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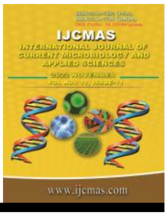

Original Research Article

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Nutritional Contribution of *Ledermanniella schlechteri* Extracted from the Cataracts of the Djoue River in Congo

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ABSTRACT

The objective of this study was to analyze the nutritional, mineral and fatty acid composition of a little known and underutilized aquatic plant available locally in the department of Brazzaville, Congo. The analytical methods used for the physicochemical analysis of *L. schlechteri* were those of the AOAC. The determination of minerals was performed by atomic absorption spectrometry. The gas chromatographic method was used to highlight the fatty acid profile. The results obtained revealed the contents of water, carbohydrates, ash, proteins and lipids respectively 97.5±1.69%, 45.45±1.64%, 13.50±1.24%, 10.7±0.65 % and finally 7.5±0.40%. Regarding the mineral content, the elements identified were calcium (1160 mg) followed by Iron (400 mg), Magnesium (360 mg) and Phosphorus (300 mg). The values of the oil indices gave respectively 1.2± 0.05 mg KOH/g oil for the acid index, 1.55± 0.1 meqO2 /kg oil for the peroxide index, 94.5±1 mg KOH/g oil for the saponification index and 93.3± 0.9% mg KOH/g for the ester index. The fatty acid composition revealed that the saturated fatty acid C16 is in the majority with 43.11% followed by C18 with C18:2w6 at 28.68%, C18:3w3 at 23.24%, C18:1w9 at 2.78% and finally C18:0 at 2.2% in the following order C16>C18w6>C18w3>C18w9>C18.

Keywords

Evaluation, Quality, Nutrients, *L. schlechteri*, green vegetable

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Introduction

Leafy vegetables play an important role in the diets of all populations in the world, particularly in Africa, Asia and Oceania, where they provide the essential part of the nutritional and medicinal needs (Bailey, 2003). They contain micronutrients (vitamins, minerals) and have good nutritional values that contribute to the well-being of the body (Tchiegang *et al.,* 2004; FAO, 1988; Rubaihayo, 1996). Leafy vegetables are economically affordable and, according to Kawashima and Soares (2003), represent an important source of nutrients for low-income people. Despite these advantages, leafy vegetables are generally neglected because of their association with consumer status, where they are often considered a "poor man's food" Kawashima and Soares (2003), But it turns out that in vegetables, we find mainly nutrients such as carotene, ascorbic acid, riboflavin, folic acid as vitamins; proteins, carbohydrates, minerals such as calcium, iron and phosphorus and; lipids. The latter represent all the vegetable oils and play an essential role in our diet Kawashima and Soares (2003). First of all, they provide a nutritional function and are a source of essential fatty acids, in particular linoleic acid, alpha-linolenic acid. Fatty acids are the main components of lipid species and are necessary in human nutrition as sources of energy and for certain metabolic and structural functions (White, 2008).

In terms of human health and nutrition area, the most important families are the n-6 (Linoleic acid) and n-3 (Alpha-linolenic acid) series (White, 2008). Each oil is characterized by its fatty acid composition according to the plant species from which it is extracted. Green vegetables are recommended since they are considered major sources of many bioactive compounds called phytochemicals (Natesh *et al.,* 2017), and which are essential for good health (Da Silva Dias João Carlos, Imai S (2017); Research has shown that high intakes of fruits and vegetables are associated with a lower risk of chronic diseases, particularly cardiovascular disease, type 2 diabetes, and certain cancers (such as those of the mouth, larynx, stomach, and intestines) (Pérez, 2002). Other research evidence supports the recommendation for increased consumption of a wide variety of vegetables, particularly dark green leafy cruciferous vegetables, which may reduce the risk of chronic diseases (Van Duyn and Pivonka, 2000 cited by Miller-Cebert *et al.,* 2009). For reasons of improving food and nutritional security, more emphasis is placed on crop diversification to meet human nutritional needs and reduce pressure on cereal production (Nath *et al.,* 2004), but also, through the consumption of aquatic plants. These, can be separated into four categories based on their growth habit: unattached floating, attached floating, submerged and emergent (Pancho and Soerjani, 1978; Noorasmah Saupi *et al.,* 2015). Taxonomically, they are aquatic angiosperms, pteridophytes (ferns), and bryophytes (mosses, anthocerotes, and liverworts) (Cook *et al.,* 1974) and also include algae (Nather Khan, 1990).

Quite often considered noxious weeds, some countries such as China, India, and Japan on the contrary, cultivate some aquatic plants such as water chestnut (Trapa spp.) and lotus (*Nelumbo nucifera Gaertn.*) for their seeds (Edwards, 1980; Noorasmah Saupi *et al.,* 2015). Others grow abundantly in natural and artificial wetland habitats from small ditches, ponds, irrigation canals, sewage lagoons, streams, rivers, water reservoirs, shallow lakes and swamps to swamps (MutaHarah *et al.,* 2005), where they are harvested from the wild as is the case in Congo. In Congo, a species of aquatic plant (*L. schlechteri*) with the vernacular name *Michiélé* is found in the Djoué River, one of the tributaries of the Congo River, and is consumed as a vegetable and remedy for some stomach ailments by the riparian populations. For the latter, this vegetable with a salty taste, but with a poorly known composition, is likely to contain important nutrients (proteins, lipids, carbohydrates, minerals, etc). Therefore, the objective of this study was: (1) to evaluate the nutritional composition of the plant and (2) to allow a better knowledge ofthe nutritional value of this vegetable in order to encourage their harvest and especially their consumption at the level of all the purses.

Materials and Methods

Plant material

The plant material used for this study was mainly composed of the *L. schlechteri* plant harvested at the cataracts of the Djoue River a tributary of the Congo River in the Brazzaville department (Figure 1). The harvest was conducted in the month of July 2018.

Methods

Preparation of the samples

After collection, the plants were washed by immersion in tap water, which was then dried in a ventilated oven (VWR. S/N: 14-06377) at 70°C for 72 h. They were then finely ground using a domestic electric grinder (brand VORWERK, France), where we obtained a powder that was first sieved through a 250 micron sieve (scientific brand Fischer 2210087), then packed in a plastic bag and finally stored in the air and light proof for further use.

Chemical composition

Water content

Two (02) grams of test sample placed in a crucible were dried in a ventilated oven at 105 °C for 24 hours. The crucible cooled in a desiccator was weighed and placed back in the oven for 1 hour. These drying, cooling and weighing operations were repeated until a constant mass was obtained according to the AOAC standard method, 934.011990.

Ash content

A 5 g sample in a crucible was incinerated at 550°C for 8 hours according to the standard method AOAC, 1997. The ash represented the mass obtained after cooling.

Content of mineral elements: P, Ca, Mg and Iron

The determination of the selected minerals was done by complexometry according to the protocol described by Didier, 1967. The ashes obtained after incineration of the plant powder were transferred into a capsule and taken up by 5mL of hydrofluoric acid. After being dried, the contents were again taken up with 1 mL of concentrated hydrochloric acid (HCl).

The solution obtained was washed and filtered with 100 mL of demineralized water for the determination of the elements Ca, Mg, Fe by atomic absorption spectrometry on an ANTHELIE LIGHT 5 SECOMAM K 314 distiller and of the element P by flame emission colorimetry.

Protein determination: KJEDALH method

In a matra, 0.5 g of plant powder were introduced, with a tip of mineralization catalyst (composed of 80 g of potassium sulfate, 31.25 g of copper sulfate and 2 g of selenium) and 10 mL of concentrated sulfuric acid.

After cold and hot mineralization for 30 minutes and 2 hours, respectively, 2 mL of distilled water with about 30 mL of NaOH at 400 g/L was added to the supernatant for distillation. The distillate was collected in an Erlenmeyer containing 20 mL of boric acid and a few drops of colored indicator (0.066% methyl red and 0.033% bromocresol green in ethyl alcohol). The solution obtained is finally dosed with N/20 sulfuric acid until the indicator turns from green to pink (pH 5.1). The nitrogen content (% N) was calculated according to the equation of formula (1); and the protein content was obtained by multiplying the obtained % N by the coefficient 6.25 (Adler-Nissen., 1986).

Lipid determination: Soxhlet extraction

A 30 g sample mass of L. schlechteri powder was weighed and placed in an extraction cartridge where the lipids were extracted at boiling point using hexane according to the Soxhlet method (AOAC, 1990) for 6 h. The measurement is performed gravimetrically after evaporation of the solvent.

Determination of oil indices

Different physico-chemical characteristics namely the acid number, ester number, saponification number and peroxide number, were determined according to the method of Codex Alimentarius, 1993.

Analysis of fatty acids by gas chromatography

Esterification was carried out by the method of Khan and Scheinmann, followed by determination by gas chromatography, AOAC Ce 1-62 (American oil chemists society, 1993). A SHIMADZU CG 14A gas chromatograph equipped with a flame ionization detector was used. A CP WA × 52 CB capillary column (25 m × 0.25 mm); DF 1 µm was used. Carrier gas flow rate (He) 1.0 mL/min. Injector temperature 200°C, detector temperature 250°C, and column temperature 190°C for 60 s, with an increase ratio of 2°C/min until reaching the maximum temperature of 250°C, remaining there for 35 min (Atnan *et al.*, 2010).

Carbohydrate content

The carbohydrate content (in % of dry matter) was estimated by differential calculation (FAO, 1988).

Statistical analysis

The experiments were carried out by repeating the analyses three times, the results obtained were presented by their mean with its standard deviation.

Results and Discussion

Chemical composition

According to Alli Smith (2009), the nutritional composition of food is the estimation of the nutritional value of human food in its chemical form. The approximate results of the chemical composition of *L. shelchteri* focused on the contents of water, ash, lipids, proteins and carbohydrates, as well as organic matter are represented in Table 1.

Water content

The results revealed a very high water content of 97.5±1.69% in wet basis (Table 1). High moisture content as obtained, could be a source of microbial proliferation see rapid spoilage of the food if not preserved quickly. Badau *et al.*, (2013) reported that the higher moisture content provides a higher activity of the water soluble enzyme and co-enzyme, necessary for the metabolic activity of the leaves.

Ash content

The ash content of *L. schlechteri* gave the value of 13.50±1.24%. This could be due to the fact that this plant grows in rocky environments such as the cataracts of the Djoue River. The ash content of *L. schlechteri* is much higher than those of *Manihot esculenta* and *Xanthosomas agittifolium* which were 6.9% and 1.71% respectively. (Ayodeji. O, Fasuyi., 2005; Kossiwa Wolali *et al.*, 2014). These high levels were observed in some vegetables like *Lagenaria siceraria* (10.35%), *Spinacia oleracea* (30.62%) and *Phytolacca dodecandra* (20.50%) (Itoua Okouango Elenga Michel *et al.*, 2015. Ash content is an index of inorganic minerals in biota (Hassan and Umar, 2006; Noorasmah Saupi *et al.*, 2015). In the plant of *L. schlechteri*, ash content represents a significant mineral fraction. The contents on the mineral composition (Table 2) of some minerals in the plant of *L. schlechteri* are thus shown.

The mineral data showed that *L. schlechteri* is a good source of mineral nutrients. The few mineral elements evaluated in *L. schlechteri* revealed a calcium (Ca) level of 1160 mg, the preponderant element, followed by Iron 400 mg, and Magnesium 360 mg and Phosphorus 300 mg. These values show the importance of this vegetable in the daily diet of children as well as adults. The value of calcium is very important for a good diet because calcium is the most abundant mineral element in the body which is involved in many reactions (cellular, enzymatic) such as the strength of bones and teeth which is constantly renewed (Jacotot, 2003). This rate is found in the range of plants and algae with high calcium content such as *Spirulina* which ranges from 130-1400 mg, *Moringaoleifera*1270 mg and the algae *Undaria pinnatifida* 1042 mg (Falquet, 2006; Tchiegang *et al.*, 2004).

As regards magnesium (Mg), the value obtained is also considerable because it is the important element in the cellular functioning, the transmission of the nervous impulse and the stimulation of the formation of antibodies.

The magnesium (Mg) content obtained from *L. schlechteri*is quite high compared to other leafy vegetables commonly consumed by the Congolese population such as 19.2 mg for *Xanthosoma sagittifolium*, 31 mg for *Manihot esculenta* and 160 mg for *Gnetum africanum* (Mbemba *et al.*, 2012; Broin, 2012; Kossiwa Wolali *et al.*, 2014).

Phosphorus count to him, gave a rate of 300 mg. Like calcium, this rate is important because its contribution plays a role in the constitution of cells and also intervenes in the absorption and transformation of certain nutrients (Jacotot, 2003).

The phosphorus content of *L. schlechteri* is a considerable content compared to other leafy vegetables such as *Xanthosoma sagittifolium* and *Manihot esculenta* whose levels are 52.9 mg and 84 mg respectively (Kossiwa Wolali *et al.*, 2014; Broin., 2012).

Finally, the determination of iron (Fe) gave the value of 400 mg, a crucial content because iron is an important element and plays a role in metabolic function such as the constitution of hemoglobin (Cnerna-Afssa., 2001). This iron content in *L. schlechteri* plant is quite considerable compared to most leafy vegetables such as *Gnetum africanum, Manihot esculenta* and *Spinacia phytolacca*.

It can be compared to those of *Moringa oleifera* and the alga *Spirulina platensis* whose rate varies from 60 to 600 (Falquet, 2006). We can conclude here, that by the rate of iron, the development of anemia in women and children for example could be countered. Let us note also for the whole of the minerals, Kala and Prakash, (2004) specify that these last ones have a bigger stability during the transformation of food than the vitamins and the proteins, what could give us a guarantee of the preservation of these micronutrients during the transformations of this plant.

Protein content

Proteins (Table.1) are large molecules consisting of nitrogen-organic compound complexes (amino acids), playing an essential role in cellular, structural functions and regulation of metabolic activities of all living organisms. Therefore, protein is of primary importance in the daily diet of consumers (Natesh *et al.*, 2017).

In our case, the protein content obtained from *L. schlechteri* is 10.75±0.65%. From this content, it can be seen that *L. schlechteri* has a high protein fraction compared to those found in *Neptunia oleracea* (3.01 to 3.23%) (Noorasmah Saupi *et al.*, 2015). *Spinacia oleracea* (5.20%), *Gnetum africanium* (4.86%) and *Xanthosoma sagittifolium* (3.63%) (Itoua Okouango *et al.*, 2019; Kossiwa Wolali *et al.*, 2014).

It can be concluded here that green leafy vegetables are the richest and cheapest sources of protein.

Fat content

Vegetables generally contain a very low fat content (Table 1), typically ranging from 0.10 to 0.20% (DeMan, 1999; Hazra and Som, 2005). However, this study showed that *L. schlechteri* possessed a crude fat content of 7.85%, a value close to that of *Manihot esculenta* leaves (7%), and comparatively higher than those of commonly consumed aquatic and terrestrial leafy vegetables in Malaysia (0.20-1.80%) for example (Noorasmah Saupi *et al.*, 2015), or other leafy vegetables such as *Spinacia oleracea* and *Moringa olifeira* respectively 0.40% and 0.6% (Itoua Okouango, Elenga Michel *et al.*, 2015 [31], Moussa Ndong *et al.*, 2007).

Carbohydrate content

Most of the energy in the diet comes from carbohydrates where The Institute of Medicine (2022) recommends that 45-65% of total calories come from carbohydrates (Slavin, 2013).

This is when Dietary Guidelines for Americans also suggests the consumption of carbohydrate-rich foods, thus including vegetables, fruits, grains, nuts, seeds, and dairy products. Dietary fiber and resistant starch are only provided by carbohydrate-rich foods (Slavin, 2013).

The carbohydrate content (Table 1) deduced in the plant of *L. schlechteri* gave the value of 45.45±1.64%; a relatively high level, but less than those of *Lagenaria siceraria* (63.22%) and *Manihot esculenta* (57.35%). Saha *et al.,* (2015), report that high carbohydrate content in food would mean high energy content, which facilitates digestion and assimilation of other foods. And are also responsible for performing daily activities of daily living (Yisa *et al.,* 2010). In view of its content, *L. schlechteri* could be a good asset for intestinal sweeping (Itoua Okouango *et al.,* 2015; Ayodeji, Fasuyi, 2005; FAO/WHO, 1993).

Chemical indices

The data of the chemical indices of *L. schlechteri* oil focused on the determination of free fatty acids, peroxides, saponifiable compounds and esters are presented in Table 3.

According to the standards set by the food codex these different indices, have a meaning in their own limit as indicators of oxidation (Marty, 2005; Barka, 2016).

The acid index (AI) is a quality criterion that allows reporting on the state of conservation of an oil and whose acidity should be low or almost zero. The value of 1.2± 0.05 mg KOH /g of oil is well below the maximum value set by the FAO Food Codex 1993 4mg potassium hydroxide /g. This tells us that the oil of *L. schlechteri*, does not contain enough free fatty acids.

Similarly for the peroxide value (PI) the value of 1.55± 0.1 meq O2/Kg of oil is much lower than the standards set by the food codex 1999 whose values range from 10 to 20 meq O2/Kg for fats with a rancid taste. This value allows to say that this oil of *L.schlechteri* is not rancid and does not oxidize easily.

The saponification index (SI) revealed a value of 94.5± 1 mg KOH /g oil, indicating that the oil contains total fatty acids in high proportion. This value is lower than the minimum value set by the Food Codex 1999 which is 100 mg KOH /g of oil which shows that the oil of *L.schlechteri* is suitable for food.

Finally, the ester index (EI) which allows to determine the structure of glycerides, its value of 93.3 mg KOH /g deduced from saponification and acid indices (Barka, 2016) gives a value in accordance with the standards of the Food Codex 1999.

Fatty acid profile

The fatty acid profile of *L. schlechteri* oil was carried out by gas chromatography (GPC). The analysis of the fatty acids present in *L. schlechteri* oil gave a chromatogram whose chromatographic profile points out the appearance of (5) five major peaks of fatty acids according to their polarity (Table 4).

The elution order revealed first 12.14 min peak 1 corresponding to palmitic acid (C16) with 43.11%. Then peaks 2 and 3 at 14.22 min and 14.90 min for Stearic (C18) and Oleic (C18:1ω9) acids with 2.2% and 2.78% respectively. Finally peaks 4 and 5 at 16.13 min and 17.53 min with percentages of 28.68% and 23.24% for Linoleic (C18:2ω6) and Linolenic (C18:3ω3) acids.

Table.1 Chemical composition of *L. schlechteri* plant

Chemical composition	**Content (%)**
Water content	97.5±1.69
Ash content	13.50 ±1.24
Lipids	7.85 ±0.40
Proteins	10.7 ±0.65
Carbohydrates	45.45 ±1.64
Total organicmatter	86.5±1.54

Table.2 Content of some minerals of *L. schlechteri*

Mineral components	**Trace elements (mg)**
Calcium (Ca)	1160
Magnesium (Mg)	360
Phosphorus (P)	300
Iron	400

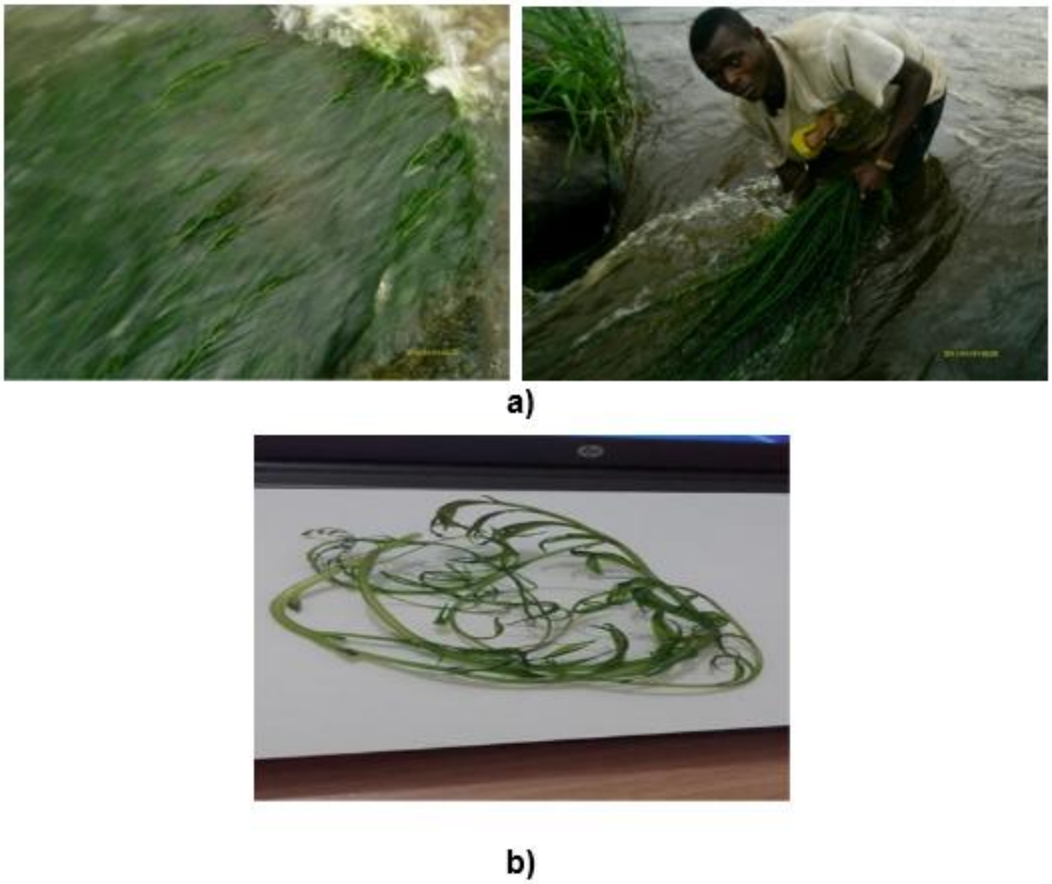
Table.3 Chemical indices of *L. schlechteri* oil

Chemical index	**Average**
Acid value (Av)	1.2 ± 0.05(mgKOH /g oil)
Peroxide Index (PI)	1.55 ± 0.1(meq of O2/Kg of oil)
Saponification Index (SI)	94.5± 1(mgKOH /g oil)
Ester Index (EI)	93.3 ± 0.9

Table.4 Fatty acid composition of *L. schlechteri* oil

Fatty acid	***L.schlechteri* (%)**
C16 : 0	43.11
C18 : 0	2.2
C18 :1ω9	2.78
C18 :2ω6	28.68
C18 :3ω3	23.24
ΣSFA	45.30
ΣMUFA	2.78
ΣPUFA	51.92
PUFA/SFA	1.15
MUFA/SFA	0.06
ω_6/ω_3	1.23

Fig.1 Extraction of *L. schlechteri* from the Djoué River (1a); Plant of *L. schlechteri* (1b)



These fatty acids are the main components of lipid species and are necessary in human nutrition as sources of energy as well as for certain metabolic functions. For saturated fatty acids, the profile of palmitic acid (C16) is preponderant of all fatty acids in *L. schlechteri* oil with a rate of 43.11%. This rate of palmitic acid coincides with that of palm oil whose value tends to 44.5% (Anthonia Bedo, 2013). Its presence as a major fatty acid has also been observed in some algae such as S. maxima with 63% (Falquet, 1996; Pang *et al.*, 1988). Stearic acid (C18) has the lowest level at 2.2% of all fatty acids present in the plant.

The low level of this acid has also been noticed in most leafy vegetables and edible oils such as Amaranth (1.60%), Sorrel (2.5%) and corn oil with (8.4%) (Anthonia Bedo, 2013; Chekroun, 2013; Debruyne, 2001; Evrard *et al.*, 2008).

In the oil of *L. schlechteri*, the sum of unsaturated fatty acids gives a proportion of 54.64%, compared to most of the food oils and fats, which have higher proportions; this is the case of the oils of Olives (55-83%), palm (39. Oleic acid (C18:1w9) is the main representative of monounsaturated fatty acids (MUFA) with 2.8% in the oil of *L. schlechteri*. The level of this fatty acid does not reach 10% and is not as representative as in most leafy vegetables like Sorrel (2.5%) and Amaranth (1.6%) (Anthonia BEDO, 2013).

However, linoleic acid (C18:2w6), a polyunsaturated fatty acid (PUFA) is about 28.68%. This rate is the highest of all unsaturated fatty acids present in *L. schlechteri* oil. This acid has been observed mostly in vegetable oils such as soybean with 50.8%, 53.51% in corn and 28.4% in peanut (Debruyne, 2001; Anthonia BEDO, 2013). This rate is very high compared to most leafy vegetables commonly consumed such as Amaranth (14.13%), Sorrel (17.2%) (Anthonia BEDO, 2013).

Regarding linolenic acid (C18:3w3 3), which is the precursor fatty acid of the n-3 series, was identified with a rate of 23.24%. Compared to food oils and fats such as olive oil (≤ 0.9%), soybean (6.8%) and corn (1.16%), (Giovacchino, 1999; Debruyne, 2001; Chekroun, 2013, this rate is very high. This considerable rate was also observed in some leafy vegetables such as Amaranth (53%) and Sorrel (48.4%) (Anthonia BEDO, 2013).

Indeed, in most vegetable oils, these two fatty acids (linoleic and alpha-linolenic) appear mainly in high concentrations. From a qualitative point of view, these fatty acids including omega-3 and omega-6 are qualified as essential because the human organism needs them and cannot produce them (Darcy-vrillon, 1993).

Their presence is beneficial because they play a role in the activity of the target functions of the organism in a direct or indirect way involved in the state of health and well-being of man (Jacotot *et al.*, 2003). Omega-6s (particularly linoleic acid) are involved in inflammatory processes that are essential for fighting infections, healing wounds, or allowing the synthesis of thyroid hormones, among other metabolic functions (Danielo, 2005).

Oils with a ratio lower and slightly higher than 1 could be nutritional oils, but those with a high PUFA/SFA ratio could not be used as food oil. But with a percentage of linoleic acid above 2%, they cannot be used as frying oil (Silou *et al.*, 2002).

The ratio omega 6/omega 3, revealed 1.23 on the oil *L. schlechteri* whereas it is recommended to take care of a balance of contributions with a ratio omega 6/ omega 3 between 1/1 and 4/1 whereas it is noted that it is often between 6/1 and 30/1 (Combe, Boué, 2001). It should be noted that the nature of the fatty acids constitutes an important factor on the oxidizability of an oil. The richer an oil is in unsaturated fatty acids, the more sensitive it becomes to oxidation (Varela *et al.*, 1988; Bouchon, 2009).

The aim of this study was achieved where the analysis on the nutritional value of *L. schelechteri* allowed highlighting the beneficial compounds for health. The chemical composition revealed the presence of lipids, proteins, carbohydrates and minerals. The present study results showed that *L. schelechteri* is a rich source in terms of mineral content, with the predominant element being calcium, followed by iron, magnesium and phosphorus. It has also been found to be very rich in water and carbohydrates. On the basis of lipid analysis by GPC, it showed us a predominance of polyunsaturated fatty acids (higher contents in C18:2 and C18:3) compared to saturated fatty acids (higher content in C16:0) and also qualifying the oil of *L. schelechteri* as a seasoning oil. We can conclude that green leafy vegetables, algae, ferns and many others of the same category, are mostly neglected, they have a good potential in terms of food value and can serve as easily accessible food resources.

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Conflict of Interest

The authors declare that we do not have any conflict of interest.

Nomenclature

AG: Fatty Acids

SFA: Saturated Fatty Acids

UFA: Unsaturated Fatty Acids

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Anthonia BEDO Année 2012-2013 Université Montpellier 2 Master 1 Biologie Santé Mention: Nutrition Rapport du Stage intitulé: Dosage des acides gras dans des aliments du Burkina Faso et estimation des apports en acides gras essentiels dans un repas type du jeune enfant burkinabè. Tableau 1 : Composition en acides gras des échantillons analysés P4.

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