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Phytochemical Profile of *Prunus africana* Stem Bark from Kenya

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Abstract

Prunus africana (Hook.f.) is an evergreen tree that grows in African mountains. *P. africana* species' bark and bark extracts are used for the treatment of benign prostate hyperplasia. The pharmacological efficacy of the extracts is believed to be due to synergistic effect of several compounds such as phytosterols, pentacyclic triterpenoids and ferulic acid esters. High demand for the bark and bark extracts of *P. africana* has led to over-exploitation of the natural population resulting to it being listed as an endangered species; Appendix II of CITES. Conservation of the species can be done through domestication. However, growth factors need to be established first to ensure success of on-farm production. With this in mind, the World Agroforestry Centre established a *P. africana* stand at Muguga, Kenya to evaluate the species phytochemical profile and yields. Phytochemistry profiling was carried out using Liquid Chromatography coupled to Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS). Myristic acid, linoleic acid, lauric acid, methyl myristate, methyl laurate and methyl linoleate were the major compounds present after analysis of essential oils in the bark samples while 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 were detected in dichloromethane and hexane extracts of the three populations. In methanolic extract we detected cyanidin-O-galactoside, cyanidin-3-O-rutinoside, procyanidin B5, robinetinidol-(4-α-8) catechin-(6,4-α)robinetinol and ursolic acid.. All these compounds have been reported to have salutary benefits in humans. The phytochemical data has important implications in coming up with strategies for sustainable harvesting and conservation of this species as well as its management on-farm.

Keywords: *Prunus africana;* Phytochemistry; Phytotherapy; Benign prostate hyperplasia

Introduction

Prunus africana is a species of the Prunus genus with a stem diameter of up to 1 meter and a height of more than 40 meters [1]. It has blackish-brown bark, shining foliage and greenish or white flowers. *P. africana* is found in mountains and underlying islands in 22 countries mostly on the eastern side of Africa [2]. It is also found in central Africa (Katanga, Congo), in West Africa, Comoros and Madagascar. It is native to the montane tropical forests of Sub-Saharan Africa and Madagascar at 1500 meters above the sea level. The range of *P. africana* has been significantly affected by past climate change and the distribution is expected to decrease significantly in coming years [3]. Unsustainable use of the species, which mainly affects large, reproductively mature trees, is likely to reduce gene flow and seed dispersal increasing isolation and reducing viability of existing populations [4]. Bark extracts of *P. africana* are used to treat benign prostate hyperplasia [5]. Prostafx, Tadenan and Pygenil are some of the herbal preparations of *P. africana* in the market. Extracts from stem and root barks contain phytochemicals with anticancer, anti-inflammatory and antiviral effects [6]. Bark extracts improve urologic symptoms in prostate cancer patients as they have apoptotic and anti-proliferative effect on the prostate [7]. The pharmacological efficacy of the wild tree bark extracts is thought to be due to synergistic effect of various compounds some of which are known and others unknown. The main compounds are; pentacyclic triterpenoids (ursolic and oleanolic acids) which inhibit glucosyl-transferase activity and have anti-edematous activity [8], phytosterols mainly β-sitosterol and β-sitostenone which have anti-inflammatory effect as they suppress the production of prostaglandins and thus prevent swelling of the prostate [9]. The bark also has ferulic acid esters (n-tetracosanol and n-docosanol) and their derivatives which have antitumor and hypocholesterolemic activity on the prostate [10]. The three compounds work synergistically to counteract the biochemical and structural changes associated with benign prostatic hyperplasia (BPH).

BPH is a progressive non-cancerous urologic condition that leads to enlargement of the prostate gland [11]. The condition common in most men over 50 and manifests itself as increased frequency in urination, pain in passing urine, inability to empty the bladder and post urinary dribbling [12]. Allopathic medical therapy for BPH includes drugs and surgical and non-surgical treatments. Drugs used include the anti-androgens terazosin hydrochloride and finasteride which are synthetic inhibitors of the 5-α-reductase enzyme [13]. Non-surgical therapy includes thermotherapy, balloon dilation and stents. Treatment by surgery involves removal of excess tissue. All these methods have a number of side effects and thus phytotherapy is the primary treatment in European countries. The bark extracts from *P. africana* also inhibit bladder hyperactivity. The use of the bark in traditional medicine includes the treatment of chest pain, urinary and bladder infections, stomach aches, kidney disease and malaria. The bark is either chewed or crushed into powder and drunk as tea [5]. The high demand of the bark extracts of *P. africana* has caused serious damage to the wild population [14]. More than 3000 tons of bark or bark extracts are exported to Europe per year. This high demand causes devastating effect to the wild population of *P. africana* which is the main source of the bark. Attempts at cultivation of *P. africana* are underway in Kenya and other countries. The bark of *P. africana* can regenerate if bark removal does not interfere with the vascular cambium and thus

harvesting is sustainable [15]. Despite the resilience of *P. africana* to debarking, in dry areas, bark re-growth is limited and large scale debarking stresses the tree even when complete re-growth occurs. Limited distribution of the species only in the afromontane islands and increased clearing for agriculture increases the threat for *P. africana*. The species was included as endangered species in Appendix II of the Convention of International Trade in Endangered Species (CITES) at the ninth conference due to the increasing international demand [16]. The species has also been assigned a vulnerable conservation status on the IUCN Red List. Import of the bark from Cameroon into the European Union was banned from November 2007 to December 2010 when CITES declared the ban lifted but with reduced quota of 150,000 kg for 2010 and 2011. In some African countries, policies have been established aiming at ensuring sustainable management of forests that contain *P. africana* species [17]. However, bark harvesting control issues persist and there is need to identify and implement sustainable management options including conservation and domestication measures. In order to optimize sustainable conservation of the vulnerable African cherry, knowledge of the phytochemical profiles and yields of domesticated *P. africana* is essential. A *P. africana* stand was established World Agroforestry Centre at Muguga for the determination of phytochemical profiles and yields. The seeds for this stand were obtained from a wild stand at Kobujoi, Nandi. This study was carried out to compare phytochemical yields of the domesticated stand, wild and on-farm remnant populations. The study results showed no significance difference in phytochemical yields for the three populations. This data will provide a basis for the right time of bark harvesting and the management strategies of on-farm *P. africana*.

Materials and Method

Study site

The *Prunus africana* stand was established by the World Agroforestry Centre at Muguga, Kenya. Muguga Regional Research Centre is situated at Kiambu County, 1° 14' S, 36° 38' E. Muguga is located approximately 2150 meters above sea level and has an average annual rainfall of 1200 mm.

Sample collection

Samples of *P. africana* stem bark for phytochemical analysis were collected from the *P. africana* stand at Muguga. Debarking was done using a sharp-edged machete at a height of 1.3 meters from the ground. The seeds used to establish the domesticated *P. africana* stand at Muguga were sourced from Kobujoi, Nandi. Reference samples were collected from Kobujoi, Nandi a natural forest and Karuri, a remnant on-farm stand. Each sample was labeled and the wet weight recorded.

Reagents and reference compounds

All solvents and reference compounds used were purchased from Sigma Aldrich Chemical Company limited, California USA unless otherwise stated and their purities ranged from 95-100%. Lupeol, a compound isolated from *Fagara tessmanii*, was donated by Ivan Addae-Mensah (University of Ghana).

Preparation of sample

The bark samples were air dried under a shade for one month after harvesting chopped into small pieces and ground to fine powders using a mill. Four hundred grams of powdered bark of each sample from the three sites was soaked in 700 mL of hexane, dichloromethane and methanol sequentially for 24 hours. The extracts were filtered using Whatman filter no. 1 using a vacuum suck pump and the filtrate concentrated under a vacuum at 40°C at reduced pressure using a Heldolph Laborata 4000-efficient rotary evaporator. After the organic solvent extraction each sample was soaked in distilled water and incubated in a water bath at 60°C for 5 hours. The aqueous extracts were then filtered using Whatman filter no. 1 and freeze-dried using SP Scientific AdVantage 2.0 benchtop lyophilizer.

Extraction of essential oils

Three hundred grams of each powdered bark sample were weighed into a distillation flask and hydro-distilled using a Dean-Stark apparatus [18]. Briefly, to each bark sample, 1.5 litres of distilled water was added and heated at 70°C and the oil collected in 5 ml hexane on the Clevenger side for 2 hrs. The condenser was set at a temperature of -15-15°C. After distillation the samples were concentrated using short path distillation apparatus and extracted using 1 ml dichloromethane. The essential oil yields after concentration ranged from 0.01 to 0.07 grams/ml.

GC-MS analysis

The samples were weighed into a 1.5 mL Eppendorf tube and each weight recorded in milligrams. One milliliter of dichloromethane was used to dissolve each sample and each mixture was vortexed for 30 seconds. The samples were then sonicated for five minutes using Branson 2510E-DTE sonicator and centrifuged for five minutes at 13000 rpm at room temperature. The samples were then transferred to 2 mL auto sampler vials and analyzed by GC-MS.

Gas chromatography–Mass conditions

Lupeol was used as internal standard for the quantification of phytosterols and ferulic acid esters in the GC-MS. Essential oils were identified and quantified by GC-MS using 1-heptene as internal standard. Before analyzing the extract using Gas Chromatography and Mass Spectrometry, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. GC-MS analysis was carried out on a GC-MS (7683 Agilent Technologies, Inc., Beijing, China) comprising a gas-chromatograph interfaced to a 5975C inert XL EI/CI mass spectrometer equipped with a 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. For GC-MS detection, an electron ionization system with ionization energy of 70Ev was used. The carrier gas was helium (99.99%) used at a constant flow rate of 1.25 ml/min, injector and mass transfer line temperature were set at 250°C and 200°C respectively, and an injection volume of 1 μl (splitless mode) was employed. The oven temperature was programmed from 35°C for 5 minutes, with an increase of 10°C/min to 280°C for 10.5 minutes, then 50°C/min to 285°C for 29.9 minutes with a run time of 70 minutes. The MS operating parameters were as follows: ionization energy, 70eV; ion source temperature, 230°C, solvent cut time, 3.3 min, relative detector gain mode, scan speed 1666 μ/sec; scan range 40-550 m/z, the interface temperature was 250°C. The total running time of GC-MS was 70 min. The relative percentage of the extract was expressed as percentage with peak area normalization.

LC-MS analysis

Methanol and aqueous sample extracts were weighed into a 1ml Eppendorf and the weight was recorded in milligrams. For methanol extracts, one millilitre of the methanol was added to each sample to re-dissolve it. Aqueous extracts were re-dissolved using 95% methanol and 5% distilled water. The samples were then vortexed for 30 seconds.

Sonication was done using a Branson 2510E-DTE sonicator for five minutes. The samples were then centrifuged for 5 minutes at room temperature at a speed of 1300 rpm and then transferred to 1.5 ml sample vials. Methanol and water extract were analyzed using liquid chromatography linked to mass spectrometry.

Liquid chromatography-mass conditions

HPLC separations were conducted on an HP 1100 capillary system with auto-sampler and a micro-pump (Agilent Technologies, Incorporation, Beijing, China). Griseofulvin was used as an internal standard to quantify compounds in LC-MS. Reverse-phase liquid chromatography was carried out with a Zorbax Eclipse Plus C18 column, 100 μm 2.1 mm, 3.5 μm being used to separate the compounds. The injection volume was set at 2 μL and the compartment of the auto-ampler was set at 4°C and nitrogen gas flow for desolvation was 400 L/h throughout the analysis. The mobile phase consisted of two components, with component A being water and component B methanol. 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 μL/min. The effluent of the first five minutes from the LC before analysis was diverted to waste to minimize ESI source contamination. Positive ion mode ESI-MS was used for the analysis. Pentacyclic triterpenoids present in the extracts were identified using METLIN metabolite data base and literature precedent and quantified using griseofulvin internal standard.

Statistical analysis

The data collected for the wild, on-farm remnant and the domesticated populations was analyzed using Winks version 7 software. Means for the quantified chemical compounds of the five individual trees of each population were calculated to establish an overview of relationships. The means were separated using Tukey's post hoc student test at 5% level of significance. The coefficients of variation within population were calculated to have a normalized comparison of variation. Inter-correlations of different constituents and correlation with tree size and environmental conditions were also calculated. ANOVA was used to test for differences between the populations.

Results

Total essential oils yields in Muguga, Karuri and Kobujoi populations

Phytochemical analysis of essential oils of the three populations was done using GC-MS. The analysis revealed the presence of linoleic acid, lauric acid, methyl laurate, methyl linoleate, methyl myristate and myristic acid among other compounds in the three populations (Figure 1). Analysis of polyunsaturated fatty acids and their methyl esters is tabulated in Table 1. Muguga population had the highest concentration of linoleic acid, methyl linoleate and methyl myristate in essential oils. Karuri population had the highest concentrations of myristic acid and lauric acid while Kobujoi had the highest methyl laurate concentration in essential oils. The concentration of linoleic acid of essential oil samples from Muguga, a domesticated stand was significantly different from Karuri, and Kobujoi (p<0.05). Muguga has the highest level of linoleic acid while Kobujoi had the lowest concentration (p<0.05). Lauric acid concentration for Karuri samples was significantly different from that of Kobujoi and Muguga samples (p<0.05). Karuri had the highest Lauric acid concentration (1152.14) while Kobujoi had the lowest concentration (4.12). The concentrations of methyl laurate in

the three populations were not significantly different (p<0.05). Methyl linoleate concentrations in the three populations were significantly different (p<0.05). Muguga had a significantly higher concentration of methyl myristate as compared to Karuri and Kobujoi (p<0.05). Myristic acid was significantly different in the three populations with Karuri having the highest concentration (p<0.05).

Hexane extract yields of Muguga, Karuri and Kobujoi populations

Phytochemical analysis of hexane extracts of the three populations was done using GC-MS and revealed 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. Analysis of these compounds is tabulated in Table 2. Muguga population had the highest amounts of campesterol, lup-20(29)-en-3-one, palmitic acid, squalene, β-sitostenone, 3-β, 5.α-stigmast-7-en-3-ol, stigmastan-3, 5-diene, myristic acid and α-tocopherol compounds in hexane extracts. Karuri populations had the highest concentrations of lauric acid and β-sitosterol. The concentrations of 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 in hexane extracts of the three populations were not significantly different (p<0.05). Muguga samples had significantly different concentration of α-tocopherol (p<0.05).

DCM extract yields of Muguga, Karuri and Kobujoi populations

Phytochemical analysis of DCM extracts of the three populations was done using GC-MS and revealed 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 (Figure 2). Hexane extracts analysis showed similar phytochemical profile to that of DCM extracts. This is because these two solvent are non-polar thus they extract similar compounds though with different concentrations due to the slight difference in polarity. These compounds were present in the three populations. These compounds are important in the treatment of BPH and their analysis is as shown in Table 3. Muguga population showed the highest concentration of campesterol, lup-20(29)-en-3-one, palmitic acid, squalene, β-Sitosterol, β-sitostenone stigmastan-3,5-diene and myristic acid. Karuri population had the highest amount of lauric acid while Kobujoi population had the highest amount of 3-β, 5α-stigmast-7-en-3-ol and α-tocopherol. The concentrations 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 in DCM extracts of the three populations were not significantly different (p<0.05). DCM extract of Muguga samples had a significantly different concentration of myristic acid compared to Karuri and Kobujoi samples (p<0.05) (Figure 3).

Methanol extract yields of Muguga, Karuri and Kobujoi populations

The phytochemical analysis of methanolic extracts of the three populations was done using LC-MS and revealed procyanidin B5, feruloyl-quinic acid, robinetinidol-(4-α-8)-catechin-(6-α)-robinetinol, prunetrin, quercetin3,3'-dimethylether-4'-glucoside, cyanidin-O-galactoside, chlorogenic acid, ursolic acid, isochamaejasmin+, cinnamtannin A2, isoliquiritin and two unknown compounds. Cyanidin-3-O-rutinoside was only present in Karuri population but isoliquiritin and isochamaejasmin+ was not present in Karuri population methanol extracts. Analysis of these compounds is tabulated

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 ± 1.359[a]	554.99 ± 22.60[b]	92.84 ± 1.81[c]

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 1: Concentration of essential oils in Muguga, Karuri and Kobujoi (mg/Kg).

Linoleic acid (1), lauric acid (2), methyl laurate (3), methyl linoleate (4), methyl myristate (5), myristic acid (6) and internal standard (IS)

Figure 1: Total ion chromatogram of the essential oils of the three.

Campesterol (1), lauric acid(2), β-sitosterol (3), lup-20(29)-en-3-one (4), palmitic acid (5), β-sitostenone (7), (3.β.,5.α)-stigmast-7-en-3-ol (8), stigmastan-3,5-diene (9), myristic acid (10), α-Tocopherol (11), internal standard (IS)

Figure 2: Total ion chromatochram of DCM extracts.

Myristic acid has a molecular weight of 228. The MS (Figure 5) displayed a molecular ion peak at 228 [48%, M+]. The base ion peak occurred at m/z 228 [48%, M].

Figure 3: Mass spectrum for Myristic acid.

in Table 4. Kobujoi population had the highest concentration of feruloyl-quinic acid, chlorogenic acid, procyanidin B5, quercetin3,3'-dimethylether-4'-glucoside, cinnamtannin A2, and isochamaejasmin in methanol extracts. Muguga population had the highest concentration of ursolic acid, isoliquiritin and unknown compound 1 in methanol extracts. Karuri population had the highest amounts of prunetrin, cyanidin-O-galactoside and robinetinidol-(4-α-8)-catechin-(6-α)-robinetinol in methanol extracts. The concentrations of feruloyl-quinic acid, chlorogenic acid, cyanidin-O-galactoside, ursolic acid, procyanidin B5 and unknown compound 2 in methanolic extracts of samples from the three populations were not significantly different (p<0.05). Karuri population had significantly different concentration of prunetrin from Muguga and Kobujoi samples whose concentrations were not significantly different (p<0.05). Analysis of Muguga and Kobujoi methanolic extracts did not reveal any presence of cyanidin-3-O-rutinoside. Kobujoi samples had significantly higher concentrations of cinnamtannin A2 (p<0.05). Concentrations of isochamaejasmin+ in the three populations were significantly different (p<0.05) with the compound absent in Karuri samples (p<0.05).

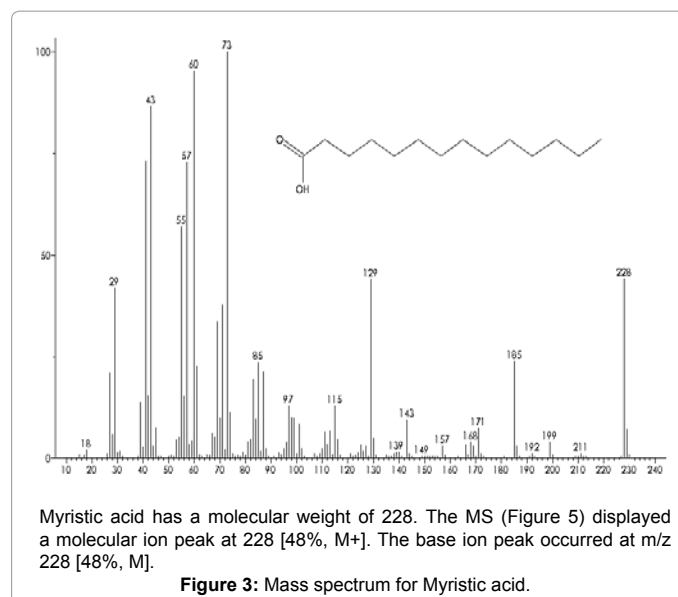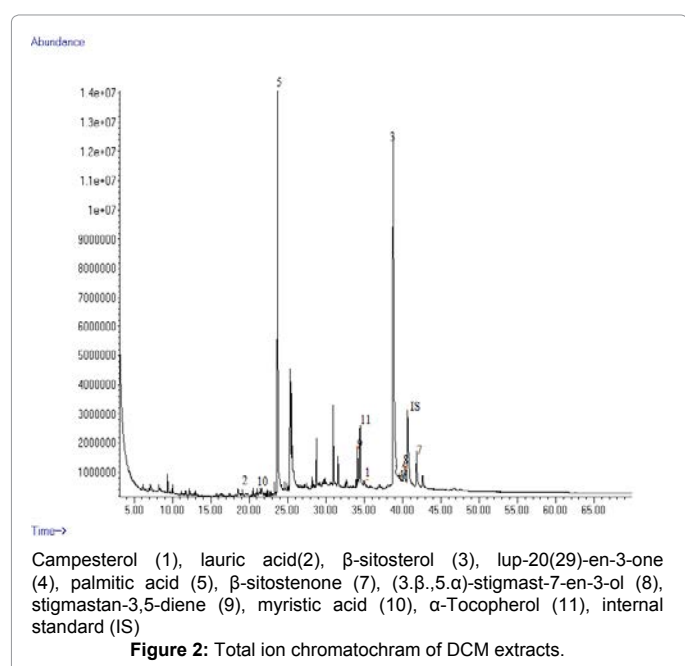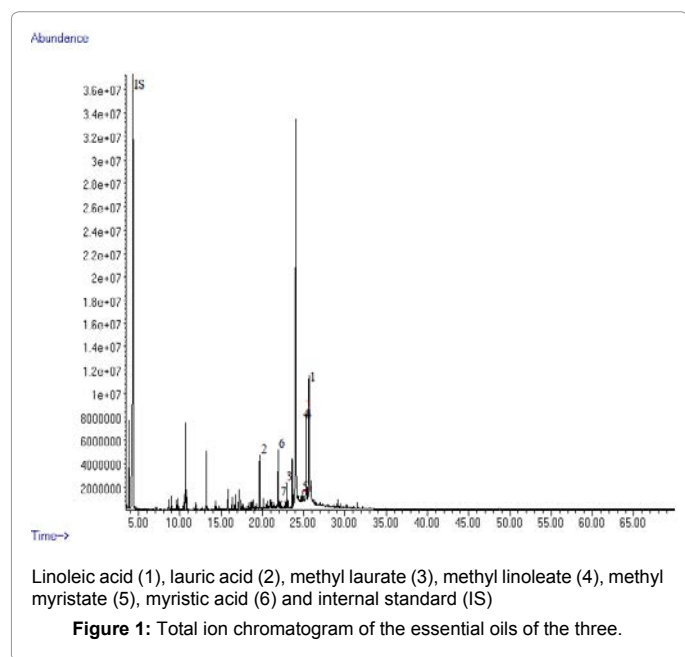## Aqueous extract yields of Muguga, Karuri and Kobujoi populations

Phytochemical analysis of aqueous extracts of the three populations was done using LC-MS and revealed procyanidin B5, robinetinidol-(4-α-8)-catechin-(6-α)-robinetinol, feruloyl-quinic acid, quercetin3,3'-dimethylether-4'-glucoside, cyanidin-O-galactoside, chlorogenic acid, ursolic acid, cyanidin-3-O-rutinoside, cinnamtannin A2, isoliquiritin, prunetrin and two unknown compounds. The analysis is tabulated in Table 5. Feruloyl-quinic acid and prunetrin was only present in aqueous extracts of Muguga population. Kobujoi aqueous extracts did not show presence of quercetin3,3'-dimethylether-4'-glucoside compound. Muguga population aqueous extracts showed the highest concentration of quercetin3, 3'-dimethylether-4'-glucoside, unknown compound 1 and unknown compound 2. Kobujoi aqueous extracts had the highest amounts of chlorogenic acid, cyanidin-O-galactoside, ursolic acid, procyanidin B5 and cinnamtannin A2. Karuri population aqueous extracts had the highest concentrations of isoliquiritin, robinetinidol-(4-α-8)-catechin-(6-α)-robinetinol and cyanidin-3-O-rutinoside compounds. The concentrations of cyanidin-3-O-

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]

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 2: Concentrations of compounds in hexane 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]

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 3: Concentration of compounds in DCM extracts of Muguga, Karuri and Kobujoi (mg/Kg).

rutinoside, procyanidin B5, unknown compound 1 and cinnamtannin A2 and in aqueous extracts of samples from the three populations were not significantly different (p<0.05). Muguga population is the only population that showed presence of prunetrin and feruloyl-quinic acid in aqueous extracts. Analysis of Karuri aqueous extracts did not reveal the presence of unknown compound 2. Kobujoi samples had significantly higher concentrations of chlorogenic acid than Muguga and Karuri (p<0.05). Concentrations of Quercetin3, 3'-dimethylether-4'-glucoside in Muguga and Karuri samples were significantly different (p<0.05) while Kobujoi aqueous extract did not have the compound. Karuri and Kobujoi population aqueous extracts did not show significant difference in the concentration of cyanidin-O-galactoside but the concentrations of the compound were significantly different from that of Muguga samples (p<0.05).

Discussion

Prunus africana bark extract has been used to suppress lower urinary tract symptoms by decreasing inflammation, reducing bladder reactivity and prostate size in patients with benign prostate hyperplasia [17,19]. The extracts are believed to counter BPH through inhibition of 5-α-reductase, anti-inflammatory activity, inhibition of prolactin levels and inhibition of prostatic fibroblast proliferation in response to growth factors [20]. The compounds analyzed in this study all play part in the treatment of BPH or alleviating the effects of BPH symptoms [8,21,22]. These compounds include polyunsaturated fatty acids, phytosterols, ketones, phenolic compounds and pentacyclic triterpenoids. Polyunsaturated fatty acids and their methyl esters were mainly present in the essential oil portion and were extracted by hydro-distillation. However, myristic acid and lauric acid were also present in hexane and DCM extracts though at lower concentrations compared to the hydro-distillation extracts. Phytosterols were observed in hexane and DCM extracts because these compounds are non-polar to mid-polar and thus were easily extracted by these solvents. Pentacyclic triterpenoids and phenolic compounds are polar compounds and thus were present in methanol and aqueous extracts. Pentacyclic triterpenoids present in *P. africana* bark extracts and mainly ursolic acid inhibits glucosyl-transferase activity and have anti-edematous activity [8,23,24]. Previous studies also showed that ursolic acid inhibits growth of melanoma cells and prostate cancer cells [25]. Phytosterols mainly β-sitosterol and β-sitostenone have anti-inflammatory effect as they suppress the production of prostaglandins and thus prevent swelling of the prostate [21,26]. The bark extracts also had ferulic acid esters and their derivatives which have antitumor and hypocholesterolemic activity on

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'-dimethylether-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]

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 4: Concentration of compounds in methanol extract 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'-dimethylether-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]

Values are expressed as Mean ± SD (n=3). Values followed by the same super script along rows are not significantly different ($p < 0.05$).
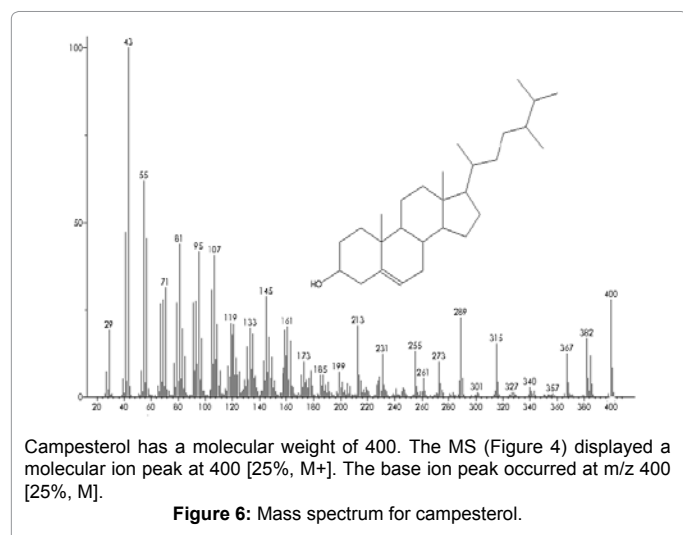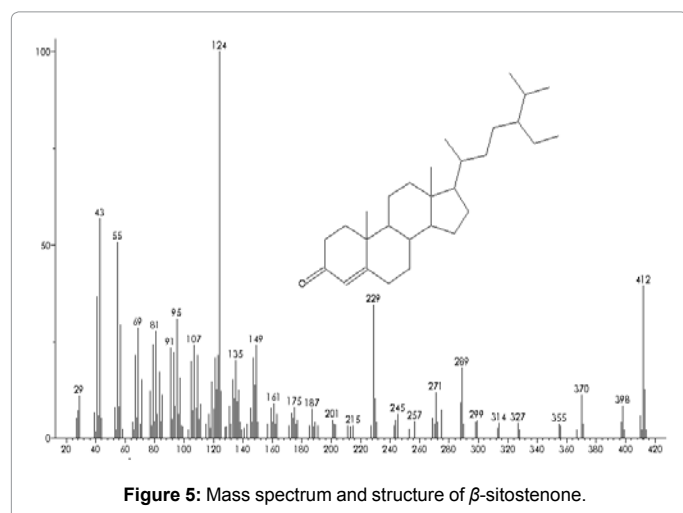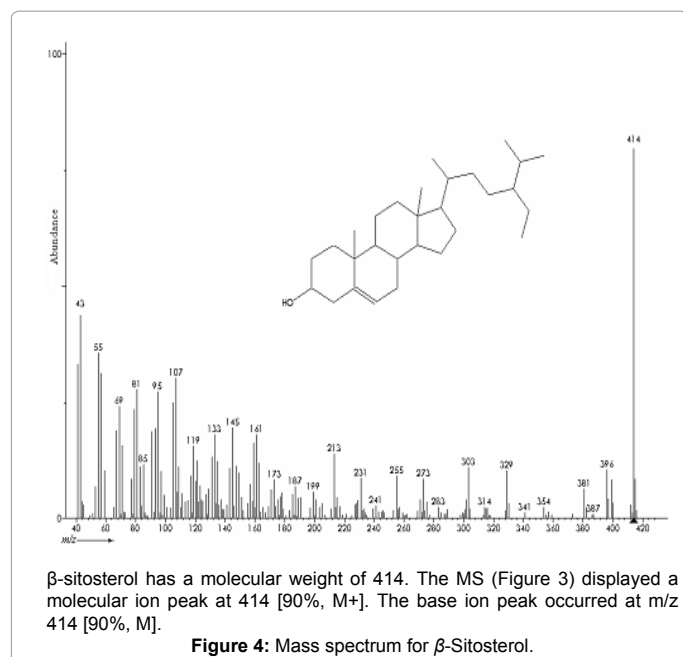**Table 5:** Concentrations of compounds in aqueous extracts from the three populations (mg/Kg).

the prostate [21]. Phenolic compounds in *P. africana* bark have also been reported to have chemo-preventive effect on estrogen dependent breast cancer [27]. β-sitostenone concentrations in DCM and hexane extracts of the three populations were lower compared to the concentration of β-sitosterol (Figures 4 and 5). This difference in the concentration of β–sitostenone compared to β-sitosterol was also reported in *P. africana* [28]. In the DCM extracts, β-Sitosterol concentration was highest in Muguga (130.20 mg/kg). The compound is believed to have anti-cancer activity and cholesterol lowering activity [29]. β-sitosterol was found in higher concentrations in *P. africana* as compared to many other species. Avocado is also a rich source of β-sitosterol [30] and the bark values are similar to those of *P. africana. Moringa oleifera* [31] and *P. spinosa* [32] have high levels of β-sitosterol and have been used in traditional medicine for their diuretic properties to increase urine flow. In this study, the concentration of β-sitosterol was not dependent on environmental factors. However, in soybeans plants grown in cold areas produced seeds with a lower content of β-sitosterol compared to plants grown in warm areas [33]. Campesterol concentration was also highest in Muguga population (Figure 6). Campesterol, stigmast-7-en-3-β-ol and β-sitosterol were also reported in hypoxis species [34,35]. These phytosterols have anticancer activity [36], cholesterol lowering activity and also anti-inflammatory effects [37]. Myristic acid and α-tocopherol concentrations in Muguga population extracts were slightly higher compared to Karuri and Kobujoi populations. This difference in concentration can be attributed to difference in soil types and environmental conditions in the three populations. Soils vary in chemical, physical and biological properties and these can lead to variation in the growth and metabolic mechanisms of plants. Epigenetic factors can be influenced by environmental conditions and thus affect DNA indirectly by switching on or off genes involved in metabolic processes hence determine secondary compounds produced by a plant. Palmitic acid was in higher concentration than myristic and lauric acid in the three populations. This variation has also been reported previously in *P.africana* where the two saturated fatty acids had lower concentration compared to other fatty acids [38,39]. The three fatty acids have also been reported in saw palmetto and pumpkin seeds [37]. Lauric acid and myristic acid have also been reported in *Artocarpus heterophyllus* [40] and in *P. amygdalus* [41] but at low concentrations. Fatty acids and sterols are believed to reduce prostate size by blocking the conversion of testosterone to dihydrotestosterone. The mechanism of action of these compounds is by inhibition of 5-α-reductase enzyme thus preventing formation of dihydrotestosterone, the modulator of prostate growth [42,43]. Friedelin was not detected in the three populations though the compound had been reported in *P. lusitanica*

[44] and in *P. africana* bark extracts [45]. Friedelin is a triterpenoid that has anti-inflammatory activity [46]. Ursolic acid is also a natural pentacyclic triterpenoid in plants and has been a component in traditional medicine [47]. Ursolic acid has been reported to have antioxidant, anti-proliferative and anti-inflammatory activities [25,47]. It also serves as starting material for the biosynthesis of more potent bioactive compounds like antitumor agents [48]. Ursolic acid has also been detected in *Eriobotrya japonica* at concentrations of up to 2000mg/kg [49]. Cyanidin-O-galactoside and cyanidin-3-O-rutinoside are polyphenols and have also been reported in plums [50,51]. Phenolic compounds have anti-oxidative activity and are thus used as anticancer agents and also have benefits for cardiovascular disease and diabetes [52-54]. Hydroxycinammic acid derivatives like chlorogenic acid and quercetin derivatives have also been identified among the phytochemicals in plums [55,50]. Chlorogenic acid has previously been reported in *P. domestica*, coffee and blue berries [8,56]. Cyanidin-O-galactoside, cyanidin-3-O-rutinoside, procyanidin B5 and robinetinidol-(4-α-8) catechin-(6,4-α) robinetinol are members of the flavonoid group and their derivatives and are believed to inhibit cell proliferation and have free radical scavenging activity [57-59]. Flavonoids have the ability to inhibit topoisomerases and protein kinases in addition to their ability to modulate apoptosis and cell differentiation and their antioxidant activity [60,61]. These properties make flavonoids important compounds in the field of cancer research. Cyanidin-O-galactoside and cyanidin-3-O-rutinoside have also been reported in Japanese plums as part of the anthocyanins found in the fruits of this species [62]. Flavonoids, particularly anthocyanins give most fruits their color [53]. The temperatures at Muguga, Kobujoi and Karuri at the time of bark harvest were 9-18°C, 8.8-23.7°C and 10-21°C respectively. Muguga was the coldest site while Kobujoi was the warmest among the three. Temperature regimes can vary in different microsites within a forest [63]. These temperature differences may cause chilling injury in plants leading to imbalances in metabolism, accumulation of toxic compounds and increased membrane permeability [1]. Intraspecies genetic variation may also lead to difference in types and quantities of secondary metabolites in plants of the same population due to genetic variation [7].

Conclusion

In conclusion, *P. africana* trees from wild, domesticated stand and on-farm remnant habitats do not vary significantly in the concentration of most of the compounds related to benign prostate hyperplasia (BPH) treatment but some phytochemicals concentration vary with habitat.

β-sitosterol has a molecular weight of 414. The MS (Figure 3) displayed a molecular ion peak at 414 [90%, M+]. The base ion peak occurred at m/z 414 [90%, M].

Figure 4: Mass spectrum for *β*-Sitosterol.

Figure 5: Mass spectrum and structure of *β*-sitostenone.

Campesterol has a molecular weight of 400. The MS (Figure 4) displayed a molecular ion peak at 400 [25%, M+]. The base ion peak occurred at m/z 400 [25%, M].

Figure 6: Mass spectrum for campesterol.

The study showed the presence of phytosterols and polyunsaturated fatty acids in high concentrations in *P. africana* bark extracts indicating their significance in the treatment of BPH. Thus habitat or whether the species is domesticated or in the wild should not be a major concern while harvesting bark for medicinal purposes. In this study, methanol and aqueous extracts had similar phytochemical profile and so was hexane and DCM but concentrations of individual phytochemicals varied in each solvent. Thus all these solvents are necessary for one to get high yields of the phytochemicals present in the *P. africana* stem bark.

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