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The phytochemical profiles and growth of *Prunus africana* in Kenya

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Abstract. *Nyamai DW, Burugu MW, Ng'ang'a MM, Muchugi AN. 2022. The phytochemical profiles and growth of* Prunus africana *in Kenya. Asian J Nat Prod Biochem 20: 63-74.* African mountain ranges are home to the evergreen tree *Prunus africana* (Hook.f.) Kalkman. Benign prostate hyperplasia can be treated with the bark or extracts from the bark of this plant. The primary goal of this research was to compare the domesticated stand at Muguga (Kenya) to samples taken from a wild stand in Kobujoi and a remnant on-farm stand in Karuri in terms of their growth characteristics and phytochemical profile. A variety of solvents, including water, hexane, dichloromethane, and methanol, were used in the compound extraction process. The phytochemical analysis was performed with the help of Liquid Chromatography and Gas Chromatography-mass spectrometry. The GC Chemstation version 11 program was used to examine the data from the GC-MS. The trees in the Muguga tamed stand varied in height from 3 meters to 14 meters, with a corresponding range in circumference at breast height from 0.9 centimeters to 104.5 centimeters. A total of 273 trees were planted in the orchard, and 92 (33%) produced fruit when the data was collected. When comparing the raw organic extract yields of the three groups, there was no statistically significant difference ($p>0.05$). Myristic acid, linoleic acid, lauric acid, methyl myristate, methyl laurate, and methyl linoleate made up the bulk of the essential oils in the bark samples across all three sources. For men with benign prostatic hyperplasia (BPH), these substances reduce cholesterol in the prostate. It was shown that dichloromethane and hexane extracts of the three populations included Campesterol, β-sitosterol, lup-20(29)-en- 3-one, palmitic acid, β-sitostenone, (3.β., 5.α)- stigmast-7-en-3-ol, stigmastan-3,5-diene, and α-tocopherol. Increased urine output and decreased prostaglandin synthesis in the prostate are effects of (3.β., 5.α)-stigmast-7-en-3-ol, β-sitosterol, and β-sitostenone. Cyanidin-o-galactoside, cyanidin-3-o-rutinoside, procyanidin B5, and robinetinidol-(4-α-8) catechin-(6,4-α)robinetinol are thought to have anticancer effects by inhibiting cell proliferation and scavenging free radicals in cancer cells. Evidence suggests that ursolic acid can reduce inflammation, prevent cell damage, and slow the growth of BPH. The essential oils of the Karuri people had noticeably ($p<0.05$) more myristic and lauric acids than those of any other population. Myristic acid, linoleic acid, methyl myristate, and α-tocopherol concentrations all varied significantly ($p<0.05$) between the Muguga population and the Karuri and Kobujoi populations. The findings show that the phytochemical composition of *P. africana* is not significantly changed by domestication ($p>0.05$), allowing for its cultivation in agricultural settings. Strategies for the sustainable collection, management, and conservation of this species through cultivation can be derived from the morphological and phytochemical data, which has crucial significance for these areas.

Keywords: Growth, phytochemical profile, *Prunus africana*

INTRODUCTION

In addition to its impressive height (more than 40 meters) and trunk diameter (up to 1 meter), the evergreen *Prunus africana* (Hook.f.) Kalkman also impresses with its broad crown (Gachie et al. 2012). Its leaves are glossy, its bark is dark brown, and its blossoms are either green or white. Although *P. africana* has a wide distribution, it is confined to the higher elevations (more than 1500 m asl.) of the Afromontane forest islands of Africa (Somalia, South Africa, Kenya, Tanzania, Sudan, Uganda, Malawi, Ethiopia, Zaire, Cameroon, Zimbabwe, Angola, and Malawi) and underlying islands like Sao Tome' and Madagascar (Hall et al. 2000). The species prefers moist, tropical, or subtropical environments. The tree has several uses in Africa, including construction materials and traditional and modern medicine.

Phytochemicals found in the stem and root bark extracts have been shown to have anticancer, anti-inflammatory, and antiviral properties (Vincenti et al. 2013; Tamasi et al. 2021). Prostate cancer patients who take bark extracts benefit from an apoptotic and antiproliferative action on the prostate, reducing their urologic symptoms (Kadu et al. 2012). Traditional practitioners have used the bark to cure various ailments, including heart problems, urinary tract infections, stomach discomfort, renal disorders, and malaria. Chewing or grinding the bark into a powder to make tea are two common consumption methods (Stewart 2003). In response to distinct geographical patterns, plant species exhibit wide ranges in chemical composition due to environmental changes.

Research conducted in Cameroon, Madagascar, and Zaire (R.D. Congo) revealed that the chemical makeup of *P. africana* extracts varies by habitat (Gachie et al. 2012). Analysis of nuclear and chloroplast DNA markers in *P. africana* reveals five unique African zones, reflecting the continent's diverse human population (Kadu et al. 2011). Only the volcanic highlands and mountains of "High Africa," an area spanning 34°S to 12°N, are home to this species. Its range in equatorial Africa spans from about 1,000 to 3,500 meters in altitude, where annual precipitation averages more than 3,000 millimeters (mm) at lower elevations and between 500 and 700 mm (mm) at higher ones (Kadu et al. 2011). Below the montane zone,

this species mostly find in rocky places and along drainage lines (Hall et al. 2000).

Because of the strong demand for *P. africana* bark extracts, the species' wild population is in significant danger (Cunningham et al. 1997). Herbal remedies for the treatment of BPH are manufactured with more than 3,000 tons of bark and bark extracts exported annually to Europe (Cunningham et al. 1997). The wild population of *P. africana*, the primary bark source, suffers greatly due to the increasing demand. A number of countries, including Kenya, are moving toward *P. africana* cultivation. Regeneration of *P. africana's* bark is possible so long as it is removed without damaging the tree's vascular cambium (Cunningham and Mbenkum 1993).

The *P. africana* is surprisingly resistant to debarking. However, large-scale debarking strains the tree even when full re-growth occurs, and in arid locations, bark re-growth is limited, to begin with. *P. africana* faces a greater threat due to its restricted distribution to a few Afromontane islands and the increased removal of land for agricultural purposes. Due to rising demand on a global scale, the species was included in Appendix II of CITES during its ninth meeting, making it legally protected as an endangered species (Betti 2008). Furthermore, the IUCN Red List has placed this species in a vulnerable conservation position.

Even though there are still control concerns and enforcement issues, it is necessary to find and implement conservation and domestication strategies as part of a sustainable management plan. Knowledge of regional variation is crucial for maximizing the sustainable conservation of the *P. africana*. This research set out to compare the growth rates, phytochemical profiles, and yields of *P. africana* bark samples collected in Muguga, Kobujoi, and Karuri. Finding no statistically significant variations in these features, the results of this study will aid in the sustainable management of domesticated species. This information will also be useful for determining when it is best to gather bark and develop conservation plans for *P. africana* in the wild and on farms.

MATERIALS AND METHODS

Study site

Located in Muguga, Kenya, the *P. africana* stand was planted by the World Agroforestry Centre. The coordinates (10°14′ S 360°38′ E) for the Muguga Regional Research Centre in Kiambu County are as follows: The elevation of Muguga is around 2,150 m asl., and the town receives about 1,200 millimeters of rain a year. The Muguga population is a cultivated stand of *P. africana* maintained through regular trimming and undergrowth removal. An on-farm remnant stand of *P. africana*, the Karuri population, is intercropped with food crops. Kiambu county is home to the Karuri and Muguga sites. Hence they have similar climates. On a farm, Karuri trees were interplanted with edible ones. The *P. africana* population in Kobujoi is located in a natural forest in northern Nandi, where it coexists with a wide range of other plant species.

Sample collection

The *P. africana* forest in Muguga provided samples of stem bark for phytochemical study. At the height of 1.3 meters off the ground, debarking was accomplished with the help of a panga that had a serrated edge. Stem bark samples were taken as a standard from Kobujoi, Nandi, and Karuri, all of which are remnant forests on farms. Five trees were chosen randomly from each population for the debarking process. The direction of the bark removal was randomized across four cardinal directions (East, West, North, and South) to prevent any potential for bias. The study did not include trees with a breast height diameter of fewer than 20 centimeters. Labels were placed on each sample after the wet weight was determined with an automated weighing scale.

Stem form, Diameter at Breast Height (DBH), tree height, and fruit set were all considered. Trees were measured in meters in height and scored on a scale from 1 to 5 for their stem shapes. In order to evaluate the growth rate of domesticated *P. africana*, we measured the diameter of the trees both at breast height and full height. We used the ring method to calculate tree ages by measuring ring widths with the help of the TSAP-WinTm program. A DBH meter was used to measure the circumference of the tree at chest height, and the results were recorded in cm. The DBH refers to a tree's circumference at the height of 1.3 to 2 meters. The DBH measurements in this investigation were performed from a height of 1.3 meters. A Suunto optical height meter was utilized to measure the height of individual trees. Tree heights served as a categorization key for the morphological data. It was assumed that all of the trees in the Muguga stand were planted simultaneously.

Preparation of tree rings for age determination

Cross-sections of trees that had been chopped down as part of a thinning effort in the Muguga forest were analyzed for their annual growth rings. All ring samples were labeled, and cross sections were cut with a chain saw at a height of 1.3 meters. Because the number of tree rings decreases with increasing height, the optimal height for ring counting was set at 1.3 meters. After the samples were cured, they were prepared for inspection using an orbital power sander to remove scratches and smooth the surface. After that, the surfaces were hand-sanded with increasingly finer grits of sandpaper (80, 120, 180, 240, and 360) to make the growth rings more apparent. Each ring's boundary was identified, and the rings were counted and marked starting from the outermost. The ring's width was observed using a pair of binocular microscopes and then measured with a computer program called TSAP-WinTm. To prevent under or over-estimation of ring width, samples were continuously modified to guarantee ring width was measured perpendicularly. The ring's breadth was measured at two different radii.

Visual cross dating

Rings can be dated to the exact year of their creation through a procedure called "*cross-dating*" (Stokes and Smiley 1968; Schweingruber 2007). The effect of

environmental conditions on the growth pattern of trees can be seen by accurate cross-dating (Worbes 1995). Using the skeleton plot method, we could visually cross-date samples by comparing the patterns of narrow and wide rings (Stokes and Smiley 1968). On the skeleton plot, the width was used to assign values between 1 and 10 to the rings, where 1 represents the narrowest ring, and 10 is the widest. Then, the rings were compared, and the narrowest ones, most indicative of the dry season, were given values close to 10. In this study, we only scored the widest rings (with a B) and ignored the average ones (Stokes and Smiley, 1968). The patterns of the wide and narrow widths must coincide with obtaining the composite skeleton map that enables dating the rings to the year they were produced (Stokes and Smiley 1968). After measuring ring width and performing visual cross-dating, the resulting data was saved on the computer in Raw picture format.

Determination of tree density

Randomly selected trees in Muguga had cross-sections taken at their DBH to collect samples for the density calculation. First, each sample's raw weight was determined with the help of an electronic balance. Then, the displacement technique was used to calculate the wet volume using water as the liquid. After drying the samples for 24 hours at 100°C, their dry weight and volume were immediately calculated. Next, the weight of the wood sample was determined by placing it in a water-filled measuring cylinder and then calculating the volume of water displaced. Finally, the density was calculated using Archimedes' principle based on the samples' dry weight and volume.

Reagents and reference compounds

Unless otherwise specified, all chemicals and solvents used were acquired from Sigma Aldrich Chemical Company limited in California, USA. Unless otherwise specified, all chemicals and reagents used were of a purity between 95% and 100% and were obtained from Sigma Aldrich Chemical Co. Ltd. Sigma Aldrich Chemical Co., Ltd. was sourced for both the crisofulvin and 1-heptene standards. Lupeol standard utilized was isolated from *Fagara tessmanii* by Ivan Addae-Mensah (University of Ghana).

Sample preparation

After being harvested, the bark samples were dried in a dark place for a month to reduce their moisture content. Next, the dried samples were sliced into smaller pieces and powdered using a fine grinder. Powdered bark from the Muguga, Karuri, and Kobujoi trees weighed 400 g and was soaked in hexane, dichloromethane, and methanol in that order for 24 hours. The extraction process required 700 ml of each solvent. After that, Whatman filter no. 1 was used with a vacuum suck pump to filter the extracts. The filtrate was then concentrated using a rotary evaporator in a vacuum at 40°C and at a decreased pressure. Next, each extract was steeped in distilled water before being boiled in a water bath at 60°C for 5 hours after the organic solvent extraction. Next, the aqueous extracts were filtered through a No. 1 Whatman filter and concentrated in an SP Scientific AdVantage 2.0 benchtop lyophilizer.

Extraction of essential oils

Each powdered bark sample was weighed at 300 g and placed in a distillation flask. Each bark sample was boiled with 1.5 liters of distilled water. After boiling, the heat was reduced to 70°C and kept at that level for two hours. To help dissolve the essential oils as they condense, 5 mL of hexane was added to the Clevenger apparatus. The condenser was cooled to between -15 and 15°C. The samples were dissolved in hexane and then placed in vials. The materials were distilled, concentrated using a short path distillation device, and extracted using one milliliter of dichloromethane.

Preparation of extracts for GC-MS analysis

Samples were weighed at 1 mg and placed in a 1.5 mL Eppendorf tube. Each hexane and dichloromethane extract was dissolved in 1 mL of dichloromethane and vortexed for 30 seconds. After centrifuging the samples at room temperature for five minutes at 1300rpm, they were sonicated with a Branson 2510E- DTE sonicator. The materials were then placed in sample vials with a capacity of 2 mL.

Instrumentation and chromatographic conditions

Gas chromatography research entails crucial optimization steps like (i) introducing sample extract into the GC column, (ii) separating components on an analytical column, and (iii) detecting target analysis utilizing a mass spectrometer detector. Using an HP-5 phenyl methyl siloxane capillary column that was 30 meters long, 320 μm in diameter and 0.25 μm in film thickness, GC-FID analysis on a GC-FID (Model: 7890B Agilent) that consisted of an auto-sampler and gas chromatograph interfaced to a Flame Ionization Detector was performed. An electron ionization device with an ionization energy of 70Ev was employed for GC-FID detection. Hydrogen (99.99%) was used as the carrier gas at a constant flow rate of 30 mL/min; the injector temperature and mass transfer line temperature were set to 270 and 280°C respectively; an injection volume of 1 μL was used (splitless); and the oven temperature was programmed from 35°C (isothermal for 5 min), with an increase of 10°C/min to 280°C for 5.4 min, 50°C/min to 285°C for 35 min.

Gas chromatography – Mass Spectrometry analysis

To determine the concentration of hexane and dichloromethane extracts in the GC-MS, lupeol was utilized as an internal standard. Using 1-heptene as an internal standard, GC-MS also identified and quantified essential oils. Before evaluating the extract using Gas Chromatography and Mass Spectrometry, the oven temperature, gas flow rate, and electron gun were initially programmed. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed using a GC-MS (7683 Agilent Technologies, Inc., Beijing, China) instrument with a gas chromatograph interfaced to a mass spectrometer and an HP-5 MS (5% phenyl methyl siloxane) low bleed

capillary column of 30 m length, 0.25 mm diameter, and 0.25 µm film thickness. An electron ionization device with an ionization energy of 70Ev was employed for GC-MS detection. For this experiment, helium (99.99 %) was utilized as the carrier gas at a constant flow rate of 1.25 mL/min, the injector temperature was set to 250°C, the mass transfer line temperature was set to 200°C, and the injection volume was 1 µL (splitless mode). The oven was set to preheat to 35°C for 5 minutes, rise by 10°C per minute to 280°C for 10.5 minutes, and finally, rise by 50°C per minute to 285°C for 29.9 minutes for a total run time of 70 minutes.

The following were the MS's settings: Interface temperature was 250°C; ionization energy was 70eV; ion source temperature was 230°C; solvent cut time was 3.3 minutes; relative detector gain mode; scan speed was 1666 µ/sec, and the scan range was 40-550 m/z. A total of 70 minutes were devoted to GC-MS operation. As a result of peak area normalization, the extract's relative % was calculated. To verify the accuracy of the data, it was compared to the compounds in a small library of chemicals maintained by the National Institute of Standards and Technology.

Identification of components

The computed fragments, molecular mass, and molecular structure were all used for identification. An explanation of the Mass Spectrum NIST's database of more than 62,000 patterns was used for the GC-MS analysis. The test materials' constituents, molecular weights, and chemical structures were identified. Library-based mass spectrometry queries were conducted on the NIST 05, Chemecol.L, NIST 11, and Adams 2.l databases. To aid in the GC-MS data system's characterization, the NIST mass spectral search programmed version 2.0 was employed. Quantification was performed by adding an internal standard to the region.

Liquid Chromatography-Mass Spectrometry analysis

Preparation of samples for LC-MS

Sample extracts were weighed in milligrams after being placed in a 1 mL Eppendorf with methanol and water. Each sample extracted with methanol had one milliliter of the methanol added to it to be re-dissolved. Dissolving aqueous extracts in a mixture of 95% methanol and 5% distilled water. After 30 seconds, the samples were vortexed again. Five minutes of sonication were performed using a Branson 2510E-DTE sonicator. Afterward, the samples were centrifuged at room temperature for 5 minutes at a speed of 1300 rpm before being poured into 1.5 mL vials. Liquid chromatography and mass spectrometry were used to examine a methanol and water extract.

LC-MS analysis

An HP 1100 capillary system equipped with an auto-sampler and a micro-pump (Agilent Technologies, Incorporation, Beijing, China) was used for the HPLC separations. In LC-MS, chemical concentrations were determined using griseofulvin as an internal standard. The chemicals were separated utilizing a Waters symmetry column, 100 µm 2.1 mm, 3.5 µm. All analyses were performed with an injection volume of 2 µL and a temperature compartment of the auto-ampler set to 4°C. Water (component A) and methanol (component B) made up the mobile phase. The solvent gradient was started at 10% B and held for 30 minutes, then programmed to 50% in 3 minutes and held for 5 minutes, then to 100% and held for 10 minutes at a flow rate of 200 L/min. To avoid contaminating your ESI source, you should send the LC's effluent from the first five minutes before analysis to the drain. The data was analyzed using ESI-MS with the positive ion mode. In order to determine the concentration of griseofulvin as an internal standard and to identify any pentacyclic triterpenoids in the extracts, we consulted the METLIN metabolite data base and reviewed the relevant literature.

Statistical analysis

Before combining the growth curves of the trees into a chronology, the ring width data were analyzed with COFECHA software to help identify the issue segments and verify cross-dating (Grissino-Mayer 2001). Winks version 7 software was used to examine data gathered from both wild and domesticated populations. The average chemical compounds of the five trees in each group were computed to provide a context for their interactions. Tukey's student-centered honestly significant difference (at the 5% significance level) was used to distinguish between the means. Variation was standardized by calculating the coefficients of variation within the population. The relationships between the various components, as well as those between the components and the tree's size and the surrounding environment, were also determined. The population differences were examined using an ANOVA test. Finally, the association between tree age and growth rates, wood density and DBH, ring number, and DBH was calculated using a Pearson's correlation analysis.

RESULTS AND DISCUSSION

Morphological characterization of *Prunus africana* trees at Muguga stand

Table 1 displays the results of an analysis conducted on the morphological data of *P. africana* trees in the Muguga stand based on the categorization of tree heights. That the trees were all the same age was based on the assumption that they had been planted simultaneously. Only two trees out of the whole population were less than 5.5 meters. In total, 146 trees range from 5.6 meters to 10.5 meters tall. The third cluster included 124 trees, ranging in height from 11.6 to 15 meters. There was a statistically significant ($p>0.05$) difference between group one and the other height groups in the Muguga stand. The stems from each group looked identical. The average DBH of the three groups increased with increasing height. Both DBH and height can be used as indicators of development.

The average DBH across all three categories was between 9.45 and 54.85 inches. The mean diameter at breast height was substantially smaller in group one compared to the other two groups ($p<0.05$). The percentage

of trees in a given group that had reached fruition was reported. Both of the Group 1 trees were still too young to bear fruit. Group 2 consisted of 146 trees, 40 of which (26.4%) were in the fruiting stage and 106 (72.6%) without fruits. Refer to appendix 43 for a blueprint of the Muguga *P. africana* display. The placement of trees in the stand is depicted here. On the West side of the plantation, trees near the *Grevillea robusta* border were subjected to longer times of shade than those near the grass border.

Visual and statistical cross dating

First, the skeleton plot technique was used for visual cross-dating in this investigation (Stokes and Smiley, 1968). Wedging ring detection was achieved by utilizing two radii on the cross sections. Fifteen of the trees out of twenty-three were correctly cross-dated, and the results showed an age range of fifteen to twenty-six years.

Relationship between age and growth rate in Muguga population

Table 2 displays the ages and growth rates (in millimeters) of seven randomly selected trees in Muguga. A non-significant relationship between age and average growth rate was found using Pearson's correlation analysis for trees randomly selected from the Muguga population (t = -0.916, DF= 16, r2=0.0498, p>0.05). There was also a negative relationship between age and development rate (r = -0.223).

Relationship between ring number and DBH of trees from Muguga population

The link between the number of rings in a tree's trunk cross section and its DBH was not statistically significant in a regression and Pearson's correlation study of trees randomly sampled from the Muguga population (DF= 14, r2=0.0858, p>0.05) (Figure 1). However, a positive correlation (r = 0.293) was found between the number of growth rings in the trunk cross-section and the DBH of the chosen trees.

Relationship between wood density and DBH of trees from Muguga population

Trees were randomly sampled from the Muguga population, and their wood density and DBH were analyzed for correlation and regression. However, the results indicated no statistical significance (DF=16, r2=0.0196, p>0.05) (Figure 2). There was a negative relationship between the density of wood section samples and tree DBH in the Muguga population (r = -0.140).

Relationship between wood density (g/cm^3) and growth rate (mm)

Wood sample density and growth rate of trees randomly selected from the Muguga population did not correlate significantly (DF=14, r2=0.0279, p>0.05) (Figure 3), according to a Pearson correlation and regression analysis. Muguga population tree growth rate was favorably linked with wood density (r = 0.167).

Crude yield extracts of individual populations

Hexane, dichloromethane (DCM), methanol, and water were used to extract bark samples from all three populations. Table 3 details the organic solvent yields. The crude bark extracts from methanol, DCM, and hexane did not differ significantly (p>0.05) amongst the three groups. Methanol crude extract yield was highest for the Karuri population and lowest for the Kobujoi population. The crude extract yields of DCM and hexane were highest for the Kobujoi population, whereas the Karuri population yielded the least. All three populations obtained their maximum yield from methanol extract.

Table 2. Relationship between age (years) and growth rate (mm)

Age (years)	Growth rate (mm)	t value	DF	r^2
1	3.53±1.69	-0.916	16	0.0498
2	3.42±1.96			
3	2.38±1.98			
4	1.82±1.14			
5	2.67±2.05			
6	1.38±0.71			
7	1.06±0.27			
8	2.07±1.84			
9	1.69±0.99			
10	4.78±3.08			
11	2.26±1.37			
12	2.36±1.13			
13	3.18±1.36			
14	1.80±0.73			
15	3.38±1.98			
16	2.00±0.75			
17	1.22±0.70			
18	1.56±0.68			

Note: Values are expressed as Mean ± SD (n=7).

Table 1. Growth characteristics of trees at Muguga (Kenya) *Prunus africana* stand

Groups (height)	No. of trees	Height (m)	Stem shape rating	DBH(cm)	Fruiting	
					Yes	No
Group 1 (0-5)	2	4.20±1.70^a	1.50±0.71^a	9.45±12.09^b	0	2 (100)
Group 2 (6-10)	146	9.22±1.16^b	1.53±0.60^a	40.17±12.19^a	40 (27.40)	106 (72.60)
Group 3 (11-15)	124	11.67±0.80^b	1.63±0.64^a	54.85±16.26^a	52 (41.94)	72 (58.06)

Note: Values are expressed as Mean ± SD (n=3). Values followed by the same super script in the same columns are not significantly different (*p*>0.05). Values in parenthesis after groups show the tree heights in each group. Values in parentheses in the fruiting column are expressed as percentages

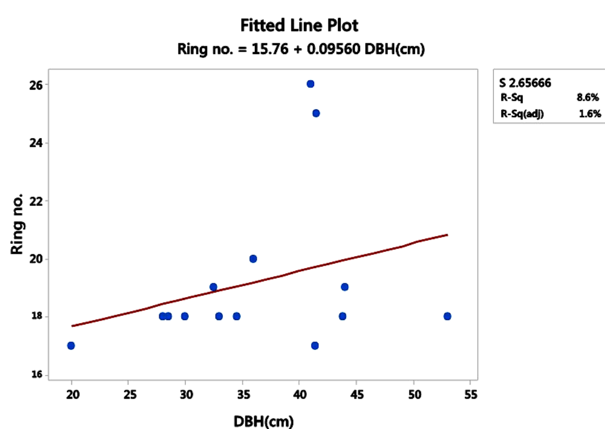

Figure 1. A fitted line plot of ring number versus DBH (cm)

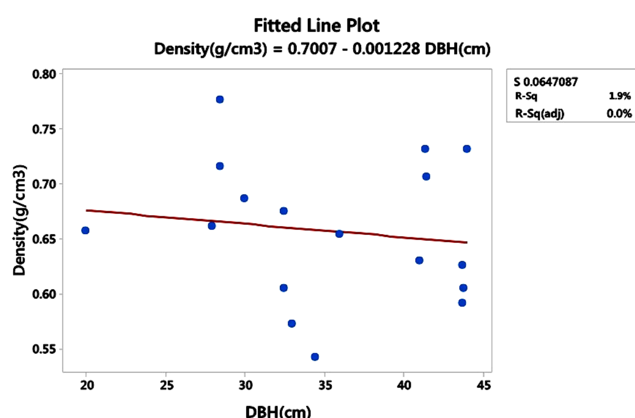

Figure 2. A fitted line plot of wood density (g/cm^3) versus DBH (cm)

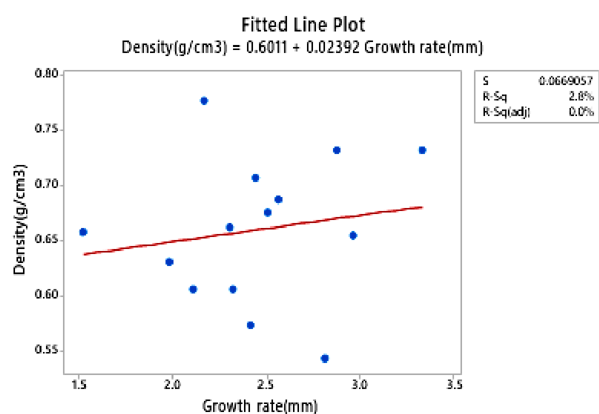

Figure 3. A fitted line plot of density (g/cm^3) versus growth rate (mm)

Table 3. Crude yields (g) for organic extracts

Population	Methanol	DCM	Hexane
Muguga	3.58±2.38^a	0.59±0.55^a	0.56±0.08^a
Karuri	4.94±0.50^a	0.88±0.41^a	0.46±0.11^a
Kobujoi	2.79±2.97^a	0.90±0.47^a	0.82±0.74^a

Note: Values are expressed as Mean ± SD (n=3). Values followed by the same super script in the same column are not significantly different (*p*>0.05)

Phytochemical yields in the three populations

Total essential oils yields in Muguga, Karuri, and Kobujoi populations

Essential oils from each of the three groups were analyzed for their phytochemical composition using GC-MS. The analysis showed that all three groups contained linoleic acid, lauric acid, methyl laurate, methyl linoleate, methyl myristate, and myristic acid. Table 4 provides a breakdown of polyunsaturated fatty acids and their methyl esters. The essential oil composition of the Muguga showed the highest content of linoleic acid, methyl linoleate, and methyl myristate. Essential oil composition showed the highest quantities of myristic acid and lauric acid in the Karuri people and the highest concentration of methyl laurate in the Kobujoi population.

Essential oil samples from Muguga, a domesticated stand, had a considerably higher linoleic acid content than those from Karuri and Kobujoi (p<0.05). When comparing, Kobujoi has the lowest linoleic acid content, and Muguga has the greatest. There was a statistically significant difference (p<0.05) between the lauric acid concentration in the Karuri samples and that in Kobujoi and Muguga samples. Lauric acid was found at the highest concentration in Karuri (1152.14) and the lowest concentration in Kobujoi (4.12). There was no statistically significant difference (p>0.05) between the methyl laurate concentrations of the three groups. There were statistically significant differences (p<0.05) in the methyl linoleate concentrations of the three groups. There was much more methyl myristate in Muguga than in Karuri or Kobujoi (p<0.05). The levels of myristic acid varied greatly between the three populations, with Karuri having the highest.

Hexane extract yields of Muguga, Karuri, and Kobujoi populations

Hexane extracts from all three populations were analyzed for their phytochemical content using GC-MS, and the results showed the presence of campesterol, β-sitosterol, lup-20(29)-en-3-one, palmitic acid, β-sitostenone, (3.β.,5.α)- stigmast-7-en-3-ol, stigmastan-3,5-diene, and α-tocopherol compounds. Table 5 provides an analysis of these chemicals. The Muguga people possessed the highest concentrations of hexane-extracted chemicals such as campesterol, lup-20(29)-en-3- one, palmitic acid, squalene, β-sitostenone, 3β,5.α-stigmast-7-en-3-ol, stigmastan-3,5-diene, myristic acid, and α-tocopherol compounds. Both lauric acid and beta-sitosterol were found in maximum quantities in Karuri communities. campesterol, lauric acid, β-sitosterol, squalene, lup-20(29)-en-3- one, β-sitostenone, stigmastan-3,5-diene, 3β,5.α-stigmast-7-en-3-ol, palmitic acid, and α-tocopherol contents in hexane extracts from the three groups were not statistically different (p>0.05). However, the levels of α-tocopherol in the Muguga samples varied considerably (p<0.05). Since one of the replicates did not contain lauric acid or myristic acid, the standard deviation for those two chemicals was quite significant.

Table 4. The concentration of essential oils in Muguga, Karuri, and Kobujoi (mg/kg)

Compound	Muguga	Karuri	Kobujoi
Linoleic acid	196.35±3.48[b]	29.13±3.47[a]	28.93±1.98[a]
Lauric acid	382.66±2.61[a]	1152.14±315.29[b]	4.12±1.07[a]
Methyl laurate	2.54±0.41[a]	3.32±0.50[a]	3.36±0.71[a]
Methyl linoleate	27.82±1.28[a]	14.83±1.59[b]	7.51±1.29[c]
Methyl myristate	26.71±0.64[b]	4.59±0.16[a]	5.80±5.20[a]
Myristic acid	287.09±1.36[a]	554.99±22.60[b]	92.84±1.81[c]

Note: Values are expressed as Mean ± SD (n=3). Values followed by the same super script along rows are not significantly different (p>0.05)

Table 5. Concentrations of compounds in hexane extracts of Muguga, Karuri, and Kobujoi (mg/kg)

Compound	Muguga	Karuri	Kobujoi
Campesterol	9.16±1.93[a]	4.51±3.75[a]	6.70±3.29[a]
Lauric acid	2.62±1.18[a]	4.84±7.56[a]	0.72±0.32[a]
β-Sitosterol	131.04±31.34[a]	160.05±3.91[a]	153.36±13.01[a]
Lup-20(29)-en-3-one	13.32±2.81[a]	10.04±5.29[a]	7.97±3.61[a]
Palmitic acid	82.24±30.049[a]	34.28±19.83[a]	55.13±58.46[a]
Squalene	34.25±14.99[a]	26.72±3.18[a]	28.34±19.13[a]
β-sitostenone	36.92±12.05[a]	19.79±0.49[a]	27.80±13.87[a]
3β,5α-Stigmast-7-en-3- ol	15.63±4.71[a]	10.46±4.64[a]	11.23±6.01[a]
Stigmastan-3,5-diene	35.99±11.50[a]	20.42±2.49[a]	27.21±16.94[a]
Myristic acid	7.21±0.50[a]	5.65±5.06[a]	2.02±0.09[a]
α-Tocopherol	13.44±2.71[b]	1.84±1.15[a]	4.88±1.38[a]

Note: Values are expressed as Mean ± SD (n=3). Values followed by the same super script along rows are not significantly different (p<0.05)

DCM extract yields of Muguga, Karuri, and Kobujoi populations

To determine the phytochemical composition of the DCM extracts from the three populations, GC-MS analysis was performed. The results showed the presence of campesterol, β-sitosterol, lup-20(29)-en-3-one, palmitic acid, β-sitostenone, (3.β.,5.α)-stigmast-7-en-3-ol, stigmastan-3,5-diene and α-tocopherol. Analysis of hexane extracts revealed a phytochemical profile comparable to that of DCM extracts. It is because, although being somewhat different in polarity, these two solvents are both non-polar. So they extract the same molecules, albeit at slightly different amounts. All three populations included these chemicals. Table 6 provides a breakdown of these chemicals, which play a significant role in managing BPH.

Campesterol, lup-20(29)- en-3-one, palmitic acid, squalene, β-Sitosterol, β-sitostenone stigmastan-3,5-diene, and myristic acid were found at the highest concentrations among the Muguga community. The lauric acid content was highest in the Karuri population, while the 3-β,5α-stigmast-7-en-3-ol, and α- tocopherol content was highest in the Kobujoi group. DCM extracts from the three populations did not differ substantially (p>0.05) concerning the amounts of campesterol, lauric acid, β-sitosterol, lup-20(29)-en-3-one, β-sitostenone, stigmastan-3,5-diene, squalene, 3-β,5α-stigmast- 7-en-3-ol, palmitic acid and α-tocopherol. Compared to Karuri and Kobujoi samples, the concentration of myristic acid in DCM extracts of Muguga samples was substantially different (p<0.05).

Methanol extract yields of Muguga, Karuri, and Kobujoi populations

Methanolic extracts from all three populations were analyzed for their phytochemical composition by means of LC-MS. The results showed the presence of procyanidin B5, feruloyl-quinic acid, robinetinidol-(4-α-8)-catechin-(6-α)-robinetinol, prunetrin, quercetin3,3'- dimethyl ether-4'-glucoside, cyanidin-o-galactoside, chlorogenic acid, ursolic acid, isochamaejasmin+, cinnamtannin A2, isoliquiritin, and two other substances. Cyanidin-3-o-rutinoside was exclusively found in Karuri population methanol extracts, although isoliquiritin and isochamaejasmin+ were absent. In methanol extracts, the Kobujoi population exhibited the highest quantity of feruloyl-quinic acid, chlorogenic acid, procyanidin B5, quercetin3,3'-dimethyl ether-4'-glucoside, cinnamtannin A2, and isochamaejasmin, as shown in Table 7. Ursolic acid, isoliquiritin, and unidentified chemical 1 were found in methanol extracts at their greatest concentration in the Muguga community. Prunetrin, cyanidin-o-galactoside, and robinetinidol-(4-α-8)-catechin-(6-α)-robinetinol levels in methanol extracts were highest in the Karuri community. There was no statistically significant difference (p<0.05) between the feruloyl-quinic acid, chlorogenic acid, cyanidin-o-galactoside, ursolic acid, procyanidin B5, and unidentified chemical 2 concentrations in methanolic extracts of samples from the three groups. Concentrations of prunetrin in the Karuri population were significantly different from those in the Muguga and Kobujoi populations (p<0.05). Cyanidin-3-o-rutinoside was not found in methanolic preparations of Muguga or Kobujoi. The levels of cinnamtannin A2 in Kobujoi were substantially greater than in the other samples (p<0.05). Isochamaejasmin+ concentrations were substantially different (p<0.05) amongst the three populations, with the chemical being absent from Karuri samples.

Table 6. The concentration of compounds in DCM extracts of Muguga, Karuri, and Kobujoi (mg/kg)

Compound	Muguga	Karuri	Kobujoi
Campesterol	12.55±3.75[a]	7.32±0.56[a]	8.10±2.51[a]
Lauric acid	1.19±0.768[a]	1.85±0.93[a]	1.71±1.24[a]
β-Sitosterol	130.20±72.95[a]	103.59±28.29[a]	117.16±20.85[a]
Lup-20(29)-en-3-one	14.04±1.89[a]	9.99±0.43[a]	8.30±3.502[a]
Palmitic acid	116.63±42.44[a]	65.55±23.54[a]	90.63±67.70[a]
Squalene	34.56±14.55[a]	22.23±6.35[a]	28.34±9.90[a]
β-sitostenone	43.21±15.52[a]	26.51±4.48[a]	30.62±6.73[a]
3β,5α-Stigmast-7-en-3-ol	18.39±7.69[a]	11.59±2.83[a]	19.57±13.93[a]
Stigmastan-3,5-diene	36.83±15.75[a]	26.13±3.66[a]	29.57±11.68[a]
Myristic acid	6.47±0.99[b]	2.89±1.27[a]	3.19±1.40[a]
α-Tocopherol	6.80±1.04[a]	7.67±2.12[a]	11.08±2.44[a]

Note: Values are expressed as Mean ± SD (n=3). Values followed by the same super script along rows are not significantly different (p>0.05)

Table 7. The concentration of compounds in methanol extract from the three populations (mg/kg)

Compound	Muguga	Karuri	Kobujoi
Feruloyl-quinic acid	1.89±0.69[a]	2.21±0.67[a]	2.56±1.45[a]
Chlorogenic acid	2.05±0.82[a]	2.07±1.33[a]	2.36±0.73[a]
Isoliquiritin	7.48±0.65[a]	0.000[b]	7.48±0.18[a]
Prunetrin	1.27±0.62[a]	2.90±0.630[b]	1.20±0.52[a]
Cyanidin-*O*-galactoside	9.87±2.79[a]	10.69±0.25[a]	7.37±0.85[a]
Ursolic acid	2.39±2.04[a]	0.78±0.26[a]	1.57±0.34[a]
Unknown compound 1	16.16±4.93[a]	12.56±2.90[ab]	6.98±1.63[b]
Procyanidin B5	1.29±0.63[a]	0.82±0.33[a]	3.10±1.60[a]
Cyanidin-3-*O*-rutinoside	0.000[a]	11.74±1.74[b]	0.000[a]
Quercetin3,3'-dimethyl ether-4'- glucoside	1.14±0.35[a]	0.62±0.18[a]	20.27±0.71[b]
Robinetinidol-(4-α-8)catechin- (6,4-α)robinetinol	0.84±0.27[b]	4.81±0.35[a]	4.22±2.43[ab]
Unknown compound 2	4.31±0.66[a]	3.63±0.69[a]	6.16±2.98[a]
Cinnamtannin A2	0.67±0.14[a]	0.74±0.03[a]	2.29±0.49[b]
Isochamaejasmin+	1.14±0.39[a]	0.000[b]	17.92±0.46[c]

Note: Values are expressed as Mean ± SD (n=3). Values followed by the same super script along rows are not significantly different (p<0.05)

Aqueous extract yields of Muguga, Karuri, and Kobujoi populations

The aqueous extracts from all three populations were analyzed for their phytochemical composition using LC-MS. The results showed the presence of procyanidin B5, robinetinidol-(4-α-8)-catechin-(6-α)- robinetinol, feruloyl-quinic acid, quercetin3,3'-dimethyl ether-4'-glucoside, cyanidin-o-galactoside, chlorogenic acid, ursolic acid, cyanidin-3-o-rutinoside, cinnamtannin A2, isoliquiritin, prunetrin and two unknown compounds. Table 8 summarizes the results of the investigation. Only the aqueous extracts of the Muguga population contained the bioactive compounds feruloyl-quinic acid and prunetrin. Quercetin3,3'-dimethyl ether-4'-glucoside was not detected in aqueous extracts of Kobujoi.

The highest concentrations of quercetin3,3'-dimethyl ether-4'-glucoside, unidentified compound 1, and unidentified compound 2 were found in aqueous extracts from the Muguga population. Chlorogenic acid, cyanidin-o-galactoside, ursolic acid, procyanidin B5, and cinnamtannin A2 were found in the greatest concentrations in Kobujoi aqueous extracts. The isoliquiritin, robinetinidol-(4-α-8)-catechin-(6-α)-robinetinol, and cyanidin-3-o-rutinoside compounds were found in the greatest amounts in the aqueous extracts from the Karuri population. In aqueous extracts of samples from the three groups, there was no statistically significant difference (p<0.05) in the amounts of cyanidin-3-o-rutinoside, procyanidin B5, unknown compound 1, or cinnamtannin A2. In aqueous extracts, only the Muguga population indicated the presence of prunetrin and feruloyl-quinic acid.

The presence of unidentified compound 2 was not detected in aqueous extracts of Karuri. The chlorogenic acid levels in the Kobujoi samples were considerably greater than in the Muguga and Karuri samples (p<0.05). Muguga and Karuri samples had substantially different quercetin- 3, 3'-dimethyl ether-4'-glucoside concentrations (p<0.05), but Kobujoi aqueous extract did not. Cyanidin-o-galactoside concentrations in Karuri and Kobujoi aqueous extracts were not substantially different from one another (p<0.05), but they were considerably different from those in the Muguga samples.

Table 8. Concentrations of compounds in aqueous extracts from the three populations (mg/kg)

Compound	Muguga	Karuri	Kobujoi
Feruloyl-quinic acid	5.64±3.24[b]	0.000[a]	0.000[a]
Chlorogenic acid	1.93±1.714[a]	4.06±2.09[a]	10.06±2.27[b]
Isoliquiritin	18.469±4.77[a]	30.97±5.11[b]	17.61±2.56[a]
Prunetrin	1.74±0.91[b]	0.000[a]	0.000[a]
Cyanidin-*O*-galactoside	1.56±1.64[b]	3.50±0.75[ab]	5.48±0.56[a]
Ursolic acid	13.73±7.89[b]	19.31±4.17[ab]	27.72±0.84[a]
Unknown compound 1	13.53±6.26[a]	11.75±2.39[a]	6.21±0.16[a]
Procyanidin B5	5.58±4.18[a]	6.99±1.44[a]	11.27±0.32[a]
Cyanidin-3-*O*-rutinoside	16.53±10.13[a]	33.03±5.85[a]	18.09±2.74[a]
Quercetin3,3'-dimethyl ether- 4'-glucoside	7.64±2.74[b]	3.01±1.00[a]	0.000[a]
Robinetinidol-(4-α-8)-catechin-(6,4-α)robinetinol	1.34±0.43[a]	5.605±0.47[b]	3.72±0.50[c]
Unknown compound 2	21.45±2.80[a]	0.000[b]	11.13±0.43[c]
Cinnamtannin A2	3.06±1.63[a]	3.54±1.33[a]	5.50±1.08[a]

Note: Values are expressed as Mean ± SD (n=3). Values followed by the same superscript along rows are not significantly different (p<0.05)

Discussion

The different shading times throughout the population likely caused the variations in height and DBH among the trees in the Muguga *P. africana* stand. Trees on the eastern side of the plantation were likewise found to be significantly shorter than their counterparts on the western side. This height variation may be due to the trees' competition for sunlight in areas where it is scarce. Given its importance to photosynthesis, light is an important environmental component that affects plant development (Canham et al. 1990; Valladares et al. 2003). All plants are subjected to some level of shade throughout their lives since light gradients can vary both within plant canopies and within the crowns of individual plants. High or low light levels might limit a plant's ability to develop, yet sunlight is necessary for photosynthesis (Grubb 1998).

There is no set time of year for *P. africana* flowers to bloom in the equatorial regions, and some plants bloom

nearly every month (Munjuga et al. 2000). Different fruiting patterns were seen because of the large variation in flowering times. The plant matures its fruit two to three months after flowering, in time with the onset of rain (Munjuga et al. 2000). Indicating that complex environment-plant interactions determine the growth rates of plant species. There is little to no correlation between short-term growth rates and individual environmental parameters (Berman and DeJong, 1997). The average growth rate of randomly selected *P. africana* individuals in the Muguga stand was inversely linked with age. Yoder et al. (1994) noted the same thing about *Pinus contorta* and *Pinus ponderosa*. It has been hypothesized that a species' photosynthesis slows with age, explaining the negative correlation between age and growth rate (Yoder et al. 1994).

Seasonal shifts influence cambial activity, affecting the width of tree growth rings and other phonological characteristics. Due to the lack of distinct tropical seasons (Hoadley, 1990), tree ring boundaries may be difficult to distinguish. Nevertheless, dendrochronology methods have successfully detected ring boundaries and date tropical trees. Seasonal shifts cause a widening or narrowing of the rings. The wetter seasons are represented by the wider rings, while the drier ones are indicated by the narrower ones (Fichtler et al. 2003; Trouet et al. 2009). In particular, P. africana does not disrupt the dormancy of cambium activity during short rains, but it reawakens cambium activity when the long rains begin (Krepkowski et al. 2011). As shown in *Podocarpus falcatus*, secondary cell enlargement and thickness can cause an increase in ring width even in the absence of cambium activity, allowing for growth even during dry periods (Deslauriers et al. 2009).

Dimensional stability, workability, and mechanical timber qualities are all aspects of wood quality that can be described in terms of wood density. It was found that the DBH of trees was inversely related to their wood density. A negative correlation was recorded in the *Picea mariana* species (Zhang et al. 1996). In the Muguga population, there was a positive correlation between the number of growth rings and the diameter of trees' breast height. A higher cambium activity is associated with maturation. A higher density of wood was associated with a faster growth rate. Earlier research has shown that dense hardwoods do not develop faster than less dense hardwoods (Zobel and van Buijtenen 1989; Zhang 1995). *Betula pendula* and *Prunus serotina* were also reported to have this characteristic (Nepveu and Velling 1983; Koch 1967). Hernandez et al. (1998) and Pliura et al. (2007) found a weak negative association between growth rate and wood density in *Populus* species, whereas other research found no correlation between the two (Debell et al. 2002; Zhang et al. 2003).

It has been found that *P. africana* bark extract helps alleviate lower urinary tract symptoms in patients with benign prostatic hyperplasia by lowering inflammation, decreasing bladder reactivity, and shrinking the prostate (Andro and Riffaud 1995; Ishani et al. 2000). Extracts are thought to reduce BPH by blocking the enzyme 5-α-reductase, reducing inflammation, lowering prolactin levels, and slowing the proliferation of prostatic fibroblasts in response to growth stimuli (Capasso et al. 2003). Each substance in this study has a role in either curing BPH or reducing its symptoms (Donovan et al. 1998; Carbin et al. 1990; Kampa et al. 2004). Phytosterols, ketones, phenolic compounds, and pentacyclic triterpenoids are all examples of such substances.

Hydro-distillation yielded an essential oil rich in polyunsaturated fatty acids and their methyl esters. Yet, hexane and DCM extracts included myristic acid and lauric acid, albeit in lower amounts than the essential oil. Since oils are volatile, it is possible that some of them were lost during the evaporation procedure used to concentrate the DCM and hexane extracts, which led to the observed disparity. Phytosterols were found in hexane and DCM extracts because they are non-polar to mid-polar molecules and are, therefore, simple to extract. Polar substances such as pentacyclic triterpenoids and phenolic compounds were found in methanol and water extracts. Management techniques that promote species production and biodiversity conservation require knowledge about phytochemical content. The delivery of crucial ecosystem services is influenced by phenotypic variety, genetic variation, and species richness, all of which are enhanced by phytochemical variation in agroforestry and cultivation (Cardinale et al. 2012). The anti-edematous and glucosyl-transferase-inhibiting properties of the pentacyclic triterpenoids found in *P. africana* bark extracts are well-known (Kokwaro, 1993; Donovan et al. 1998; Mothana et al. 2006). Researchers have shown that ursolic acid can stop the spread of cancer cells, including melanoma and prostate cancer cells (Nataraju et al. 2007).

For the concentrations examined here, there was no statistically significant variance. Prohibiting prostaglandin production, phytosterols like β-sitosterol and β-sitostenone reduce inflammation and prevent prostate enlargement (Raicht et al. 1976; Carbin et al. 1990). In addition to ferulic acid esters and their derivatives, which exhibit anticancer and hypocholesterolemic effects on the prostate, the bark extracts are also contained (Kampa et al. 2004). Researchers have found chemopreventive effects of phenolic compounds in *P. africana* bark on estrogen-dependent breast cancer (Noratto et al. 2009).

Compared to β-sitosterol, the concentration of β-sitostenone was lower in DCM and hexane extracts from the three populations. The lower levels of β-sitostenone in *P. africana* were also noted by Catalano et al. (1984). While analyzing DCM extracts, the highest concentration of β-sitosterol was found in Muguga (130.20 mg/kg). The chemical has been studied for its potential anticancer and cholesterol-lowering effects (Awad and Fink 2000). Compared to several other species, *P. africana* has significantly greater quantities of β-sitosterol. Bark values in avocado trees are comparable to those in *P. africana* trees, and avocados are a major source of β-sitosterol (Duester 2001). Traditional medicine uses the diuretic effects of *Moringa oleifera* (Anwar et al. 2007) and *P. spinosa* (Wolbiš et al. 2001) due to their high levels of β-sitosterol. β-sitosterol concentrations in this investigation were shown to be independent of environmental controls. However, soybeans found a lower concentration of β-

sitosterol in seeds generated by plants grown in colder climates (Yamaya et al. 2007). The Muguga had the highest levels of cholesterol and campesterol, as well. Aside from β-sitosterol, campesterol and stigmast-7-en-3-β-ol have also been found in Hypoxis species (Pegel 1979; Moghadasian 2000). The phytosterols in question have been shown to fight cancer (Choi et al. 2003), lower cholesterol, and reduce inflammation (Quilez et al. 2003).

Compared to the Karuri and Kobujoi populations, the Muguga population had greater quantities of myristic acid and -tocopherol in their extracts. The soil types and ambient circumstances of the three populations explain the variation in concentration. Plants' development and metabolic pathways may differ because of the range of chemical, physical, and biological characteristics in soils. Indirectly affecting DNA through the regulation of transcription of genes involved in metabolic activities, environmental variables can affect epigenetics, which in turn controls the production of secondary chemicals in plants.

Across all three groups, palmitic acid was most prevalent, followed by myristic acid and then lauric acid. The two saturated fatty acids were found to have a lower concentration in *P. africana* than the other fatty acids, and this variation had previously been observed (Ganzera et al. 1999; Abe et al. 2009). Also found in saw palmetto and pumpkin seeds are the aforementioned fatty acids (Ganzera et al. 1999). *Artocarpus heterophyllus* (Chowdhury et al. 1997) and *P. amygdalus* (Munshi and Sukhija 1984) have been shown to contain trace amounts of lauric acid and myristic acid. Sterols and fatty acids may lower prostate growth by preventing testosterone from being converted to dihydrotestosterone. These drugs inhibit the 5-α-reductase enzyme, which stops the production of dihydrotestosterone, the prostate growth regulator (Edeoga et al. 2005; Bent et al. 2006).

Friedelin was not detected in the three populations though the compound had been reported in *P. lusitanica* (Sainsbury 1970) and *P. africana* bark extracts (Catalano et al. 1984). Friedelin is a triterpenoid with anti-inflammatory activity (Antonisamy et al. 2011). Ursolic acid is also a natural pentacyclic triterpenoid in plants and has been a component in traditional medicine (Amico et al. 2009). Ursolic acid has been reported to have antioxidant, antiproliferative, and anti-inflammatory activities (Nataraju et al. 2007; Amico et al. 2009). It also serves as starting material for the biosynthesis of more potent bioactive compounds like antitumor agents (Ma et al. 2005). Ursolic acid has also been detected in *Eriobotrya japonica* at concentrations of up to 2000 mg/kg (Zhou et al. 2011).

Despite reports of friedelin in *P. lusitanica* (Sainsbury, 1970) and *P. africana* bark extracts, we were unable to identify this chemical in any of the three populations (Catalano et al. 1984). Friedelin is an anti-inflammatory triterpenoid (Antonisamy et al. 2011). Another component of traditional medicine, ursolic acid, is found naturally in plants as a pentacyclic triterpenoid (Amico et al. 2009). Some studies have found that ursolic acid possesses anti-inflammatory, antiproliferative, and antioxidant properties (Nataraju et al. 2007; Amico et al. 2009). Furthermore, it is used as a precursor in producing other, more powerful bioactive chemicals, such as anticancer drugs (Ma et al. 2005). The *E. japonica* has also been found to contain ursolic acid at levels as high as 2,000 mg/kg (Zhou et al. 2011).

Polyphenols such as cyanidin-o-galactoside and cyanidin-3-o-rutinoside have also been found in plums (Kim et al. 2003; Usenik et al. 2008). Phenolic chemicals are employed as anticancer treatments due to their anti-oxidative activity; they also help with cardiovascular disease and diabetes (Utsunomiya et al. 2005; Belkaid et al. 2006; Noratto et al. 2009). Phytochemicals in plums include hydroxycinnamic acid analogs, such as chlorogenic acid and quercetin derivatives (Raynal et al. 1989; Kim et al. 2003). *P. domestica*, coffee, and blueberries have all been linked to chlorogenic acid (Donovan et al. 1998; Prior and Cao 2000). Robinetinidol-(4-α-8) catechin-(6,4-α) robinetinol, cyanidin-o-galactoside, cyanidin-3-o-rutinoside, and procyanidin B5are products of the flavonoid family having antiproliferative and free radical-scavenging properties (Rukunga and Waterman1996; Cai et al. 2004; Jacob et al. 2012). Inhibiting topoisomerases and protein kinases are just one of the many functions flavonoids serve; they also influence apoptosis and cell differentiation and have antioxidant activity (Kuo 1997; Pinhero and Paliyath 2001). As a result of their characteristics, flavonoids are valuable molecules in the study of cancer. Some anthocyanins in Japanese plums include cyanidin-o-galactoside and cyanidin-3-o-rutinoside (Wu and Prior 2005). Most fruits get their color from flavonoids, especially anthocyanins (Usenik et al. 2009).

Similar profiles were observed among the three populations, and there was no appreciable difference in the component concentrations between organic and aqueous extracts. However, more catechin chemicals were found in samples of tea grown in agroforests and other mixed-crop areas compared to forest samples (Ahmed et al. 2013). The various tea administration methods are responsible for these variations. A recent study found the phytochemical profile of both domesticated and wild *Amburana cereansis* to be very comparable. Results from this study lend credence to the concept of using cultivated medicinal plants in the production of herbal remedies (Canuto et al. 2012). It protects the native ecosystem and guarantees a steady flow of high-quality raw materials (Canuto et al. 2012).

While in Muguga, temperatures ranged from 9 to 18ºC, while in Kobujoi and Karuri, they ranged from 8.8 to 23.7ºC and 10 to 21ºC, respectively, during the time of bark harvest. The coldest location was Muguga, and the hottest was Kobujoi. Some microsites inside a forest may have significantly different temperature regimes than others (Longman and Jenik 1987). Chilling injury caused by these temperature variations may cause metabolic disruptions, toxin buildup, and plant membrane permeability increases (Gachie et al. 2012). In addition, different plants within the same population may produce different amounts and types of secondary metabolites due to intraspecific genetic variation (Kadu et al. 2012).

In conclusion, variation in tree height and DBH across trees growing in the same habitat suggests that shading periods impact the growth rate of *P. africana*. Even among members of the same *P. africana* species growing in the

same environment, there was a wide range of flowering times and hence, a wide range of fruiting times. Potentially age-related decreases in photosynthesis and increases in respiration demand explain the age-related deceleration in *P. africana's* growth rate. This study found that crude yields of bark extract from the *P. africana* species did not vary greatly with the habitat of the individual trees used for the extraction. The phytochemical profile of the methanol and aqueous extracts was identical to that of the hexane and DCM extracts. However, the quantities of the various phytochemicals differed across the solvents. High yields of the phytochemicals in *P. africana* bark require using all these solvents. The concentration of most compounds associated with BPH treatment does not significantly differ across *P. africana* trees sourced from the wild, domesticated stand, and on-farm remnant environments. In contrast, the concentration of other phytochemicals changed with habitat. Therefore, whether the species is wild or cultivated when collecting bark for medicinal purposes is not very important.

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