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Research Article

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Comparison of N- Docosanol and β - Sitosterol Content in the leaf and Stem Bark of *Prunus africana* Collected from different Geographical Zones in Tanzania

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ABSTRACT

The study was designed to investigate quantitative differences of sterols and ferulic esters in the leaves and stem barks of Prunus africana if leaves can substitute destructive exploitation of stem barks in the production of anti-Benign Prostatic Hyperplasia herbal formulations.

Plant collection from the Northern, Southern and Western region of Tanzania was From May to July, 2012 while Chromatographic analyses were carried out at the Institute of Traditional medicine, Muhimbili University of Health and Allied Sciences from September 2012 to March 2013. The quantitative comparison was done by determining the quantities of β-sitosterol and n-docosanol as marker compounds using Camag HPTLC analysis. The analysis revealed that, leaves had higher concentration of β-sitosterol than stem barks. The concentration of β-sitosterol in the leaves of P. africana ranged between 0.1518 – 0.2277 µg. In the stem barks the concentrations of β-sitosterol was low compared with amount detected in the leaves. The concentration of β-sitosterol in the stem barks ranged between 0.0969-0.2184 µg. The concentration of n-docosanol in the leaves was higher than in the stem barks of P. africana. In the leaves, the concentration ranged between 0.33991 – 0.4989 µg and in the barks, the concentration ranged between 0.1484 – 0.2069 µg. Generally, the concentration of β-sitosterol and n-docosanol in the leaves were much higher than in the stem barks of P. africana. For a sustainable harvesting and conservation reasons, it is therefore ideal to substitute stem barks by leaves in the preparation of herbal medicines used in the treatment of BPH. Further study on other essential phytochemicals responsible for anti-BPH efficacy should be done to emphasize on complementing harvesting of stem barks with leaves.

Key words: *Benign Prostatic Hyperplasia, Prunus africana, bark extracts, leaves extracts, Tanzania.*

INTRODUCTION

Prunus Africana (Hook.f.) Kalkman is a medicinal plant widely distributed in montane regions of Africa[1,2]. It is the most abundant tree in open areas along forest margins and in disturbed areas[3]. The montane habitat in Cameroon and Madagascar has higher population of this plant species. In Tanzania, *P. africana* grow in Arusha, Kilimanjaro, Pare, East and West usambara, Uluguru mountains, Mufindi and Mahenge escarpments, Udzungwa mountains, Southern highlands and west and central Tanzania. According to Kapinga[4] Tanzania is one of the seven countries in Africa where this species is over harvested for medicinal purposes, thus there is an urgent need for conservation of this species.

The liquid extracts from *P. africana* bark are used in the treatment of benign prostatic hyperplasia and prostate gland hypertrophy. Leaves are used as an inhalant for fever or are drunk as an infusion to improve appetite. The extract from the barks of this species is used as a remedy for stomach-ache; and also the bark extract is used as a purgative for cattle[5]. Its medicinal use in the treatment of Benign

Prostatic Hyperplasia (BPH) has led this species to be highly exploited for exportation. Consequently, it is a highly endangered species in Africa which need to be conserved. *Prunus africana* is rich in sterols, triterpenoids and ferulic esters. The documented secondary metabolites from this species include; β-sitosterol, β-sitostenone, campesterol, ursolic acid, oleanolic acid, crateaegolic and epimaslinic acid. Furthermore, n-tetracosanol and n-docosanol have been reported from the stem bark of this species [6,7, 8]. These phytochemicals have been implicated to be responsible for the claimed efficacy of *P. africana* against BPH.

The high demand for stem barks which are used in the formulation of herbal drugs for the treatment of BPH is threatening this plant species to extinction. Therefore conservation efforts and/or changing the current harvesting methods are highly needed to rescue this species from becoming extinct. Consequently, this study was designed to investigate the quantitative differences of the amounts of sterols and ferulic esters in the leaves and stem barks of *P. africana*. This is with the aim of establishing whether leaves can substitute stem barks in the preparation of anti-BPH herbal formulae. Therefore, this article reports the quantitative comparison of sterol and ferulic esters in the leaves and stem barks of *P. africana* collected from different geographic areas in Tanzania.

MATERIALS AND METHODS

Chemicals: Methanol (absolute) was purchased from Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands), Chloroform (HPLC grade), Dichloromethane (HPLC grade), acetone (Synthesis grade), Ethyl acetate (HPLC grade) and petroleum ether (HPLC grade) were supplied by KAS medics (Dar es Salaam, Tanzania). Standard drugs: n-docosanol 98% and Ursolic acid 98% were purchased from Sigma-Aldrich Chemie Gmbh, Germany, and β-sitosterol was obtained from chemical library at the Institute of Traditional Medicine phytochemistry Lab at Muhimbili University of Health and Allied Sciences (MUHAS).

Collection and identification of *Prunus africana*

All the plant materials (stem bark and leaves) of *P. africana* were harvested during dry season in June, 2012. The collection was done from seven geographical zones in Tanzania namely; Lushoto T-3, Sumbawanga T-7, Mufindi T-7, Rombo T-2, Kiloreni-Kindoroko Mwanga T-2, Suji T-2 and Monduli T-2. Identification and authentication of the plant species was done by Joseph N Otieno, an ethnobotanist from the Department of Medical Botany and Agronomy, Muhimbili University of Health and Allied Sciences, Tanzania. The voucher specimens of all collections are kept in the Herbarium at the Institute of Traditional Medicine.

Preparation and extraction of plant materials

The stem barks and leaves of *P. africana* were separately dried at room temperature and milled using an electric miller. Portions of plant materials were separately soaked in methanol (99.8%) for 24 hours and filtered. The filtrates were concentrated in *vacuo* using rotary evaporator to obtain the stem bark and leaf methanolic extracts. The extracts were kept in the fridge set at 4^{o}C ready for further analyses.

Chromatographic analyses: Development of Calibration Curves for n-docosanol and β-sitosterol

The calibration curves of n-docosanol and β-sitosterol were obtained using Camag HPTLC Scanner 3 (Camag, Swizerland), controlled by external computer with WINCAT software. The Camag Linomat IV was used as an applicator and the detection was done under UV radiation using Mercury Lamp. Samples were loaded on a TLC silica gel 60 F_{254}, 10 x 20 cm aluminum barked plate. 0.5 mg of standard compounds namely n-docosanol and β-sitosterol were separately dissolved in 1ml of dichloromethane and chloroform respectively. Then the stock solutions were serially diluted to the concentrations levels of 0.1, 0.15, 0.2, 0.25, 0.3, 0.4 and 0.45 mg/ml. Thereafter, 25 µl of each level was loaded on TLC as 8 mm band length, and at a distance of 15 mm. The TLC plates were developed in the CAMAG glass chamber containing chloroform-methanol (7:3 v/v) mobile phase. After development the plates were dried at room temperature for about 15 minutes then derivatized with Vanillin-Sulphuric acid. Thereafter, TLC plates were dried in an oven at 110 °C for 15 minutes then scanned at ʎ = 254 nm. Peak heights and peak areas for standards were recorded after the finger prints observed from the densitograms. The slit dimensions

were 6.00 mm x 0.450 mm and the scanning speed was 20mm s^{-1}. Thereafter linear curves of abundance (Peak area) versus concentration (in ng/ml) were plotted ready for use in the quantification of the two compounds in the crude extracts of *Prunus africana*.

Quantitative analyses of extracts using High Performance Thin Layer Chromatography (HPTLC)

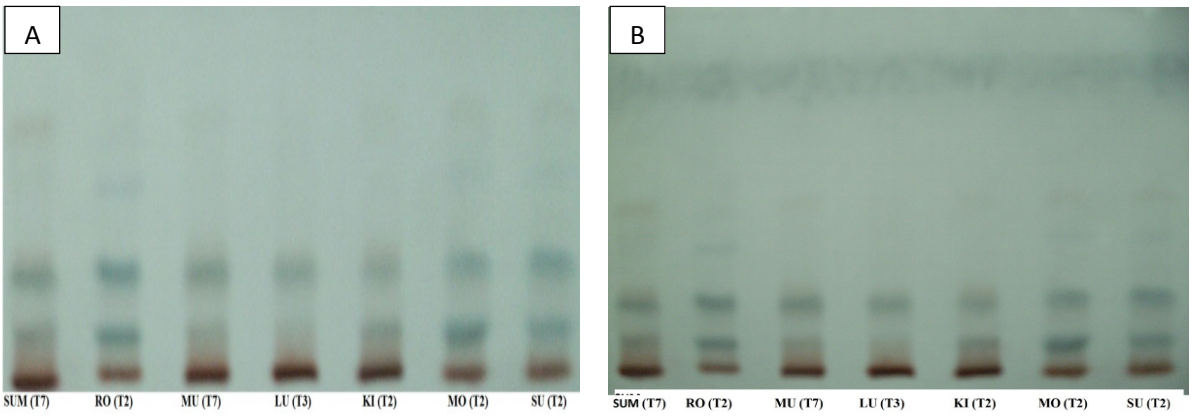
The quantitative analyses of n-docosanol and β-sitosterol in the stem bark and leaf extracts of *P. africana* were done using Camag HPTLC Scanner 3 (Camag, Swizerland), controlled by external computer with WINCAT software. The stock solutions of extracts were prepared by dissolving 0.5 mg in 1 ml of methanol (HPLC grade). Thereafter, 10µl of each stock solution was loaded on TLC as 8 mm band length, and at a distance of 15 mm. The plates were developed in chloroform-methanol (7:3 v/v) mobile phase in the CAMAG glass chamber previously saturated with the mobile phase vapor for 15 minutes at room temperature [(25± 2) °C]. After development the plates were dried at room temperature for about 15 minutes then sprayed with Vanillin-Sulphuric acid followed by drying in the oven at 110 °C for 15 minutes. Thereafter, TLC plates with visible bands were scanned at λ = 254 nm. The slit dimensions were 6.00 mm x 0.450 mm and the scanning speed was 20mm s^{-1}. The data resolution was 100 µm/step and CATS' software was used to perform quantitative analysis by recording the chromatograms in fluorescence mode using K_{400} optical filter at 254 nm under Hg lamp, and extrapolating the obtained data with the respective calibration curve of each standard compound.

RESULTS AND DISCUSSION

Qualitative analyses of phytochemical contents in the leaves and stem barks of *P. africana*

The qualitative TLC profile of the leaves and stem bark extracts of *P. africana* showed that, the two parts of this plant species contains similar compounds. The only difference was in terms of bands intensity which translates to differences in concentration of individual compounds (Fig. 1a and 1b).

Figures 1A and B: TLC plates of stem bark (A) and leaf (B) extracts of *Prunus africana* collected from Sumbawanga (SUM), Rombo (RO), Mufindi (MU), Lushoto (LU), Kiloleni (KI), Monduli (MO) and Suji (SU)



Quantitative determination of β-sitosterol in the leaves and stem barks of *P. africana*

The concentration of β-sitosterol in the leaves and stem barks extracts of *P. africana* was determined using Camag HPTLC Scanner 3 controlled by external WINCAT software. This is a fast yet efficient and precise chromatographic technique for quantitative determination of bioactive compounds in the extracts. The calibration curve for β-sitosterol had a good linearity (R^2>0.905) throughout the range of 1000-6000 ng/ml. This confirmed further that overall accuracy of the method was good (Fig 2a and 2b). The RF value for β-sitosterol was 0.67 as reflected in all densitograms (Fig. 2a, 3a and 3c).

Figure 2a: Densitogram for a standard β-sitosterol (λ= 254 nm)

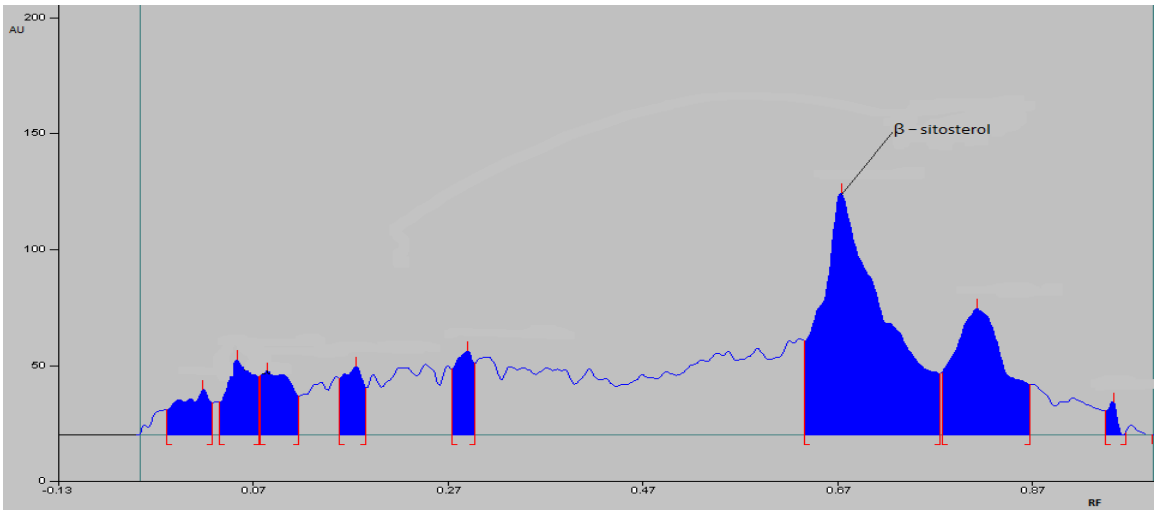


Figure 2b: Calibration curve for β-sitosterol

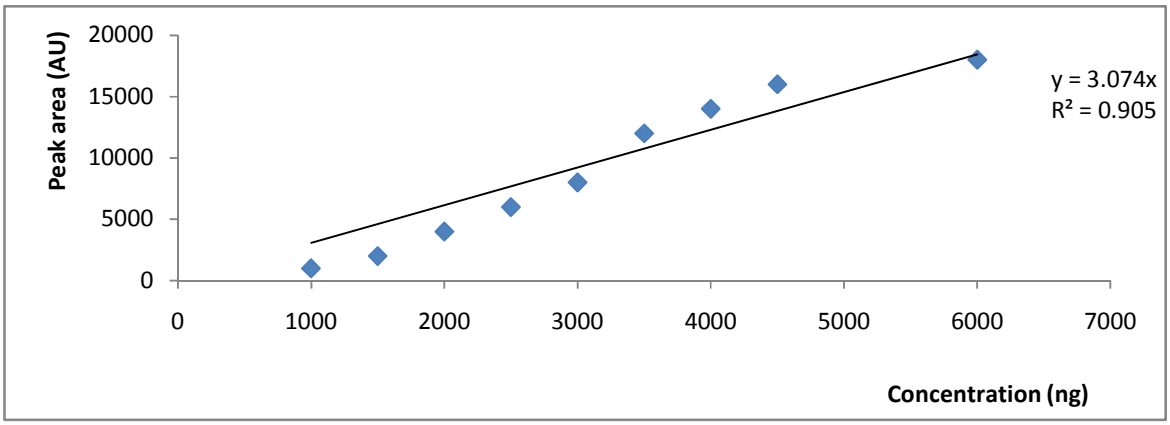
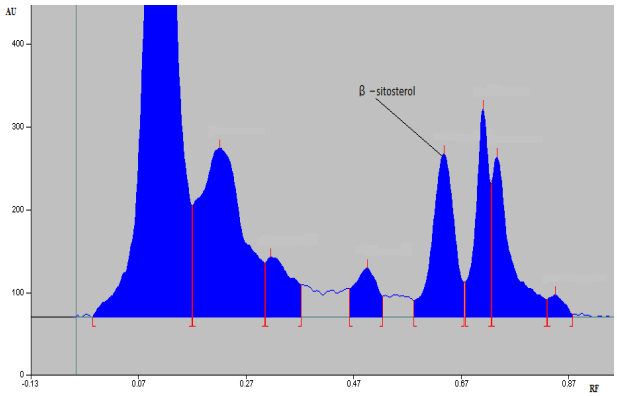


Figure 3a:Densitogram of β-Sitosterol of the leaf extract from Monduli (T2)-A and Suji (T2)-B



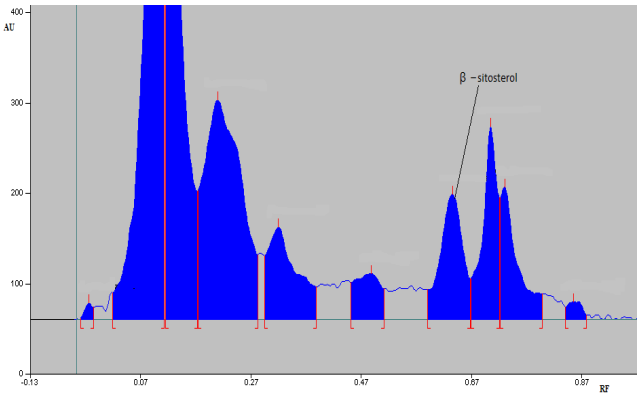
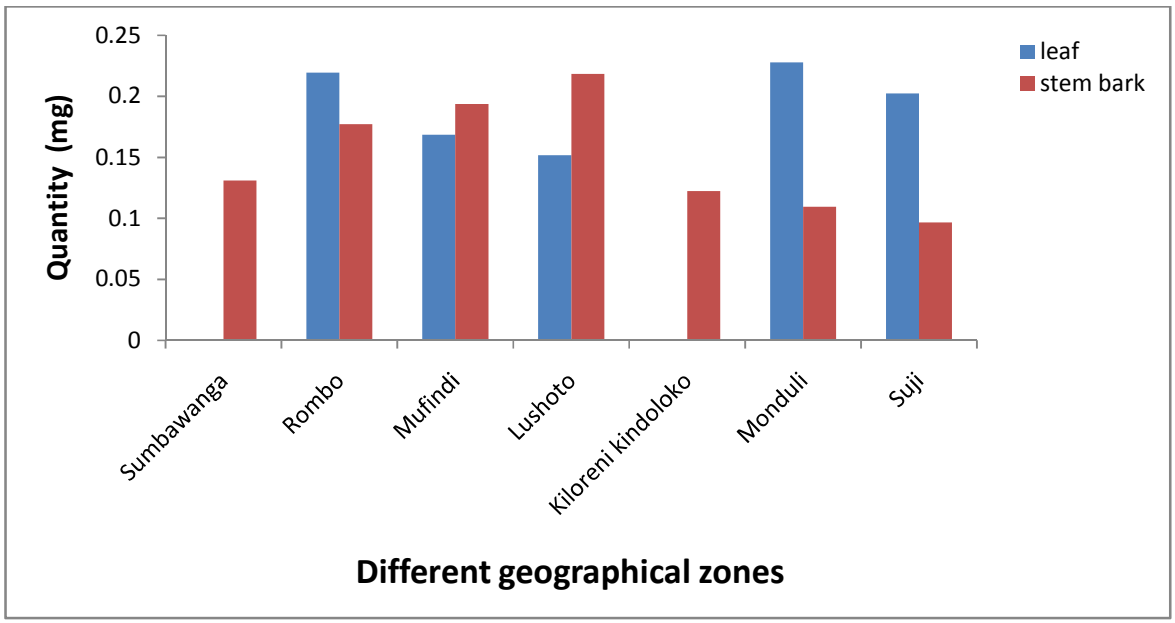


Figure 3c: The histogram showing the quantity of β-sitosterol (phytosterol) contents in the leaf and stem bark *P. africana* from different geographical zones



The presence of β-sitosterol was detected in *P. africana* leaves collected from different geographic areas with the exception of samples from Sumbawanga (LSUM) and Kiloreni-Kindoloko (LKK). Leaves from Monduli (LMO) had higher β-sitosterol content (0.2277 µg) than samples from other geographic areas. This was followed by leaf samples from Rombo (0.2193 µg) and Suji (0.2024 µg) respectively. *Prunus africana* leaves from Lushoto had the least concentration of β-sitosterol, registering 0.1518 µg (Fig. 3c). The concentration of β-sitosterol in the stem barks of *P. africana* was generally low compared with the figures registered in the leaves. Stem barks from Lushoto (BLU) had higher concentration of β-sitosterol than samples collected from other geographic areas. The stem barks had 0.2184 µg, quite higher that the sample from Sumbawanga which had 0.0969 µg of β-sitosterol (Fig. 3c).

When comparing the concentration of β-sitosterol in the leaves and stem barks of *P. africana*, a clear difference could be observed from the peak areas of the densitograms, as well as peak heights. Therefore, this suggests that leaves can substitute stem barks in the preparation of herbal drugs which aims at optimal concentration of β-sitosterol as an active ingredient. Sterols, such as β-sitosterol in *P. africana* have been reported to inhibit the production of prostaglandins in the prostate, thereby suppressing the inflammatory symptoms associated with BPH and chronic prostatitis. Through such activities, the absorption of cholesterol is blocked leading to reduced blood cholesterol levels (9).

Quantitative determination of n-docosanol in the leaves and stem barks of *P. africana*

Prunus africana is known to contain ferulic esters, a class of secondary metabolites reported to lower cholesterol levels in the blood, from which testosterone is produced (10). N-docosanol and n-tetracosanol are ferulic esters which have been reported from the barks of *P. africana*. N-docosanol was detected in all leaf samples of *P. africana* collected from different regions in Tanzania. The concentration levels ranged between 0.3991 – 0.4989 µg. The leaves from Kiloreni-kindoloko (LKK) had higher amount of n-docosanol than all samples registering 0.4989 µg, followed by leaf samples from Lushoto (LLU) which contained 0.4900 µg. The collection with least amount of n-docosanol was that obtained from Sumbawanga (LSU) which had 0.3991 µg (Fig. 5a, b and c).

Figure 5a& b:Densitogram of n-docosanolin the leaf extract of *P.africana* collected from Sumbawanga (T7)-A and Mufindi (T7)-B

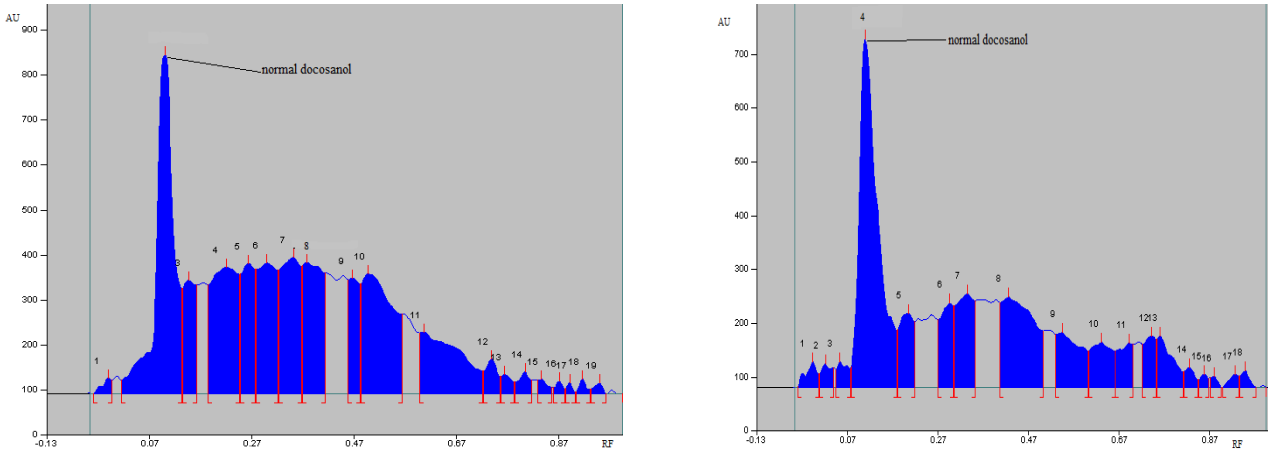
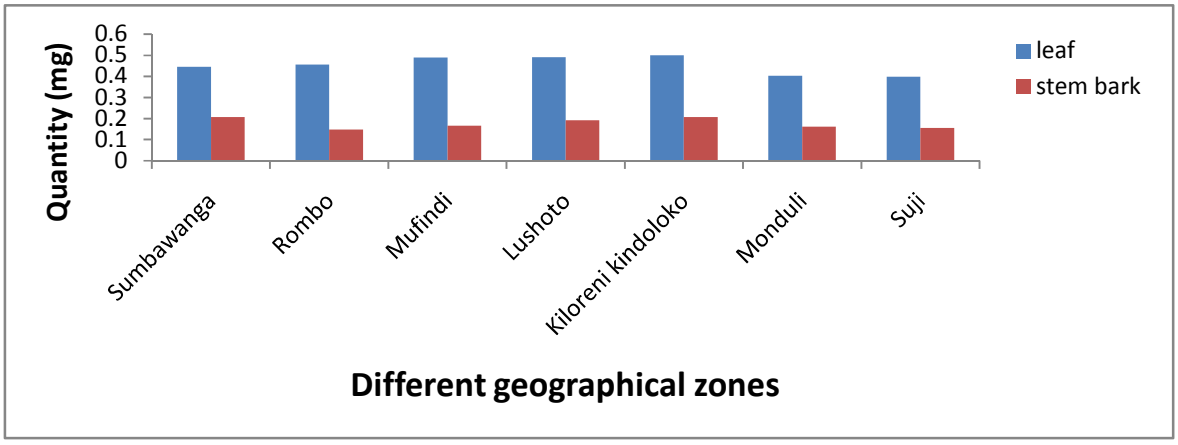
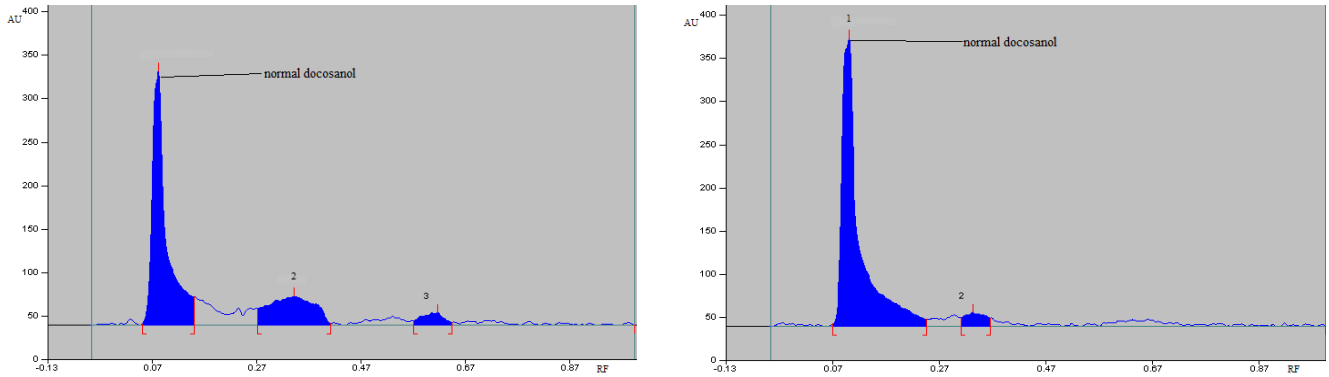


Figure 5c: The histogram showing the quantity of n-docosanolin leaf and stem bark *P. africana* from different geographical zones.



Stem barks of *Prunus africana* collected from all geographic areas contained n-docosanol at a varying level. However, the concentration of this phytochemical was three times lower compared to its concentrations in the leaves. The abundances ranged from 0.1484-0.2069 µg. The stem barks from Kiloreni-Kindoloko had the highest abundance (0.2069 µg) of n-docosanol followed by samples from Sumbawanga which had 0.2068 µg, and Lushoto 0.1924 µg (Fig.5c, d and e).

Figure 5d & e:Densitogram of n-docosanol in the bark extract of *P. africana* collected from Sumbawanga (T7)-C and KiloleniKindoroko (T2)-D



The higher concentration of β-sitosterol and n-docosanol in the leaves suggests that, leaves can substitute stem barks in the preparation of herbal formulae which requires higher concentration of these phytochemicals. For instance, stem barks of *P. africana* are highly exploited as raw material in the formulation of herbal medicines used in the treatment and/or management of Benign Prostatic Hyperplasia (BPH). Since the main phytochemicals of interest are sterols (one of which is β-sitosterol), ferulic esters (n-docosanol) and pentacyclic triterpenoids (Ursolic acid), then leaves can be the best raw materials of such formulations. In this study, leaves have revealed to have twice to thrice amount of phytochemicals of interest than stem barks. This study has therefore provided the basis for a shift of interest from stem barks to leaves, as this shall promote conservation of this highly sought after medicinal plant in the tropics. Further work should be done to determine the amount of pentacyclic triterpenoids in the leaves and stem barks of this species so that, emphasis on the sustainable harvest of raw materials from this species is encouraged to protect *P. africana* from becoming extinct.

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REFERENCES

1. Cunningham, M., Cunningham A.B, Schippmann, U. Trade in *Prunus africana* and the Implementation of CITES. Results of the R&D Project 808 05 080. German Federal Agency for Nature Conservation. (1997)
2. Hall, JE., Brien, O., Sinclair F. *Prunus africana,* A monograph. University of Wales Bangor, Mount Cameroon Project, IRCRAF. Sciences, S. O. A. F. Publication No 18, University of Wales Bangor, School of Agricultural and Forest Sciences 104 (2000)
3. Ndam, N. Recruitment patterns of *Prunus africana* (Hook f.) Kalkman on Mount Cameroon: a case study at Mapanja. In: A Strategy for the Conservation of *Prunus africana* on Mount Cameroon. Technical Papers and Workshop Proceedings. February 21–22 (1996)
4. Kapinga, C., Hussein, I. Country report on implementation of review of significant trade (RST) recommendations for *Prunus africana*. Proceedings of the workshop organized by ICUN to review implementation of recommendations on the trade of *Prunus africana* in East Africa/Africa held at Naivasha, Kenya. (2008)
5. Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., Simons, A. 2009. Agroforestry tree Database: a tree reference and selection guide version 4.0. (2013)
6. Longo, R and Tira, S,. Constituents of Pygeumafricanumbark. Plantamedica 1981; 14: 195–203.
7. Catalano, S., Gerretti, M, Marsili, A., Morelli, I. New constituents of *Prunus africana* bark extract. Journal of Natural Products . **47:** 910–911 (1984)
8. Fourneau C,, Hocquemille,r R., Cavé, A.. Triterpenes from Prunus Africana bark. Phytochemistry **42:** 1387–1389 (1996)
9. Breza, J., Dzurny, O., Borowka, A., Hanus, T., Petrik, R., Blane, G., Chadha-Boreham H. Efficacy and adaptability of Tedenan (*Pygeum africanum* extract) in the treatment of benign prostatic hyperplasia (BPH), a multicenter trial in central Europe. Current Medical Research Opinion. **14:** 127–139 (1998)
10. Bombardelli, E., Morazzoni, P. *Prunus africana* (Hook. f) Kalkm. Fitoterapia. **68:** 205–218 (1997)