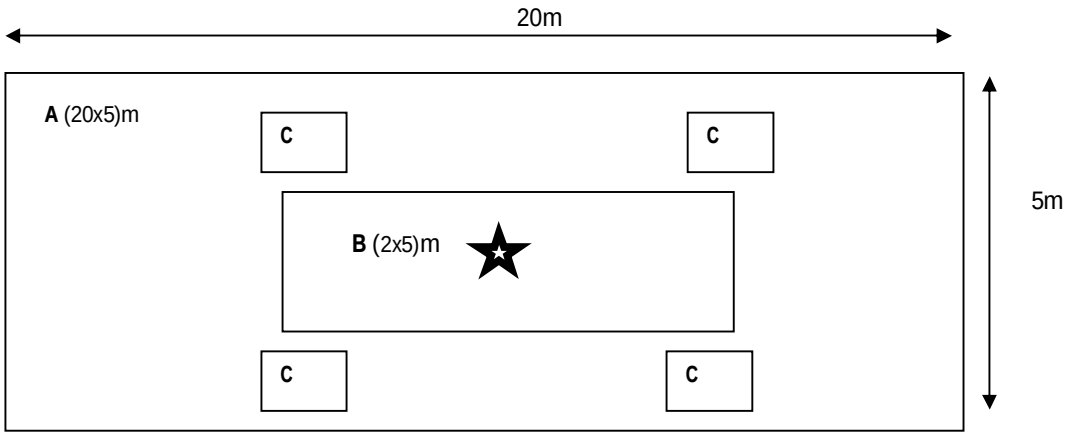


Figure 5: A modified Nested-intensity sample plot used to collect vegetation data. The star indicates approximate location of Osyris trees in the sample plots *(Source: Author's Survey)*

3.5 Determination of indicative species for predicting *Osyris lanceolata* occurrence

Data was collected from individual plots and subplots in the nested-intensity sample plots:

- Main plot A: was used to record all multi-stemmed shrubs and trees that were present. Multi-stemmed shrubs and trees were identified according to (Beentje, 1994). If the species could not be identified, its vernacular name was used, and a specimen was collected for later identification at the national herbarium, and the name was cross-referenced with previous checklists. Trees height, diameter at 0.3 m above the ground, DBH at 1.3m, and stem form data were recorded. Dummy variables taking values of 1-4 were used to categorize the stem form according to straightness. Canopy closure was estimated visually by three persons independently

of each other by standing in the centre of the plot and looking upwards and then averaged (Murphy and Lodge, 2002) (Appendix i).

- Subplot B was used to record height, DBH and canopy cover of only *Osyris lanceolata* trees which was deliberately located at the middle of the main plot by ensuring that all plot measurements starts with Osyris. To measure the tree height and DBH, hypsometers and diameter tapes were used respectively (Appendices ii).

- Subplots C was used to identify and enumerate ground cover vegetation i.e. herbaceous species, grass and seedlings (a seedling was considered as any woody plant less than 0.5 m in height. Estimation of percent ground cover was the proportion of the ground covered by vegetation when viewed from above. Names of herbaceous species, grass and seedlings were identified in the field, following (Beentje, 1994). If the species could not be identified, its vernacular name was used, a specimen was collected for later identification at the national herbarium, and the name was cross-referenced with previous checklists (Appendices iii).

3.6 Determination of Soil Variables Influencing O. lanceolata Distribution

Soil samples were collected under *O. lanceolata* trees at depths of 0-25cm and 25-50cm using a soil auger. Samples were bulked and homogenized according to their different depths to obtain two soil samples under the tree.

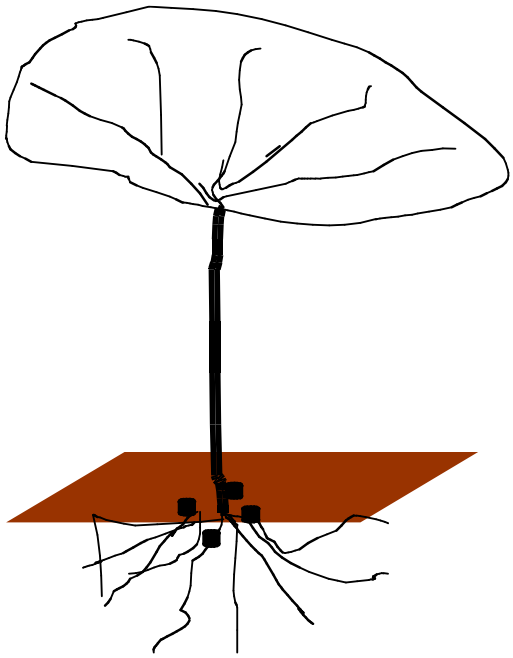


Figure 6: Soil sampling points under *Osyris lanceolata* tree *(Source: Author's Survey)*

Additionally, other soil samples were collected from the corners of main plot A using the same depths, and then they were bulked and homogenized to make two more samples. Soil samples under *O. lanceolata* tree were labeled (A1), whereas soil from the main plot A were marked (B1). More information included in the label was the sampling date, site and depth. Description of the topography, gradient, land use, drainage, soil classification among other information was recorded (Appendix iv).

These soil samples were taken to KEFRI laboratory for preparation through air drying, grinding using a pestle and mortar to make the sample homogenous, and sieving using a 2mm sieve to remove any plant debris and stones present in the sample. Nutrients concentration and physical properties were analyzed using laboratory procedures described by Okalebo *et al*., (2002).

3.7 Laboratory Soil Analysis

3.7.1 Determination of soil moisture

Fresh soil samples placed in a cool box to prevent moisture loss during transportation from the field were used for this analysis. A top loading analytical balance was used to weigh an empty can (W_1). A scoop of fresh soil was added and their weight recorded (W_2). The

samples were then oven dried at 105° C for 72 hours and allowed to cool in a desiccator for 30 minutes. The weight of dry soil and can was then measured (W_3). The moisture content of the soil was calculated as a percentage of the dry soil weight as shown below:

% Soil Moisture Content = $W_2 - W_3 / (W_3 - W_1) \times 100$

Whereby,

W_1 - Weight of can

W_2 - Weight of can + fresh soil

W_3 - Weight of can + dry soil

3.7.2 Determination of soil texture/soil particle analysis

Using analytical weighing balance, 50g of air dried soil samples that had passed through 2mm sieve were weighed into a 500ml plastic shaking bottles and 300ml water added followed by 50ml of calgon solution and then tightly closed. A blank without any soil sample but with 300ml water was included as a control. These samples were shaken in a reciprocal shaker overnight. The following day, the soil suspension was transferred into 1000 ml graduated cylinder and made-up to the mark of 1000 ml with water. The contents were stirred thoroughly using a plunger for 2 minutes and then the percent sand was measured using a hydrometer for 40 seconds. The second reading which is percent clay was taken 2 hours after settling down of sand suspension.

Calculation:

Reading of sample – Reading of blank/weight of sample taken*100

NB: This formula applies for sand or clay only. For silt, addition of sand and clay minus the normal 100% gives the silt reading.

3.7.3 Soil pH and Electro conductivity (E.C.)

Soil pH (water) and electro conductivity (E.C.) were measured according to the procedures of Anderson and Ingram (1993) and Okalebo *et al* (2002), respectively. A 20g soil sample was weighed into 100ml plastic bottles and 50 ml distilled water added. The contents were shaken for 30 minutes, allowed to stand for 30 minutes. The pH (water) was measured using a pH meter (Model 691) and electrical conductivity measured with conductivity meter (Model TOA Cm-20S).

3.7.4 Total nitrogen

Nitrogen content in the soil sample was determined using Kjeldhal method as prescribed by Anderson and Ingram (1993) and Okalebo *et al* (2002). A sample of 0.3g of finely ground soil was weighed and transferred to clean labeled digestion tubes. A digestion mixture of 4.4 ml prepared by dissolving 0.42g of selenium (Se) powder and 14g of lithium sulphate in 350 ml of 30% hydrogen peroxide and mixed well. Concentrated sulphuric acid 420ml was slowly added while cooling in an ice bath. The mixture (4.4ml) was transferred to the digestion tubes with samples and placed in a block digester (Skalar Block Digester System, Model SA 5640). The digestion tubes were heated at 360ºC for 2 hours until the solution was clear. The contents were allowed to cool. After cooling 25ml of distilled water was added and contents transferred into a 50ml volumetric flask and topped up to the 50ml mark with distilled water. The contents were allowed to settle and a clear solution taken from the top of the tube for nitrogen analysis. An aliquot of 10ml of the solution was transferred to the distillation tubes and 10ml of 40% sodium hydroxide added for distillation. The extract was steamed immediately into 5ml of 1% boric acid and 4 drops of mixed indicator. Distillation was continued for 2 minutes from the time the indicator turned green. The distillate was removed and titrated with 0.1 M HCl until the colour changed from green through grey to a definite pink.

Calculation of Nitrogen content in soil sample:

% N in soil sample = corrected ml of N/140HCl ×0.1 / Weight of sample

Whereby;

Corrected ml of N/140HCl = Burette reading – The ml of N/140HCl required for the blank.

3.7.5 Extractable phosphorus

Available phosphorus was extracted with sodium bicarbonate as described by Olsen *et al* (1982) and analyzed according to the method of Anderson and Ingram (1993). Air-dry soil (5g) was weighed into a 250ml shaking bottle and 50ml of 0.5M of pH 8.5 sodium bicarbonate solution added. The contents were shaken on a mechanical shaker for 30 minutes at 200-300 rpm.

The suspension was filtered through Whatman No. 42 filter paper and an aliquot of the filtrate (10ml) pipetted into a 50ml volumetric flask. To this aliquot 5ml of 0.8M boric acid was added and finally, 10ml of ascorbic acid reagent. The contents were diluted to the 50ml mark with distilled water, mixed well and let to stand for 1 hour to allow full colour development. A blank was made by substituting the sample with 5 ml of distilled water and carrying through the procedure. A standard series containing 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5ppm phosphorus was complexed similarly and absorbance values of all the solutions were measured at 880 nm (Model UV Spectronic 21-Milton Roy Co).

3.7.6 Potassium

Potassium was determined spectro-photometically (Anderson and Ingram, 1993) using Corning M 410 flame photometer model. 5g of air-dried soil samples were weighed into a 250ml clean plastic bottle and 100ml of 1.0M neutral ammonium acetate solution added and the contents were shaken on a mechanical shaker for 30 minutes (Neutral ammonium acetate was used to extract maximum cations that occupy exchange sites on the soil surface). The contents were filtered through Whatman No. 42 filter paper. 5ml of the filtrate was pippeted into a 50ml volumetric flask where it was added 1ml of 26.8 % Lanthanum chloride solution and the contents made to the mark with the ammonium acetate solution. Standards containing potassium at concentrations 0.0, 2.5, 5.0, 7.5 and 10ppm potassium were prepared similarly to fall within the measurable range of the calibrated flame photometer. The flame emission intensities were measured at 766nm for potassium.

3.7.7 Oil Content Determination

The researcher had to obtain authorization to harvest *O. lanceolata* trees from KWS and only four trees per site were permitted to be used for the research. Selection of trees to be harvested was through the assistance of rural community to apply Traditional Ecological Knowledge (TEK) which they put into practice in resource management thus identify trees of about 10 years for extraction of oil (Mauro *et al*, 2000). Trees above 10 years were not available due to past illegal practice of poaching the species from its natural habitat for trade. In Gachuthi forest, four trees with 2.7 cm, 2.5 cm, 2.8 cm and 2.6 cm diameter at 30cm above ground were harvested and separated into three different tree components (root, stem and bark). Similarly, trees from Kibwezi forest with 5.0 cm, 4.7 cm, 4.9 cm and 5.2 cm diameter at 30cm above ground were harvested and separated into tree components making a total of

24 samples (Melanie *et al*., 2003). Roots were cleaned off field dirt and all the samples stored in airtight polythene bags. The 24 samples were then transferred to the laboratory. The samples were air-dried under room temperature (22^{o}C) for 15 days to avoid loss of volatile essential oils (QCHD, 2001). The three components were grinded separately into a fine powder using a Dietz model, D-73265 grinder. The ground samples were stored in airtight plastic containers to prevent further moisture loss. Determination of moisture content was done by weighing sample into a glass petri-dish and drying it in an oven at 105^{o}C till a constant weight was attained.

% Moisture Content = $(W_2 - W_3) / (W_3 - W_1) \times 100$

Whereby,

W_1 - Weight of petri-dish

W_2 - Weight of petri-dish + fresh sample

W_3 - Weight of petri-dish + dry sample

Using the standard procedures of Association of Official Analytical Chemist (AOAC 1990), hydro distillation method was used to extract oil from the plant samples. Distillation of oil from the samples was done by weighing 100g of the fine powder into round bottomed flask using an electronic weighing balance. Some anti- bumps to help in boiling and 1000ml of distilled water was added to the sample. The round-bottomed flask was placed in the mantle heater to heat up to 90^{o}C. A Clevenger with 10ml of distilled water and hexane was fixed in a centered neck. A cooling machine was fixed to the Clevenger at about -15^{o}C to cool the water. The distillation process was about 2 hours after start of boiling. After 2 hours the sample was removed and water was separated from hexane. The hexane containing the oil was passed through sodium sulfate anhydrous to remove remaining water through the use of a glass pipette and glass wool to filter oil; it was then stored in a brown sample bottle to avoid light at -10^{o}C in a fridge. Fig: 7 below show the hydro distillation method in the laboratory:

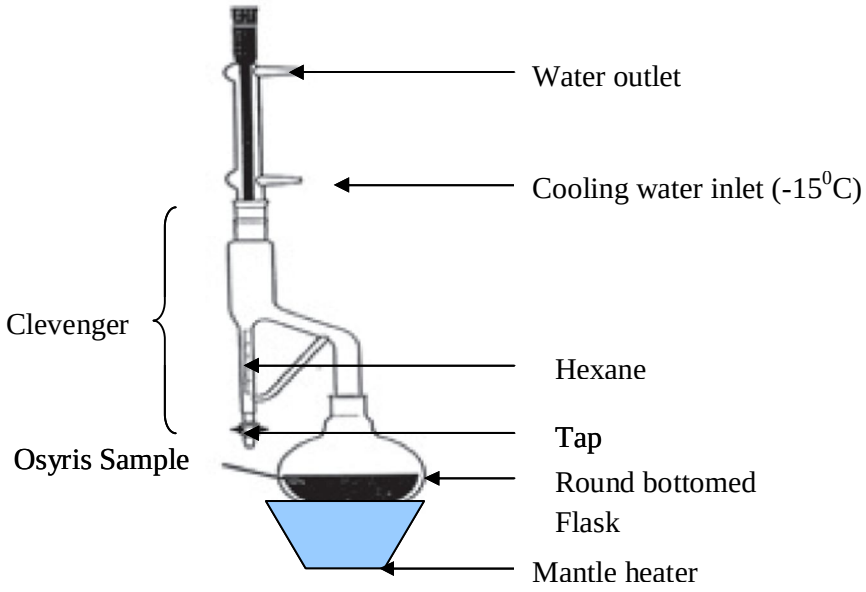


Figure 7: Clevenger-type laboratory-scale hydro distillation apparatus

3.7.8 Quality of Oil

To determine the quality of oil, 10µl of the distilled oil was pipetted into 1.5 ml sample bottle and diluted with 1 ml of hexane. 200µl of the diluted sample was transferred into an insertion for Gas Chromatography-Mass Spectra (Agilent Technologies, model, 7890 A) for identification of compounds. To quantify the amount of α and β santalol in each sample of oil, an external standard of a C_{15} sesquiterpene caryophyllene was made using the following series: 1, 25, 50, 75, and 100 ng/µl and calibration curve used for calculation. The method used for compound analysis was hexane volatiles, at 35^{o}C – 280°C for 50 minutes per sample.

3.7.9 Oil Yield Content

Percentage weight recovery method was used to calculate the percentage oil yield from root, stem and bark of *O. lanceolata* tree. A short path distillation apparatus was used to distill off hexane in the oil. A sensitive analytical balance was used to weigh an empty bottle and recorded as W_1. Hexane plus oil was placed in the bottle, hexane was evaporated using a water bath at 50^{o}C and at 535 kPa pressures. The bottle with oil was weighed again and recorded as W_2.

To calculate percentage yield;

% yield = W_2 (weight of bottle +oil) – W_1 (weight of bottle) /W_3 (weight of sample used in distillation) *100

3.7.10 Data Analysis

Table 1: Summary of Data Analysis Process

Objective	**Hypotheses**	**Variables**		**Tool of Statistics**
		Independent	**Dependent**	
To determine indicator species for predicting *O. lanceolata* occurrence in the highland and lowland natural forest.	There is no significant relationship in occurrence of *O. lanceolata* and indicative species in highland and lowland natural forests.	Indicative species	*O. lanceolata* occurrence	Canonical Correspondence Analysis (CCA) using CANOCO Microsoft Excel
To determine soil physical and chemical parameters where *O. lanceolata* grows naturally in highland and lowland natural forests.	There is no significant difference on physical and chemical parameters where *O. lanceolata* naturally grows in highland and lowland natural forests.	Soil variables Soil moisture Soil texture pH Electro conductivity Nitrogen Phosphorous Potassium	*O. lanceolata* distribution	T- test using SPSS
To evaluate differences in oil yield among tree components in highland and lowland forest.	There is no significant difference in oil yield among tree components in the highland and lowland dry forests.	Tree components Root Stem Bark	Oil yield	ANOVA using SPSS
To determine variation of *O. lanceolata* oil quality in the highland and lowland forest.	There is no significant variation of *O. lanceolata* oil quality between highland and lowland dry forests.	Site Highland Lowland natural forests	Variation of *O. lanceolata* oil quality	ANOVA using SPSS

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Indicative species for Predicting *O. lanceolata* Occurrence

This chapter presents and discusses the findings of the current study as was guided by the specific objectives. Vegetation data in the field was collected in terms of species and habit for determination of indicative species for predicting Sandalwood occurrence. The density stocking of trees in Gachuthi forest was 700 trees/ha which was equivalent to Kibwezi forest. The number of multi-stemmed shrubs in Gachuthi was 200 shrubs/ha compared to Kibwezi forest with a higher density of 500 shrubs/ha. In addition, the seedlings density in Gachuthi was 250 seedlings/ha which is higher than Kibwezi with 100 seedlings/ha. The density stocking of Osyris in Gachuthi forest was 100 trees/ha, average height 3.3m, DBH 1.96cm and diameter at 30cm was 2.7cm. In Kibwezi forest Osyris density stocking was 200 trees/ha, height 4.3m, DBH 3.1cm and diameter at 30cm was 5.1cm.

Vegetation data for plots with Osyris and with no Osyris was analysed using CANOCO and Microsoft excel software. In Gachuthi forest, 19 species of herbs and grass were found in plots with Osyris and 28 species in plots with no Osyris. In Kibwezi forest, 13 species of herbs and grass were found in plots with Osyris and 7 species in plots with no Osyris (Table 2). Occurrence of multi-stemmed shrubs in Gachuthi forest were 22 species in plots with Osyris and 21 species in plots without Osyris as compared to Kibwezi 14 species in plots with Osyris and 10 species in plots without Osyris in Table 3. The occurrence of trees in Gachuthi forest were 14 species in plots with Osyris and 17 species in plots without Osyris compared to Kibwezi forest where 20 species occurred in plots with Osyris and 7 tree species occurred in plots without Osyris (Table 4).

Table 2: Herbs and Grass

	Gachuthi Plots		**Kibwezi Plots**	
	With Osyris	**Without Osyris**	**With Osyris**	**Without Osyris**
Abutilon mauritianum	√	√	√	×
Achyranthes aspera	√	√	√	×
Ageratum conyzoides	√	√	×	×
Asparagus racemosus	√	√	√	√
Barlelia acanthoides	×	×	√	√
Bidens pilosa	√	√	×	×

Chenopodium pumilio	×	√	×	×
Chloris sp	√	×	×	×
Cissus quadrangularis	×	×	√	√
Clematis brachiata	×	√	×	×
Commelina benghalensis	√	√	×	×
Conyza sumatrensis	√	√	×	×
Cyathula sp	×	√	×	×
Cynodon dactylon	×	√	√	√
Cyperus sp	×	×	√	×
Cyphostemma maranguen	×	√	×	×
Digitaria abyssinica	×	√	×	×
Duosperma kilimandscharicum	×	×	√	×
Fuarstia Africana	√	√	×	×
Galinsoga parviflora	√	√	×	×
Glycine wightii	√	√	×	×
Gutenbergia condifolia	√	√	×	×
Hyparrhenia rufa	√	√	×	×
Hypoestes forskahlii	√	√	√	√
Ipomea wightii	×	√	√	×
Justicia diclipteroides	×	×	√	×
Ocimum gratissimum	√	√	×	×
Oplismenus hirtellus	√	√	×	×
Oxalis obliquifolia	×	√	×	×
Pennisetum clandestinum	×	√	×	×
Periploca linearifolia	×	√	×	×
Seddera hirsute	×	×	√	×
Setaria verticillata	√	√	×	×
Sida tenuicarpa	×	×	×	√
Solanum incanum	√	√	√	√
Zehneria scabra	√	√	×	×
Total	19	28	13	7

Source: field data (2013)

Key: √-Presence ×-Absence

Out of 19 species of herbs and grass found in Gachuthi forest occurring in plots with Osyris 18 species repetitively occurred in plots without Osyris. These species were *Abutilon mauritianum, Achyranthes aspera, Ageratum conyzoides, Asparagus racemosus, Bidens pilosa, Commelina benghalensis, Conyza sumatrensis, Fuarstia Africana, Galinsoga parviflora, Glycine wightii, Gutenbergia condifolia, Hyparrhenia rufa, Hypoestes forskahlii, Ocimum gratissimum, Oplismenus hirtellus, Setaria verticillata, Solanum incanum* and *Zehneria scabra*. *Chloris sp* was an exceptional because it occurred only in plots with Osyris.

In Kibwezi forest 13 species occurred under Osyris but only 6 species out of them repeatedly occurred in plots without Osyris. These were *Asparagus racemosus, Barlelia acanthoides, Cissus quadrangularis, Cynodon dactylon, Hypoestes* forskahlii and *Solanum incanum*. The other species only found under Osyris were *Abutilon mauritianum, Achyranthes aspera, Cyperus sp, Duosperma kilimandscharicum, Ipomea wightii, Justicia diclipteroides* and *Seddera hirsute*.

Table 3: Multi-stemmed shrubs

Species	**Gachuthi Plots**		**Kibwezi Plots**	
	With Osyris	**Without Osyris**	**With Osyris**	**Without Osyris**
Acacia brevispica	×	×	√	×
Adenium spp	×	×	×	√
Aspilia mossambicensis	√	√	√	√
Clutia abyssinica	√	×	×	×
Commiphora eminii	×	×	√	×
Crotalaria mauensis	√	√	×	×
Croton dichogamus	×	×	√	×
Dodonaea viscose	×	×	×	√
Dombeya burgessiae	√	×	√	√
Erythrococca bongensis	√	√	×	×
Euphorbia schffleri	×	×	√	×
Grewia similis	√	×	√	×
Helichrysum sp.	√	√	×	×
Hibiscus diversifolius	√	√	√	×
Indigofera swaziensis	×	√	√	√
Lantana trifolia	√	√	×	×
Leucas grandis	√	√	√	√
Lippia javanica	√	√	×	×
Maerua oblongifolia	×	×	√	√
Maytenus undata	×	√	√	×
Microglossa pyrifolia	√	√	×	×
Mystroxylon aethiopicum	√	√	×	×
Plectrunthus barbatus	×	×	×	√
Pterolobium stellatum	√	√	×	×
Rhus natalensis	√	√	√	√
Scutia myrtina	√	√	×	×
Syphorstermma viminale	×	×	√	√
Trimeria grandifolia	√	√	×	×
Triumfetta tomentosa	√	√	×	×
Vangueria madagascariensis	√	√	×	×

Vernonia brachycalyx	√	√	×	×
Vernonia lasiopus	√	√	×	×
Total	22	21	14	10

Source: field data (2013)

Key: √-Presence ×-Absence

There were 22 species of multi-stemmed shrubs found occurring in plots with Osyris and 19 species among them repeatedly occurred in plots without Osyris in Gachuthi forest. These species were *Aspilia mossambicensis, Crotalaria mauensis, Erythrococca bongensis, Helichrysum sp., Hibiscus diversifolius, Lantana trifolia, Leucas grandis, Lippia javanica, Microglossa pyrifolia, Mystroxylon aethiopicum, Pterolobium stellatum, Rhus natalensis, Scutia myrtina, Trimeria grandifolia, Triumfetta tomentosa, Vangueria madagascariensis, Vernonia brachycalyx* and *Vernonia lasiopus*. Species found under Osyris only were *Clutia abyssinica, Dombeya burgessiae* and *Grewia similis*.

In Kibwezi forest 14 species of multi-stemmed shrubs were found under Osyris but only 7 species repeatedly occurred in plots without Osyris. These species were *Aspilia mossambicensis, Dombeya burgessiae, Indigofera swaziensis, Leucas grandis, Maerua oblongifolia, Rhus natalensis* and *Syphorstermma viminale*. The species which were found to co-occur with Osyris were *Acacia brevispica, Commiphora eminii, Croton dichogamus, Euphorbia schffleri, Grewia similis, Hibiscus diversifolius* and *Maytenus undata*.

Table 4: Trees

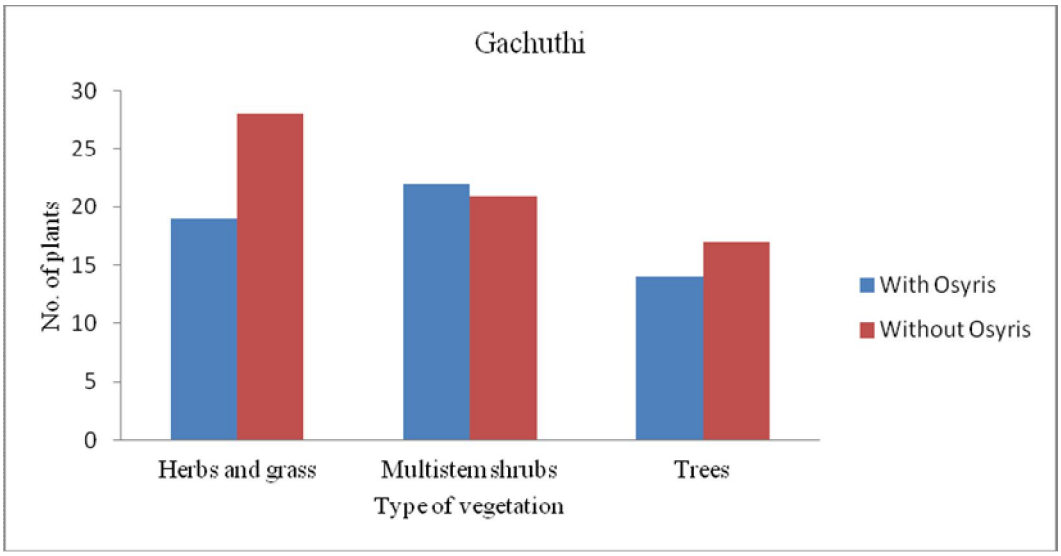
Species	Gachuthi Plots		Kibwezi Plots	
	With Osyris	**Without Osyris**	**With Osyris**	**Without Osyris**
Acacia mearnsii	×	√	×	×
Acacia robusta	×	×	√	×
Antidesma venosum	×	×	√	×
Balanites maughamii	×	×	√	×
Calodendrum capense	√	√	×	×
Cassipourea malosana	√	√	×	×
Celtis Africana	×	√	×	×
Clausena anisata	√	√	×	×
Combretum sp	×	×	×	√
Combretum sp	×	×	×	√
Commiphora baluensis	×	×	√	×
Commiphora spp	×	×	√	×
Croton megalocarpus	×	×	√	×
Cussonia hostii	×	×	√	×
Diospyros consolatae	×	×	√	√
Ehretia cymosa	×	√	×	×
Elaeodendron buchananii	√	√	×	×
Euclea divinorum	√	√	√	×
Euphorbia candelabrium	×	×	√	×
Fagaropsis angolensis	√	×	×	×
Ficus vasta	×	×	√	√
Haplocoelum foliolosum	×	×	√	×
Heteromorpha trifoliate	×	×	√	×
Hymenodictyon parvifolium	×	×	√	×
Juniperus procera	×	√	×	×
Mystrxylon aethiopicum	×	×	√	×
Nuxia congesta	√	√	×	×
Ochna ovate	×	×	√	×
Olea europaea ssp. Africana	√	√	√	√
Pappea capense	×	×	√	√
Pittosporum viridiflorum	√	√	×	√
Ritchiea albersii	×	√	×	×
Schrebera alata	√	√	×	×
Steganoteenia oraliacea	×	×	√	×
Teclea simplicifolia	√	√	√	×
Turraea abyssinica	√	√	√	×
Waburgia ugandensis	√	×	×	×
Zanthoxylum usambarense	√	√	×	×
Totals	14	17	21	7

Source: field data (2013)

Key: √-Presence ×-Absence

There were 14 tree species found occurring with Osyris and 12 out of them repeatedly occurred in plots without Osyris in Gachuthi forest. These tree species were *Calodendrum capense, Cassipourea malosana, Clausena anisata, Elaeodendron buchananii, Euclea divinorum, Nuxia congesta, Olea europaea ssp. Africana, Pittosporum viridiflorum, Schrebera alata, Teclea simplicifolia, Turraea abyssinica* and *Zanthoxylum usambarense*. Only 2 species of trees were exceptional because they occurred in plots with Osyris. These were *Fagaropsis angolensis* and *Waburgia ugandensis*.

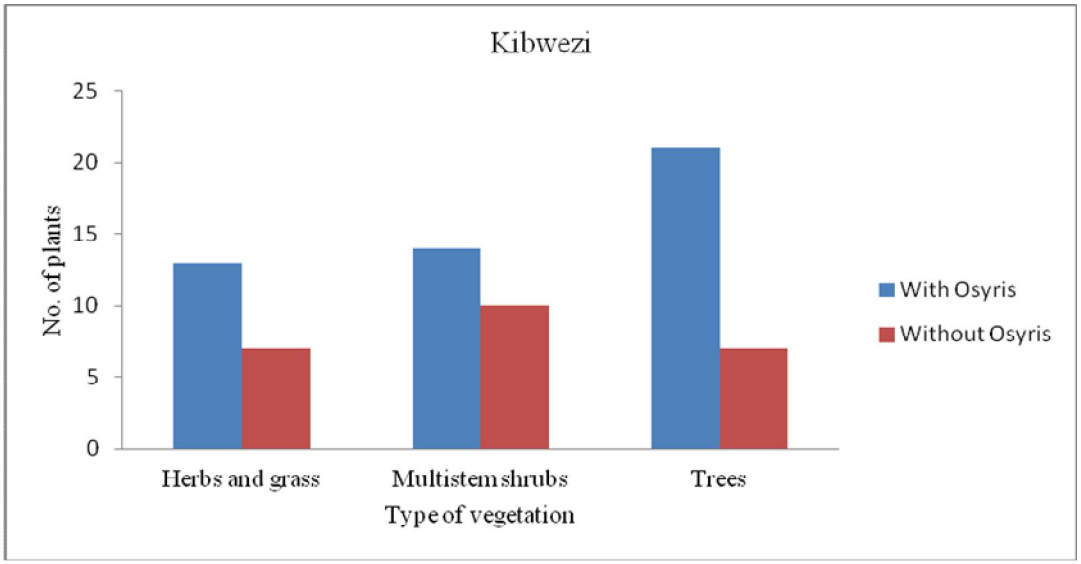
In Kibwezi forest there were 21 species of trees occurring in Osyris plots and only 4 species occurred also in plots without Osyris. These tree species were *Diospyros consolatae, Ficus vasta, Olea europaea ssp. Africana* and *Pappea capense*. The tree species that occurred with Osyris only were *Acacia robusta, Antidesma venosum, Balanites maughamii, Commiphora baluensis, Commiphora spp, Croton megalocarpus, Cussonia hostii, Euclea divinorum,Euphorbia candelabrium, Haplocoelum foliolosum, Heteromorpha trifoliate, Hymenodictyon parvifolium, Mystrxylon aethiopicum, Ochna ovate, Steganoteenia oraliacea, Teclea simplicifolia* and *Turraea abyssinica*.



Source: field data (2013)

Figure 8a: Distribution of Vegetation in Gachuthi Forest

In Gachuthi forest, plots with Osyris had lower number of herbs and grasses (19) compared to plots without Osyris (28). The number of multi-stemmed shrubs in plots with Osyris was 22 and those without Osyris were 21. The number of trees in plots with Osyris were 14 compared to 17 in plots without Osyris.



Source: field data (2013)

Figure 8b: Distribution of Vegetation in Kibwezi forest

In Kibwezi forest, plots with Osyris had 13 herbs and grass species compared to 7 in plots without Osyris. The number of multi-stemmed shrubs in plots with Osyris were 14 compared to 10 in plots without Osyris. A higher number of trees (21) was found in plots with Osyris than in plots without Osyris (7).

4.1.1 Indicative Species Occurrence under Osyris plots in Gachuthi and Kibwezi Forests

Among herbs and grass species in Gachuthi forest, only *Chloris sp* occurred under Osyris plots compared to Kibwezi with 6 species namely *Asparagus racemosus, Barlelia acanthoides, Cissus quadrangularis, Cynodon dactylon, Hypoestes* forskahlii and *Solanum incanum*. Multi-stemmed shrubs under Osyris plots in Gachuthi were *Clutia abyssinica, Dombeya burgessiae* and *Grewia similis* compared to Kibwezi with *Acacia brevispica, Commiphora eminii, Croton dichogamus, Euphorbia schffleri, Grewia similis, Hibiscus diversifolius* and *Maytenus undata*. The only common multi-stemmed shrub species in both sites was *Grewia similis*. Trees found under Osyris plots in Gachuthi were *Fagaropsis angolensis* and *Waburgia ugandensis* compared to Kibwezi with *Acacia robusta, Antidesma venosum, Balanites maughamii, Commiphora baluensis, Commiphora spp, Croton megalocarpus, Cussonia hostii, Euclea divinorum, Euphorbia candelabrum, Haplocoelum*

foliolosum, Heteromorpha trifoliate, Hymenodictyon parvifolium, Mystrxylon aethiopicum, Ochna ovate, Steganotenia oraliacea, Teclea simplicifolia and *Turraea abyssinica*.

There was high diversity and abundance among herbaceous vegetation in Gachuthi compared to Kibwezi forest (Table 2). This could be attributed to the difference of abiotic factors found in lowlands and highlands. In Kibwezi, the forest lies within an altitude of 914 masl, with rainfall amount ranging annually between 250 mm and 350 mm. Kibwezi soils are from recent volcanic materials and have very high percentage of sand which has very poor moisture holding capacity (Okalebo, *et al.*, 2002). Due to the high temperatures of about 30°C and high rate of evaporation, the herbaceous diversity and abundance is limited to wet seasons only. In contrast Gachuthi forest lies between an altitude of 2040 and 2200 masl, with a minimum temperature of 12°C and maximum temperature of 25°C. Annual rainfall is between 990 and 1500 mm. The soils are deep and well developed from volcanic rocks hence have a high moisture holding capacity, which sustains a high diversity of herbaceous vegetation throughout the year. These results concur with the findings of Hogan (2012) that difference in soil, water, air temperature, and sunlight access causes diversity in the types of plants that grow in different areas.

Irving and Cameron (2009) have classified *O. lanceolata* in a group of photosynthetic hemi parasites that depend on other plants for nutrient uptake through developed haustoria between the parasite and the host plant. It is estimated that Sandal trees can be parasites on over 300 species of plants, ranging from herbs and grasses, multi- stem shrubs and trees. In East Africa, previous studies have found Osyris tree with various hosts such as *Dodonea viscosa,Tecomaria capensis, Catha edulis, Apodytes dimidiata, Brachytegia spiciforms, Rhus natalensis* and *Casuarina equisetifolia* (Mwang'ingo *et al.*, 2010). This is consistent with the present study since *Rhus natalensis* and *Grewia similis* were common hosts in both forests while *Dodonea viscosa* was found only in Kibwezi forest.

Kibwezi lowland dry forest had 700 trees/ha stocking density which was the same as in Gachuthi highland forest. This could be attributed to the strict conservation measures enforced jointly by the KFS, KWS and DSWT to restrict the local community from over-exploitation of Kibwezi forest. Conservation started around 2007, with an agreement between the three organizations to place an electric fence surrounding the forest to control human activities that had threatened the forest. Unlike Gachuthi forest, the control of human

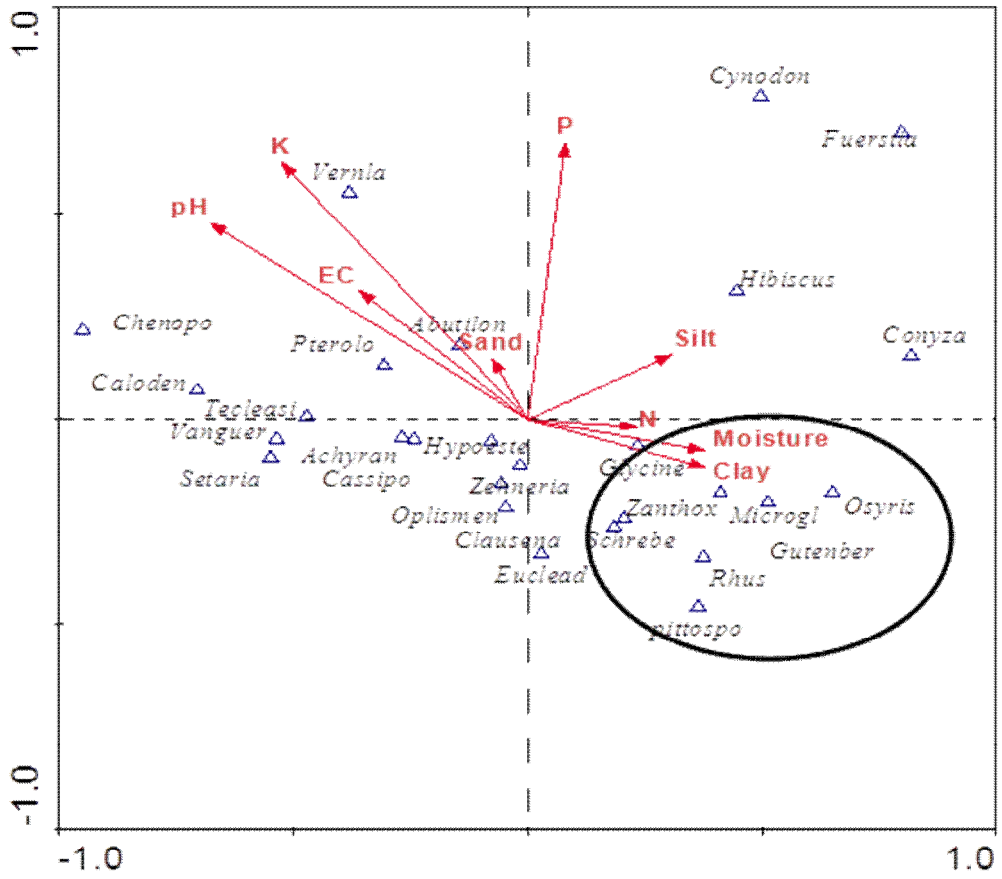
activities is by KEFRI, KFS and Community Forest Association (CFA) that has not completely managed to prevent grazing and poaching of trees from the forest.

4.1.2 Biotic and Abiotic Environmental Factors Influencing the Occurrence of *Osyris lanceolata*

Ecosystems are complex; they consist of interacting biotic and abiotic components. Abiotic environmental variables often influence biotic composition. In this study, Canonical Correspondence Analysis (CCA) for windows 4.15 (Ter Braak, 1997) was used to analyse ecological data from interaction of Osyris indicator species and soil variables with reference to *O. lanceolata* tree. Canonical Correspondence Analysis is a multivariate statistics, which integrates ordination with regression and permutation methodology to allow sound statistical modelling of ecological data. By drawing perpendicular lines from the sites, we thus obtain a ranking of values of the reference species at the site. The imaginary axis defines the direction in the diagram along which the value of species changes hence species that lie close to the point of a reference are therefore likely to have high abundance towards the reference species.

In this study, a biplot was obtained by adding natural vegetation species data with a relative frequency (RF) of at least 25% in sampled plots and soil variables to an existing diagram of Gachuthi and Kibwezi forest with *O. lanceolata* as the reference tree (species relative frequency in Table 12 and 13).The RF of Osyris in Gachuthi forest within sampled plots was 29.17% compared to Kibwezi forest with 75%. Species are shown by the first seven letters of their genus names (full genus names are shown in Table: 2, 3 and 4)

Gachuthi forest Biplot

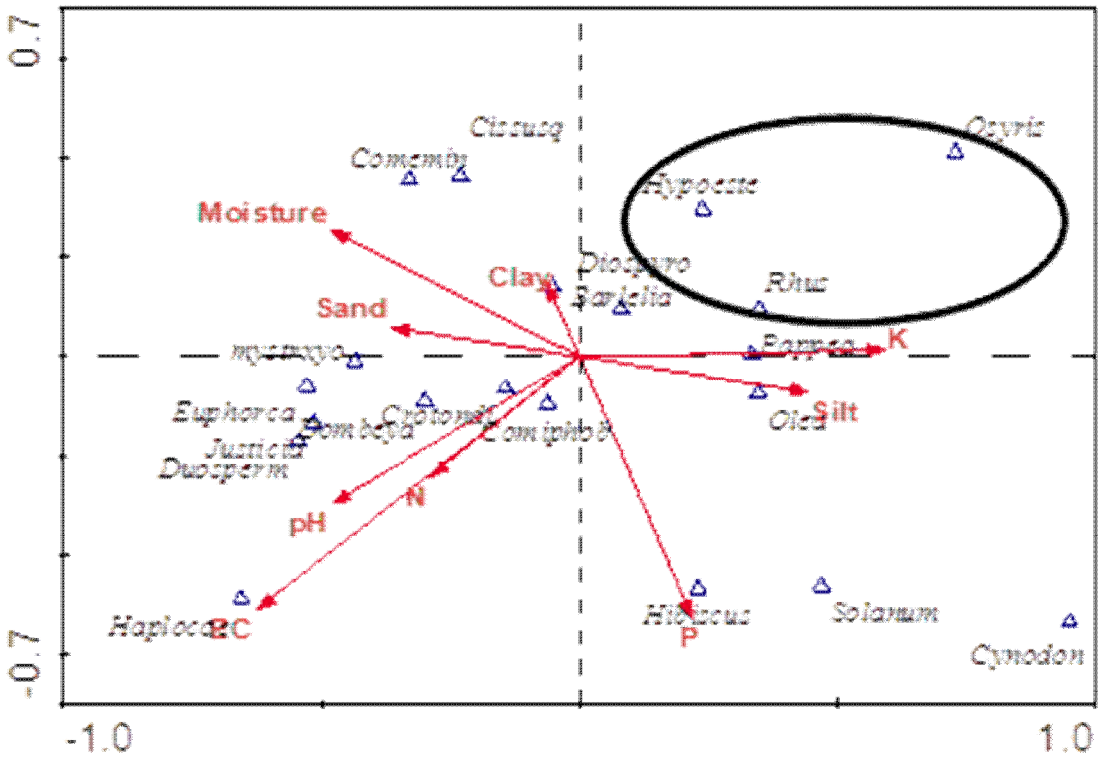


Source: Field data (2013)

Figure 9a: Interactions among environmental variables with *O. lanceolata*

The interactions among environmental variables are shown in Figure 9a. The red arrows represent the soil variables whereas the triangles represent vegetation diversity in the forest. This biplot indicates that *Osyris* in Gachuthi positively relates particularly to soils with high moisture content and high clay percent. The circled cluster of vegetation in the biplot, are indicator species found in abundance co-existing positively with *O. lanceolata*. These species include *Glycine wightii* and *Gutenbergia condifolia* (herbs), *Microglossa pyrifolia* and *Rhus natalencis* (shrubs), and *Pittosporum viridiflorum, Screbera alata, Zanthoxylum usambarense* as tree species. The species that are far in the biplot from reference position decreases in abundance with increasing distance from the reference species.

Kibwezi forest Biplot



Source: Field data (2013)

Figure 9b: Interactions among environmental variables with *O. lanceolata*

The interaction of vegetation species with soil variables in the environment at Kibwezi forest is shown in Figure 9b. *O. lanceolata* most likely correlates positively to soils with high levels of potassium and certainly reacts negatively to soils with high moisture content, high clay and sand percentage. The vegetation clusters found to co-exist positively with *Osyris* were few compared to Gachuthi forest and they included mainly *Hypoestes aristata* and *Rhus natalensis,* described as a herb and a shrub respectively. Other species in the biplot that lie close to *O. lanceolata* position though outside the circle are *Barlelia acanthoides* (herb), *Diospyros consolatae* and *Pappea capense* (trees).

4.1.3 Abiotic and biotic factors associated with occurrence of *Osyris lanceolata* in Gachuthi and Kibwezi forests

From the biplots in Figures 9a and 9b, *O. lanceolata* can grow in different soil types as demonstrated by presence of the species in both highlands and lowlands. These findings are consistent with previous studies that the species can grow in many different types of soil but is more prevalent in nitisols followed by acrisols and more abundant in volcanic than non-volcanic soil habitats (Mukonyi *et al*., 2011). In Gachuthi forest, the species particularly prefers soils with high moisture content and high clay content. From previous findings, soils rich in nutrient and with high moisture such as nitisols probably do not support strong heartwood development in Sandalwood (Maundu and Tengnas, 2005).

Kibwezi soils were formed from recent volcanic materials and are known as volcanic ash soils (FAO, 1984). These soils are very shallow and rocky though they are very high in potassium from the rocks. The main role of potassium in plants growth is to provide the appropriate ionic environment for metabolic processes in the cytosol, and as such functions as a regulator of various processes including growth (Gachene and Kimaru, 2003). In this forest, the *O. lanceolata* particularly prefers soils with high levels of potassium from rocks and most likely supports heartwood formation.

Plate 1: *Osyris lanceolata* at Kibwezi forest growing on volcanic rocks

4.2.1 Indicator Species for *O. lanceolata*

From the biplots, Figure 9a and 9b, *O. lanceolata* both in Gachuthi and Kibwezi forest, have only one common plant indicator which is *Rhus natalensis* and it concurs with other findings. Other circled plant species in the two biplots are different which could be attributed to the different biophysical factors in highlands and lowlands areas. Most probably, whenever *R. natalensis* occurs in the natural forest one is likely to find Osyris tree. Sandalwood has been found to co-exist with various hosts such as *Dodonea viscosa, Tecomaria capensis, Catha edulis, Apodytes dimidiata, Brachytegia spiciforms, Rhus natalensis and Casuarina equisetifolia* (Mwang'ingo *et al.*, 2005).

4.2 Soil physical and Chemical Properties

In this study, soil sampling was done in Gachuthi and Kibwezi forest to determine the effect of biophysical soil properties on yield and quality of oil. Gachuthi forest soils are deep and well developed hence it was easy to sample at two depth levels of 0-25 cm and 25-50 cm. In Kibwezi forest, soil sampling was difficult because of the spreading rocky areas and therefore, only a depth of about 0-25 cm was attained around *O. lanceolata* trees.

Plate 2: Soil sampling using a panga at Kibwezi forest (soils are shallow).

Soil physical and chemical variables in the two study areas were compared using t- test. Variable means of pH, E.C. (ms/cm), % N, P (ppm), K (meq), % sand, % clay, % silt and % moisture content between Gachuthi and Kibwezi sites were tested for normality using Levene's test for Equality of Variances. Values in the same row followed by a different letter are significantly different at $p<0.05$.

Table 5: Soil site data

Soil variable	Site	F	P	Mean
pH	Gachuthi	13.0	.000	6.41±0.074 a
	Kibwezi		.000	7.41±0.043 b
EC (m/cm)	Gachuthi	18.3	.000	0.070±0.009 a
	Kibwezi		.000	0.412±0.038 b
N (%)	Gachuthi	19.0	.000	0.375±0.009 a
	Kibwezi		.000	2.01±0.039 b
P (ppm)	Gachuthi	1.46	.000	7.23±1.345 a
	Kibwezi		.000	30.17±1.345 b
K(meq)	Gachuthi	5.2	.000	1.73±0.13 a
	Kibwezi		.000	3.30±0.33 b
Sand (%)	Gachuthi	7.7	.000	29.42±1.335 a
	Kibwezi		.000	82.89±.973 b
Clay (%)	Gachuthi	5.9	.000	54.71±1.648 a
	Kibwezi		.000	13.56±1.629 b
Silt (%)	Gachuthi	1.6	.000	15.87±.959 a
	Kibwezi		.000	4.89±1.051 b
Moisture	Gachuthi	15.6	.000	8.26±.240 a
(%)	Kibwezi		.000	17.75±.889 b

Source: laboratory analysis (2013)

The soil site data for physical and chemical properties shown in Table 5 reveal significant difference between the two study sites. Sand in Gachuthi was 29.42% compared to 82.89% in Kibwezi samples. Clay in Gachuthi samples was 54.71% compared to Kibwezi soils with 13.56%. Silt in Gachuthi soils was 15.87% whereas in Kibwezi it was 4.89%. Soil moisture content in Gachuthi was 8.26% compared to Kibwezi 17.74%.

The mean value of pH in Gachuthi soils was 6.40 compared to Kibwezi samples 7.41. Electro Conductivity in Gachuthi soils was 0.070 ms/cm compared to Kibwezi at 0.412 ms/cm. Nitrogen in Gachuthi soils samples was 0.37% compared to Kibwezi samples 2%. Phosphorous in Gachuthi samples was 7.23 ppm compared to Kibwezi soils 30.17 ppm. Potassium in Gachuthi samples was 1.74 meq compared to Kibwezi soil which was 3.36 meq.

Gachuthi and Kibwezi soil chemical properties (Table 5), showed significant difference in phosphorous analysis. Soils from Gachuthi and Kibwezi area are classified as nitosols and andosols respectively (Gachene and Kimaru, 2003). Kibwezi being a lowland region has different biophysical environmental factors affecting the soil characteristics. Kibwezi soils were formed from volcanic materials which are still weathering (Gachene and Kimaru, 2003;

FAO, 1984). Through the effect of weathering agents such as plant root penetration, temperature changes and rainfall incidences, soils are developed from geological surface materials. Field observation showed evidence of early primary succession with epiphytic plants attached on rocks such as orchids, lichens, ferns and bryophytes. Soil samples collected under the Osyris trees from Kibwezi were dark in colour, rich in organic matter from fallen litter and high rate of decomposition, low bulk density and very porous owing to high sand content in the area.

Gachuthi area in the highlands has also different biotic and abiotic environmental factors i.e. temperature, rainfall, fauna and flora, with different geological parent material affecting soil characteristics. The area sampled in the forest had deep soil profile (>150cm), and high moisture holding capacity due to high clay content with a stable structure and showed uneven fertility measured variables.

The chemical soil properties data indicates that nitrogen was 0.37% in Gachuthi and 2% in Kibwezi which is high to very high (Okalembo *et al*, 2002). Gachuthi high levels of nitrogen could be attributed to high organic matter in forest whereas in Kibwezi forest it could also be attributed to high organic matter from plants litter which decomposes at a higher rate due to high temperatures and microbial activity by termites in the area. Phosphorous in Gachuthi forest was 7.23 ppm which is low and deficient compared to 30.17 ppm in Kibwezi forest which is high and adequate for plant growth (Okalembo *et al*, 2002). In Gachuthi forest, phosphorous could been limited by the high aluminum levels in nitisols (Gachene and Kimaru, 2003) compared to Kibwezi high phosphorous content which could be attributed to weathering of volcanic rocks spreading in the sampled area. In Gachuthi, potassium was 1.74 meq compared to 3.36 meq in Kibwezi which is adequate for plant growth in both forests. The high levels of potassium in Kibwezi could be attributed to weathering of volcanic rocks which contributes to high potassium in soils (Okalembo *et al*, 2002). Soil pH in Gachuthi forest was 6.40 which is slightly acidic compared to 7.4 in Kibwezi slightly alkaline. This indicates that Sandalwood can grow under varying conditions of soil pH. This conforms to previous findings that Sandalwood can grow in different soil pH ranging from 6.0-7.5 (Maundu and Tengnas, 2005).

4.2.1 Osyris lanceolata occurrence in Gachuthi

The effect of presence and absence of *O. lanceolata* in soil variables within Gachuthi forest was compared using t-test at $p<0.05$.

Table 6: Effect of *O. lanceolata* presence and absence on soil variables

Soil variables	pH	EC(m/cm)	N (%)	P(ppm)	K(meq)	Sand (%)	Clay (%)	Silt (%)	Moisture (%)
Presence	6.23	0.050	0.40	5.11	1.15	21.43	62.36	16.21	8.56
Absence	6.51	0.060	0.38	7.24	2.05	21.71	57.71	14.49	7.20

Source: laboratory analysis (2013)

Presence and absence factor of Osyris in Gachuthi forest is illustrated in Table 6.Values of pH in plots with Osyris and no Osyris was 6.23 and 6.51 respectively, electro conductivity(E.C.) was 0.050 ms/cm and 0.060 ms/cm, nitrogen was 0.40% and 0.38%, phosphorous was 5.11 and 7.24, potassium was 1.15 meq and 2.05 meq, percent sand was 21.43% and 27.71%, clay was 62.36%and 57.71%, silt was 16.21% and 14.49% and percent moisture was 8.56% and 7.20%. Therefore, there were no significant differences ($p<0.05$) shown within Gachuthi forest site owing to presence or absence of *O. lanceolata* among the measured soil variables.

Results obtained from *O. lanceolata* presence and absence factor in the plots sampled at Gachuthi site (Table 6) reveals that the differences among soil variables measured was not significant except for variables like levels of potassium and percent moisture content. These findings show that *O. lanceolata* does not influence biotic and abiotic properties at micro site level. Plots with *Osyris* have low potassium 1.15 meq level compared to 2.05 meq in Osyris absence. This could be attributed to the nutrient uptake from soils by *Osyris* and its surrounding plants associates which is a new finding. The different moisture content levels could be attributed to low percent sand (21.3%) where Osyris was present allowing high water holding capacity compared to 27.71% where Osyris was absent. This is a new finding at micro site level which contrasts with earlier studies that Sandalwood avoids high moisture content (Maundu and Tengnas, 2005).

4.2.2 Horizon A and B

Gachuthi soil sampling effective depth was 0-25 cm (Horizon A) and 25-50 cm (Horizon B). To test if there was any difference between the two soil horizons, a t-test was used to compare them.

Table 7: Chemical and physical properties of A and B soil horizons of Gachuthi forest

Soil Variables	pH	E.C.(m/cm)	N (%)	P(ppm)	K(meq)	Sand (%)	Clay (%)	Silt (%)	Moisture (%)
Hor. A	6.45	-	-	7.23	1.74	29.42	54.71	15.87	8.23
Hor. B	6.45	-	-	5.32	1.55	20.32	64.90	14.77	7.48

Source: laboratory analysis (2013)

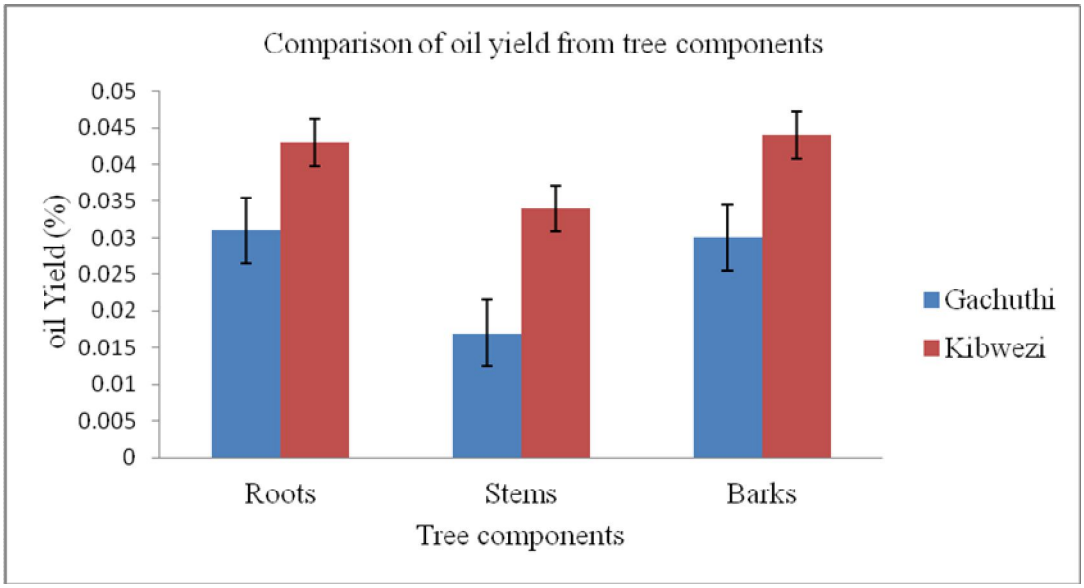
Chemical and physical properties of A and B horizons in Gachuthi forest are shown in Table 7. Both pH levels in A and B were 6.45, E.C. and %N could not be computed because the standard deviations of both groups were 0, phosphorous was 7.23ppm and 5.32ppm in A and B respectively, potassium was 1.74 meq in A and 1.55 meq in B, percent sand was 29.42% in A and 20.32% in B, Clay was 54.71% in A and 64.90% in B, Silt was 15.87% in A and 14.77% in B, percent moisture was 8.23% in A and 7.48% in B. There was no significant difference ($P<0.05$) between means of horizon A and B in all soil variables (Table 7).

4.3 Oil Yield Content in lowlands and highlands

Oil extracted from *O. lanceolata* roots, stems and barks were used in this study to determine both quantitative and qualitative oil yields of the different tree parts. Three parts, the roots, stem and the bark were used for oil extraction to determine the most appropriate part to obtain oil and therefore avoid unsustainable destructive harvesting of whole plant through uprooting. Analysis of variance (ANOVA) was used to compare means from Gachuthi and Kibwezi at 95% confidence level ($p<0.05$).

4.3.1 Oil Yield from Root, Stem and Bark in Kibwezi and Gachuthi Forest

The mean oil yield from roots stems and barks from both sites is shown in Figure 10. Samples collected from Gachuthi forest had lower oil yield in the stem (0.017%) compared to that of the root (0.031%) and bark (0.030%). Similar pattern was observed in Kibwezi samples where the root and bark had 0.043% and 0.044% respectively. Gachuthi stem had 0.017% compared to Kibwezi stem 0.034%. There was no significant difference in oil yield from the roots and barks of both forests. The stems had a significant lower oil yield compared to root and bark. An extra-ordinary higher score of 2.26% oil yield was observed in one of the root samples from Kibwezi which could have been attributed to the age of samples. This sample was not included in the data analysis as it was considered an outlier.



Source: laboratory analysis (2013)

Figure 10: Comparison of oil yield from roots, stems and barks

These findings are consistent with previous studies that most of the root portion after a certain age is heartwood and yields the highest amount of oil, and is not evenly distributed within different sections of a tree particularly the stem (Sindhu *et al.*, 2010). In India, research on *Santalum album* concluded that oil in the stem tissues can range from nil to 90 % (Sajan, 2012). Currently, there is no sufficient research done about the oil yield from the bark

in Kenya, but studies done in India on mature trees of 20 years found that the bark had 4 - 10% yield when distilled (Sajan, 2012). This is contrary to the current study because the barks from Kibwezi and Gachuthi had a mean oil yield of 0.045% and 0.030% respectively.

The observed difference could be due to several factors including environmental, genetic, tree age and the method of oil extraction used. In Kibwezi, most of the mature trees ranging from 15-45 years had been smuggled before conservation measures were put in place.

Plate 3. Mature and illegally harvested sandalwood outside Kibwezi KWS office

There are no sufficient experimental studies that have been undertaken to ascertain the appropriate harvesting age of the East African sandalwood. However, data collected from illegally harvested specimens showed age ranging from 15 to 45 years (Mukonyi *et al*., 2011).

4.3.2 Comparison of oil yield and moisture content in Osyris samples

Table 8: Mean Oil Yield and Moisture Content

Variable	Site	F	P	Mean
% Oil Yield	Kibwezi	0.241	.023	0.040±0.005 a
	Gachuthi		.025	0.026±0.004 b
% Moisture	Kibwezi	3.637	.000	2.85±0.17 a
Content	Gachuthi		.000	1.99±0.08 b

Source: laboratory analysis (2013)

Comparison of mean oil yield and moisture content from Kibwezi and Gachuthi forest (Table 8) shows the mean oil yield from Kibwezi forest was 0.040% compared to Gachuthi at 0.026%. Moisture content from Gachuthi Osyris samples was 1.99% while those of Kibwezi were 2.85%. There is a significant difference between the two study areas. The variation in oil yield from the two study areas could be attributed to the significant difference in soil physical and chemical properties in both study areas. Kibwezi study area has sandy soils which are shallow and rocky contributing to the high levels of phosphorus and potassium soil nutrients; however, it had the highest oil yield which conforms to previous studies that Osyris trees grown in such soils produce more and better oil (Mukonyi *et al*., 2011). In addition, stressful conditions such as sandy soils, high temperatures and high rate of evapotranspiration, low rainfall and altitude enhance heartwood formation which is the most valuable part in oil production (Subasighe, 2013).

On the other hand, Gachuthi forest has soils with high clay percent which are deep and fertile (FAO, 1984). On the contrary, nutrient rich soils with high moisture do not support heartwood development and Osyris trees grown in such soil yield low oil (Mukonyi *et al*., 2011). From observation, there is a significant difference between heartwoods from Osyris tree samples harvested from the two study areas. This concurs with similar studies that there is a strong relationship between heartwood development with the soil type, rainfall and the level of sun exposure on the canopy of sandalwood trees (Radomiljac *et al*., 1998). Observations made in the field revealed that *O. lanceolata* found in Gachuthi were multi-stemmed from the root collar unlike those of Kibwezi forest where trees started branching above 30 cm. Variation in oil yield from Gachuthi and Kibwezi Osyris tree samples could also be attributed to genetic differences of the two populations, this conforms to similar studies in Tanzania (Mwang'ingo *et al*, 2010).

There was a significant difference in moisture content between *O. lanceolata* harvested from Kibwezi and Gachuthi forest. The high moisture content in *O. lanceolata* from Kibwezi forest could be attributed to the exposure of abiotic stresses that cause imbalances in natural status of the environment. Therefore, plants develop natural adaptations to stress conditions with a selection of biochemical and physiological interventions that involves the function of many stress associated genes that help in retention of moisture within plants (Bernacchia and Furini, 2004). For example, plant species in Kibwezi forest are exposed to environmental stress such as draught, extreme temperatures and light conditions i.e. high light or shading.

As a result closure of stomata leads into reduction of transpiration and photosynthesis through decreased leaf expansion in plant species hence retention of moisture (Githae *et al*., 2011).

4.4. Oil Quality

Sandalwood species differ in their chemical composition and quality of oil. Widespread uses of different parts of Osyris in traditional medication system have resulted in considerable chemical analysis of the plant and their active ingredients. The phytochemical investigation of genus Osyris has resulted to 108 compounds with varying chemical structural patterns (Sajan, 2012, Hettiarachichi *et al*, 2010). Phytochemical composition of genus Osyris include volatile constituents that are important in essential oils and are known as sesquiterpenes. The quality of Sandalwood oil is determined by the level of Alpha (α) and Beta (β) santalol assayed in Sandalwood extracts which is compared to the international oil quality standards with reference to α and β santalol levels, (Howes *et al*., 2004).

Gas chromatographic analysis of Osyris extracts was performed using hexane volatile method at 35^{o}c-280^{o}c for 50 minutes. Helium gas was used as a carrier gas and GC-MS facilitated in identification of different compounds in the oil extracts at different time detection (Sajan, 2012). Chromatograms from GC-MS analysis showing presence of α and β santalol in Gachuthi and Kibwezi roots, stems and barks oil are shown in figures 11a,11b,11c,11d,11e and 11f. Areas were recorded for all detectable peaks, and percent concentration was calculated by taking area of peak divided by total chromatogram area ·multiplied by100.

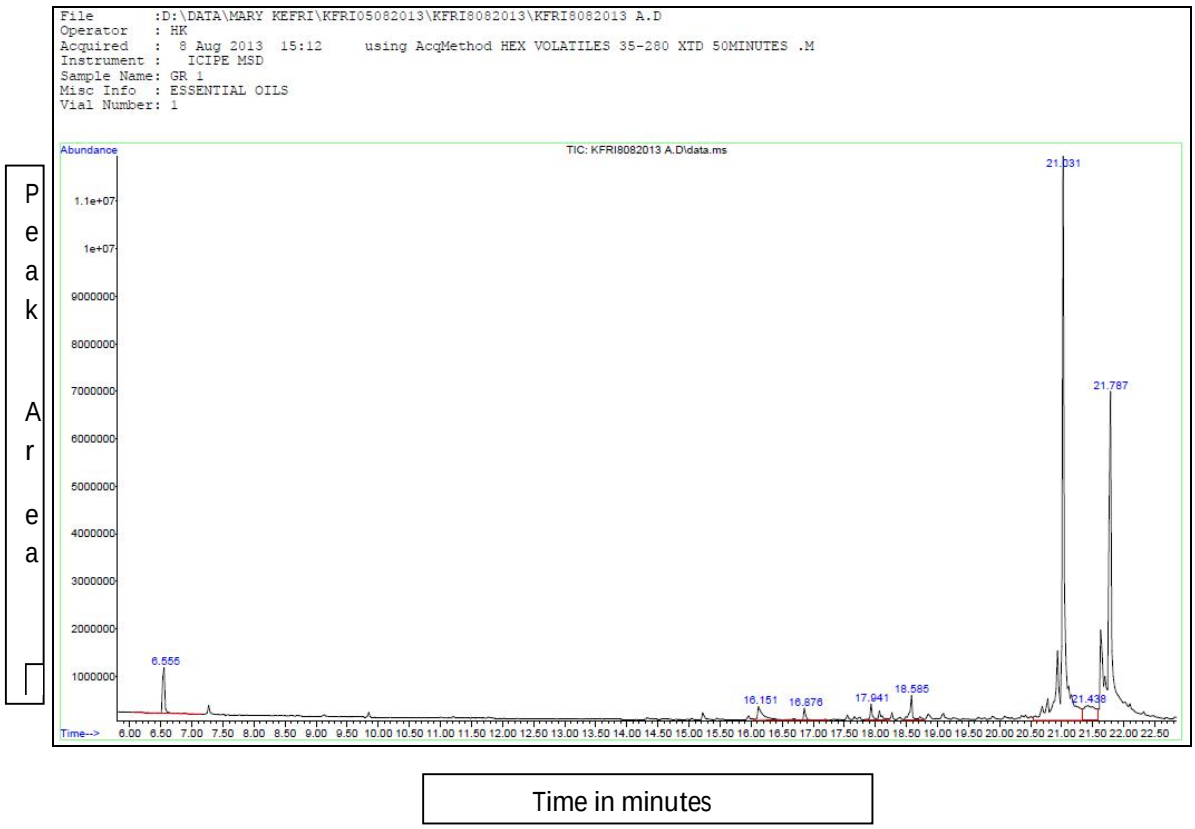


Figure 11a: Gachuthi root

Gachuthi root oil extract in figure 11a showing β santalol peak detection between 20 – 22 minutes with an area of 0.3086% but α santalol peak was not detected in this chromatograph.

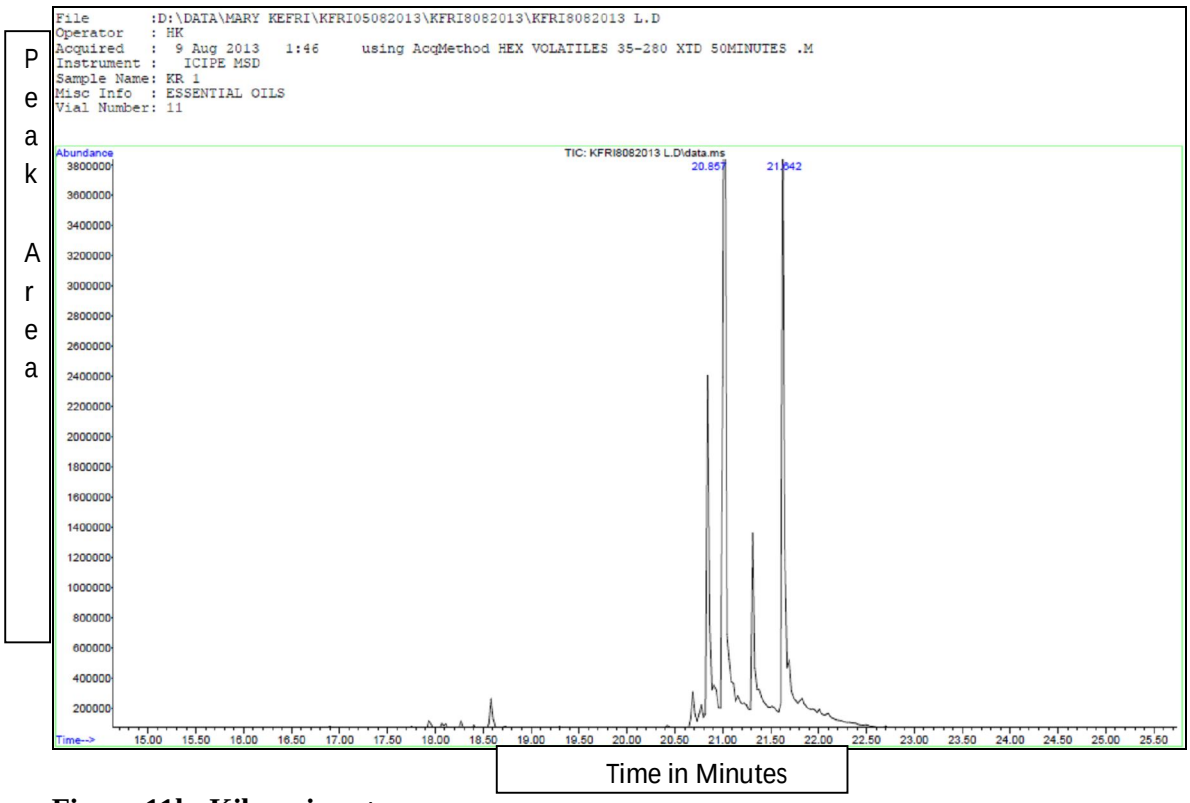


Figure 11b: Kibwezi root

Kibwezi root oil extract in figure 11b showing α and β peaks detection between 20 – 22 minutes with a peak area of 10.8037% and 8.6786%, respectively.

```
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Acquired   :  8 Aug 2013  16:12     using AcqMethod HEX VOLATILES 35-280 XTD 50MINUTES .M
Instrument :   ICIPE MSD
Sample Name: GS 1
Misc Info  : ESSENTIAL OILS
Vial Number: 2
```

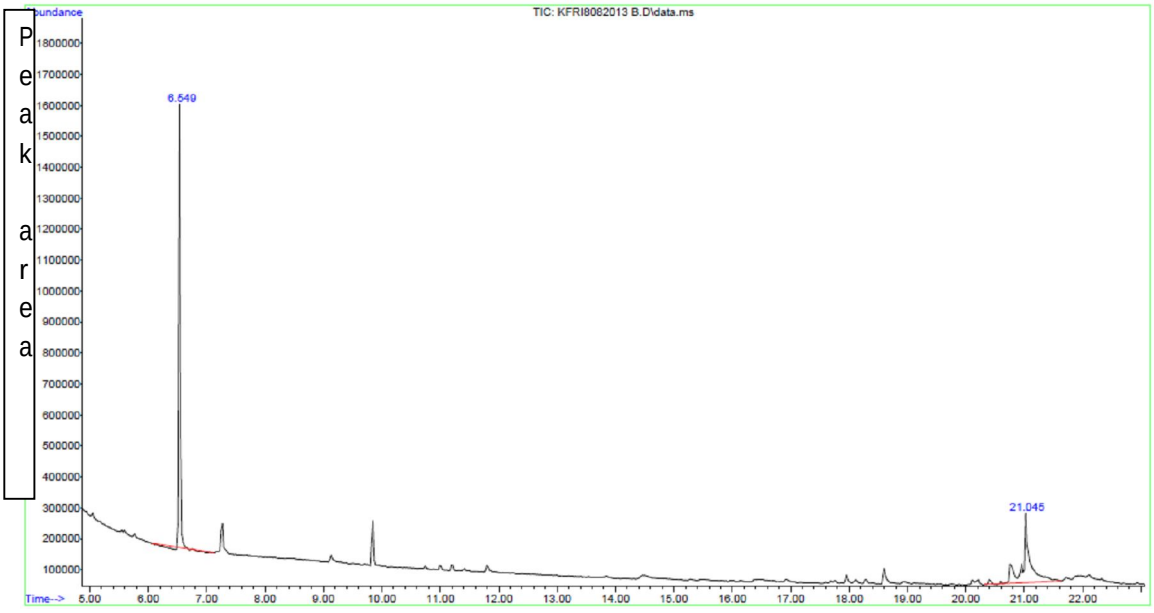

Peak area

Time in minutes

Figure 11c: Gachuthi stem

Gachuthi stem oil extract in figure 11c showing α and β peak detection between 20 – 22 minutes with peak area of 3.0927% and 0.4610%, respectively.

```
File       :D:\DATA\MARY KEFRI\KFRI05082013\KFRI8082013\KFRI8082013 S.D
Operator   : HK
Acquired   :  9 Aug 2013   7:45     using AcqMethod HEX VOLATILES 35-280 XTD 50MINUTES .M
Instrument :   ICIPE MSD
Sample Name: KS 3
Misc Info  : ESSENTIAL OILS
Vial Number: 17
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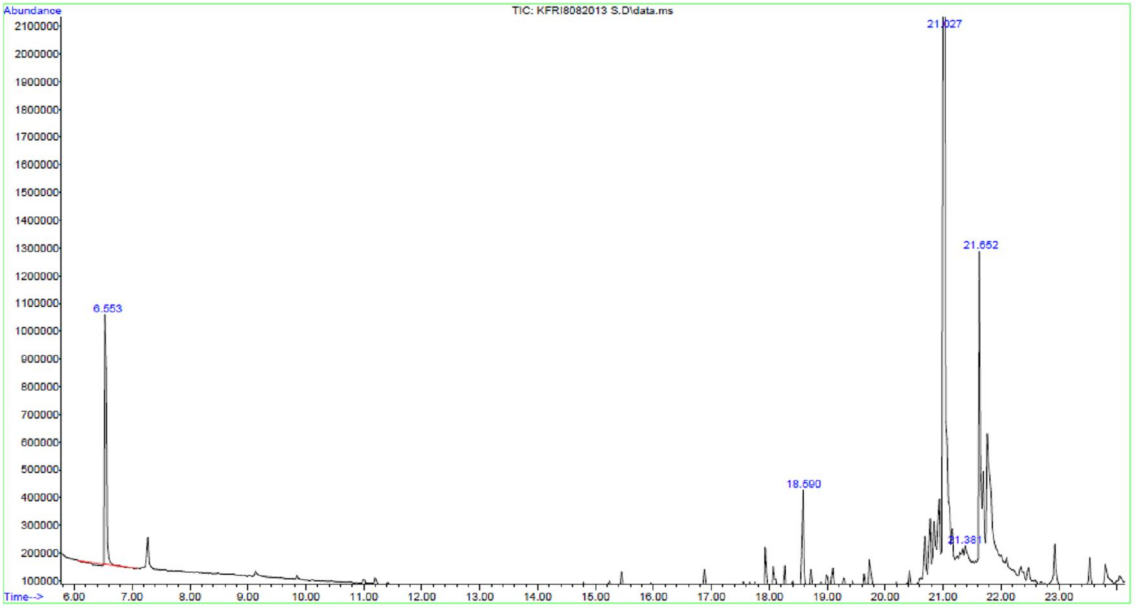

Peak area

Time in minutes

Figure 11d: Kibwezi stem

Kibwezi stem oil extract in figure 11d showing α and β peaks detection between 20 – 22 minutes with peak area of 4.6140% and 2.1519%, respectively.

```
File      :D:\DATA\MARY KEFRI\KFRI05082013\KFRI8082013\KFRI8082013 G.D
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Instrument :   ICIPE MSD
Sample Name: GB 3
Misc Info  : ESSENTIAL OILS
Vial Number: 7
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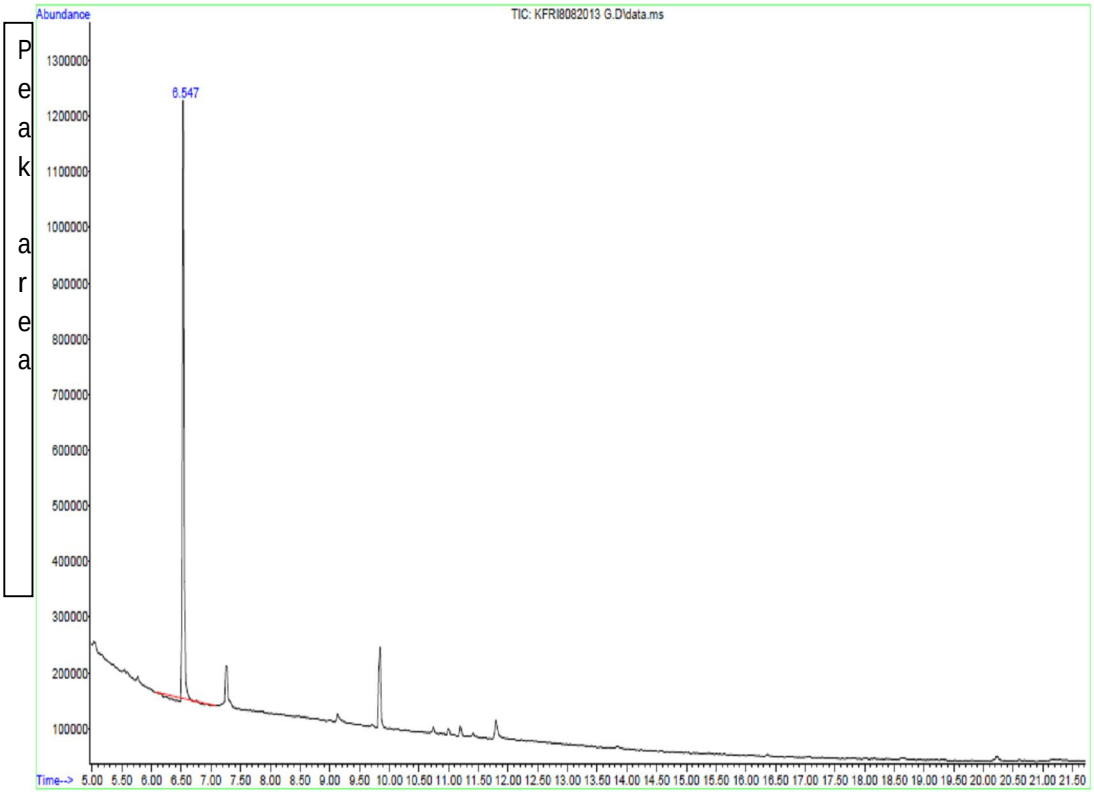


Figure 11e: Gachuthi bark

Gachuthi bark oil extract in figure 11e showing α peak detection between 20 – 22 minutes with an area of 0.0620% but β peak was not detected.

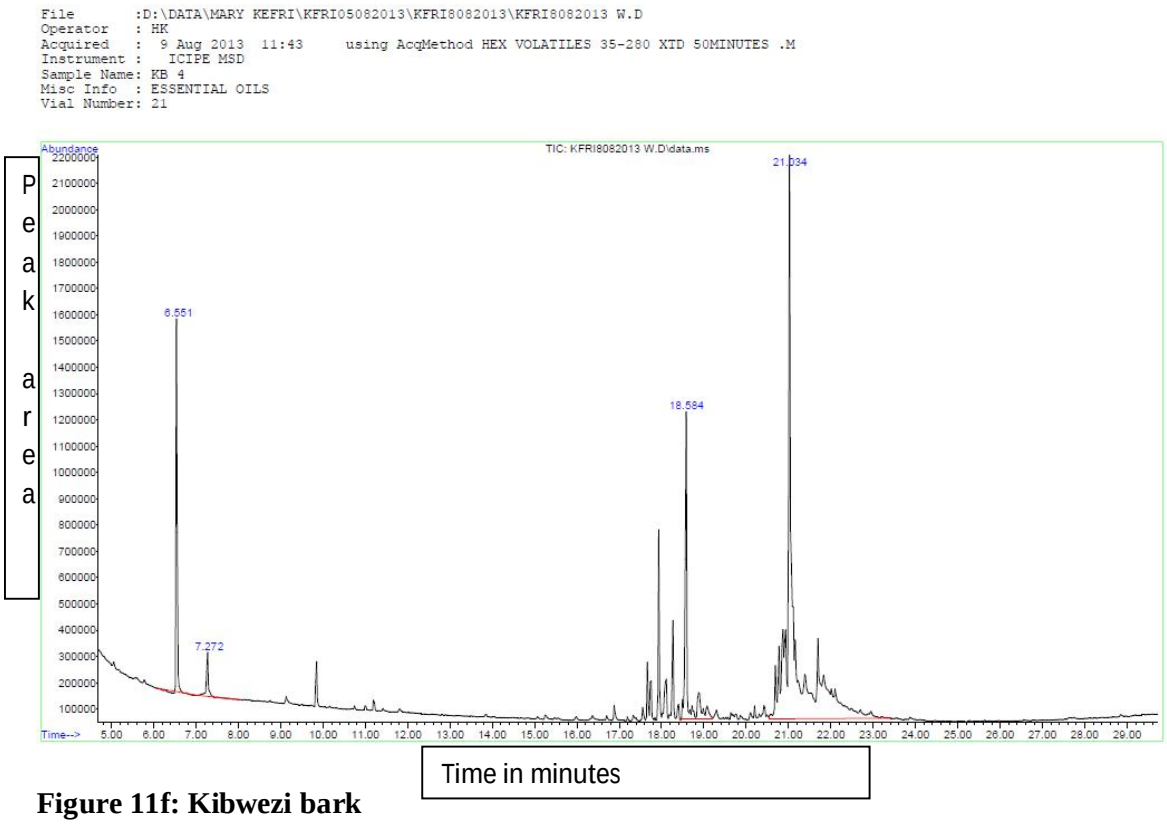


Figure 11f: Kibwezi bark

Kibwezi bark oil extract in figure 11f showing α and β peaks detection between 18 – 22 minutes with an area of 14.2435% and 6.1349%, respectively.

Table 9a: Laboratory analysis of oil extracts from Gachuthi and Kibwezi forest

	Name of Compound	Gachuthi			Kibwezi		
		Root	Stem	Bark	Root	Stem	Bark
1	(E)-cis-epi-b- Santalol	√	×	×	√	×	×
2	(Z)-a-Farnesene	√	√	×	√	×	×
3	(Z)-cis-a- Santalol	×	×	×	√	×	×
4	Alpha-Caryophyllene	×	×	×	×	×	√
5	Alpha-Farnesene	√	×	×	×	×	×
6	Alpha-Santalol	√	×	×	√	×	×
7	Xi-Lanosta	×	×	×	×	√	×
8	1S-alpha-Pinene	×	×	×	√	√	√
9	Bergamotene<alpha-cis->	√	√	√	√	√	√
10	Bergamotol	√	√	×	√	√	√
11	Bergamotol<(Z)-alpha-trans->	√	√	√	√	√	√
12	Bisabolene<(Z)-alpha->	√	×	×	√	√	×
13	Bisabolene<beta->	√	×	×	×	×	×
14	Carene<delta-2->	×	×	√	√	√	√
15	Carene<delta-3->	√	√	√	√	×	×
16	Carvacrol	×	√	√	√	√	√
17	Caryophyllene	×	×	×	×	√	×

18	Cedrene<alpha->	√	×	×	×	×	×
19	Cedrol	√	×	×	×	×	×
20	Cedrol<epi->	×	×	×	×	√	√
21	Cineole<1,8->	×	√	√	×	×	×
22	Cis- Lanceol	√	×	×	×	√	√
23	Cis-alpha-Bisabolene	×	×	×	×	×	√
24	Curcumene<beta->	√	×	×	√	√	√
25	Curcumene<gamma->	√	×	√	√	√	√
26	Farnesene<(E)-beta->	×	√	×	√	√	√
27	Farnesene<(Z)-beta->	×	√	×	×	√	×
28	GermacreneB	×	×	×	√	√	√
29	Italicene	√	×	×	√	×	√
30	Italicene<iso->	×	×	×	√	×	×
31	Lanceol<Z->	√	×	×	√	×	√
32	Limonene	×	×	×	√	√	√
33	Ocimene	×	×	√	×	×	×
34	Ocimene<(E)-beta->	×	×	×	√	×	√
35	Pinene	×	×	×	×	√	×
36	Pinene<alpha->	√	√	√	√	√	√
37	Pinene<beta->	√	√	√	√	√	×
38	Santalene<alpha->	√	√	√	√	√	√
39	Santalene<beta->	√	√	√	√	√	√
40	Santalene<epi-beta->	√	×	×	√	√	√
41	Santalol	×	×	√	×	×	×
42	Santalol<(E)-beta->	√	√	×	√	√	√
43	Santalol<(Z)-alpha->	×	√	√	√	√	√
44	Santalol<(Z)-beta->	√	√	×	×	×	×
45	Santalone	×	×	×	√	×	×
46	Santolina Triene	√	×	√	√	√	√
47	Sesquiphellandrene<beta->	√	×	√	√	×	×
48	Sesquisabinene	×	×	√	×	×	×
49	Sesquithujene	√	√	×	√	×	×
50	Sesquithujene<7-epi->	√	×	×	√	√	√
51	Sinensal<beta->	√	√	×	×	√	√
52	Trans-alpha-Bergamotene	√	×	×	√	√	√
53	Trans-a-Bergamotene	×	×	×	×	√	√
54	Trans-b- Ocimene	×	×	√	×	×	×
55	Zingiberene<alpha->	×	√	×	√	×	√
	Total	30	19	17	35	30	30

Source: laboratory analysis (2013)

Compound presence (√) or absence (x) in *O. lanceolata* tree components

A total of 55 compounds were identified in Kibwezi and Gachuthi oil extracts (Table 9a). In Gachuthi oil extracts, 30 compounds were detected in the roots, 19 in the stem and 17 in

the bark whereas in Kibwezi 35 were in roots, 30 in the stem and 30 in the bark. ANOVA was used to analyse data at ($P<0.05$). The concentration for the different constituents was expressed as peak per area (Mwang'ingo *et al*., 2003). The analysis showed only 7 compounds with an occurrence of 12 times out of 24 in oil extract GC-MS analyses in root, stem and bark of both forests (Table 11). These compounds were Bergamotene<alpha-cis>, Bergamotene<(Z)alpha-trans>, Farnesene<(E)-beta>, Pinene<alpha>, Santalene<alpha>, Santalene<beta> and Santalol(Z)alpha shown in Table 9b.

Table 9b: Compounds mean percent Concentration in Root, Stem and Bark found in both forests

		Mean % Concentration of Compounds						
Site	Tree Component	Bergamotene <alpha-cis>	Bergamotene <(Z)alpha-trans>	Farnesene <(E)-beta>	Pinene< alpha>	Santalene <alpha>	Santalene <beta>	Santalol (Z)alpha
Kibwezi	Root	2.31	34.76	1.18	0.64	0.78	5.05	17.79
Gachuthi	Root	0.64	20.64	1.91	2.23	0.32	0.50	Trace
Kibwezi	Stem	0.41	18.06	0.25	0.13	0.06	0.40	4.03
Gachuthi	Stem	2.63	5.11	Trace	3.67	0.32	0.51	0.77
Kibwezi	Bark	2.63	16.44	6.66	0.04	1.46	3.07	5.77
Gachuthi	Bark	1.24	1.18	0.64	5.38	0.55	0.52	Trace

Source: laboratory analysis (2013)

4.4.1.1 Bergamotene<alpha-cis> and Bergamotene<(Z)alpha-trans>

Bergamotene<alpha-cis> appeared in root, stem and bark of the trees sampled in Gachuthi and Kibwezi. Kibwezi bark and Gachuthi stem had the highest concentration (2.63%). The lowest concentration was found in Kibwezi stem (0.41%) and Gachuthi roots (0.64%). There is no significant difference of this compound among all tree components analysed from both forests.

Bergamotene<(Z)-alpha-trans> appeared in root, stem and bark of all tree components sampled in both study areas. The highest concentration (34.76%) was found in Kibwezi roots followed by Gachuthi roots with 20.64%. The lowest concentration was found in Gachuthi stem (5.11%) and bark which was (1.18%). There was significant difference in Gachuthi stem and bark compared to Kibwezi stem and bark.

4.4.1.2 Farnesene<(E)-beta>

Farnesene<(E)-beta> had the highest concentration (6.66%) found in Kibwezi bark followed by Kibwezi root 1.91%. The compound was absent in Gachuthi stem but the root and bark had very low mean concentration (1.17%) and 0.64% respectively.

4.4.1.3 Pinene<alpha>

The compound was present in all tree parts but Gachuthi samples had highest concentration. Gachuthi roots, stems and bark had concentrations of 2.23%, 3.67% and 5.38%, respectively. Kibwezi roots, stem and bark had concentrations of 0.64%, 0.13% and 0.04%. There was significant difference in Pinene<alpha> concentration between Gachuthi and Kibwezi forests.

4.4.1.4 Santalene<alpha> and Santalene<beta>

Santalene<alpha> was present in root, stem and bark samples of both forests with concentration of 0.32% root and stem, 0.55% in bark of Gachuthi. Kibwezi had 0.78% in root, 0.06% in stem and 1.46% in bark. There was no significant difference of both forests.

Santalene<beta> was present in all tree parts from both study forests. Kibwezi roots had the highest concentration of 5.05% followed by bark with 3.07%. Lowest concentration was

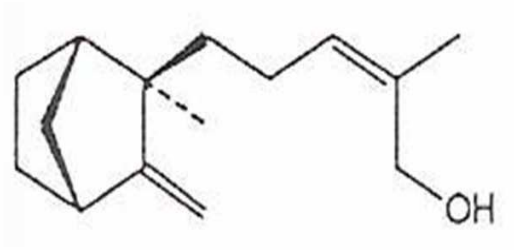
found in Gachuthi which was 0.50% in root and stem, and 0.52% in bark. There is no significant difference between samples collected from Gachuthi and Kibwezi.

4.4.1.5 Santalol(Z)alpha

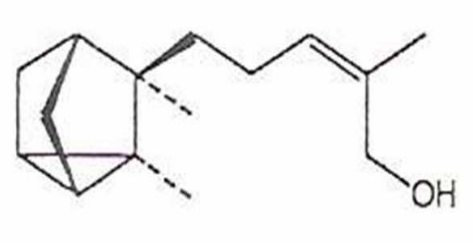
Santalol(Z)alpha was present in Gachuthi stem only with a concentration of 0.77% but it was present in all sample components from Kibwezi samples. Kibwezi roots, stems and bark had a concentration of 17.79%, 4.03 and 5.77%, respectively. There was a significant difference in concentration of Santalol (Z)alpha in samples from both study areas.

4.4.2 Importance of Sesquiterpenes in Sandalwood oil

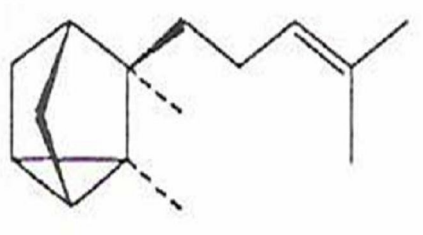
The compounds found in Sandalwood oil play an important role in prevention of pathogens in both plants and animals. These compounds known as sesquiterpenoids have been associated with plant pathogen defense strategies (Hammerschmidt *et al*, 2006). Sesquiterpenoids are defined as the group of 15 carbon compounds derived by the assembly of 3 isoprenoid units and they are found mainly in essential oils from plants. Isoprenoid are a class of organic compounds composed of two or more units of hydrocarbons, with each unit consisting of five carbon atoms arranged in a specific pattern. Isoprenoids play wide and varying roles in the physiological processes of plants and animals. They also have a number of commercial uses particularly to augment the odors of perfumes. Sesquiterpenoids are also active against a variety of *Candida albicans* which is a dimorphic fungus that grows both as yeast and filamentous cells and are a causal agent of opportunistic oral and genital infections in humans. Sesquiterpenes with monoterpenes are the most diverse group of isoprenoids and in plants; they function as pheromones and juvenile hormones (Hammerschmidt *et al*, 2006). Sesquiterpene structures present several acyclic, mono-, bi-, tri-, and tetracyclic systems. Some of natural sesquiterpenoids are shown in figure 12;



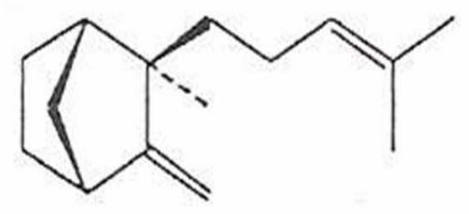
α- santalol



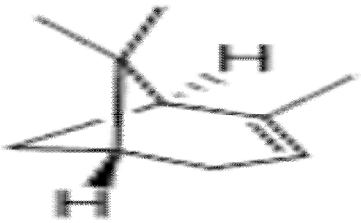
β-santalol



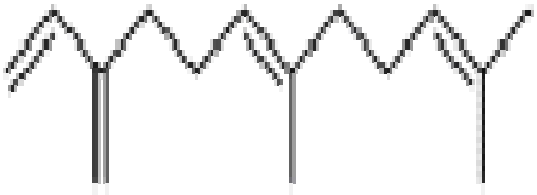
α- santalene



β-santalene



α – pinene



Farnese (E) beta

Source: Gas Chromatographic- Mass Spectra, Agilent Technology 7890 A (2013)

Figure 12: Sesquiterpenes

The warm, sweet, woody fragrance of Sandalwood essential oil is a precious tool in perfumery. The main component of alcoholic part in Sandalwood essential oil is santalol which is usually described as α-santalol and β-santalol. These small molecules possess antibacterial and sedative properties resulting in the importance of Sandalwood oil.

In this study, α-santalol and β-santalol constituents were both present in the extracted oil from *O. lanceolata* tree (Table 10.) In Gachuthi samples, β-santalol occurred with a frequency of 1 in the root and stem with a concentration of 0.31% and 0.47% respectively compared to Kibwezi with a frequency of 2 in root, 1 in stem and bark with a higher concentration of 10.11%, 2.15% and 6.13%, respectively.

α- Santalol occurred with a frequency of 1 in the stem and bark of Gachuthi samples with a concentration of 3.09% and 0.06% respectively compared to Kibwezi with a frequency of 4 in the root, stem and bark and a higher concentration of 35.21%, 5.82% and 14.24%, respectively.

Table 10: β-santalol and α-santalol range and frequency in Gachuthi and Kibwezi oil extracts

Site	Plant part	β-santalol % concentration	Frequency	α-santalol % concentration	Frequency
Gachuthi	Root	0-0.31	1	0	0
Gachuthi	Stem	0-0.47	1	0-3.09	1
Gachuthi	Bark	0	0	0-0.06	1
Kibwezi	Root	0-10.11	2	10.80-35.21	4
Kibwezi	Stem	0-2.15	1	0.01- 5.82	4
Kibwezi	Bark	0-6.13	1	2.30-14.24	4

Source: laboratory data (2013)

A comparison study on *Santalum album* (Indian Sandalwood) and *Osyris lanceolata* (East African Sandalwood from Tanzania) to establish an alternative to replace diminishing resources of Indian Sandalwood showed that α- santalol in *S. album* ranged from 46.05 to 57.06% compared to *O. lanceolata* with a range of 20.28 to 25.06%. The range of β- santalol was 28.14 to 38.84% in *S. album* and 20.27-21.44% in *O. lanceolata*, (Bhat *et al.*, 2004).

In the current study, the Kibwezi *O. lanceolata* root, stem and bark had a concentration of α-santalol 35.21%, 5.82% and 14.24%, respectively and β- santalol concentration was 10.11%, 2.15% and 6.13%, respectively. The difference between Kibwezi and East African Sandalwood from Tanzania could possibly be attributed to several factors such as different environmental conditions, genetic and tree age.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study showed low distribution of Osyris trees in Gachuthi situated in the highlands with a low density compared to Kibwezi situated in low dry part of Kenya. Out of 24 established plots in each of the study site, only 7 plots in Gachuthi had Osyris compared to Kibwezi forest which had 18 plots with Osyris. Most of the herbs and grass species in Gachuthi forest, only *Chloris sp* occurred in Osyris plots compared to Kibwezi with 6 species namely *Asparagus racemosus, Barlelia acanthoides, Cissus quadrangularis, Cynodon dactylon, Hypoestes* forskahlii and *Solanum incanum*. Multi-stem shrub trees in Osyris plots in Gachuthi were *Clutia abyssinica, Dombeya burgessiae* and *Grewia similis* compared to Kibwezi with *Acacia brevispica, Commiphora eminii, Croton dichogamus, Euphorbia schffleri, Grewia similis, Hibiscus diversifolius* and *Maytenus undata*. The only common multi-stem shrub tree species in both sites was *Grewia similis*. Trees found in Osyris plots in Gachuthi were *Fagaropsis angolensis* and *Waburgia ugandensis* compared to Kibwezi with *Acacia robusta, Antidesma venosum, Balanites maughamii, Commiphora baluensis, Commiphora spp, Croton megalocarpus, Cussonia hostii, Euclea divinorum, Euphorbia candelabrium, Haplocoelum foliolosum, Heteromorpha trifoliate, Hymenodictyon parvifolium, Mystrxylon aethiopicum, Ochna ovate, Steganoteenia oraliacea, Teclea simplicifolia* and *Turraea abyssinica*.

From the analysis of ecological data it was clear that Osyris trees in Gachuthi were positively related to moist clay soils when compared to those of Kibwezi where soils with high potassium levels were preferred. In this study there were different Osyris indicator species, with only *Rhus natalensis* as a common species in both study areas. Evaluations done on Osyris indicator species in this study showed that they had a relationship with the distribution and density of Sandalwood within Kibwezi forest. It was apparent from this study that different abiotic factors of an ecosystem results into different biotic composition of an area. The reason is that Gachuthi vegetation composition is diverse with high density when compared to Kibwezi which is mainly dominated by Acacia commiphora woodlands of varying density.

Sandalwood can grow in wide range of soil pH levels from 6.0 to 7.5, and different soil types such as nitisols, acrisols and volcanic soils. In Kibwezi, most probably heartwood development was triggered by stressful conditions such as shallow soils, high temperatures, high evaporation rate and low amount of rainfall. Gachuthi forest is situated in highland area receiving high rainfall in fertile soils and lower temperature compared to Kibwezi forest. There is a possibility that the environment difference of the two study areas could have an effect on heartwood development, oil yield and quality. High potassium levels in Kibwezi and moist clay soil in Gachuthi played an important role in biochemical constituents of oil in both study areas. This study showed that there was an effect of soil chemical and physical properties on quantity and quality of Sandalwood oil from trees growing in different ecological zones.

Oil yield from different tree parts of *O. lanceolata* did not vary significantly though the roots contained the highest yields compared to stem and the bark. This therefore indicates that uprooting of Osyris trees to obtain roots could be necessary. Stems of *Osyris* trees had the lowest yield compared to roots and bark. This study showed that there is need to domesticate and establish Sandalwood plantations for sustainable utilization in order to meet the rising demand of Osyris trees. This study demonstrated that the best area for Sandalwood plantation establishment could possibly be in arid environments with biophysical factors that favour heartwood development. *Osyris lanceolata* tree is under protection of CITES Appendix-II listings to discourage its poaching and trading across the borders of these countries. Therefore, conservation of existing natural stands and regeneration in the natural habitats will most likely contribute to attaining of mature age of Sandalwood trees. In this study, observation made on Osyris tree samples from the two study sites showed a difference in heartwood development which could have been a result of age difference.

Kibwezi tree samples had the highest moisture content compared to Gachuthi samples. It was clear from this study that Osyris trees in arid zones most likely develop natural adaptation to stress conditions with an arrangement of biochemical and physiological interventions that involves function of many stress associated genes hence resulting into higher oil yield of high quality.

The most common sesquiterpenes with an occurrence of 50% in both study areas were Bergamotene<alpha-cis>, Bergamotene<(Z)alpha-trans>, Farnesene<(E)-beta>, Pinene<alpha>, Santalene<alpha>, Santalene<beta> and Santalol(Z)alpha. This study

revealed that Bergamotene<(Z)alpha-trans> had the highest concentration in the roots among all the tree samples analysed in Gachuthi and Kibwezi natural populations. Oil quality depends on the level of constituents in the oil with more emphasize to α and β santalol amount. This study showed that there was significant difference in concentration of sesquiterpenes found in Gachuthi and Kibwezi oil. Gachuthi oil had low levels of α and β santalol compared to Kibwezi samples with high levels though in varied amounts in roots, stem and bark.

5.2 Recommendations

i) The current study objective on evaluation of indicator species demonstrated that there is a strong relationship in occurrence of *O. lanceolata* and *R. natalensis* as indicator tree species in both highlands and lowlands natural forests. Therefore, *O. lanceolata* site suitability for domestication can be predicted using *R. natalensis*.

ii) This study showed that soils in both highlands and lowlands natural forests had significant difference in physical and chemical properties measured. Due to the limited number of sites used in the current study, there is need for more studies on relationship between soil variables and *O. lanceolata* occurrence in natural ecosystems.

iii) This study found out that Kibwezi Sandalwood had well developed heartwood, which resulted to high oil yield compared to Gachuthi low yield. This study recommends harsh environmental conditions for establishment of Sandalwood plantations to achieve well developed heartwood, high yield and quality oil.

iv) This study showed no significant differences in oil production among stem, bark and roots. Therefore, uprooting the whole *O. lanceolata* trees to obtain roots for oil extraction is not necessary.

v) This study showed a significant difference in oil quality from extracted oil from both Gachuthi and Kibwezi natural forests which could be attributed to difference in edaphic factors. Therefore, domestication of *O. lanceolata* could be most suited in dry environments.

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APPENDICES

Appendix i

Vascular Plant Species

BIOPHYSICAL ENVIRONMENTAL FIELD DATA FOR *Osyris lanceolata* USING NESTED INTENSITY PLOTS SHEET A

Name of the site:
Approximate Distance from nearest Landmark/ Road (m) %slope
GPS coordinates: Altitude Southings Eastings

Main Plot (5x20) m; Stratum No; For recording all vascular Plant Species

Tree No.	Tree species	Local Name	Height	D (0.3) m	DBH	Stem form (Score 1-4)	Canopy Cover	Remarks
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								

Stem Straightness: 1=Straight, 2=Slightly Crooked, 3=Crooked and 4= Highly` Crooked

Assessment done by: Date: Signature:

Checked by: Date: Signature:

Appendix ii

Osyris lanceolata trees

BIOPHYSICAL ENVIRONMENTAL FIELD DATA FOR *Osyris lanceolata* USING NESTED INTENSITY PLOTS SHEET A-1

Name of the site:

Approximate Distance from nearest Landmark/ Road (m) %slope

GPS coordinates: Altitude Southings Eastings

Sub-plot (2m by 5 m): Stratum No; **for Recording *Osyris lanceolata* only**							
Tree No.	**Height**	**Collar Diameter**	**D (0.3)m**	**DBH**	**No. of Stems**	**Canopy cover**	**Remarks**
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Assessment done by: Date: Signature:

Checked by: Date: Signature:

Appendix iii

Seedlings, herbs and Grasses

BIOPHYSICAL ENVIRONMENTAL FIELD DATA FOR *Osyris lanceolata* USING NESTED INTENSITY PLOTS SHEET A-2

Name of the site:

Approximate Distance from nearest Landmark/ Road (m) **%slope**

GPS coordinates: Altitude **Southings** **Eastings**

Sub-plot (0.5by 2m) Stratum No.: is for assessing seedlings, herbs and Grasses.								
			% Cover (Only Herbs and Grasses					
No.	Species	Local Name	Plot 1	Plot 2	Plot 3	Plot 4	Mean	Remarks
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								

Assessment done by: Date: Signature:

Checked by: Date: Signature

Appendix iv

Soil Sampling

BIOPHYSICAL ENVIRONMENTAL FIELD DATA FOR *Osyris lanceolata* USING NESTED INTENSITY PLOTS SHEET A-3

Name of the site:

Approximate Distance from nearest Landmark/ Road (m) **%slope**

GPS coordinates: Altitude **Southings** **Eastings**

Stratum No.:		Remarks
Date		
Location		
Topography		
Land use		
Human Influence		
Vegetation		
Drainage		
Soil Classification		
Soil Texture		
Field Soil pH		
Soil sample reference	Depth (cm)	
Main Plot (5x20m)	0 – 25	
	25 – 50	
Sub plot (2x5m) selected Osyris tree	0 – 25	
	25 -50	

Assessment done by: Date: Signature:

Checked by: Date: Signature

Table 12: Relative frequency (RF) of Osyris and other species in Gachuthi forest

Plot No	Ehretia	Acacimea	Zanthox	pittospo	Nuxiaco	Rhus	Cassipo	Tecleasi	Schrebe	Euclead	Osyris	Olea
1	0	0	0	1	0	0	0	0	0	0	0	0
2	1	1	1	0	0	0	0	0	0	0	1	0
3	0	0	0	1	1	1	1	1	0	0	0	0
4	0	0	1	0	0	0	0	0	1	1	1	0
5	0	0	0	0	0	1	1	0	1	1	0	1
6	0	0	1	1	0	0	0	1	1	1	0	0
7	1	0	1	0	1	0	1	1	0	1	1	0
8	0	0	0	1	0	1	0	0	0	1	0	0
9	0	0	0	0	0	1	0	0	1	0	1	0
10	0	0	1	0	0	0	1	1	0	1	1	0
11	0	0	0	0	0	0	0	1	0	1	1	0
12	0	0	1	1	0	0	0	1	0	0	0	0
13	0	0	1	0	0	0	0	1	0	0	0	0
14	0	0	0	0	0	0	1	1	1	0	0	0
15	0	0	0	1	0	1	0	0	1	1	0	0
16	0	0	1	1	0	1	1	1	1	1	1	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	1	0	1	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	1	0	1	0	0	0	0
20	1	0	0	0	0	0	0	1	0	0	0	0
21	0	0	0	0	0	0	0	1	0	1	0	0
22	0	0	0	0	0	0	1	1	0	0	0	1
23	0	0	0	0	0	0	1	1	0	0	0	1
24	0	0	0	0	0	0	1	1	1	1	0	0
No.	**4**	**1**	**9**	**7**	**2**	**7**	**8**	**16**	**8**	**11**	**7**	**3**
R F	**16.67**	**4.17**	**37.5**	**29.17**	**8.33**	**29.17**	**33.33**	**66.67**	**33.33**	**45.83**	**29.17**	**12.5**

Table 12: Cont.

Plot	Grewia	Scutia	Vanguer	Trimeria	Clausena	Fagaro	Erythro	Caloden	mystrxyo	Dombeya	Elaeode	Zehner
1	1	0	0	0	1	0	0	0	0	1	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	1	0	0	1	0	0	0	0	0	0	0	0
4	0	0	0	0	1	0	0	0	0	1	0	0
5	1	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	1	0	0	1	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	1	0
9	0	1	1	1	0	0	0	0	0	0	0	0
10	0	0	0	0	1	1	1	1	1	1	0	0
11	0	0	1	0	0	0	0	1	0	1	1	1
12	0	0	0	0	1	0	1	0	0	0	0	0
13	0	0	0	0	0	0	0	1	0	0	0	1
14	0	0	0	0	1	0	1	1	0	0	0	0
15	0	0	0	0	1	0	0	0	1	0	0	0
16	0	0	1	0	0	0	0	0	1	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	1	1	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	1	0	0	0	1	1	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	1	0	0	0	0	1	0	0	0	0
23	0	0	0	0	1	0	1	1	1	0	0	0
24	0	0	1	0	0	0	0	0	0	0	0	0
No.	**3**	**3**	**7**	**2**	**8**	**1**	**5**	**7**	**4**	**4**	**2**	**2**
R F	**12.5**	**12.5**	**29.17**	**8.33**	**33.33**	**4.17**	**20.83**	**29.17**	**16.67**	**16.67**	**8.33**	**8.33**

Table 12: Cont.

Plot No	Lippia	Lantana	Triumfe	Clutia	Helichry	Solanum	Abutilon	Crotalar	Aspiliia	Indigo	Ipomea
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	1	1	0	0	0	0	0	0	0	0	0
4	0	0	1	0	0	0	0	0	0	0	0
5	1	0	0	0	0	0	0	0	0	0	0
6	0	1	0	1	1	1	0	0	0	0	0
7	0	0	0	0	0	0	1	0	0	0	0
8	0	1	0	0	0	0	1	0	0	0	0
9	0	0	0	0	0	0	1	0	0	0	0
10	0	1	0	0	0	0	1	0	0	0	0
11	1	0	0	0	0	0	0	1	1	0	0
12	0	0	0	0	0	0	1	0	0	0	0
13	0	0	0	0	0	0	1	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	1	0	0	0	0
16	1	0	0	0	1	0	1	0	1	1	1
17	1	0	0	0	0	1	1	0	0	0	0
18	0	0	1	0	0	0	1.	1	0	0	0
19	0	0	0	0	0	0	1	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	1
21	0	0	0	0	0	0	1	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	1	0	0	0	1
24	0	0	0	0	0	0	0	0	0	1	0
No.	**5**	**4**	**2**	**1**	**2**	**2**	**12**	**2**	**2**	**2**	**3**
R F	**20.83**	**16.67**	**8.33**	**4.16**	**8.33**	**8.33**	**50.0**	**8.33**	**8.33**	**8.33**	**12.5**

Table 12: cont.

Plot No	Turraea	Waburgia	Juniper	Celtis	Maytenus	Ritchi	Vernonia	Leucas	Ocimum	Microgl	Pterolo	Vernla	Hibiscus
1	0	0	0	0	0	0	1	1	1	1	1	1	1
2	0	0	0	0	0	0	0	1	0	1	1	0	1
3	0	0	0	0	0	0	0	0	0	0	0	1	1
4	0	0	0	0	0	0	1	1	0	1	0	0	1
5	0	0	0	0	0	0	0	0	1	0	0	0	0
6	0	0	0	0	0	0	0	0	1	1	0	0	1
7	0	0	0	0	0	0	0	0	0	1	1	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	1	0	0	1
10	0	0	0	0	0	0	0	0	0	1	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	1
12	1	0	0	0	0	0	0	0	0	0	1	1	0
13	0	0	0	0	0	0	0	0	0	0	1	0	0
14	1	0	0	0	0	0	0	0	0	0	1	1	0
15	0	0	0	0	0	0	0	1	0	1	0	1	1
16	0	1	1	1	1	0	0	0	0	1	0	0	1
17	0	0	0	0	0	0	1	0	1	0	0	1	1
18	0	1	0	0	0	0	0	1	1	1	0	0	1
19	0	0	0	0	0	0	0	0	0	0	0	1	1
20	0	0	0	0	0	0	0	0	0	0	0	1	0
21	0	0	0	0	0	1	1	0	0	0	0	1	0
22	0	0	0	0	0	0	0	0	0	0	1	1	0
23	0	0	0	0	0	0	0	0	0	1	1	0	1
24	0	1	0	0	0	0	0	0	0	0	1	1	0
No.	**2**	**3**	**1**	**1**	**1**	**1**	**4**	**5**	**5**	**11**	**9**	**11**	**13**
R F	**8.33**	**12.5**	**4.17**	**4.17**	**16.67**	**4.17**	**16.67**	**20.83**	**20.83**	**45.83**	**37.5**	**45.83**	**54.17**

Table 13: Relative frequency (RF) of Osyris and other species in Kibwezi forest

plot No.	Rhus	Maerua	Diospyro	Pappea	Dodonaea	Olea	Cobretum	Pittospo	Heteromo	Dombeya	Comphoh	Ficusv
1	1	1	1	1	1	1	1	1	1	0	0	0
2	1	0	1	0	0	1	1	0	0	0	1	0
3	0	0	0	1	0	1	0	1	0	0	0	1
4	1	0	0	0	1	1	0	0	0	0	0	0
5	1	0	0	0	1	0	0	1	0	0	0	0
6	1	1	0	0	1	0	1	1	0	0	0	0
7	1	0	0	1	0	0	0	0	1	0	0	1
8	1	1	0	0	0	0	0	0	0	0	0	0
9	1	0	0	1	1	0	0	1	0	0	0	0
10	1	1	0	1	0	0	0	1	0	0	0	0
11	1	0	1	1	1	0	0	0	0	0	0	0
12	1	0	1	1	0	1	0	0	0	0	0	1
13	1	1	0	1	0	1	0	0	0	1	0	0
14	0	0	0	1	0	1	0	0	0	0	0	0
15	0	0	0	0	0	1	0	0	1	0	0	0
16	0	0	1	0	0	0	0	0	0	0	0	0
17	0	0	1	1	0	0	0	0	0	1	1	0
18	1	0	1	0	0	0	0	0	0	1	0	0
19	0	0	0	0	0	0	0	0	0	1	0	0
20	0	0	0	0	0	0	0	0	0	1	0	0
21	0	0	0	1	0	0	0	0	0	1	0	0
22	1	0	1	0	0	0	0	0	0	1	0	0
23	0	1	0	0	0	0	0	0	0	1	0	0
24	0	0	1	1	0	1	0	0	0	1	0	0
No.	**14**	**6**	**9**	**12**	**6**	**9**	**3**	**6**	**3**	**9**	**2**	**3**
R F	**58.33**	**25.00**	**37.50**	**50.00**	**25.00**	**37.50**	**12.50**	**25.00**	**12.50**	**37.50**	**8.33**	**12.50**

Table 13: Cont.

Plot	Osyris	Syphoste	Combresp	Comiphob	Adenium	Maytenus	Euclead	Cussonia	Acaciabr	mystrxyo	Tecleasi	Ochnaova
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	1	0	0	0	0	0	0	0	0	0	0	0
4	1	1	1	0	0	0	0	0	0	0	0	0
5	1	0	1	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	1	0	0	1	0	0	0	0	0	0	0	0
8	0	0	0	0	1	0	0	0	0	0	0	0
9	1	0	0	0	0	1	1	1	0	0	0	0
10	0	1	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	1	0	0
12	1	0	0	0	0	0	1	0	0	0	1	0
13	1	1	0	1	0	0	0	0	0	0	0	1
14	1	0	0	0	0	0	0	0	0	0	0	0
15	1	0	0	1	0	0	0	0	0	1	0	0
16	1	0	0	0	0	0	0	0	0	0	0	0
17	1	0	0	1	0	0	0	0	0	0	0	0
18	1	0	0	1	0	0	0	0	0	1	0	0
19	1	0	0	1	0	0	0	0	1	0	0	0
20	1	0	0	1	0	0	0	0	1	0	0	0
21	1	0	0	1	0	0	1	0	0	0	1	0
22	1	0	0	0	0	0	0	0	0	1	1	0
23	1	0	0	1	1	0	0	0	0	0	0	0
24	1	0	0	1	0	0	0	0	0	1	0	0
No.	**18**	**3**	**2**	**10**	**2**	**1**	**3**	**1**	**2**	**5**	**3**	**1**
R F	**75.00**	**12.50**	**8.33**	**41.67**	**8.33**	**4.17**	**12.50**	**4.17**	**8.33**	**20.83**	**12.50**	**4.17**

Table 13:Cont.

plot	Crotondi	Antidven	Grewia	Stegaora	Hymepar	Haplocoe	Euphorca	Euphorsc	Acaciaro	Balanite	Comemin	Crotonme
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	1	1	0	0	0	0	0	0	0	0	0	0
14	1	1	1	0	0	0	0	0	0	0	0	0
15	1	0	0	1	1	0	0	1	0	0	0	1
16	0	0	1	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	1	0
18	1	0	0	0	0	1	0	0	0	0	0	1
19	0	0	0	0	0	1	1	0	1	0	0	0
20	0	0	0	0	0	1	0	0	0	1	0	0
21	1	0	0	0	0	0	1	0	0	0	1	0
22	1	0	0	1	0	1	1	0	0	0	1	0
23	1	0	0	1	0	0	0	0	0	1	1	0
24	1	0	0	0	0	0	1	0	0	0	1	0
No.	**8**	**2**	**2**	**3**	**1**	**4**	**4**	**1**	**1**	**2**	**5**	**2**
R F	**33.33**	**8.33**	**8.33**	**12.50**	**4.17**	**16.67**	**16.67**	**4.17**	**4.17**	**8.33**	**20.83**	**8.33**

Table 13: Cont.

plot No.	Indigo	Plectrun	Ipomea	Hibiscus	Abutilon	Solanum	Aspiliia	Leucas
1	1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	1
6	0	1	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	1	0	0
10	1	0	0	0	0	0	0	0
11	0	0	1	0	0	0	0	0
12	1	0	0	1	0	0	0	0
13	1	0	0	1	0	0	0	0
14	0	0	0	1	0	0	0	0
15	0	0	0	0	1	0	0	0
16	0	0	0	0	1	0	0	0
17	0	0	0	0	1	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
20	0	0	0	1	0	0	0	0
21	0	0	0	0	0	0	0	0
22	0	0	0	1	0	0	0	0
23	0	0	0	0	0	0	1	0
24	0	0	0	0	0	0	0	0
No.	**4**	**1**	**1**	**5**	**3**	**1**	**1**	**1**
R F	**16.67**	**4.17**	**4.17**	**20.83**	**12.50**	**4.17**	**4.17**	**4.17**

Appendix v

Table 11: 50% Occurrence of all compounds identified in roots, stems and barks

0 means-absent, 1 means -present

Site	Tree part	(E)-cis-epi	(Z)-a-Farn	.alpha.-Fa	.alpha.-Sa	Bergamot	Bergamot	Bergamot	Bergamot	Bisabolen	Bisabolen	Carene<d	Carene<d	Carvacrol	Caryophy	Cedrene<
Gachuthi	GR1	1	1	1	1	1	0	0	1	1	0	0	1	0	0	0
Gachuthi	GR2	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1
Gachuthi	GR3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Gachuthi	GR4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Gachuthi	GS1	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0
Gachuthi	GS2	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
Gachuthi	GS3	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0
Gachuthi	GS4	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
Gachuthi	GB1	0	0	0	0	1	0	0	1	0	1	1	0	1	0	0
Gachuthi	GB2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GB3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GB4	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
Kibwezi	KR1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0
Kibwezi	KR2	0	0	0	0	1	0	0	1	0	0	1	1	1	0	0
Kibwezi	KR3	0	0	0	0	1	1	0	1	1	0	0	0	1	0	0
Kibwezi	KR4	0	1	0	0	1	0	0	1	0	0	0	0	1	1	0
Kibwezi	KS1	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0
Kibwezi	KS2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Kibwezi	KS3	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Kibwezi	KS4	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0
Kibwezi	KB1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
Kibwezi	KB2	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Kibwezi	KB3	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
Kibwezi	KB4	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0
		1	2	1	2	17	6	0	17	3	1	4	5	9	1	1

Table 11: (Cont.)

Site	Tree part	Cedrol	Cedrol<e	Cineole<1	cis- Lance	cis-.alpha	cis-b-Ocin	Copaene<	Crinamine	Curcumer	Curcumer	Farnesene	Farnesene	Germacre	Italicene	Italicene<	Lanceol<Z
Gachuthi	GR1	1	0	0	1	0	0	0	0	1	1	1	0	0	1	0	1
Gachuthi	GR2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Gachuthi	GR3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GR4	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Gachuthi	GS1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GS2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Gachuthi	GS3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GS4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GB1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Gachuthi	GB2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GB3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GB4	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
Kibwezi	KR1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Kibwezi	KR2	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	1
Kibwezi	KR3	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0
Kibwezi	KR4	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	0
Kibwezi	KS1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kibwezi	KS2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kibwezi	KS3	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0
Kibwezi	KS4	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Kibwezi	KB1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
Kibwezi	KB2	0	0	0	1	1	0	0	0	1	1	1	0	1	1	0	0
Kibwezi	KB3	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Kibwezi	KB4	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	1
		1	2	4	4	1	1	1	1	11	6	12	1	1	6	1	3

Table 11: (Cont.)

Site	Tree part	Limonene	Ocimene	Ocimene<	Pinene	Pinene<a	Pinene<b	Santalene	Santalene	Santalene	Santalol	Santalol<	Santalol<	Santalol<	Santalone	Santolina	Sesquiphe	Sesquisab
Gachuthi	GR1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0
Gachuthi	GR2	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0
Gachuthi	GR3	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GR4	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0
Gachuthi	GS1	0	0	0	0	1	1	1	1	0	0	1	1	0	0	0	0	0
Gachuthi	GS2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GS3	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0
Gachuthi	GS4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GB1	0	1	0	0	1	1	1	1	0	1	0	0	0	0	1	1	0
Gachuthi	GB2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Gachuthi	GB3	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GB4	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
Kibwezi	KR1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0
Kibwezi	KR2	1	0	1	0	1	1	1	1	1	0	1	1	0	1	1	0	0
Kibwezi	KR3	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0
Kibwezi	KR4	0	0	0	0	1	0	1	1	0	0	0	1	0	0	0	1	0
Kibwezi	KS1	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0
Kibwezi	KS2	1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
Kibwezi	KS3	1	0	0	1	1	1	1	1	1	0	1	1	0	0	1	0	0
Kibwezi	KS4	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0
Kibwezi	KB1	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0
Kibwezi	KB2	0	0	1	0	1	0	1	1	0	0	0	1	0	0	1	0	0
Kibwezi	KB3	1	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0
Kibwezi	KB4	0	0	0	0	1	0	1	1	0	0	1	1	0	0	1	0	0
		5	1	3	1	20	9	12	15	7	1	6	13	1	1	7	3	1

Table 11: (Cont.)

Site	Tree part	Sesquithu	Sesquithu	Sinensal<	trans-.alp	trans-a-Be	trans-b- O	Zingibere	(Z)-cis-a-	.alpha.-Ca	.Xi.-Lanos	1S-.alpha.
Gachuthi	GR1	0	1	1	1	0	0	0	0	0	0	0
Gachuthi	GR2	1	0	0	0	1	0	0	0	0	0	0
Gachuthi	GR3	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GR4	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GS1	1	0	1	0	0	0	1	0	0	0	0
Gachuthi	GS2	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GS3	1	0	1	0	0	0	0	0	0	0	0
Gachuthi	GS4	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GB1	0	0	0	0	0	1	0	0	0	0	0
Gachuthi	GB2	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GB3	0	0	0	0	0	0	0	0	0	0	0
Gachuthi	GB4	0	0	0	0	0	0	0	0	0	0	0
Kibwezi	KR1	0	0	0	0	0	0	0	0	0	0	1
Kibwezi	KR2	1	1	0	0	0	0	0	1	0	0	0
Kibwezi	KR3	0	0	0	1	0	0	1	0	0	0	0
Kibwezi	KR4	0	0	0	0	0	0	1	0	0	0	0
Kibwezi	KS1	0	0	1	0	0	0	0	0	0	0	0
Kibwezi	KS2	0	0	0	0	1	0	0	0	0	0	0
Kibwezi	KS3	0	1	0	0	0	0	0	0	0	1	0
Kibwezi	KS4	0	0	1	1	0	0	0	0	0	0	1
Kibwezi	KB1	0	1	1	1	0	0	0	0	0	0	0
Kibwezi	KB2	0	1	0	0	1	0	1	0	1	0	0
Kibwezi	KB3	0	1	1	0	0	0	0	0	0	0	1
Kibwezi	KB4	0	1	0	0	0	0	1	0	0	0	0
		4	7	7	4	3	1	5	1	1	1	3

Pearson correlation for 50% occurrence compounds

	Bergamotene <(Z)alpha-trans>	**Farnesene <(E)-beta>**	**Pinene <alpha>**	**Santalene <alpha>**	**Santalene <beta>**	**Santalol(Z) alpha**
Bergamotene <alpha-cis>	-0.104	0.336	-0.038	0.579	0.367	0.048
Bergamotene <(Z)alpha-trans>	1	-0.018	-0.532	0.102	0.276	0.593
Farnesene <(E)-beta>		1	-0.208	0.540	0.432	0.169
Pinene <alpha>			1	-0.121	-0.263	-0.352
Santalene <alpha>				1	0.369	0.066
Santalene <beta>					1	0.803

- Correlation significant at 0.05 level
- Independent variables: Root, Stem and Bark
- Dependant variables: Compounds