



## Fatty Acid Composition of *Lebrunia bushiae* Staner and *Tephrosia vogelii* Hook.f. Seed Oils

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors BB, FMK, AOM, KM, AM, IM, JNK conceived experiment and designed the experiments. Authors BB, FMK, AOM, and KM performed experiments. Authors KM, AM, IM, JNK analyzed the data. Authors BB, FMK, KM, JNK wrote the paper. All authors read and approved the final manuscript

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### ABSTRACT

**Aim:** The nutritive and medicinal values of vegetable oils rely on the nature of their fatty acids (FAs). This study aimed to determine the nature and content of FAs in *Lebrunia bushiae* Staner and *Tephrosia vogelii* seed oils which are used by the local population of South Kivu province in DRC mainly for medical purposes.

**Materials and Methods:** The seeds were harvested from Bunyakiri, zone close to the National Parc Kahuzi-Biega, in South-Kivu province/DRC. The oils were manually expressed, and the FAs composition characterized by gas chromatography (GCMS).

**Results:** Ten major FAs were detected in *Lebrunia bushiae* seed oil comprising of 4 saturated and 6 unsaturated of which oleic acid (18:0;43.0%), linoleic acid (18:1;11.74%) and erucic acid (22.1;14.07%) predominate. In the *Tephrosia vogelii* oil, 5 saturated and 5 unsaturated FAs were detected comprising in majority of linoleic acid (40.34%), oleic acid (19.97%), alpha linolenic acid (7.62%), palmitic acid (13.98%) and stearic acid (5.78%).

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**Conclusions:** The yield in oils from these two plants is sufficient for being exploited. The composition of *Tephrosia* oil presents high nutritive value while the nutritive value of *Lebrunia* oil may be limited by a high content of erucic acid, making it much valuable for medicinal interest unless its erucic acid content is reduced to make it edible.

*Keywords:* *Lebrunia bushiae*; *Tephrosia vogelii*; oil; fatty acids.

## 1. INTRODUCTION

Vegetable oils are natural products of plant origin consisting of ester mixtures derived from glycerol with chains of fatty acids (FAs) containing commonly 14 to 20 carbon atoms with different degree of unsaturation. They play important functional and sensory roles in food products, act as carriers of fat-soluble vitamins (A, D, E, K), and provide energy and essential FAs responsible for growth.

In Africa, during the last decade, the production of vegetable oils from three most important crops (palm, cotton, peanuts) shows an upward trend due to the increase in the African population and rising standards of living [1,2]. As the global world population is steadily growing, population needs for oil and meal will also steadily continue to grow. The desire for personal care products in many Sub-Saharan countries has led to vegetable oils, being imported from Asian spaces, even palm oil.

The Democratic Republic of Congo (DRC) is by far the African country with the highest biodiversity [3] and its flora is among the richest in the world. At least 50,000 plant species were currently inventoried of which 10% are oil-bearing potentials [4]. Today, not all these resources are exploited for industrial market or personal care, yet the country faces food shortage and health problems of all kinds. It is more than important that these resources be featured to better use.

*Lebrunia bushiae* Staner is a tree of Clusiaceae plant family called Bukerenge or Mashai in Eastern DRC. Its seed oil is traditionally used medicinally for various ailments including parasitic infections, and pulmonary disorders such as bronchitis and cough. It is also used in cosmetics to treat dermatological diseases such as damaged skin, eczema, scabies, wound, snakes and insect bites among others; and in veterinary medicine, the oil is used for some rabbit diseases [5].

*Tephrosia vogelii* Hook.f. is another plant marked of community interest and used traditionally as antiparasitic, bactericidal, abortifacient, insecticide, and also for fishing [5-8].

This study is one of the ongoing investigations aimed at evaluating nutritive and medical values of local plants by measuring their yield in oil and the content of FAs.

## 2. MATERIALS AND METHOD

### 2.1 Plant Seeds Collection and Treatment

The plant seeds were collected from Bunyakiri area in the North of Bukavu city close to Kahuzi-Biega National Park (KBNP) in Kivu region, Eastern DRC. Voucher specimens of the two plants are kept in the herbarium of the Centre of research in natural substances of Lwiro

(CRSN/Lwiro) in South Kivu, DRC. Seeds were manually harvested and then taken to CRSN/Lwiro where they were sun dried before toasting in oven at 100°C for thirty minutes. After shelling by hand, the seed almonds were crushed with a coffee-mill (model Corona 01 Landers and CIA, SA) to produce fine paste from which the oil was extracted.

## **2.2 Extraction and Determination of Seed Oil Content**

The fine seed almonds paste was extracted using Soxhlet procedure [9] by repeated washing with petroleum ether (boiling point of 40-60°C). After 8 hours, the Soxhlet extraction flask containing oil and solvent mixture was removed. The oil dissolved in petroleum ether was filtered on paper filter and the solvent evaporated under vacuum in a rotary evaporator model Eyala of Tokyo Rikakikai Co. Ltd. The remaining solvent traces were removed by heating the flask containing oil in a water bath 100°C. The oil obtained was thereafter hermetically stored in closed bottles and kept in a refrigerator till further analysis. The percentage of seed oil content was determined by dividing the weight of obtained oil on the weight of fine seed almonds paste used.

## **2.3 Identification and Quantification of Fatty Acids**

The identification of FAs was realized by Gas Chromatography [10]. Oil samples of approximate 50mg were transferred to thick-walled 15ml glass tubes, taking care to avoid water contamination. The tubes were filled with an accurately determined amount of the saturated FA, as internal standard. This was added to the tubes by sampling 50.0µl of a solution of C19:0 chloroform into the tubes, and then allowing the chloroform to evaporate. Anhydrous methanol containing hydrogen chloride was added to the methanol as dry gas in a concentration of 2mol/l, to allow the hydrolysis of oil triglycerides. The tubes were securely closed with teflon-lined screw caps. After keeping the tubes in an oven at 90°C for two hours, the samples were methanolysed by the replacement of glycerol in the triglyceride by methanol. In this way, all FAs were converted to FA methyl esters (FAMES). After cooling to room temperature, approximately half the methanol was evaporated by bubbling nitrogen-gas through the mixture, 0.5ml distilled water was then added. The FAMES were extracted from the methanol/water-phase with 2 x1.0ml hexane by vigorous shaking by hand for one minute, followed by centrifugation at 3000rpm. The FAMES extracted were recovered in a 4 ml vial with teflon-lined screw cap. One µl of the FAMES extracted was automatically injected splitless on a capillary column.

The quantitatively most important FAs were identified in the samples, using a standard mixture basing on previous experience of relative retention times of FAMES and mass spectrometry (MS) [11,12]. The peaks were integrated by Chromeleon software and the resulting area values exported to Excel, where they were corrected by response factors. These empirical response factors, relative to 18:0, were calculated from the 20 FAMES, present in known proportions in the standard mixture GLC-68D from Nu-Chek-Prep (Elysian, Minn, USA). An average of 10 runs of the standard mixture was used for these calculations. The response factors for the FAMES for which there were no standards, were estimated by comparison with the standard FAMES which resembled each of those most closely in terms of chain length and number of double bonds. The relative amount of each FA in a sample was expressed as percentage of the sum of all FAs in the sample. Mean values and their standard deviations (mean±SD) were calculated for each fatty acid from 4 replicates. The smallest peaks with areas of less than 0.1% of the total area of all peaks were not considered. Correlations were performed using GraphPad.Prism.v5.01 statistical software.

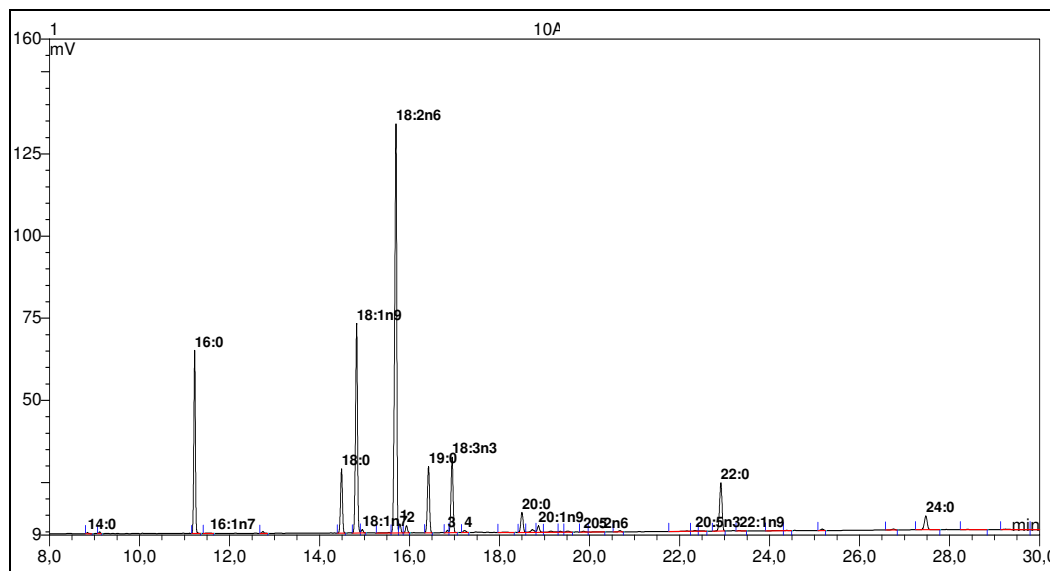
### 3. RESULT

The results are summarized in Table 1 and Fig. 1. The oil of *Tephrosia vogelii* contains 5 unsaturated and 5 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 crude oil yield from *Lebrunia bushiae* seeds was about 77.6%. Ten major FAs comprising of 4 saturated and 6 unsaturated were detected. The four predominant components were oleic acid (43.0%), erucic acid (14.07%), linoleic (11.74%) and heneicosylic acid (11.41%). Of total FAs, saturated fatty acids constituted 9.0%, monounsaturated 77.2%, and polyunsaturated 13.8% respectively. Nervonic acid (24:1 n9), which is a very long chain acid is remarkably represented in this oil with 8.8%. Erucic acid is absent in the oil of *Tephrosia vogelli*.

**Table 1. Yield of fatty acids in crude oil from *Tephrosia vogelii* and *Lebrunia bushiae* seeds**

Fatty acids Name	Short hand	<i>Tephrosia vogelii</i>		<i>Lebrunia bushiae</i>	
		%(Mean	± SD)	%(Mean	± SD)
Palmitic acid	16:00	13.98	±0.03	2.96	± 0.37
Stearic acid	18:00	5.78	±0.02	2.32	± 0.02
Oleic acid	18:1n9	19.97	±0.05	43.00	± 0.81
Linoleic acid	18:2n6	40.34	±0.08	11.74	± 0.19
Alpha-linolenic acid	18:3n3	7.62	±0.03	0	
Arachidic acid	20:00	2.05	±0.00	1.04	± 0.04
Eicosenoic acid	20:1n9	0.66	±0.00	0	
Eicosapentaenoic acid	20:5n3	0.12	±0.02	0	
Heneicosylic acid	21:00	0		11.41	± 0.65
Behenic (docosanoic) acid	22:00	5.76	±0.03	2.67	± 0.10
Erucic acid	22:1n9	0		14.07	± 0.06
Docosahexaenoic acid	22:6n3	0		2.02	± 0.10
Nervonic acid	24:1n9	0		8.78	± 0.11
Lignoceric (tetracosanoic)	24:00	1.55	±0.03	0	



**Fig. 1. Chromatogram of fatty acid methyl esters from *Tephrosia vogelii* crude oil. GCMS conditions: Agilent 6890N auto sampler 7683B series; Stationary phase: Polyethylene-glycol (PEG); Mass Selective Detection: Ionization energy, 70 eV; source temperature: 230 °C; The injector temperature: 260 °C; Column: CP WAX 52CB 25m X 0.25mm (i.d), thickness of 0.2µm; Standard Mixture (20FAME): GLC-68A from NuCheckPrep Elysian, WO**

## 4. DISCUSSION

### 4.1 Nature and Content of Fatty Acids

The oil yield in the seeds of *Lebrunia bushae* was 77.6% compared to 7.62% for *Tephrosia vogelii* seeds. The oil content in *Lebrunia bushae* is about two times higher compared to other commercial oil-bearing plants such as cottonseed (36%), sesame (44%), peanut (40%), and palm oil (40%) [13-17]. Yield of *Tephrosia vogelii* seeds is comparable to soja oil (7.8%) and colza oil (7.9%) [13]. The fatty acid profile showed that unsaturated acids represent about 79.6% and 68.7% of the total content for *Lebrunia bushae* and *Tephrosia vogelii* respectively. The two main FAs are oleic acid (43%) in *Lebrunia* oil and linoleic acid (40.3%) in *Tephrosia* oil. The ratio of unsaturated to saturated fatty acids is significantly high (10) in comparison with other vegetable oils like palm oil (1), raphia oils (1 to 3) [18]. The oil from *Tephrosia vogelii* is higher in omega-3 fatty acids (ALA) compared to other commercial oils such as peanut oil (0.4%), sesame oil (0.4%) and sunflower oil (0.5%) and in the same range with soybean and rapeseed which are commercial sources of alpha-linolenic acid (ALA) [13]. For the omega-6, this plant contains 40.3% which is lower than rapeseed and soybean but comparable and even superior to other edible commercial oils such as sesame, peanut and sunflower crops. The nature and content of FAs from *Tephrosia* are comparable to the findings on the Chinese species [7].

## 4.2 Nutritional and Medical Value

A special attention concerns the presence of erucic acid (14.07%) in *Lebrunia* oil but absent in *Tephrosia* oil. Fatty acids are widely consumed worldwide by the food industry, in medicine, cosmetics and soap industries [19-21]. The high oil content of *Lebrunia bushiae* indicates that the plant can be economically exploited.

Nutritionally, the concentration of linoleic acid in *L. bushiae* (11.74%) is comparable to the value of olive oil (9.40 %), palm oil (10.2 %) [13] but less than *Raphia sese* (38.5%) and *Raphia laurentii* (33.9%) [17]. *Tephrosia* seeds contain the same amount of linoleic acid as *R. sese* and *R. laurentii*.

Food rich in linoleic acid prevent against cardiovascular disorders, atherosclerosis and increased blood pressure [21]. In several studies in animals or human cells, omega-3 fatty acids and conjugated linoleic acid have beneficial effects on one or more current health problems such as cardiovascular disease, hypertension, rheumatoid arthritis, certain cancers and neurological disorders, insulin resistance, diabetes and obesity [22]. Symptoms of deficiency in linoleic acid include eczema, hair loss, and male infertility. In cosmetics, oils rich in linoleic acid help to regenerate and moisturize the skin [19], which justifies its importance in cosmetology.

*L. bushiae* is richer in oleic acid (43.0%) than *Tephrosia* (20%). Oleic acid makes up 59-75% of pecan oil [23], 61% of canola oil [24], 36-67% of peanut oil [25], 60% of macadamia oil, 20-85% of sunflower oil and 14% of poppy seed oil [26]. Comparing to this, we find that *L. bushiae* and *Tephrosia* oils could have great economic values and considered as a new source of oleic acid. Oleic acid is the most ever widely consumed fatty acid and is a fatty acid of interest on health as it lowers total cholesterol level, prevents the development of cardiovascular diseases and is source for the production of antioxidants [27,28]. Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil [29]. It may hinder the progression of adrenoleukodystrophy (ALD), a fatal disease that affects the brain and adrenal glands [30].

The oil of *L. bushiae* proved richer in nervonic acid (8.75%), which is remarkable. Nervonic acid is abundant in King Salmon (Chinook) with 140mg/100g, yellow mustard seed 83mg/100g, flaxseed 64mg/100g, Sockeye salmon 40mg/100g, sesame seed 35mg/100g, and macademia nuts 18mg/100g [31]. This fatty acid is essential to help maintain brain health. Epidemiological studies have shown that in areas where mustard, known to be rich in nervonic acid, is much used, there is less cardiovascular diseases. There is more interest in using nervonic acid for the treatment of neurological diseases associated with dementia, especially Alzheimer's disease, multiple sclerosis, and adrenoleukodystrophy [32,33]. Nervonic acid is a natural component of breast milk and is currently used as a supplement in infant formulas to help brain development in children less than five years [34]. Thus, the oil of *L. bushiae* finds supplement reason to be considered indispensable source of fatty acids for health.

The content of Behemic acid is around 2.75% in *L. bushiae*, and 2.83% in *Tephrosia*. Behenic acid name (also docosanoic acid) comes from the Ben oil tree, *Moringa oleifera*. At 9%, it is a major component of Ben oil (or behen oil). In appearance, it consists of white to cream color crystals or powder with a melting point of 80°C and boiling point of 306°C. Behenic acid is also present in some other oil-bearing plants, including rapeseed (canola) and peanut oil. It is estimated that one ton of peanut skins contains 5.9kg of behenic acid

[35]. Commercially, behenic acid is often used to give hair conditioners and moisturizers their smoothing properties, and also in lubricating oils as solvent evaporation retarder in paint removers. Despite its low bioavailability compared with oleic acid, behenic acid is a fatty acid that raises blood cholesterol in humans and is therefore not a suitable substitute for palmitic acid in manufactured triacylglycerols.

*L. bushiae* would be also interesting for its content (2.04%) in docosahexaenoic acid (DHA). DHA is the most abundant omega-3 fatty acid in the brain and retina. DHA comprises 40% of the polyunsaturated fatty acids (PUFAs) in the brain and 60% of the PUFAs in the retina. Fifty percent of the weight of a neuron's plasma membrane is composed of DHA [36]. DHA is richly supplied during breastfeeding, and DHA levels are high in breast milk regardless of dietary choices. DHA deficiency is associated with cognitive decline [32]. Researches on DHA supplementation and attention deficit hyperactivity disorder (ADHD) have shown mixed results. One study of pure DHA supplementation on children with ADHD found no behavioral improvements [34], while another study found fish oil containing both EPA and DHA did improve behavior [37].

According to the World Health Organization, evidence is "convincing" that consumption of palmitic acid increases risk of developing cardiovascular diseases, placing it in the same evidence category as trans fatty acids [38]. The two plants are poor in palmitic acid, making them suitable for health.

However, the oil of *L. bushiae* proved richer in erucic acid (14.07%). This plant could be considered as the source of this acid. Erucic acid is used to produce emollients, surfactants, and other chemicals. But medical implications are conflicting. Erucic acid enters into the composition of Lorenzo's oil, used in the treatment of neurobiological disorders such as thrombocytopenia and adrenoleukodystrophy, properties probably related to high content in erucic acid that contains the oil [39]. This acid is also used as an ingredient in products inhibiting appetite (anorexic); this property can be exploited in the treatment of obesity.

Nevertheless, the yield of erucic acid is higher than the limits fixed by the regulations of the European Commission [40] stating that the content should not exceed 5% in edible oils, due to the toxicity of this acid. The effects of erucic acid from edible oils on human health are controversial. No negative health effects have ever been documented in humans [41]. However, for the reasons of toxicity, it is not advisable for nursing mothers or babies to eat food containing erucic acid [42]. The levels of erucic acid in human foods are restricted, in part, over concerns that it may adversely affect heart tissue [43], but is not metabolized there [44]. Food grade is regulated to a maximum of 2% erucic acid by weight in the USA and 5% in the EU with special regulations for infant food. Thus the edibility of *Lebrunia* oil is questionable unless the erucic acid is removed or reduced to the acceptable limits.

On the other hand, it is known that when plant sterols (phytosterols) are ingested, they are not well absorbed by the body, but inhibit cholesterol absorption in intestine, so cholesterol levels in the blood are lowered. Free phytosterols extracted from oils are insoluble in water, relatively insoluble in oil, and soluble in alcohols. Beta-sitosterol, lanosterol, and stigmasterol have been found in fruit coat of *Tephrosia* species [45,46]. In our study, we did not search for phytosterols content in *Lebrunia* or *Tephrosia* oil, but their presence may add supplement value to their nutritional and medicinal quality. However, despite well documented LDL-cholesterol lowering effects of plant sterols, no scientifically evidence of any beneficial effect on cardiovascular overall mortality is documented.

## 5. CONCLUSION

The present study has documented a rich profile of healthy fatty acids found in the *L. bushiae* and *Tephrosia* seed oils supporting their popularized use for various health problems. However, due to its high content of erucic acid that is beyond the level recommended by the European regulations, the use of *Lebrunia* oil as edible oil should be limited unless a supplement treatment is conducted to lower the content of erucic acid in food preparations particularly for children.

## CONSENT

Not applicable.

## ETHICAL APPROVAL

Not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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