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Oil yield and Sterols content in Seeds and Barks of Wild and Domestic Plants Growing in Kahuzi-Biega National Park and surroundings

[Rendement en huile et teneur en stérols des graines et écorces de plantes sauvages et domestiques poussant dans le parc national du Kahuzi-Biega et ses environs]

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Abstract

Kahuzi-Biega National Park in the Democratic Republic of the Congo is home to various plants as sources of edible oil for food preparations and medicinal therapy. This study aimed to assess and compare the oil yield and phytosterol content of seeds and barks of eight plants growing in this park and its surroundings. These were *Albizia grandibracteata*, *Hagena abyssinica*, *Millettia dura*, *Piptadeniastrum africanum*, *Prunus africana*, *Prunus salsii*, *Sesbania sesban* and *Tephrosia vogelii*. Common procedures for petroleum extraction and gas chromatography were used. The seeds of *Prunus salsii* contain the highest yield of crude oil (45.8%). *Millettia dura* and *Piptadeniastrum africanum* have the highest total sterol content (27-54 mg / g oil). Among the thirteen *phytosterols* identified, the most abundant are *β-sitosterol*, *stigmasterol*, *campesterol* and 5-*avenasterol*. The properties of these *phytosterols* support the traditional use of these plants as sources of oils for cooking and health issues.

Keywords: plants, seed oils, phytosterols, Kahuzi-Biega National Park, D.R. Congo

Résumé

Le Parc National de Kahuzi-Biega, en République démocratique du Congo, abrite diverses plantes servant de source d'huile comestible pour les préparations alimentaires et la thérapie médicinale. Cette étude visait à évaluer et comparer le rendement en huile et la teneur en phytostérols des graines et écorces de huit plantes poussant dans ce parc et ses environs. Il s'agissait d'*Albizia grandibracteata*, *Hagena abyssinica*, *Millettia dura*, *Piptadeniastrum africanum*, *Prunus africana*, *Prunus salsii*, *Sesbania sesban* et *Tephrosia vogelii*. Des procédures courantes d'extraction à l'éther de pétrole et de chromatographie en phase gazeuse ont été utilisées. Les graines de *Prunus salsii* contiennent le rendement le plus élevé en huile brute (45,8 %). *Millettia dura* et *Piptadeniastrum africanum* ont la teneur en stérols totaux la plus élevée (27-54 mg/g d'huile). Parmi les treize phytostérols identifiés, les plus abondants sont le β-sitostérol, le stigmastérol, le campestérol et le 5-avénastérol. Les propriétés de ces phytostérols soutiennent l'utilisation traditionnelle de ces plantes comme sources d'huiles pour la cuisine et pour des questions de santé.

Mots-clés : plantes, huiles, phytostérols, Parc National de Kahuzi-Biega, R.D. Congo

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1. Introduction

The human diet often contains edible vegetable oil and animal fats as sources of sterols and fatty acids that the body needs for vital metabolic functions. Studies have shown the positive protective effect of vegetable oils against various cardiovascular diseases. The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapy (Sahu et al., 2021). Also, in rural and remote localities, residents are still dependent on herbal medicine (Qureshi et al., 2017).

Phytosterols are currently widely used as active compounds in various food preparations and drugs to treat and prevent different ailments among them the cardiovascular disease. Plant sterols are among active principles helping in the findings of new lead compounds in the fields of anti-inflammatory and antiarthritic drug (Mamillapalli et al., 2020; Singh et al., 2011).

Medicinal properties of *Parkia biglandulosa* (Mimosaceae) are reported due to the presence of metabolites like sterol and other. This plant is used against wide range of ailments (Rohini et al., 2020). Accumulating evidence reveals that phytosterols and diets enriched with them can control glucose and lipid metabolism, as well as insulin resistance (Prasad et al., 2022).

Butea Monosperma which extract described possessing significant Hepato protective and antipyretic property, the phytochemical analysis showed the presence of constituents such as flavonoids, glycosides and sterols (Sathish et al., 2011). New formulations have given rise to various consumer products, such as vegetable spreads, designed to lower total blood cholesterol and LDL cholesterol (Moreau et al., 2018). In assessing the role of herbal drug as an immunity booster during Covid-19 Pandemic (Aware & Rohane, 2021) stated that the bioactive compounds of ginger are known to inhibit viral replication. These bioactive compounds are nevirapine, b-sitosterol, 6 gingediol, germacrene, methyl-6-shogaol, 6-gingerol, a-linalool, 6-shogaol, gingerdion, zingiberene, etc… Among these the most potent inhibitors of reverse transcriptase (RT) enzyme is b-sitosterol, which is predicted to be used as non-nucleoside reverse transcriptase (NNRTIs) HIV-1 inhibitors.

Kahuzi-Biega National Park (KBNP), located in the Kivu region, Eastern Democratic Republic of the Congo (DRC), is home to many plants, potential oils sources for food preparations and medical therapy (WCS NR, 2022; Fern, 2014; Grade et al., 2008; Hawthrone & Jongkind, 2006). Different authors have conducted various studies to look for plants with significantly high commercial values (Silva et al., 2020; Kazadi, 2011; Kazadi et al. 2015a; Kazadi et al. 2015b; Bavhure et al., 2014).

In plants, phytosterols represent only a tiny fraction of the total lipid composition (Singh et al., 2014); they are often present at low concentrations in vegetable oils (0.80-12 mg / g). The qualitative and quantitative analysis of sterols is essential to identify lipid mixtures in the event of suspected adulteration. Also, the sterol profile of a commercial product is a valuable indicator of the quality and origin of the oil sold (Azadmard-Damirchi & Torbati, 2015). The current research aimed to determine the oil yield and the total sterols present in the seeds and barks of eight plants growing in and around KBNP.

2. Material et methods

2.1. Sites location map and description

The fieldwork investigations were made and samples for analysis collected in the study site showed in Figure 1. This study site lies in DR Congolese Albertine Rift area.

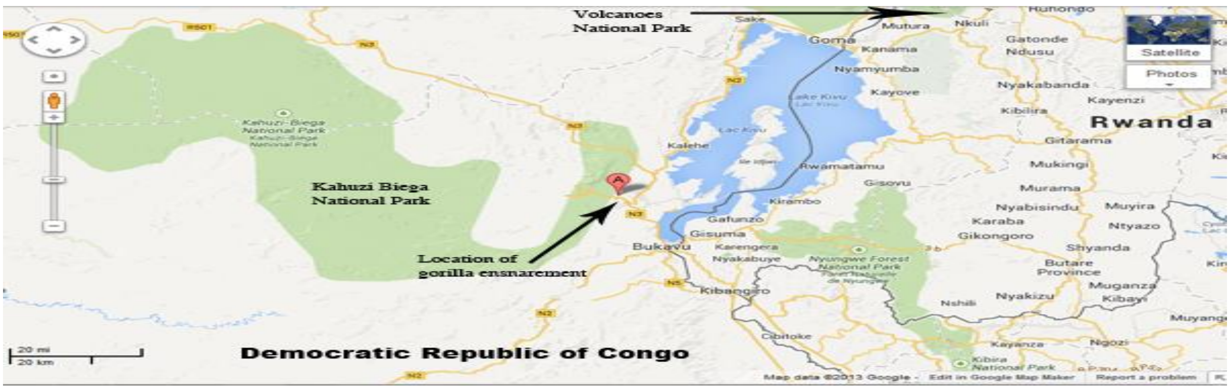

Figure 1. Map of the study site showing the park and surroundings (Google)

2.2. Material

Table 1 lists the names and collecting locations of plant materials analyzed for oil yield and content.

Table 1. Names and collecting locations of plant materials analyzed for oil yield and content

Plant materials		Code	Local name	Family	Part	Location
Albizia grandibracteata	*bark*	A.gB	Mushebei	Fabaceae	Bark	Lwiro
Hagena abissinica wild	*bark*	H.awB	Mwizuzu	Rosaceae	Bark	KBNP
Hagenia abissinica domestic	*bark*	H.adB	Mwizuzu	Rosaceae	Bark	Lwiro
Millettia dura	*seed*	M.dS	Nshunguri	Fabaceae	Seed	Lwiro
Millettia dura	*bark*	M.dB	Nshunguri	Fabaceae	Bark	Lwiro
Piptadeniastrum africanum	*seed*	Pi.aS	Mungu	Fabaceae	Seed	Lake Kivu
Piptadeniastrum africanum	*bark*	Pi.aB	Mungu	Fabaceae	Bark	Lake Kivu
Prunus Africana	*bark*	Pr.aB	Muhumbahumba	Rosaceae	Bark	Lwiro
Prunus salsii bark	*bark*	Pr.sB	Obulunus	Rosaceae	Bark	Lwiro
Prunus salsii	*seed*	Pr.sS	Obulunus	Rosaceae	Seed	Lwiro
Sesbania sesban	*seed*	S.sS	Munyegenyege	Fabaceae	Seed	Lwiro
Tephrosia vogelii	seed	T.vS	Mukulukulu	Fabaceae	Seed	Lwiro

2.3. Methods

2.3.1. Determination of oil yield in each plant material

The collected plant specimens indicated in Table I were brought to the phytochemistry laboratory of CRSN / Lwiro in the DRC for botanical identification and the preparation of dry powders. The raw fresh seeds and barks were dried before being ground to obtain fine powders. The oil content was determined according to the ISO 659: 1998 method (ISO, 1998) in the laboratory of Dr. Matthäus Bertrand at the Max Rubner-Institut (MRI), Germany (Silva et al., 2020). Briefly, about 2 g of plant material were ground in a ball mill and extracted with petroleum ether in a Twisselmann apparatus for six hours. A rotary evaporator removed the solvent at 40°C and 25 Torr. The lipoid was dried with a stream of nitrogen and stored at -20°C until use.

The lipoid mass was weighed, and the percentage in the initial sample calculated using the following formula with Pl = mass of lipoid and M = mass of sample powder used.

$$\%Lipid = \left(\frac{Pl}{M}\right)x100$$

2.3.2. Detection and quantification of sterols in the extracted oils

For the detection of sterols, 1 mL of oil dissolved in 1 mL of glacial acetic acid was added to 2 mL of an ice-cold mixture of acetic anhydride / concentrated H_2SO_4 (20: 1 v / v); the instant appearance of green indicates the presence of sterols.

The sterol composition was determined according to ISO / FIDS 12228: 1999 (E). Briefly, 250 mg of oil was saponified with a solution of ethanolic potassium hydroxide by boiling at reflux. The unsaponifiable material was isolated by solid-phase extraction on an aluminum oxide column (Merck, Darmstadt, Germany) on which fatty acid anions are retained during the passage of the sterols. The sterol fraction was separated from the unsaponifiable material by thin-layer chromatography (Merck, Darmstadt, Germany); after that, the sterols were assayed by GLC using betulin as an internal standard.

The compounds were separated on an SE 54 CB column (Macherey-Nagel, Düren, Germany; length 50 m, ID 0.32 mm, film thickness 0.25 µm). Other parameters were: hydrogen as a carrier gas; 1:20 division ratio; injection and detection temperature adjusted to 320 ° C; temperature program, 245 ° C to 260 ° C at 5 ° C / minutes. The peaks were identified using standard compounds (β-sitosterol, campesterol, stigmasterol), or a mixture of sterols isolated from rapeseed oil (brassicasterol), or a mixture of sterols isolated from sunflower oil (Δ7-avenasterol, Δ7-stigmasterol and Δ7 -campesterol). Each sample was analyzed in triplicate.

3. Results

3.1. Oil yield

The figure 2 shows that there are, 45.8% in *Prunus salsii* seed (Pr.sS), 30% in *Millettia dura* seed (M.dS), 22.9% in *Piptadeniastrum africanum* bark (Pi.aB), 13.5% in *Tephrosia vogelii* seed (T.vS), 11.3% in *Piptadeniastrum africanum* seed (Pi.aS), 11% in *Sesbania sesban* seed (S.sS), 10.3% in *Millettia dura* bark (M.dB), 1.1% in *Hagena abissinica* wild bark (H.awB), 0.67% in *Hagenia abyssinica* domestic bark (H.adB), 0.61% in *Prunus salsii* bark (Pr.sB), 0.59% in

Albizia grandibracteata bark (A.gB) and 0.46% *Prunus Africana* bark (Pr.aB). The content is richer in seeds compared to bark except for *Piptadeniastrum africanum*: Pi.aB (22.9%)> Pi.aS (10.3%).

However, the concentrations of 5-avenasterol, cholesterol, and 7-stigmasterol in Pi.aS are higher than in Pi.aB. The main sources would be M.dB and Pi.aB for β-sitosterol and camposterol; M.dB for

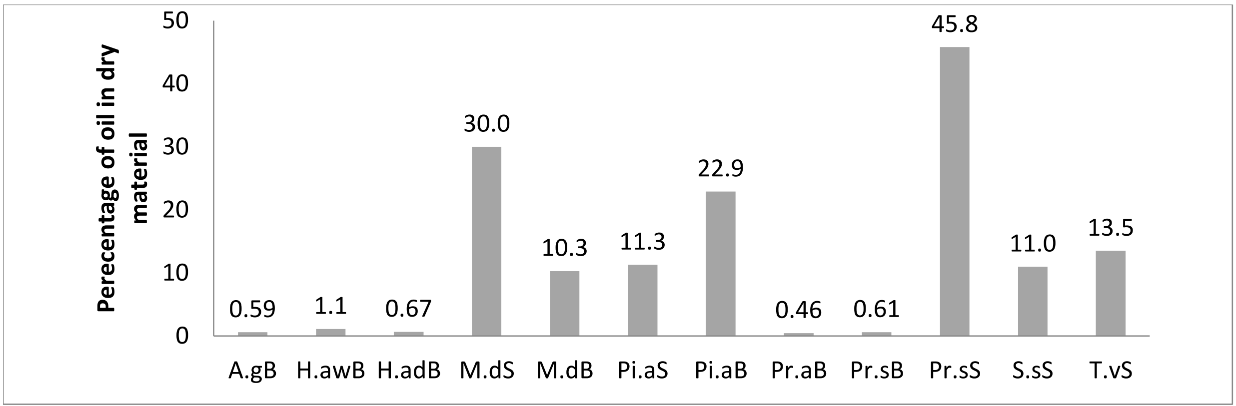

Figure 2. The yield of oils in different wild and domestic plants from KBN Park.

Legend: A.gB (*Albizia grandibracteata* bark); H.awB (*Hagena abissinica* wild bark); H.adB (*Hagenia abyssinica* domestic bark); M.dS (*Millettia dura* seed); M.dB (*Millettia dura* bark); Pi.aS (*Piptadeniastrum africanum* seed); Pi.aB (*Piptadeniastrum africanum* bark); Pr.aB (*Prunus Africana* bark); Pr.sB (*Prunus salsii* bark); Pr.sS (*Prunus salsii* seed); S.sS (*Sesbania sesban* seed); T.vS (*Tephrosia vogelii* seed).

stigmasterol, campostanol, 7-camposterol, sitostanol, 5,24-stigmastadienol and brassicasterol; P.iaB for 7-avenasterol; T.vS for 7-stigmastadienol. The eight

3.2. Sterols identified in each oil

Table 2 shows the sterols content (mg/kg oil) in plants oils.

Table 2. Sterols content (mg/kg oil) in plants oils

Sterols	M.dS	M.dB	Pi.aS	Pi.aB	Pr.sS	S.sS	T.vS
ß-Sitosterol	2337.8	25471.4	3595.7	19312.0	1850.7	2235.9	6637.0
Stigmasterol	1084.5	18470.6	1705.5	1603.0	10.5	3965.0	1295.5
Campesterol	138.3	3662.9	1054.5	3582.0	68.9	277.6	487.8
5Avenasterol	132.7	0.0	147.3	84.4	30.6	84.4	151.1
Cholesterol	41.5	458.6	626.4	330.6	14.8	51.7	23.9
Campestanol	37.9	481.0	15.8	92.7	5.2	0.0	5.8
Sitostanol	35.6	2781.7	209.0	579.4	25.8	107.9	90.0
7Campesterol	32.5	1855.1	82.7	52.5	0.0	84.7	12.2
7Avenasterol	19.1	0.0	44.6	1210.0	67.3	189.3	78.8
5,23-Stigmastadienol	18.5	117.3	0.0	58.5	22.1	0.0	52.7
7Stigmasterol	17.0	0.0	94.7	0.0	14.3	698.3	0.0
5,24-Stigmastadienol	10.0	489.3	84.7	72.1	59.0	33.9	26.2
Brassicasterol	0.0	272.7	0.0	26.1	1.7	0.0	0.0
Total sterols	3905.0	54060.7	7660.1	27002.5	2170.5	7729.0	8861.0

Legend: A.gB*(*Albizia grandibracteata bark*);* H.awB*(Hagena abissinica* wild bark*);* H.adB*(Hagenia abyssinica domestic* bark*);* M.dS *(Millettia dura* seed*);* M.dB *(Millettia dura* bark*);* Pi.aS *(Piptadeniastrum africanum* seed*);* Pi.aB *(Piptadeniastrum africanum bark);* Pr.aB *(Prunus Africana* bark*);* Pr.sB *(Prunus salsii* bark*);* Pr.sS *(Prunus salsii* seed*);* S.sS *(Sesbania sesban* seed*);* T.vS *(Tephrosia vogelii* seed*)*

4. Discussion

The Total sterols content (mg/kg oil) in plants oils is richer in seeds compared to bark except for Pi.aB (22.9%)> Pi.aS(10.3%) (table 2). Also, in general, the concentration of sterols is higher in bark than in seed. For example, the concentration of M.dB is approximately 10-times the concentration of M.dS. plants studied detain many other medicinal and nutritional values reported in the literature.

Albizia species appear in trees or shrubs, with 145 species listed in all tropical regions. In addition to their use as lumber, many critical biological activities have been reported on crude extracts and purified substances of various species of this genus (Rajemiarimoelisoa et al., 2015). For example, *A. lebbeck, A. julibrissin, A.*

gummifera, *A. chinensis*, *A. adianthifolia*, and *A. procera* are essential in traditional medicine. These species are used in folk medicine to treat rheumatism, stomach ache, cough, diarrhea, wounds, and intestinal worms.

The alcoholic and hydroalcoholic extracts of Albizia species have shown anticonvulsant, sedative, anti-inflammatory, antitumor, antifungal, antibacterial and antiparasitic activity. *A. lebbeck* is known to be helpful in the treatment of allergic conjunctivitis and atopic allergy (Grade et al., 2008; Barbosa et al. 2014; Nurul et al., 2011). Among the various compounds isolated from Albizia, saponins were the most common and the most abundant. *Albizia grandibracteata* has a low lipid yield (0.59%) and not a good source of sterols.

Hagenia abyssinica Bruce (Rosaceae) is a tree of about 6-20 m high, also has a low lipid yield, 0.67% in domestic bark and 1.1% in wild bark. Its extracts are known for having antibacterial activity (Hauman, 1952).

Millettia dura Dunn (Fabaceae) is a small tree. The specific epithet "dura" reflects the locality from which the first botanical collection was made, the Dura river in the Kibale forest in Uganda. Its geographic distribution is the DRC, Ethiopia, Kenya, Rwanda, Tanzania and Uganda (ICRAF, 2008). Its fruits are reported as food for elephants in Kibale National Park, Uganda (Rode et al., 2006).

According to the results obtained (table 2), *Millettia dura* gave 30% oil in the bark and 10% in the seeds containing 54060 mg/kg of sterols in the bark and 3905 mg/kg in the seeds. Previous studies have shown that the oil content of *Millettia dura* was 27.81%, containing oleic acid (32.3%) and essential fatty acids Omega-3, α-linolenic acid (21.2%), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). *M. dura* oil is rich in active vitamin E compounds (tocopherols, tocotrienol and plastochromanol) (Kazadi et al., 2015b). These compounds, in supplements with fish oil (omega-3 fatty acids) and Vitamin E are also reported in the treatment of psoriasis (Sondhi et al., 2021).

Piptadeniastrum africanum (Hook) is a large buttress rainforest tree that can reach 45-50 m high, which carries seeds of around 20-25 mm generally attached to the pod margin by a thin wire (Nyananyo, 2006). It is widespread in different types of forests in West and Central Africa and shared in many regions. It is commonly called African greenheart, with germs releasing from the strain; the sapwood, when fresh, is pale reddish-yellow or pinkish white and relatively large. In Nigeria, the tree is called Kiryar kurmi in Hausa, Ofie in Igbo and Agbo in Yoruba (Hutchinson & Dalzeil, 1972). The tree is gaining importance as a commercial wood tree, considered an excellent replacement for oak (*Quercus spp.*) in Europe (Hawthrone & Jongkind, 2006; Phongphaew, 2003).

The wood of *P. africanum* is essential in the construction/carpentry industry to construct bridges, floors, cupboards, canoes, etc. (Fern, 2014; Burkill, 1995; Youkparigha et al., 2019).

The wood has an unpleasant odor of ammonia when freshly cut and when it is wet. According to the results obtained (table 2), *P. africanum* has the highest sterol levels, 27002.5 mg/kg in the bark and 7660 mg/kg in the seeds. Ethnopharmacological investigations have indicated that decoctions made with the bark of the plant are used to treat coughs, bronchitis, headaches, mental disorders, hemorrhoids, genitourinary infections, sore throats, stomach, dysmenorrhea, male impotence, among others (Fern, 2014; Burkill, 1995; Youkparigha et al., 2019).

The aqueous, ethanolic and residual extracts obtained from the bark powders have an antifungal activity on a clinical strain of *Trichophyton mentagrophytes*. The three extracts (ethanolic, aqueous and residual) showed inhibitory activity with a minimum fungicidal concentration (MFC) between 0.195 mg/mL and 12.5 mg/mL. *P. africanum* was selected in the ethnobotanical survey carried out in the Haut-Sassandra region (Ivory Cost) for its frequency of use against skin infections (Kanga et al., 2017). Its richness in oil can support some of the indications.

Prunus africana (Hook f.), Kalkm (synonym: *Pygeum africanum* Hook f.) belongs to the Rosaceae family. Cameroon is home to a large part of the population of this species, which is threatened by extinction and, therefore, included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Awono et al., 2015).

This high-altitude species is of economic, social and scientific importance for the local people and the international community. Locally, it is a source of timber (Craft), firewood, income and it contributes to the traditional pharmacy. Scientifically and internationally, its bark is used by Western industries to treat benign prostate hyperplasia (Awono et al., 2015).

Prunus salsii STANDLEY, from Guatemala, was introduced in Kivu eastern of DRC in association with Eucalyptus. It is a fast-growing tree, usually 9-15 m, sometimes reaching 35 m (Hauman, 1952).

P. salasii has the highest lipid yield (45.8%) and oil content. Prunus genus has several economically essential members, including the cultivated almond, peach, plum, cherry and apricot. In addition, many species flower prolifically and are grown as ornamentals.

Sesbania sesban L. (Fabaceae) seeds yielded 11% of oil and only 0.1% of sterols. A previous study (Kazadi et al., 2015b) obtained a 7.2% oil yield. The content in linoleic acid, an omega-6 fatty acid, was found 58.82%, while alpha-linolenic acid, an omega-3, accounted for 5.88%. It also contains a high relative content of tocopherols (10.9 mg/100 g) (Kazadi et al., 2015b). In addition to the common fatty acids, very long-chain FAs with 20 carbons or more were detected in *S. sesban* oil. Thus, *S. sesban* oils could have significant economic value as oleic acid sources compared to 59-75% found in the pecan oil, 61% in canola oil, or 20-85% reported for peanut oil and sunflower oil (Dambatt & Ogah, 2000).

Tephrosia vogelii Hook.F. (Fabaceae), common names in English, include fish bean and Mukulukulu in eastern DRC. The seed yielded 14% (Adriaens, 1944) and 17.23% (Kazadi et al., 2015b) of oil.

The plant is among plants of community interest and used traditionally as antiparasitic, bactericidal, abortifacient, insecticide and fishing (Defour, 1995 ; Xin et al., 2009). It is a potential source of rotenone, an important nonresidual insecticide and a fish poison (Blommaert, 1950 ; Lambert et al., 1993).

The oil of *Tephrosia vogelii* contains five unsaturated and five saturated FAs: linoleic acid (18:2n6; 40.34%), oleic acid (18:1n9; 19.97%), alpha-linolenic (18:3n3;7.62%), eicosenoic acid (20:1n9; 0.66%), eicosapentaenoic acid (20:5n3; 0.66%) and palmitic acid (16:0;13.98%), stearic acid (18:00; 5.78%), arachidic acid (20:00;2.05%), behenic acid (22:00;0.12%), lignoceric acid (24:00;1.55%).

The fatty acid profile showed that unsaturated acids represent about 68.7% of the total content. One study (Bavhure et al. 2014) also showed the relatively high presence of ß-Sitosterol and other sterols in Tephrosia oils that could have considerable economic value and be considered a new source of oleic acid.

5. Conclusion

Not all studied plant species are equally rich in oil yield and sterols content. The bark powder of *Piptadeniastrum africanum* and *Millettia dura* are the most significant rich sources of sterols, particularly in β-sitosterol, campesterol and stigmasterol. Wild species would yield more oil than domestic species. However, all eight plants have interesting, medical and industrial values.

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