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Research Article

Influence of Soil Nutrients, Tree Age, and Sandalwood Provenances on Sandalwood Oil Yield and Quality

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East African Sandalwood (*Osyris laceolata*) is an important tree species used in perfumery and pharmaceutical industries. In Kenya, the tree is illegally poached and smuggled mostly to India as a substitute for Asian sandalwood. Therefore, there is a need to domesticate E. A. sandalwood to ease pressure on natural stands. The aim of this study is to determine ecological factors influencing Osyris oil yield and quality to guide the selection of provenances for on-farm domestication. Soil and woody samples were obtained from 12 provenances and used for soil and oil analysis, respectively. The results showed that only tree age significantly influenced the oil yield ($r = 0.31$, $p = 0.04$). The GC-MS quality results recorded nine common and most abundant compounds across the study sites. These were Z-alpha-trans-bergamotol, alpha bisabolol, lanceol cis, beta bisabolene, alpha santalol, beta santalol, cis-alpha-copaene-8-ol, isopropyl myristate, and isopropyl palmitate. Baringo and Mbooni provenances had the highest number of compounds (8), followed by Homabay (7) while the majority (Chyulu, Kitui, Loita, Maralal, Marsabit, Muranga, and Narosura) had six and Ol Donyo Sabuk and Namanga had the least (5). The species diversity is therefore important for breeding, domestication, and conservation purposes.

1. Introduction

Sandalwoods are a commercially and culturally important group of plant species belonging to the family Santalaceae [1]. Sandalwoods provide high-value essential oils used as fragrances and cosmetic ingredients. In Kenya, *Osyris lanceolata* (East African Sandalwood) is not region-specific and can adapt to different environmental conditions [2]. *Osyris lanceolata* (Hochst. and Steud.) in the family Santalaceae is a hemiparasite dioecious tree species that requires a host plant to grow to its full potential [3]. The species grows in transitional wet and dry zones and is found in most of the agroclimatic zones of Kenya [4]. In Kenya, sizeable populations have been recorded in Taita Taveta, Makueni, Kitui, Embu, Machakos, Kiambu, Laikipia, Pokot, Uasin Gishu, Narok, Samburu, Murang'a, Kajiando, Migori, Nyandarua, Marsabit, and Baringo counties. In its natural range, the species grows at an altitude of 900–2250 m above sea level (asl) in areas with a mean annual rainfall of 600–1600 mm. Naturally, it can grow in areas whose natural vegetation is dry woodland in ecological zone III and moist to dry forest in zone II. *Osyris lanceolata* requires well-drained soil and does not tolerate waterlogging [3, 4].

Wild-harvested *Osyris lanceolata* from Kenya is illegally poached and smuggled mostly to India, where its oil is used as a substitute for Asian sandalwood, the oil of which is an ingredient in the multimillion dollar cosmetic, perfumery, and flavor industries [5]. In Kenya, the global market per kilogram of O. *lanceolata* heartwood is estimated to be about 3.0 US dollars [6] compared to *Santalum album* at 100 US dollars [7]. Sandalwood oil is composed of natural compounds referred to as sesquiterpenoids, which give the essential oil its distinctive aroma. *Osyris lanceolata* has biochemical composition of 55 constituents in oil such as (E)-cis-epi-b-santalol, (Z)-a-Farnesene, (z)-cis-a-santalol, and Alpha-caryophyllene among others as compared to

S. *album* with between 250 and 300 such as (Z) α santalol, (Z) β-santalol, and (Z)- epi-β- santalol [8]. In pharmaceutical industries, sesquiterpenoids are used as a composition of medicines for treating bronchitis, dry persistent coughs, laryngitis, diarrhea, dermatitis arthritis, and insomnia among other diseases [9]. In perfumery industries, sesquiterpenoids are effective in aromatherapy treatment because of the sweet powerful long-lasting aroma. The sandalwood sesquiterpenoids make an excellent composition for scenting soaps and all kinds of perfumes [10]. Both O. *lanceolata* and S. *album* contain α and β santalols which are responsible for fragrance in sandalwood oil [8].

Domestication of Osyris is being encouraged to reduce overexploitation of the natural populations in Kenya by providing sandalwood markets with sustainable stocks grown in plantations [7]. High oil yields would make such enterprises more profitable and attractive to investors. Studies conducted in Tanzania revealed different provenances had contrasting oil yield that varied in quality [11]. This study sought to establish how age, soil, and agro-ecological factors influenced oil yield and quality to add to the body of knowledge that would guide in selection of provenances and prescription of appropriate on-farm husbandry for the species. The objective of this study was to determine the effect of age, soil, and agro-ecological factors on oil yield and quality of Osyris growing in natural stands.

1.1. Materials and Methods

1.1.1. Study Sites. This study was undertaken at 12 sites, namely, Baringo, Chyulu Hills, Homabay, Kitui, Loita, Maralal, Marsabit, Mbooni, Murang'a, Namanga, Narosura, and Ol Donyo Sabuk. Figure 1 shows the distribution of study sites.

The study sites are in different Agro-Ecological Zones (AEZ) ranging from high and medium potential to semiarid and arid climatic zones. Table 1 shows a summary of agroclimatic characteristics of the study sites. II.

1.2. Soil Sampling and Analysis. At each site, soil samples were collected under O. *lanceolata* trees to a depth of 0–25 cm using a soil auger. Soil was taken at three random sampling points around the *Osyris* tree and mixed to make a composite sample. The soil samples were analysed for texture, pH, electrical-conductivity (EC), total nitrogen, total carbon, extractable phosphorus, calcium, magnesium, potassium, zinc, copper, manganese, and iron properties using standard laboratory procedures as described by Okalebo et al. [12].

1.3. Sampling of Osyris lanceolata Trees. As the harvesting of sandalwood in its natural stands is legally proscribed, authorization to harvest O. *lanceolata* trees was obtained from relevant authorities. Four trees per site were permitted to be used for this research, which is adequate for research in the extraction and quality analysis of oil [13]. Mature trees were harvested at the base using a pruning saw, labeled, and packed in gunny bags for transportation in line with procedures described in Howes et al. and Brand et al. [14, 15].

1.4. Age Determination of O. lanceolata Trees. To determine the age of Osyris trees, a dendrochronological method was used as described by Kankare et al. [16]. Dried *Osyris* wood blocks were cut horizontally at the base to a uniform size of 2 cm thickness using an electric saw. The rings present in the wood samples were counted using a magnifying glass, and the number of rings counted was assumed to correspond to the age in years.

1.5. Extraction of O. lanceolata Oil and Yield Determination. Wood samples were air-dried at room temperature (22°C) for 20 days to avoid loss of volatile essential oils [17]. Smaller pieces of wood, about 1 cm^3, were cut using an electric saw so they could be accommodated in the grinder. They were then ground into fine powder using a Dietz model D-73265 grinder. The fine powder was stored in airtight plastic containers to prevent further moisture loss. One hundred grams of fine powder were weighed using an analytical weighing balance and placed into a separating funnel, where 300 ml of hexane (analar grade) was added and left to stand for 72 hours to give a yellow-coloured extract. The extract was removed from the separating funnel and concentrated with a rotary evaporator (Yamato model: CF 301) under vacuum. The oil obtained was weighed, and the yield was calculated as per Equation (1). The oil was then transferred into 20 ml brown sample bottles to avoid light and stored in the freezer at −10°C awaiting further analysis.

$$\%\ \text{yield} = \frac{W2 - W1}{W3} * 100, \tag{1}$$

where W1–weight of empty bottle, W2–weight of the bottle plus oil, and W3–weight of the ground sample used in extraction.

1.6. Determination of Oil Quality. To determine the quality of oil, 10 μl of the distilled oil was pipetted into a 1.5 ml sample bottle and diluted with 1 ml of hexane. Additionally, 200 μl of the diluted sample was transferred into an insertion for gas chromatography-mass spectra (Agilent Technologies, model 7890 A) for the identification of compounds. The method used for compound analysis was hexane volatiles at 35°C–280°C for 50 minutes per sample**.** Helium gas was used as a carrier gas, and GC-MS facilitated the identification of different compounds in the oil extracts at different time points of detection [18]. Areas were recorded for all detectable peaks, and percent concentration was calculated by taking the area of the peak divided by the total chromatogram area multiplied by 100.

1.7. Data Analysis. The statistical tool used for analysis of variance (ANOVA) was R version 4.1.0 for Windows at 95% confidence level ($p < 0.05$). The packages used for analysis are: agricolae, tidyverse, ggplot2, ggpubr, devtools, prcomp, and ggbiplot. The means were separated using Duncan's

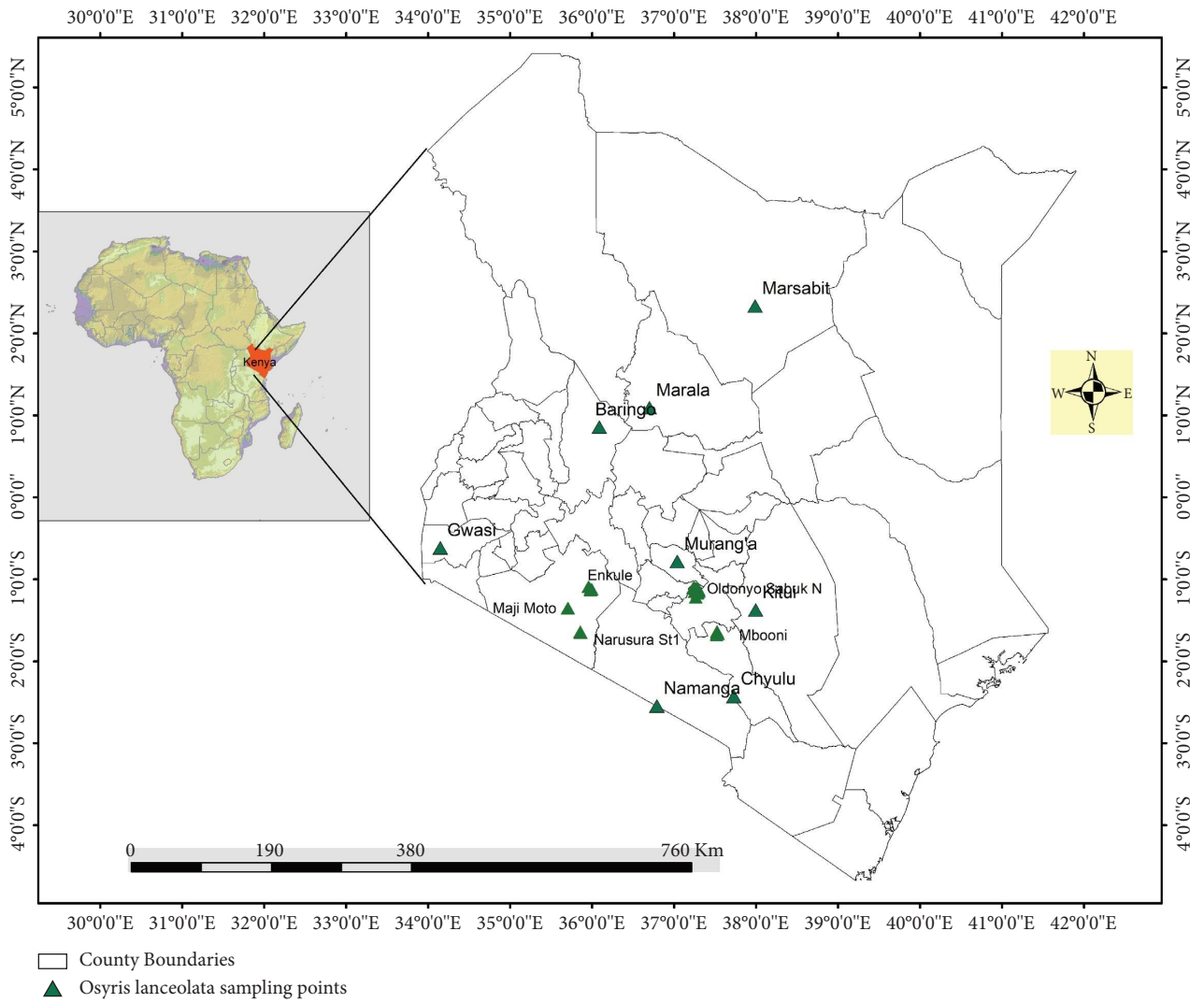

Figure 1: Map of Kenya showing study sites.

Table 1: Agroclimatic and soil characteristics of the study site.

Sites	Mean annual temperature (°C)	Mean annual rainfall (mm)	Altitude (masl)	AEZ	Soil texture
Homabay	22.5	1646	1606	II	Sandy clay loam
Narosura	17.8	1045	2222	III	Clay
Loita	17.8	1045	2411	III	Sandy loam
Baringo	24.6	1000	2001	III	Clay
Marsabit	23.5	375	1074	VI	Clay
Maralal	18.2	879	1965	IV	Clay
Murang'a	19.7	996	1177	III	Clay
Mbooni	24.0	800	1426	IV	Sandy clay loam
Chyulu hills	24.0	495	2188	V	Sandy loam
Kitui	21.9	881	1121	IV	Sandy loam
Namanga	23.0	534	1327	IV	Sandy loam
Ol Donyo sabuk	18.9	800	2144	IV	Clay

multiple comparison test. Regression analysis was used to determine the strength of existing relationships among variables, while principal component analysis *R* (PCA) was used for correlation of multivariate data such as soil, age, provenance, and oil data, where negative loadings indicated negative correlation and positive loadings indicated positive correlation. The influence of tree age in selected provenances on oil yield was fitted as a covariate.

2. Results and Discussion

2.1. Variation of Soil Nutrients in Different Study Sites. Soil pH levels significantly varied in all study sites ($p = 0.001$). In Baringo, Maralal, Narosura, Marsabit, Namanga, and Ol Donyo Sabuk soils were acidic, while Mbooni, Murang'a, Kitui, Loita, and Chyulu were neutral. Only Homabay had alkaline soil. EC levels significantly differed amongst all study sites ($p = 0.009$) as shown in Table 2. Carbon levels differed significantly across all study sites ($p = 0.001$). The highest carbon levels were recorded in Chyulu and Homabay, whereas the lowest levels of carbon were recorded in Maralal as shown in Table 2.

Nitrogen levels also differed significantly ($p = 0.009$) with the highest levels recorded in Chyulu and the lowest levels in Kitui. Phosphorus levels varied significantly ($p = 0.001$), with the highest levels in Homabay and Chyulu; all other sites had phosphorus levels below 15 ppm. Similarly, potassium levels varied significantly ($p = 0.001$), with Homabay having the highest levels while Namanga had the lowest levels (Table 2).

These results show that Osyris naturally occurs in contrasting soil types and different agro-ecological zones with varying soil nutrient levels as demonstrated by the presence of the species at sites ranging from high- to medium-potential agricultural sites to semiarid and arid regions [19]. These findings are consistent with previous studies of Osyris cutting across different AEZ with varying soil nutrient levels [11].

2.2. Influence of Soil Nutrients on Oil Yield. There was no significant difference on oil yield as influenced by soil nutrient levels in all sites as indicated by nonsignificant regressions and Pearson correlations (Table 3). This study contrasts with a previous study by Golubkina et al. [20], where increased soil nutrients showed beneficial effects on plant growth, development, and oil content in aromatic plants. A similar study done by Argyropoulou et al. [21] concurred with Golubkina's results, where growth performance and oil yield among aromatic plants such as spearmint and rosemary increased with the addition of soil nutrients. The current study also contradicts the study by Argyropoulou et al. [21] on the cultivation of aromatic plants and the addition of nitrogen and potassium, which established a significant increase of oil quantity and quality in the aromatic plants. However, the significant difference in soil nutrients across all study sites could contribute to differences in the total woody biomass of individual trees.

2.3. Influence of Age on Oil Yield. The age of sampled trees ranged from 8 to 40 years. In this study, there is a very weak but positive significant influence of age on oil yield in *Osyris* trees ($R = 0.31$, $p = 0.04$). The strength of the relationship is shown by $R^2 = 0.096$, where 9.6% of observation made in oil yield can be attributed to Osyris's age while over 90.4% is explained by other factors. Similar studies in Western Australia by Brand and Prank [22] on *Santalum spicatum* plantations indicated significant difference in oil yield as influenced by age. Increased oil yields in older trees could be contributed by the well-developed mature heartwood. These findings concur with the current study which showed significant increase in oil yield with increasing age of Osyris trees (Figure 2).

Bush and Pronk [23] revealed that S. *album* and S. *yasi* oil yield was also significantly positively influenced by the age of the tree from which the oil was extracted. Their study further revealed that *Santalum* spp. trees less than 16 years old growing in the Pacific under much wetter conditions than the current study were not mature enough to produce essential oil due to the low formation of heartwood at that age. Mashra et al. [24] also reported a significant increase in oil yield with age; however, they concluded that there was no uniform pattern in heartwood formation and oil content with the increasing age of the tree which may be attributed to varying edaphoclimatic conditions.

2.4. Effect of Provenance on Oil Yield. The results of this study indicate that *Osyris* oil yield was not significantly different among the various provenances sampled ($p = 0.19$). Loita provenance, however, recorded the highest oil yield amongst the provenances (3.56%); this was followed by Baringo, which recorded an oil yield of 3.49% as shown in Table 4 below. Narosura provenance recorded the lowest oil yield of 1.50%, which was 57.9% lower than the Loita provenance. The provenances which recorded higher oil yield were all found in agro-ecological zone III, except Mbooni and Ol Donyo Sabuk which fall under agro-ecological zone IV. The differences in oil yield among the different provenances can partially be attributed to the age of the trees selected for oil quantification. Age of trees selected in the various provenances significantly influenced the oil yield ($f_{(1,41)} = 2.97$, $p = 0.008$).

In Kenya, no previous studies have evaluated the effect of provenances on *Osyris* oil yield. A study by Mwang'ingo [25] reported that *Osyris* populations in Tanzania varied significantly in the amount of oil produced. A similar study [11], which assessed oil yield and quality variation between sexes in *Osyris* study, concluded that there were no significant differences in oil yield between the different sexes. However, it reported that oil yield was significantly different ($p = 0.001$) among populations with the highest oil yield being 9.32%. The study concluded that the reasons for observed trends in the quantity and quality of oil are still uncertain. The observed variations could have resulted from environmental and genetic factors. Moniodis et al. [26] similarly reported a significant difference in oil yield in different provenances and soil types.

2.5. Interaction between Tree Age, Soil Variables, and Oil Yield. Figure 3 explores the interaction of different variables under the current study. The study revealed that the first and second principal components accounted for 48.3% of the overall variation. The study further revealed that there was a positive correlation amongst soil variables such as carbon, nitrogen, pH, EC, manganese, magnesium, and potassium. However, there was no correlation between oil yield and most soil variables. There was a negative correlation between

Table 2: Soil chemical properties variation in all the study sites.

Sites	pH	EC (ms/cm)	%C	%N	P (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)
Baringo	5.48 ± 0.13d	0.0101 ± 0.056b	4.56 ± 1.15bc	0.36 ± 0.11bc	6.33 ± 1.52c	997.45 ± 210.48b	556.23 ± 37.97bc	2305.07 ± 525.74bcde	20.36 ± 3.77b	496.74 ± 229.53abc	274.69 ± 56.34ab	0.67 ± 0.33d
Chyulu	7.11 ± 0.33ab	0.545 ± 0.488a	8.12 ± 2.59a	1.04 ± 0.44a	81 ± 31.96b	631.98 ± 180.78de	710.54 ± 399.91abc	6818.24 ± 155.92a	43.63 ± 7.37a	39.77 ± 1.348d	345.38 ± 159.79a	7.53 ± 3.01bc
Homabay	7.64 ± 0.23a	0.159 ± 0.027b	7.19 ± 2.26ab	0.66 ± 0.23b	114.00 ± 11.35a	1294.49 ± 29.83a	675.73 ± 47.14abc	5200.10 ± 250.39a	20.07 ± 2.439.43 ± 4.03cde	518.46 ± 187.84abc	247.47 ± 44.93ab	16.37 ± 3.38a
Kitui	6.67 ± 0.44bc	0.330 ± 0.137ab	2.64 ± 0.40cd	0.12 ± 0.05	7.60 ± 0.89c	290.62 ± 161.95f	463.32 ± 174.59c	2690.06 ± bcde	9.43 ± 4.03cde	110.41 ± 53.76d	164.01 ± 38.28bc	5.59 ± 2.96bcd
Loita	6.61 ± 0.96cd	0.0108 ± 0.002b	4.90 ± 2.98bc	0.38 ± 0.37	9.6 ± 3.05c	960.61 ± 2.37bc	575.74 ± 124.75bc	3132.99 ± 1747.52bcd	18.13 ± 5.94bc	824.41 ± 374.62a	213.24 ± 28.24abc	4.63 ± 1.57cd
Maralal	5.89 ± 0.08cd	0.070 ± 0.001b	1.15 ± 1.10d	0.23 ± 0.06	12 ± 5.68c	971.54 ± 178.90bc	954.26 ± 504.33ab	2641.02 ± 704.14bcde	1.00 ± 0.00e	482.41 ± 189.04abc	235.01 ± 59.28ab	0.52 ± 0.08d
Marsabit	6.56 ± 0.20bc	0.102 ± 0.052b	3.32 ± 1.31cd	0.31 ± 0.09bc	14.00 ± 8.57c	612.57 ± 258.20de	1059.73 ± 143.32a	3588.50 ± 658.41a	14.11 ± 11.99bcd	803.12 ± 196.02a	209.39 ± 13.73abc	10.07 ± 5.82b
Mbooni	6.79 ± 0.27b	0.109 ± 0.042b	1.47 ± 1.31d	0.20 ± 0.25c	8.5 ± 6.02c	600.49 ± 90.63de	676.08 ± 357.48abc	1779.64 ± 984.91de	3.22 ± 2.67e17.33 ± 7.13bc	175.54 ± 174.59cd	156.78 ± 140.43bc	1.06 ± 0.45d3.67 ± 1.04cd
Murang'a	6.78 ± 0.23b	0.138 ± 0.036b	3.37 ± 0.59cd	0.30 ± 0.28bc	4.66 ± 1.15c	670.44 ± 149.04cde	757.06 ± 188.99abc	2566.27 ± 539.96bcde	17.33 ± 7.13bc	356.39 ± 185.17bcd	170.16 ± 75.29bc	3.67 ± 1.04cd
Namanga	6.55 ± 0.03bc	0.066 ± 0.012 b	1.49 ± 0.44d	0.03 ± 0.04c	3.66 ± 1.52c	130.27 ± 124.35f	303.94 ± 18.13c	1366.26 ± 195.63e	7.30 ± 2.89de	40.29 ± 8.13d	55.63 ± 14.46c	2.36 ± 0.41cd
Narosura	5.97 ± 0.82cd	0.102 ± 0.33 b	4.96 ± 2.51bc	0.35 ± 0.06bc	8.00 ± 3.91c	894.25 ± 342.68bcd	552.37 ± 229.67bc	2032.26 ± 900.52cde	16.55 ± 1.06bcd	648.41 ± 388.99ab	353.77 ± 158.87a	4.31 ± 1.57 cd
Ol Donyo Sabuk	6.68 ± 0.47bc	0.110 ± 0.031 b	3.56 ± 0.88c	0.32 ± 0.26bc	6.25 ± 4.27c	371.70 ± 96.91ef	1053.24 ± 227.17a	3407.80 ± 809.63bc	4.42 ± 6.07e	559.07 ± 219.08ab	212.34 ± 48.03abc	18.29 ± 5.46a
	$f(11, 31) = 5.12$, $p = 0.001$	$f(11, 31) = 2.90$, $p = 0.009$	$f(11, 31) = 16.98$, $p = 0.001$	$f(11, 31) = 5.05$, $p = 0.001$	$f(11, 31) = 33.95$, $p = 0.001$	$f(11, 31) = 11.40$, $p = 0.001$	$f(11, 31) = 3.09$, $p = 0.006$	$f(11, 31) = 10$, $p = 0.001$	$f(11, 31) = 14.88$, $p = 0.001$	$f(11, 31) = 6.29$, $p = 0.001$	$f(11, 31) = 2.94$, $p = 0.008$	$f(11, 31) = 12.89$, $p = 0.001$

Values followed by different letters are statistically different according to Duncan's test.

Table 3: Influence of soil nutrients on oil yield presented as the correlation coefficient R and the coefficient of determination resulting from Pearson correlations.

Parameters	R	r^2	p value	Significance
%Nitrogen	0.20	0.041	0.19	n.s.
Phosphorus	0.086	0.007	0.58	n.s.
Potassium	0.046	0.002	0.77	n.s.
pH	0.11	0.012	0.48	n.s.
EC	0.052	0.003	0.74	n.s.
%Carbon	−0.012	0015	0.43	n.s.
Calcium	−0.035	0.001	0.82	n.s.
Magnesium	−0.015	0.0002	0.92	n.s.
Manganese	0.032	0.001	0.84	n.s.
Zinc	0.081	0.007	0.61	n.s.
Iron	−0.29	0.008	0.06	n.s.
Copper	0.06	0.004	0.70	n.s.

Note. Significance codes: 0 "***," 0.001 "**," 0.01 "*," 0.05 "." and n.s. not significant.

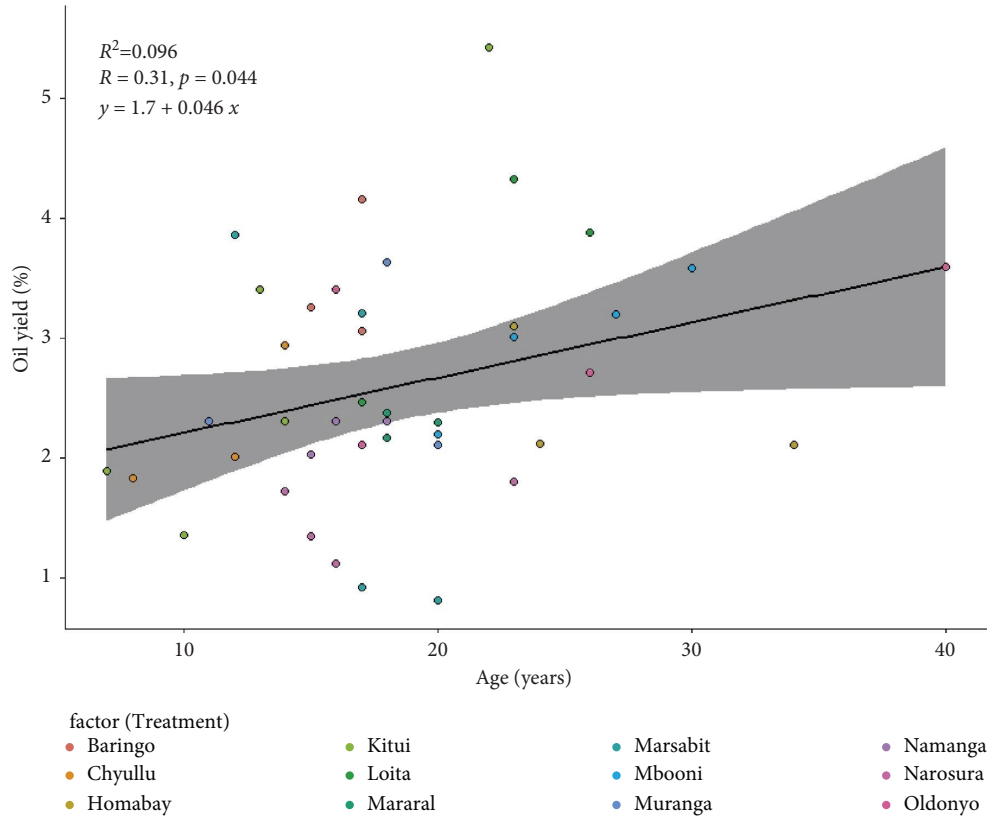

Figure 2: Age versus oil yield. NB: Meaning of shaded part: This layer adds both the best-fitting regression line and also the 95% confidence interval for the line shown in grey shading. The outer edges of the shaded area represent the confidence bands, indicating the 95% confidence intervals for the mean of the Y-variable (Oil yield) at each value of the X-variable (tree age).

oil yield and soil EC. Oil yield is positively correlated to tree age and clay content in the soil. Ecosystems are complex and consist of interacting biotic and abiotic components [4].

2.6. Oil Quality Potential in Different Provenances in Kenya. The phytochemical composition of genus *Osyris* includes volatile constituents that are important in essential oils and are known as sesquiterpenes. The superiority of sandalwood oil is determined by the level of alpha (α) and beta (β) santalol relative to the international oil quality standards for these compounds [27]. The GC-MS analysis in this study recognized compounds belonging to groups of hydrocarbons, fatty acids, esters, alcohols, amines, sesquiterpenes, among others, in Osyris oil. The GC-MS analysis on oil extracts from 12 sampled Osyris populations showed numerous compounds occurring at various peak intervals. However, the most common and abundant compounds from the sampled populations were Z-alpha-trans-bergamotol, alpha bisabolol, lanceol

Table 4: Oil yield potential in different provenances in Kenya.

Provenances	Oil yield ± SE (%)
Baringo	3.49 ± 0.59a
Chyulu	2.27 ± 0.49ab
Homabay	2.44 ± 0.57ab
Kitui	2.89 ± 1.61ab
Loita	3.56 ± 0.97a
Maralal	2.28 ± 0.11ab
Marsabit (karare)	2.20 ± 1.56ab
Mbooni	2.99 ± 0.58ab
Murang'a	2.68 ± 0.83ab
Namanga	2.22 ± 0.16ab
Narosura	1.50 ± 0.32b
Ol Donyo sabuk	2.96 ± 0.68ab
$f_{(11,31)} = 1.47$	$p = 0.19$ n.s.

NB: means denoted by the same letter are not significantly different.

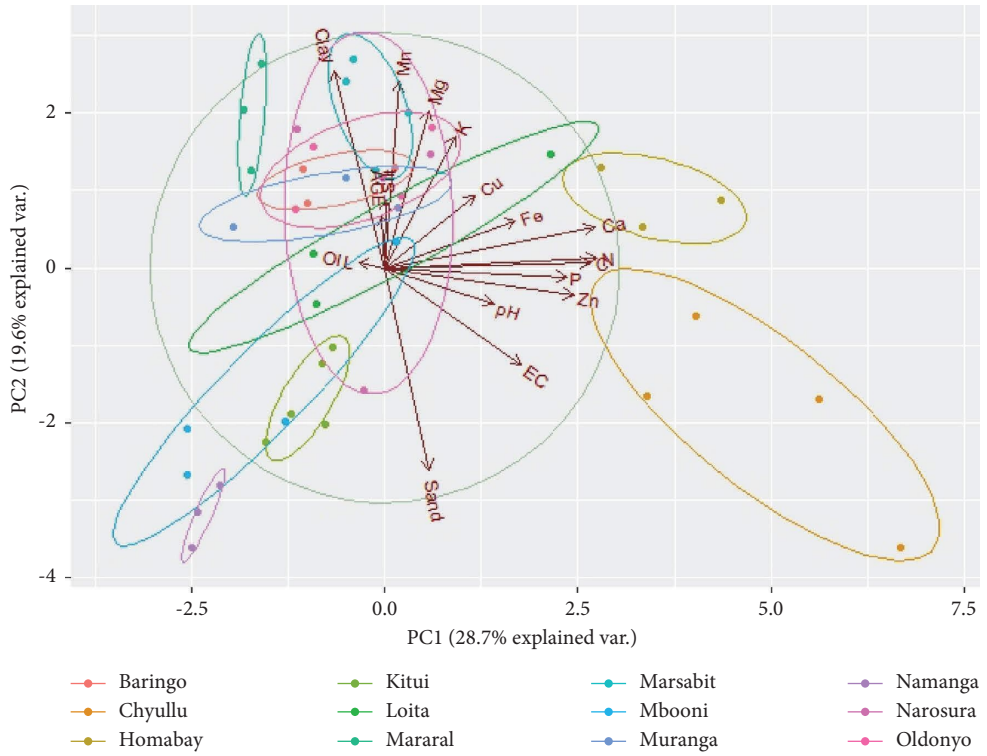

Figure 3: Principal component analysis of soil nutrients, texture, oil yield, and tree age at different Sandalwood study sites. NB: The circled clusters in the biplot represent the provenances.

cis, beta bisabolene, alpha santalol, cis-alpha-copaene-8-ol, isopropyl myristate, and isopropyl palmitate (Table 5 and 6).

2.7. Z-Alpha-Trans-Bergamotol. The compound was found present in all sampled provenances except Ol Donyo Sabuk. Oil extracted from Baringo provenance had the highest concentration of 3.73%, followed by Homabay 2.20% and Loita 1.89%. There was no significance difference ($p = 0.34$) among the oil extracted from provenances which were sampled.

2.8. Alpha Bisabolol. The highest concentration of this compound was 0.87% found in Loita. This compound was absent in Kitui, Maralal, Namanga, and Ol Donyo Sabuk. There was no significant difference ($p = 0.34$) among oil extracted from the sampled provenances.

2.9. Cis Lanceol. The compound was present in all sampled provenances. The highest concentration was 8.12% found in Chyulu, followed by Homabay at 7.20%, and the lowest was 1.15% from Maralal provenance. However, there was no significant difference ($p = 0.41$) among oil extracted from the sampled provenances.

Table 5: The relative presence of common compounds in oil extracts from 12 Sandalwood provenances in Kenya.

Name of compounds	Baringo	Chyulu	Homabay	Kitui	Loita	Maralal	Marsabit	Mbooni	Muranga	Namanga	Narosura	Ol Donyo Sabuk
Z-alpha-trans-bergamotol	√	√	√	√	√	√	√	√	√	√	√	×
Alpha Bisabolol	√	√	√	×	√	×	√	√	√	×	√	×
Lanceol cis	√	√	√	√	√	√	√	√	√	√	√	√
Beta Bisabolene	√	√	√	√	×	√	√	√	√	√	√	√
Beta santalol	×	√	×	×	×	×	×	×	×	×	√	×
Alpha santalol	√	×	×	×	×	×	×	√	×	×	×	√
Cis-alpha-copaene-8-ol	√	√	√	√	√	√	√	√	×	×	√	√
Isopropyl myristate	√	√	√	√	√	√	√	√	√	√	√	√
Isopropyl palmitate	√	×	√	√	√	√	×	√	√	√	×	×
Total no. of compounds	8	7	7	6	6	6	6	8	6	5	7	5

Source: GC- MS analysis. Compound presence (√) or absence (×) in oil extracts from sampled provenances in Kenya.

Table 6: Mean% concentration of most abundant compounds across different provenances in Kenya.

Provenances	Bergamotol	α-bisabolol	Lanceol cis	β-Bisabolene	β-santalol	α-santalol	cis-alpha-copaene-8-ol	Isopropyl myristate	Isopropyl palmitate
Baringo	3.73	0.4	4.57	0.37	0	4.85	0.03	0.57	1.18
Chyulu	0.33	0.13	8.12	0.13	0.14	0	0.63	0.24	0
Homabay	2.2	0.08	7.2	0.19	0	0	1.26	1.76	0.38
Kitui	0.12	0	2.64	0.09	0	0	0.1	0.23	4.42
Loita	1.89	0.87	4.9	0	0	0	2.44	0.37	0.48
Maralal	0.28	0	1.15	0	0	0	0.48	0.27	0.44
Marsabit	0.23	1.05	3.32	0.05	0	0	5.51	0.39	0
Mbooni	0.003	0.31	1.48	1.06	0	0.3	1.26	0.58	0.26
Murang'a	0.027	0.37	3.37	1.11	0	0	0	0.62	0.99
Namanga	0.027	0	1.49	0.04	0	0	0	0.53	0.16
Narosura	0.47	0.09	4.96	0.36	1.78	0	0.99	0.21	0
Ol Donyo sabuk	0	0	3.56	0.09	0	0.08	1.76	0.76	0
$p = 0.05$	$p = 0.34$	$p = 0.34$	$p = 0.41$	$p = 0.34$	$p = 0.001$	$p = 0.002$	$p = 0.67$	$p = 0.20$	$p = 0.15$

Source: GC-MS Analysis

2.10. Beta Bisabolene. The highest concentration of this compound was 0.37% found in Baringo provenance, followed by Narosura 0.36%. The compound was absent in oil extracted from Loita and Maralal provenances. However, there was no significant difference ($p = 0.34$) among oil extracted from the sampled provenances.

2.11. Alpha Santalol. This compound was only present in three provenances, namely, Baringo (4.85%), Mbooni (0.30%), and Ol Donyo Sabuk (0.08%). There was a significant difference ($p = 0.001$) among oil extracts from the sampled provenances.

2.12. Cis-Alpha-Copaene-8-Ol. The compound was present in all sampled provenances except Murang'a and Namanga. The highest concentration was 5.51% in Marsabit, followed by 2.44% in Loita provenances. There was no significant difference ($p = 0.67$) in all oil extracts from sampled provenances.

2.13. Isopropyl Myristate. The compound was present in all oil extracts from sampled provenances. The highest concentration was 1.76% in Homabay and the lowest 0.21% in Narosura. There was no significant difference ($p = 0.15$) in all oil extracts from sampled provenances.

2.14. Isopropyl Palmitate. The highest concentration was 4.42% in Kitui. The compound was absent in Chyulu, Marsabit, Narosura, and Ol Donyo Sabuk provenances. There was no significant difference in oil extracts from all sampled provenances.

2.15. Beta Santalol. This compound was not among the common and abundant compounds, it was only detected in Narosura (1.78%) and Chyulu (0.14%) provenances.

All sesquiterpenes in sandalwood oil are valueable. According to Arn and Acree [28], β bisabolene belongs to organics known as sesquiterpenoides which are terpenes with 3 consecutive isoprenes units used in fragrance

industries. Oduor intensive compounds include α santalol which play crucial role in perfumery industries [29]. Jirovetz [30] revealed that α and β santalol have antimicrobial activities against klebsiella pneumonia and α santalol induces apoptosis in human prostate cancer cells [31]. Copaene is an attractant synergistic with quercetin, a flavor used as an ingredient in dietary supplements contained in beverages and foods [32]. According to Christian [33], isopropopyl myristate is a pollar emollient used in cosmetics and topical pharmaceutical preparations for dry skin moisturizers. Isopropyll palmitate is a fatty acid ester used as a treatment for head lice, in flea, and tick killing products for pets [34]. Isopropopyl myristate is also used as a mouthwash disinfectant to remove bacteria from oral cavity [34]. This fatty acid ester is also used in the perfumery and pharmaceutical industries as an emollient, emulsifier, and plasticizer physical stabilizer in preparations of products such as creams, lotions, and eye makeups, [32]. According to Sharifi [35], Bergamotol, cis lanceol, and α-bisabolol are sesquiterpene alcohols and they inhibit antifungal activity against *Onychomycosis, Candida albicas,* and *Cryptococuccus neoformans.* These sesquiterpenoids compounds have anti-inflamatory, tissue-remodelling effects, and aromatherapy treatment, hence their wide use in pharmaceutical and perfumery industries.

Tables 5 and 6 below show the presence/absence and relative abundance of the most common compounds for oil extracted from 12 *Osyris* provenances in Kenya.

A similar study conducted on the quality of Osyris oil from roots, stems, and barks from Gachuthi (humid) and Kibwezi (semihumid) areas by Gathara [36], revealed that concentration of sesquiterpenes in extracted oil varies between sites. Other similar studies by [11] on oil yield from different populations of *Osyris lanceolata* (African sandalwood) in Tanzania results were as follows: Bereko (7.25%), Gubali (8.45%), Lushoto (6.26%), Image (3.42%), Nundu (7.87%), and Sao Hill (7.25%). The percentage composition of santalol was Bereko (32.17%), Gubali (6.14%), Lushoto (12.09%), Image (2.18%), Nundu (2.29%), and Sao Hill (1.59%). Another similar study by Gathara [36] on *Osyris lanceolata* oil yield among lowland (Kibwezi) and highlands (Gachuthi) populations in Kenya oil yield in roots (0.043%), stems (0.034%), and barks (0.044%) at Kibwezi, whereas in Gachuthi yield in roots was (0.031%), stems (0.017%), and barks (0.030%). The percentage composition of α santalol in Kibwezi showed roots having (35.21%), stems (0.034%), and barks (14.24%), whereas β santalol in roots was (10.11%), stems (2.15%), and barks (6.13%) in Kibwezi. At Gachuthi, α santalol results showed roots (0%), stems (5.09%), and barks (0.6%), whereas β santalol in roots (0.31%), stems (0.47%), and barks (0%). This conforms with the current study, where the highest oil yield in stems was in Loita (3.56%) and the least at Narosura (1.5%). The constituents of α santalol were highest in Baringo (4.85%) and β santalol in Narosura (1.78%). The study further revealed that diverse concentrations of sesquiterpenes may be related to environmental factors, genetic differences, and tree age.

The objective of the current study was to support a programme on breeding, domestication, and conservation of *Osyris lanceolata* from its natural habitat to a farm in Kenya. Similar work done by Page [37] on *Santalum austrocaledonicum*, identified superior germplasm which was used in domestication work in Vanuatu. The oil quality results shows that Baringo, Mbooni, and Ol Donyo Sabuk populations can be selected for domestication as unique populations with Alpha santalol, while Narosura and Chyulu provenances can be targeted due to Beta santalol. However, the results of this study are based on samples collected in natural stands, and studies on planted Osyris should be done to confirm that these unique characteristics are maintained in established plantations. In addition, studies on younger Osyris woody samples ranging from18 years and below should be done for comparison of oil yield and quality to guide on the optimal age of rotation. The studies should also focus on how ecological and soil characteristics influence the volume of the heartwood to provide additional information to support the domestication of the species. The current study was not able to sample the population on a farm as domestication is on the infancy stage in Kenya. Research on domestication of Osyris populations in Kenya should be conscious of challenges of genetic fragmentation [37] of Osyris across varying AEZs. The study shows that the natural populations in Kenya have a lower oil yield than those of Tanzania (11) and are of inferior quality compared to *Santalum album* and *Santalum austrocaledonicum* [37, 38]. It is therefore recommended that the domestication process would benefit from the introduction of Osyris populations from Tanzania and germplasm from populations of genus Santalum from the Asian continent to Kenya in order to shift focus from the wild Osyris populations to high-value sandalwood grown on farms.

3. Conclusion

In this study, soil nutrients varied significantly across the study sites, though there was no correlation between Osyris trees oil yield and most soil variables. These results of soil analysis show that Osyris can be planted in a wide range of soil types across different agro-ecological zones. The age of Osyris trees influenced the oil yield weakly but significantly, though the yield did not vary significantly amongst provenances. Loita provenance recorded the highest oil yield (3.56%), followed by Baringo (3.49%), whereas Narosura recorded the lowest yield (1.5%). Osyris trees which recorded high oil yield fall under agro-ecological zones (AEZs) III and IV. The GC-MS oil quality results recorded nine common and most abundant compounds across the study sites which includes Z-alpha-trans-bergamotol, alpha bisabolol, lanceol cis, beta bisabolene, alpha santalol, beta satalol, cis-alpha-copaene-8-ol, isopropyl myristate, and isopropyl palmitate. These compounds are used as a phytochemical composition of commercial essential oils which are responsible for flavors and fragrances industries. However, the superiority 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. In this study, alpha santalol was detected in oil extracts from Baringo, Mbooni, and Ol Donyo Sabuk provenances and

concentration varied significantly. Beta santalol was only detected in Narosura and Chyulu provenances. Based on the foregoing Baringo, Mbooni, Ol Donyo Sabuk, Narosura, and Chyulu provenances could offer the best germplasm for on-farm propagation and domestication.

Data Availability

Data supporting the development of this manuscript can be availed by the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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