JOURNAL OF ADVANCEMENT IN
MEDICAL AND LIFE SCIENCES

Journal homepage: http://scienceq.org/Journals/JALS.php

Research Article Open Access

The Occurrence of Very Long-Chain fatty acids in oils from Wild Plant species Originated from Kivu, Democratic Republic of the Congo

M. Kazadi[1], P.T. Mpiana[2*], M.T. Bokota[3], KN Ngbolua[2], S. Baswira[4] and P. Van Damme[5]

[1]Dapartement de Biologie, Centre de Recherches en Sciences Naturelles, Lwiro, Sud Kivu, D.R.Congo
[2] Faculté des Sciences B.P. 190, Université de Kinshasa, Kinshasa XI, D.R. Congo
[3]Faculté des Sciences, Université de Kisangani, Kisangani, D.R. Congo
[4]Department de Chimie, Institut Supérieur Pédagogique de Bukavu, D.R. Congo
[5]Department of Plant Production, Tropical & Subtropical Agriculture & Ethno-botany, Gent University, Belgium.

*Corresponding author: P.T. Mpiana, Contact no: +243818116019, E-mail: ptmpiana@yahoo.fr
Received: September 8, 2014, Accepted: October 28, 2014, Published: October 29, 2014.

ABSTRACT

Fatty acids C20-C26 are important for use in oleo-chemical industry whereas they also allow assessing chemotaxonomic relationships among plant taxa. There are however, comparatively few common vegetable fats which contain them in appreciable amounts.Using gas chromatography this type of very long-chain fatty acids was analyzed in oils from Pentaclethra macrophylla (Fabaceae), Millettia dura (Fabaceae), Tephrosia vogelii (Fabaceae),Cardiospermum halicacabum (Sapindaceae), Maesopsis eminii (Rhamnaceae), Podocarpus usambarensis (Podocarpaceae) and Myrianthus arboreus and M. holstii (Moraceae),wild plant species from Kahuzi-Biega National Park and adjacent areas in D.R. Congo. These plants are used by the local population mainly for nutrition and medical purposes.The percentage of very-long chain fatty acids in the analyzed oils ranged from 1.2 to 21.3%. P. macrophylla revealed the highest rate and M. holstii showed the lowest rate. These acids consist of arachidic, eicosenoic, eicosadienoic, eicosapentaenoic, erucic, behenic and lignoceric acids. Traces of non-methylene interrupted fatty acids were identified (0.1 – 0.2%) in three of the analyzed species and at a significant level (7.4%) in P. usambarensis. Some species had an arachidic acid content similar to that of groundnut oil and could be used as an alternative source of this acid.

Keyword: Wild plants, fatty acids, Kahuzi-Biega National Park, Democratic Republic of the Congo.

INTRODUCTION

The common fatty acids (FAs) regularly met in plant oils include lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids. Very long-chain fatty acids (VLCFAs) (≥20 carbon atoms) are more commonly found in fish oil rather than in plants [1,2]. VLCFAs are important in the oleo-chemical industry where they cover about 6% of the industries requirements in fatty acids (FAs)[3,4].They are used specifically in the manufacturing of some products such as high-grade candles. In nutrition, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (20 and 22 carbon, respectively) are recognized as essential Fas [4]. There are comparatively few common vegetal fats that contain appreciable amounts of VLCFAs. Groundnut oil which contains around 2% of arachidic acid [5, 6] is known as an important vegetal source of this FA [7] and is an oil required for nutritional use. This illustrates that it is still quite useful to look for new sources of VLCFA oils.

In order to promote the conservation of certain plant species by highlighting possible useful products they might contain, oils from a number of wild plant species were analyzed [8]. These plants naturally occur in Kahuzi-Biega National Park (KBNP) and surrounding areas in the Democratic Republic of Congo (DRC) where a number of them are threatened with extinction [9].

In the present work, eight promising species: *Cardiospermum halicacabum* (Sapindaceae),*Maesopsis eminii (Rhamnaceae), Millettia dura* (Fabaceae), *Myrianthus arboreus* and *M. holstii (*Moraceae*), Pentaclethra macrophylla* (Fabaceae), *Podocarpus usambarensis* (Podocarpaceae) and *Tephrosia vogelii* (Fabaceae) (Moraceae) from Kahuzi-Biega National Park and adjacent areas in DRC were analyzed in order to see whether they can be potential source of these specific FAs. These plant species are wild plant species used by the local population mainly for nutrition and medical purposes.

MATERIALS AND METHODS:

Seed samples from the above mentioned plant species were collected from KBNP and surrounding in South–Kivu Province, Eastern DRC. Mature seed samples were collected from beneath the trees and put in plastic bags. Only healthy seeds whose kernels were still protected by their seed coat were collected to avoid infected seeds. At least 500 g of seeds were collected from 5 to 7 trees for each plant species. To confirm identification, voucher specimens of these plant species were brought at Herbarium of the "*Centre de Recherche en Sciences Naturelles de Lwiro"* (CRSN/Lwiro) in South–Kivu, DRC and at the Herbarium of the Department of Botany of Makerere University, Uganda. Oil

extraction, identification and quantification of FAs was done following the American Oil Chemists Society's methods [10]. Oil samples were extracted from seed kernels by petroleum ether in a Soxhlet apparatus [11]. Identification and quantification of fatty acids was done using Gas Chromatography (GC) using a Chromatograph Hewlett-Pakard 5890A model with an auto-sampler Hewlett-Pakard 7673A model in the Department of Chemistry, University of Bergen, Norway, by Dr. Otto Grahl-Nielsen. Oil samples were weighed to approximately 50 mg, and were transferred to thick-walled 15 mL glass tubes, avoiding water contamination. Tubes were prepared with an accurately determined amount of nonadecanoic acid (19:0; Nu Chek Prep, Elysian, Minn., USA) a saturated fatty acid used as internal standard. The latter was added to the tubes by pipetting 50 µL of its solution in chloroform into the tubes, and then allowing the chloroform to evaporate. This pipetting was carried out with a Handystep electronic, motorized repetitive pipette. Then 750 µL anhydrous methanol containing hydrogen chlorides was added to the methanol as dry gas, in a concentration of 2 mol/l to allow hydrolysis of the oil triglycerides. Tubes were securely closed with teflon-lined screw caps. After keeping the tubes in an oven (model Boekel from Arthur H. Thomas Co.) at 90 °C for two hours, the samples were then methanolysed by the replacement of glycerol in the triglyceride by methanol. By doing so, all fatty acids were converted to fatty acid methyl esters (FAMEs). After cooling to room temperature, approximately half the methanol was evaporated by nitrogen-gas bubbling, and 0.5 mL distilled water was added.

FAMEs were then extracted from the methanol/water phase with 2 x 1.0 mL hexane by vigorous shaking for one minute each time, followed by centrifugation at 3000 rpm. FAMEs extracted were recovered in a 4 mL vial with teflon-lined screw cap. The concentration of the FAMEs in the extracts was adjusted by dilution with hexane to obtain levels suitable for gas chromatography.

One µL of the adjusted extract was automatically injected split less (the split was opened after 4 min), on a capillary column. Samples were analyzed in random order with a standard solution, GLC 68D from Nu Chek Prep (Elysian, Minn., USA) containing 20 FAMEs.

The 20 - 40 quantitatively most important fatty acids were identified in the samples, by way of the standard mixture following previous experience [12] of FAMEs relative retention times. The smallest peaks, i.e. those with areas of less than 0.1% of total peak area, were not considered. Peaks were automatically integrated by Chromeleon software and the resulting area values exported to Excel, where they were corrected by response factors [13]. These empirical response factors, relative to 18:0, were calculated from the 20 FAMEs, present in known proportions in the standard mixture. 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 fatty acid in a sample was expressed as percentage of the sum of all fatty acids in the sample [12, 14].

The data analysis was performed with at least 3 replicates, mean values and standard deviations (mean ± SD) calculated and all data subjected to analyses of variance (ANOVA). The least significant differences of means (LSD) test at 5% probability level were also carried out. All analyses were done using the GenStat computer package program (GenStat release 7.1, Copyright 2003, Lawes Agricultural Trust; Rothamsted Experimental Station, Seventh Edition).

RESULTS AND DISCUSSION

Twenty-four FAs were determined and identified in the studied plant species as shown in Table 1.

Table 1: Fatty acid (FA) composition (wt. % of total of FA) of oil from plants of Kahuzi-Biega National Park and surrounding areas in D.R. Congo. All FAs showed significant variation among species (p<0.001)

FA & symbol	*Cardiospermum halicacabum*	*Maesopsis eminii*	*Millettia dura*	*Myrianthus arboreus*	*Myrianthus holstii*	*Pentaclethra macrophylla*	*Podocarpus usambarensis*	*Tephrosia vogelii*	LSD	CV%
Myristic 14:0	-	-	-	0.1	0.1	-	-	0.1	0.01729	19.0
Palmitic 16:0	10.7	8.3	4.1	3.9	4.0	4.9	4.0	14.0	0.1535	0.9
Palmitoleic 16:1n7	0.2	-	-	0.2	0.2	0.1	-	-	0.02615	10.5
Stearic 18:0	8.5	17.8	3.3	2.8	3.3	1.9	3.5	5.8	0.2304	1.1
Oleic 18:1n9	36.8	36.7	31.8	9.8	8.6	30.1	39.6	19.6	0.2382	0.4
Oleic 18:1n7	0.7	0.3	0.5	2.5	1.2	0.5	0.3	0.3	0.03344	1.8
LA 18:2n6	35.1	26.8	20.2	77.2	80.2	40.6	29.7	40.3	0.5279	0.7
ALA 18:3n3	0.6	3.8	21.2	0.5	0.5	0.1	9.0	7.6	0.05142	0.4
Arachidic 20:0	2.4	2.3	0.8	0.1	0.2	2.0	0.4	2.0	0.008787	1.2
Eicosenoic 20:1n9	0.5	2.2	2.4	0.3	0.4	2.4	2.0	0.7	0.10636	4.1
Eicosadienoic 20:2n6	-	0.1	0.2	0.3	0.2	0.2	2.9	0.1	0.01547	2.1
Eicosatrienoic 20:3n3	-	0.4	-	0.1	0.1	-	0.1	-	0.004386	3.5
EPA 20:5n3	0.2	0.2	0.3	0.4	0.1	0.2	0.2	0.1	0.08086	18.0
Behenic 22:0	1.5	0.6	7.3	-	0.1	6.3	0.3	5.8	0.06019	1.0
Erucic 22:1n9	-	-	0.7	0.2	-	0.3	-	-	0.01446	3.6
Lignoceric 24:0	2.0	0.2	2.6	0.1	0.1	9.8	0.2	1.5	0.09896	2.7
DHA 22:6n3	-	-	0.1	0.1	-	-	-	-	0.01382	23.0
Nervonic 24:1n9	-	-	0.1	-	-	0.1	-	-	0.013086	19.4
c9, t12	0.4	0.1	0.7	0.6	0.3	0.3	0.1	0.8	0.012148	1.1
t9, t12	0.4	0.1	0.7	0.5	0.3	0.2	0.1	0.7	0.01531	1.6
c9, c12, t15	-	-	1.2	0.1	-	-	0.2	0.2	0.011512	1.8
t9, c12, c15	-	-	1.7	-	-	-	0.2	0.3	0.008787	1.0
c5, c11, c14	-	-	0.1	0.2	0.2	-	5.4	-	0.05273	5.0
c5, c11, c14,c17	-	-	-	0.1	0.1	-	2.0	-	0.008152	2.2
%FA Tot.	82.0	79.7	78.8	63.8	79.4	82.0	84.4	61.3		

LA = linoleic acid, ALA = α-linolenic acid, DHA = Docosahexaenoic acid, EPA = Eicosapentaenoic acid, N (replicate) = 3.
LSD: Least significant differences of means (5% level), CV%: coefficients of variation, (-) = was found in trace.

As it can be seen in the table, the composition of the FAs from the oils are significantly different ($p<0.001$). All The oils contain linoleic (18:2), oleic (18:1), stearic (18:0), palmitic (16:0) and α-linolenic (18:3) acids as well as a number of VLCFAs. Total FA contents in oils from the studied plant species ranged from 61.3% for *Tephrosia vogelii* to 84.4% for *Podocarpus usambarensis*.
VLCFA fractions in analyzed oils ranged from 1.2 to 21.3% as indicated in Figure 1.

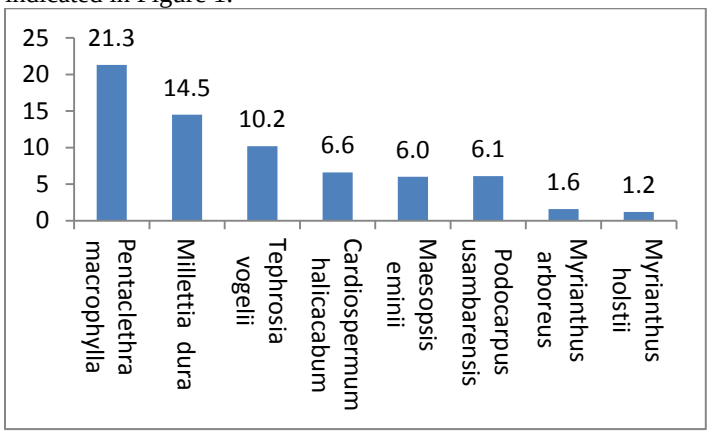

Figure 1: Very Long-chain FAs % in oils of plants from Kahuzi-Biega National Park and surroundings areas in D.R. Congo.

The highest value is observed for *Pentaclethra macrophylla* and the lowest for *Myrianthus holstii*. Oils of *Pentaclethra macrophylla*, *Millettia dura* and *Tephrosia vogelii* have respectively, 21.3, 14.5 and 10.2% of their total FAs made up of acids with chain lengths of ≥ 20 carbons. Moreover traces of non-methylene interrupted FAs are identified in 3 species (*Millettia dura*, *Myrianthus arboreus* and *M. holstii)* and in substantial levels (7.4%) in *Podocarpusus ambarensis*.

In fact, *Pentaclethra macrophylla* seed oil has the highest fraction (21.3%) of VLCFAs (Figure 1) with the latter including lignoceric (9.8%), behenic (6.3%) and eicosenoic acids (2.4%) (Table 1).*Milletia dura* seed oil had the second highest fraction (14.5%) of VLCFAs, including lignocericacid 2.6%, gadoleicacid 2.4%, erucic acid 0.7% and remarkably high percentage (7.3%) of behenic acid. This behenic acid was also reported by Ezeagu *et al*.[15]in the seed oil of the *Milletia thonningii* species with a content (8.93%) nearly that was similar to that find in *M. dura* seed oil in the current study. *M. pinnata* has been cultivated in India as a source of lamp oil and a natural medicine for 3,000 years [16]. Ezeagu *et al*.[16] evidenced in *M. thonningii* samples from Nigeria a FA profile very alike to that of our own *M. dura* results. Behenic, lignoceric and gadoleic acid seem to be characteristic for *Milletia* genus. The VLCFA fraction of *Tephrosia vogelii* seed oil (10.2 %) also contained behenic acid (5.8%) and lignoceric acid (1.5%; Table 1).

Cardiospermum halicacabum, *Maesopsis eminii*, *Pentaclethra macrophylla* and *Tephrosia vogelii* had an arachidic acid content similar to that of groundnut oil [5] with respectively 2.4; 2.3; 2.0; and 2.0%. FA profile of *M. eminii* oil resembles to that of peanut cultivars oil as reported by Davis *et al*. [5].

In *C. halicacabum's* seed oil, more than 6% of total FAs were of VLCFAs (Table 1). Surprisingly, we did not find the 11-eicosenoic (gadoleic) acid as major FA as reported in previous works in samples originating from Brazil and Pakistan [17-19]. Nevertheless, Chisholm and Hopkins [20] had reported other works where this same FA was absent in *C.halicacabum's* seed oil. These differences may be due to seasonal variation or soil [21] because in one species of plant it can have meaningful difference in quantity and quality of substances restrained according to the different soil properties [22].

In *M. arboreus* and *M. holstii* oils the high amount of linoleic acid may be useful as chemotaxonomic criteria for this genus. The *Myrianthus* species analyzed in this study showed similar FA composition both qualitatively and quantitatively. They all have linoleic acid as predominant acid respectively at 77.2 and 80.2% followed by oleic acid 12.3 and 9.8%, palmitic acid 3.9 and 4%, and stearic acid 2.8 and 3.3%. These two *Myrianthus* species are harvested in geographically distinct localities, Irangi and Tshibati, situated more than 100 Km apart. Nevertheless this resemblance is only about usual acids i.e. linoleic acid, oleic acid, palmitic acid, stearic acid. On the contrary, the VLCFAs are quantitatively different between two species. This is so for EPA with respectively 0.372 and 0.071 %, arachidic acid 0.001 and 0.122%, erucic acid 0.171 and 0.001% and DHA 0.089 and 0.001%.

About Non-methylene-interrupted FAs the presence of all cis-Δ5-unsaturated polymethylene interrupted FAs (Δ5-UPIFA), 5,11,14-20:3 (sciadonic acid) and 5,11,14,17-20:4 (juniperonic acid) in *P. usambarensis*, were expected, since these two acids together with other polymethylene interrupted FAs, are often present in all gymnosperms in levels around 5% of the total FAs, with the former usually being the most abundant [23,24]. So far, 5-UPIFAs have been determined in 6 out of the approximately 100 *Podocarpus* species [24-26].

CONCLUSION

Cardiospermum halicacabum, *Maesopsis eminii*, *Pentaclethra macrophylla* and *Tephrosia vogelii* have arachidic acid content similar to that of groundnut oil and can be serve as alternative sources of that acid. As for *Myrianthus arboreus* and *M. holstii*, additional studies are necessary by increasing sampling to improve the understanding of statistic resemblance and differences of fatty acids profiles and other criteria of these species. Further investigations on *Cardiospermum halicacabum* in current study area could help to understand the fact that the sample from this study area contained as trace the VLCFA 11-eicosenoic acid which is reported as major fatty acid in previous works in such species.

ACKNOWLEDGEMENT

We would like to acknowledge the support of CRSN/Lwiro and the authorization to collect plant samples in the Kahuzi-Biega National Park through its partnership with this institution. The Belgian Technical Cooperation provided support to carry out this work through the fellowship program no L06RDC/581. We are also indebted to Mac Arthur Foundation through MUIENR, Makerere University for additional funding. We would like also to extend our thanks to Prof. Otto Grahl-Nielsen of the Department of Chemistry, University of Bergen, Norway for experimental analysis.

REFERENCE:

1. M.I. Gurr, 1999. *Lipids in nutrition and health: a reappraisal*. Maypole Scientific Services, Isles of Scilly, UK. The Oily Press Bridgwater, PJ Barnes & Associates.
2. V. Wijendran, K.C. Hayes, 2004. Dietary n-6 and n-3 fatty acid balance and cardiovascular health. Annu. Rev. Nutr. 24, 597–615.

3. F. Hirsinger, 1986. Oleochemical raw materials and new oilseed crops. *Oléagineux* 41(7), 345-352.
4. M. Opperman, W. Marais, A.J. SpinnlerBenade, 2011. Analysis of omega-3 fatty acid content of South African fish oil supplements. Cardiovasc. J. Afr. 22(6), 324-329.
5. J.P. Davis, L.O. Dean, W.H. Faircloth, T.H. Sanders, 2008. Physical and chemical characterizations of normal and high-oleic oils from nine commercial cultivars of peanut.Journal of the American Oil Chemists' Society 85 (3), 235-243.
6. V.Dubois, S.Breton, M.Linder, J.Fanniand M.Parmentier, 2007. Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. *European Journal of Lipid Science and Technology*, 109, 710–732.
7. A. Zamora, 2005. Fats, oils, fatty acids, triglycerides - chemical structure. Scientificpsychic.com, Available on:www.scientificpsychic.com/ fitness/ fattyacids. html. accessed 29th January 2012.
8. M. Kazadi, 2011. Study of oil from wild plants of Kivu, DR Congo: Oil content and physicochemical characteristics of oils from wild plants of Kivu region, Democratic Republic of Congo. Ed.: LAP Lambert, Germany.
9. A.J. Plumptre, G. Eilu, C. Ewango, P. Ssegawa, D. Nkuutu, R. Gereau, H. Beentje, A.D. Poulsen, E. Fischer, D. Goyder, T.R. Pearce, D. Hafashimana, 2003. The biodiversity of the Albertine Rift. Section 7: Plants. Albertine Rift Technical Reports Series 3, 68-77.
10. AOCS, 2004. Official methods and recommended practices of the American Oil Chemists Society. Champaign
11. V.J. Barthet, T. Chornickand, J.K. Daun, 2002. Comparison of methods to measure the oil contents in oilseeds. *Journal of Oleo Science* 51, 589-597.
12. V.T. Namulawa, J. Mbabazi, J. Kwtegyeka, 2011. Fatty acid profiles of the eggs and juvenile muscle of Nile perch (*Lates niloticus* L. 1758) caught from Lake Victoria, Uganda. African Journal of Pure and Applied Chemistry 5(6), 127-135.
13. O. Grahl-Nielsen, M.O. Hammill, C. Lydersen, S. Wahlstrøm, 2000. Transfer of fatty acids from female seal blubber via milk to pupblubber. Journal of Comparative Physiology 170(4), 277-283.
14. J. Kwetegyeka, J. Masa, B.T. Kiremire, G.B. Mpango, O. Grahl-Nielsen, 2011. Fatty acids of polar lipids in heart tissue are good taxonomic markers for tropical African freshwater fish. African Journal of Aquatic Science 36(2), 115–127.
15. I.E. Ezeagu, K.J. Petzkeb, E.Lange, C.C. Metges, 1998. Fat content and fatty acid composition of oils extracted from selected wild-gathered tropical plant seeds from Nigeria. Journal of the American Oil Chemists' Society 75(8), 1031-1035.
16. Earth Equity, 2008. Plant Profiles: *Millettia pinnata*, the Pongam tree and *Moringa oleifera*. *Earthequity.com*, Available on:www.earthequity.com.au/profiles.html. accessed 19th March 2012.
17. I. Ahmad, 1992. Phytochemical investigation of the genus *stocksia* of the family Sapindaceae of Pakistan. *PhD Thesis in Chemistry*, Institute of Chemistry, University of the Punjab, Pakistan.
18. C.Y. Hopkins, R. Swingle, 1967.Eicosenoic acid and other fatty acids of Sapindaceae seed oils. Lipids 2 (3), 258-260.
19. K.L. Mikolajczak, J.C.R. Smith, L.W. Tjarks, 1970. Cyanolipids of *Cardiospermum halicacabum* L. and other sapindaceous seed oils. Lipids 5 (10), 812-817.
20. M.J. Chisholm, C.Y. Hopkins, 1958. Fatty acids of the seed oil of *C. halicacabum*. Canadian Journal of Chemistry 36 (11), 1537–1540.
21. C.C. Akoh, C.V. Nwosu, 1992. Fatty acid composition of melon seed oil lipids and phospholipids. Journal of the American Oil Chemists' Society 69 (4), 314-316.
22. C. Calvaruso, M.P. Turpault, P. Frey-Klett, 2006. Root-Associated Bacteria Contribute to Mineral Weathering and to Mineral Nutrition in Trees: a Budgeting Analysis. Applied Environmental Microbiology 72 (2), 1258–1266.
23. S. Mogrand, A. Badoc, B. Patouille, C. Lacomblez, M. Chavent, C. Cassagne, J.-J. Bessoule, 2001. Taxonomy of gymnospermae: multivariate analysis of leaf fatty acid composition. Phytochem. 58, 101-115
24. R.L. Wolff, W.W. Christie, 2002. Structures, practical sources (gymnosperm seeds), gas liquid chromatographic data (equivalent chain lengths), and mass spectrometric characteristics of alcis D5-olefinic acids. Eur. J. Lipid Technol. 104, 234-244.
25. R.L. Wolff, F. Pedrono, A.M. Marpeau, F.D. Gunstone, 1999. The seed fatty acid composition and the distributions of D5-olefinic acids in the triacylglycerols of some taxares (Cephalotaxus and Podocarpus). J. Am. Oil Chem. Soc. 76, 469-473.
26. E. Bagcie, Y. Karagacliy, 2004. Fatty Acid and Tocochromanol patterns of Turkish pines. Acta Biol. Cracoviensia – Ser. Bot. 46, 95-100.

Citation: Mpiana P.T., et al. (2014). The Occurrence of Very Long-Chain fatty acids in oils from Wild Plant species Originated from Kivu, Democratic Republic of the Congo. J. of Advancement in Medical and Life Sciences. V2I1. DOI: 10.15297/JALS.V2I1.03