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Nutritional Value and Phytochemical Screening of Four Wild Edible Plants Used as Functional Foods in Kabare, South-Kivu (DRC)

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Abstract

Wild food plants are among the oldest food sources for nutrition and traditional medicine in the Democratic Republic of Congo. The aim of this study was to compare the nutrient content as well as the results of phytochemical screening of organs from 4 plant species utilized by the local community as *functional foods*. Five plant samples (three leaf samples and two seed samples) from four species of wild edible plants were analysed. They are: *Aframomum laurentii* (leaves), *Ensete ventricosum* (leaves/seeds), *Portulaca oleracea* (leaves), *Myrianthus holstii* (seeds). The use frequency by the population, the seasonal availability of the plants and the desire to increase the number of organs were the three selection criteria. Laboratory analyses were carried out in strict compliance with the standards required by the literature, and PAST and SPSS v27 software were used for statistical data analysis. The results show that the leaves are richer in carbohydrates than the seeds, which in turn had high lipid and protein content. The maximum and minimum contents per metabolite were as follows: for carbohydrates: *Portulaca oleracea* (leaves) (38.9%) and *Myrianthus holstii* (seed) (17.3), for lipids: *Myrianthus holstii* (seed) (6.7%) and *Portulaca oleracea* (leaves) (1.4%) and for proteins: *Myrianthus holstii* (seed) (29.5%) and *Ensete ventricosum* (leaves) (15.8%). The phytochemical screening revealed the presence of following key bioactive metabolites (phenols, alkaloids, flavonoids). These results confirm the use of these

plants as food and medicinal products by the community. Further studies are essential to confirm our results.

Keywords

Spontaneous Food Plants, Nutrient Content, Functional Food, South Kivu

1. Introduction

In the world, it has been demonstrated that people have been using plants for a long time to meet their many basic needs, namely food, ornamental, clothing and shelter, as well as for the treatment of many diseases such as malaria, abdominal pain, diabetes, digestive disorders, cancer, snake bites, etc. [1]-[3]. Due to their multiple uses, plants remain essential to human life on earth, particularly because of their role in photosynthesis [4]. A person is considered to be in good health when they have a well-balanced diet and can be sure of receiving healthcare in the event of illness. To achieve this, it is imperative to have a sustainable food system, defined by the FAO (2024) as one that ensures food security and nutrition for all without compromising the economic, social and environmental foundations necessary to ensure food security and nutrition for future generations. Plants are also used in the preparation of traditional (natural or alternative) medicines, as there are places in the Democratic Republic of Congo where hospitals are rare [5]. Under these conditions, the community, perhaps not in its entirety, resorts to wild plants because it has difficulty accessing quality modern healthcare due to limited purchasing power. According to [2], the conservation of these plants in the form of ethnobotanical gardens is a necessity for farmers on the one hand and for scientists on the other, in that this technique contributes to the fight against the extinction of some very important plant species, especially during the dry season. [4] has also demonstrated that, apart from the edaphic changes that wild plants undergo when they are moved from their natural environment to a new one, these plant species have three advantages: they contain natural compounds, unlike pharmaceutical products, which are artificial and have therefore undergone several modifications that negatively affect the original composition of the plant. In addition, they constitute a source of income when sold to those in need or to tourists visiting ethnobotanical gardens. Finally, these plants play a pure cultural role because they are rich in a significant body of endogenous knowledge in which our communities believe. Furthermore, on a social, ecological and nutritional level, local communities use wild plants for food security, the prevention of malnutrition and sustainable agriculture.

Finally, from an economic perspective, these plants are an effective source of income when sold after harvest and are easily accessible to poor communities living in the surrounding areas [6]. Thus, it is necessary to assess the nutritional value of the wild edible plants consumed by rural communities for being confident of their contribution to normal growth and human health.

2. Materials and Methods

2.1. Study Area

This study was conducted in South Kivu Province, in eastern DRC. South Kivu is one of 26 provinces in the DRC. It is located in the eastern part of the country and covers an area of 65,070 km^2, or about one-third of the country. It is bordered to the north by the North Kivu Province, to the west by Maniema Province, to the south by Tanganyika and Maniema Provinces, and to the east by the neighboring republics of Rwanda, Burundi and Tanzania. The province is subdivided into eight territories: Fizi, North Idjwi, South Idjwi, Kabare, Kalehe, Mwenga, Shabunda, and Walungu. It is located approximately between 1˚36' and 5˚ south latitude on the one hand, and 26˚47' and 29˚20' east longitude on the other [6] [7].

2.2. Plant Material

This study was conducted on four local varieties of wild edible plants used in the territory of Kabare and its surroundings in the province of South-Kivu. The four species are used for several purposes such as nutrition and traditional medicine for the treatment of various diseases, including women's infections and skin diseases [5]. The *Ensete ventricosum* (Musaceae), is renowned for the quality of the product obtained from preparation in an aqueous medium in terms of colour and taste, while the other three, namely *Aframomum laurentii* (Zingiberaceae), *Portulaca oleracea* (Portulacaceae) and *Myrianthus holstii*, are domesticated for their high yield and disease resistance and medicinal use (based on our surveys).

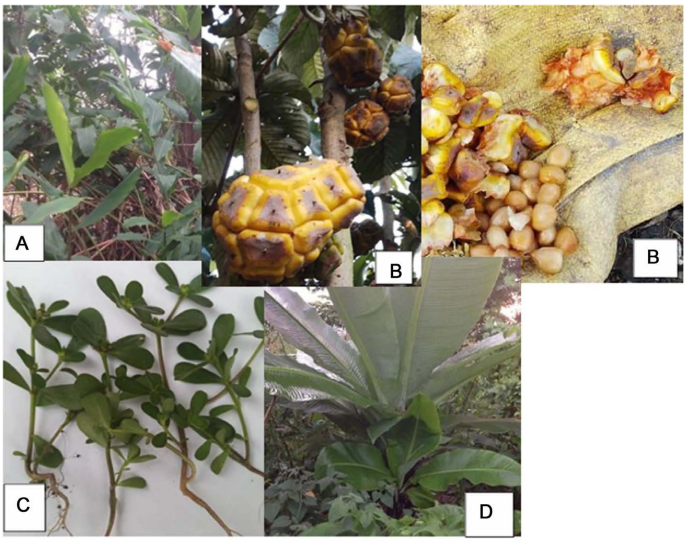
2.3. Methods

2.3.1. Collecting Samples

Five plant samples (three leaf samples and two seed samples) from four species of wild edible plants were analysed in this study. They were collected in three villages: Kadjuchu, Lwiro and Tschivanga in the Kabare territory of South Kivu. Sampling was carried out at ten-day intervals over a month during the ripe season for each plant. After collection, samples of more than ten kilograms were transported to the malacology laboratory of the Lwiro Natural Sciences Research Centre (CRSN-Lwiro). At the laboratory, the seeds were extracted and dried in the open air to prevent them from rotting while awaiting sample processing (**Figure 1**). The figure below gives different plant samples used during this study.

2.3.2. Sample Preparation

Samples of the four wild edible plants were collected, washed, cut into slices (approximately 5 mm) separately, then dried in an oven (Binder GMBH, Model: M-115, Germany) at 70˚C until a constant weight was achieved. The dried samples were then finely ground using a laboratory mill and stored in airtight plastic jars. They were then kept in desiccators for further analysis at the malacology laboratory of the Centre for Natural Science Research (CRSN) in Lwiro.



Legend:

A: *Aframomun laurentii* (leaves); B: *Myrianthus holstii* (Fruit & seeds) ;
C: *Portulaca oleracea* (leaves); D: *Ensete ventricosum* (leaves)

Figure 1. Plants' species samples.

2.3.3. Physico-Chemical Analysis

1) Moisture content

The moisture content was determined using the Association of Official Analytical Chemists [8] method, official method 930.15, in which empty crucibles were washed and then dried in an oven for 72 hours in desiccators, after which they were weighed (W_1). Approximately 40 g of the sample were weighed accurately (W_2) in a previously weighed crucible, each step being carried out in desiccators. The crucible containing the sample was then placed in an oven (Binder GMBH, Model: M-115, Germany) at 70˚C for 42 hours, until a constant mass was obtained. After cooling to room temperature in the desiccators, the crucibles were weighed again (W_3). The moisture content was determined using the following equation

$$\text{Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad (1)$$

2) Ash content

The ash content was determined using the [9] method, official method 923.03. We weighed 5 clean crucibles and dried them at 105˚C in an oven for 2 hours. They were then cooled in desiccators and weighed (W_1). After that, 3 g of sample was weighed into a pre-weighed crucible (W_2). The crucibles containing the samples were then incinerated in a muffle furnace (Stuart SF, United Kingdom) at 550˚C for 2 hours, until a light grey ash was obtained. After cooling, they were weighed (W_3). To calculate the ash content, we used the formula:

$$(\%) = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

3) Crude fat content

The crude fat content was determined using the Soxhlet extraction method [10], official method 2003.06. After drying the aluminum cups containing boiling stones in an oven at 105˚C for 30 minutes, they were cooled in desiccators at room temperature and then weighed (W_1). We then weighed 5 grams of sample into extraction cartridges and extracted with 70 ml of diethyl ether. After extraction, we removed the cups from the extractor and dried them in an oven at 105˚C for 30 minutes to evaporate the solvent. We then cooled them in desiccators for 30 minutes and immediately weighed them (W_2). The amount of fat obtained was expressed as a percentage of the initial weight of the sample using the following formula:

$$\text{Crud fat content}(\%) = \frac{W_2 - W_1}{SW} \times 100$$

where **SW**: sample weight.

4) Crude fiber content

The crude fiber content was determined according to the International Organisation for Standardisation (ISO 5498) (2002) method [8] [10] [11]. We weighed 2 grams of sample, transferred it to a 600 ml beaker, and boiled it with 200 ml of 1.25% sulphuric acid for 30 minutes. After digestion with 20 ml of 28% caustic soda (NaOH) for 30 minutes, we filtered the mixture using a vacuum pump through a crucible containing a layer of sea sand, then washed the residues five times with hot distilled water. The residue was then washed three times under vacuum, each time with 30 ml of 1% sulphuric acid solution, followed by distilled water, 1% soda solution, distilled water and finally acetone, then dried by suction. We then dried the residue in an oven at 130˚C for 2 hours, cooled it in desiccators and weighed it (W_1). After incinerating the samples in a muffle furnace at 550˚C for 2 hours, we cooled them again in desiccators and weighed them (W_2) [8] [10]. To calculate the fiber content, we used the following formula:

$$\text{Crude fiber content}(\%) = \frac{W_1 - W_2}{W_3} \times 100$$

where

W_1 = Weight of crucible + residues before incineration.

W_2 = Weight of crucible + ashes after incineration.

W_3 = Weight of initial sample.

5) Crude protein content

The crude protein content was determined using the Kjeldahl method [9], in which 1 g of sample was weighed into a digestion flask, then 12 ml of concentrated sulphuric acid (H_2SO_4) was carefully added. Next, we took 3 ml of 30% hydrogen peroxide (H_2O_2) and added it gradually after mixing thoroughly. Once the violent reaction had stopped, the flasks were shaken, then 3 g of catalyst (a mixture of copper sulphate and potassium sulphate) was added, and digestion was carried out by heating to 420˚C. Once the digestion was complete, we connected the flasks

to another receiving flask containing 2% boric acid and indicators (methyl red and bromocresol green). The solution in the digestion flask was then made alkaline by adding 40% sodium hydroxide (NaOH), which released ammonia (NH_3) into the receiving flask, where it reacted with the boric acid to form a borate ion. Finally, we titrated the distillate with a standard 0.1 M HCl solution, and the crude protein content was calculated based on Total Nitrogen, in accordance with the AOAC method (2016).

$$\textbf{(\%) Total Nitrogen} = [V_{HCl} - V_{HCl} \times N_{HCl} \times 1.401]/P$$

where: V_{HCl} = Volume of hydrochloric acid for the sample (ml), N_{HCl} = Normality of hydrochloric acid = 0.9516, Vo = Volume of hydrochloric acid added to the blank (ml), Conversion factor = 6.25, Constant = 1.401, Test sample = 1 gram.

6) Total carbohydrate content

To determine the percentage of total carbohydrates, we added up the crude protein, crude fat, moisture and ash contents of the sample, then subtracted them from 100%. The following formula was therefore established:

Total carbohydrates (%): 100 − (M + P + F + A) where: M: moisture content, P: protein content, F: fat content and A: ash content [8] [9].

7) Mineral analysis

- 3 g of each powdered plant organ sample were calcined at 550˚C in a muffle furnace, then dissolved in 20% hydrochloric acid and boiled to solubilise the ash.
- The solution was cooled, then filtered through filter paper (Whatman 42 mm) into a 100 ml acid-washed volumetric flask. The residue was dissolved and transferred to the flask, and the volume adjusted with distilled and deionised water.
- A blank solution was prepared in the same way [8] [10]-[12].
- The minerals calcium, magnesium, iron, zinc, manganese and copper were determined by atomic absorption spectroscopy (Shimadzu, model AA-6800, Tokyo, Japan). Phosphorus was measured using a UV-VIS spectrophotometer (Thermo Scientific, model Evolution 220, USA), according to the AOAC method (1990) method.
- Sodium and potassium contents were determined by flame photometry (Jenway, model pfp7, United Kingdom) in accordance with the Osborne method [13].

2.4. Phytochemical Screening

The analysis of secondary metabolites focused on the following species: *Aframomun laurentii*, *Portulaca oleracea*, *Ensete ventricosum*, and *Myrianthus holstii.*

Phytochemical screening was performed to identify ten secondary metabolites, including saponins, alkaloids, flavonoids, tannins, terpenoids, steroids, glucosides, phenols, quinones and lipoids. These bioactive compounds were analysed using the qualitative analytical methods described for the detection of each active

ingredient sought, which was identified either by the appearance of a characteristic colour, or by the formation of a precipitate, flocculation, turbidity or opacity, as appropriate [14]. The presence or absence of any of the above constituents in the tested extracts was determined according to the type of chemical reaction in the presence of an appropriate reagent. The test is negative (-) when there is no active compound in the extract. This absence is confirmed by no change in colour or no formation of a precipitate. The reaction is considered weakly positive (+) when there is slight opacity or low-intensity colouring followed by weak precipitation. On the other hand, the reaction is moderately positive (++) in the case of marked turbidity or distinct colouring. Finally, the test result is considered strongly positive (+++) when there is strong precipitation, flocculation or intense colouring.

Statistical analysis

Data on the primary and secondary metabolites and mineral content of five organs from four species of wild edible plants, analysed in triplicate, were subjected to analysis of variance (ANOVA) using PAST and SPSS v27.0. In cases of significant differences between varieties, comparisons of means were performed using Duncan's test. Decisions were made at a significance level of 5% ($p < 0.05$).

3. Results

The comparative analysis of nutritional value and secondary metabolites covered the organs consumed by the population on the one hand and the leaves used for natural remedies on the other. The results of this study are summarized in the following tables.

3.1. Physicochemical Parameter Content

Table 1 shows the average content for each physicochemical parameter of the organs of plant species analyzed.

Table 1. Nutrition value of plant organs species (%) DM.

Selected plants	Moisture (%)	Total Ashes	Crude Fat	Total protein	Total Fiber	Carbohydrates	Total
Aframomum laurentii (leaves)	20.2	16.5	1.8	17.1	6.7	37.7	100
Ensete ventricosum (leaves)	26	8.6	1.6	15.8	10.5	37.5	100
Portulaca oleracea (leaves)	27.9	8.5	1.4	16.7	6.6	68.9	100
Ensete ventricosum (seeds)	18.3	19	5.5	28.7	1.1	27.4	100
Myrianthus holstii (seeds)	26	18.8	6.7	29.5	1.7	17.3	100

The are differences in average means for the independent samples. For matched

samples, the single sample T-test Protein-carbohydrates ($t = -1.429$, $p = 0.226$), indicates no difference. However, the comparison between Protein-Lipids in the samples ($t = 9.139$, $p < 0.001$), indicates a significant difference, therefore samples are richer in protein than in lipids. The same for carbohydrates-lipids ($t = 5.387$, $p = 0.006$), significant difference has been observed, therefore samples are richer in carbohydrates than in lipids.

3.2. Mineral Content

The following table shows the results of chemical analysis of the mineral matter in the different samples.

Table 2. Average mineral matter content (mg/100g DM) of plant organs.

Selected species	Ca	Mg	P	K	Na	Fe	Zn	Cu	Mn
Aframomum laurentii (*leaves*)	11. 2	13.4	126.5	1198.8	20.1	2.8	8.5	0.8	0.7
Ensete ventricosum(leaves)	97.1	12.4	115.4	1159.2	19.0	2.7	3.8	0.6	0.9
Portulaca oleracea (*leaves*)	97.3	15.5	98.9	1267.0	15.6	2.1	5.1	0.5	0.7
Ensete ventricosum (seeds)	123.3	11.9	110.3	897.7	17.8	2.5	4.4	0.6	1.0
Myrianthus holstii (seeds)	11. 9	15.6	94.0	1598.3	21.6	2.8	5.1	0.7	1.1

There is a significant difference between the average means of all minerals in the selected sample plant species ($p < 0.001$).

3.3. Qualitative Analysis of Secondary Metabolites

The following table summarizes the results of qualitative phytochemical analysis of ten secondary metabolites in the leaves of the different samples (Table 3).

Table 3. Results of phytochemical screening of samples of the species analysed.

Compounds sought	*Portulaca oleracea* (leaves)	*Aframomum laurentii* (leaves)	*Ensete ventricosum* Leaves	*Myrianthus holstii* leaves
Saponins	++	++	+++	+
Alcaloids	+++	+	++	+++
Flavonoids	+++	++	+	+++
Tannins	++	+	+	-
Terpenoids	++	++	++	+++
Steroids	+++	++	++	+++
Glucosides	+++	+	+	+++
Phenol	+++	+++	+++	+++
Quinone	++	+	+	+
Lipids	+++	++	++	+

Key: +++: Strongly positive test; ++: Moderately positive test; +: Weakly positive test; -: Negative test.

4. Discussion

The results in Table 1 show that the leaf samples had higher carbohydrate content than the seed samples, which in turn had higher lipid and protein content than the leaf samples. Thus, *Aframomum laurentii* (leaves) (57.7%), *Ensete ventricosum* (leaves) (57.5%), *Portulaca oleracea* (leaves) (68.9%), (seeds) (57.4%) and *Myrianthus holstii* (seeds) (47.3%) for carbohydrate content; *Ensete ventricosum* (seeds) (5.5%) and *Myrianthus holstii* (seeds) (6.7%), *Aframomum laurentii* (leaves) (1.8%), *Ensete ventricosum* (leaves) (1.6%), *Portulaca oleracea* (leaves) (1.4%) for lipid content and *Ensete ventricosum* (seeds) (8.7%) and *Myrianthus holstii* (seeds) (9.5%), *Aframomum laurentii* (leaves) (7.1%), *Ensete ventricosum* (leaves) (5.8%), *Portulaca oleracea* (leaves) (6.7%) for protein content. This situation is normal because, in principle, the seeds of a plant generally contain more lipids and proteins than the leaves, while the leaves have a higher proportion of carbohydrates[15] In fact, the leaves are mainly responsible for photosynthesis and therefore store carbohydrates in the form of starch, while the seeds are intended for reproduction and contain reserve nutrients for the development of the new plant, particularly proteins and lipids [1] [6] [16] [17] These same results indicate that for the carbohydrate content of all organs, whether leaves or seeds, all recorded values below the range recommended by some authors, ranging from 71% to 76%.This characteristic may explain the use of *Myrianthus holstii* seeds (47.1%) in the management of diabetics to balance sugar levels. The carbohydrate content of all organs, both leaves and seeds, analysed in this study deviates from the average value obtained by [18] who, in a study of the applicability of a triple bagging system for preserving the nutritional quality of cowpea seeds (Vigna unguiculata L. Walp) based on a central composite design, arrived at an average carbohydrate content of 71.23%. This discrepancy could be explained by the extraction method, which may have certain imperfections. With regard to the results for lipids, proteins and minerals, the lipid contents of 5.5% and 6.7% obtained for *Ensete ventricosum* (seed) and *Myrianthus holstii* (seed) respectively are higher than those obtained by [19], who chemically analyzed the seeds of three local varieties of (*Ensete ventricosum*)(Welw.) used in Ethiopia to determine their nutritional value and obtained values ranging from 0.52% to 0.64%. These results are lower than the limit proposed by Joseph (1995) cited by [13] which varies from 71% to 76%. This difference in results is thought to be due to the origin of the seeds, as seasonal variations influence the results of the physico-chemical composition of plant organs. The results for the lipid content of leaves and seeds combined range from 1.4% to 6.7%, which is close to the levels obtained by Bagalwa *et al.* (2015), who, when conducting phytochemical screening of wild edible plants of Gorilla beringei graueri and Pan troglodytes schweinfurthii in Kahuzi Biega Park, obtained lipid values ranging from 1.5% to 6.6%. The protein content ranged from 5.8% to 9.5%, which is not far from the protein range obtained by [17] who obtained a protein content of 6.5% after nutritional analysis of Moringa oleifera leaves used in the manufacture of biscuits made from local ingredients in Kikzit

in Bas Congo in the Democratic Republic of Congo. The average protein content obtained by [6] of 4.5% during the bromatological analysis of five plant species consumed by gorillas in Kahuzi Biega Park is not so far from the value of 5.8%. For matched samples, the single sample T-test Protein-carbohydrates (t: −1.429, p: 0.226), indicates no difference. However, the comparison between Protein-Lipids in the samples (t: 9.139, $p < 0.001$), indicates a significant difference, therefore samples are richer in protein than in lipids. The same for carbohydrates-lipids (t: 5.387, p: 0.006), significant difference has been observed, therefore samples are richer in carbohydrates than in lipids. The statistical results support the difference in terms of plants nutritional function among local community.

The mineral composition (see **Table 2**) showed a significant difference ($p < 0.05$) for calcium, magnesium, iron and zinc Ca (from 11.2 to 123.3 mg/100g), Mg (from 11.9 to 15.6 mg/100g), Fe (from 2.1 to 2.8 mg/100g), Zn (from 3.8 to 8.5 mg/100g), while for Cu (from 0.5 to 0.8 mg/100g), P (from 94.0 to 126.5 mg/100g), K (from 897.7 to 1598.3 mg/100g), Na (from 15.6 to 21.6 mg/100g) and Mn (from 0.7 to 1.1 mg/100g), there were no statistically significant differences ($p > 0.05$). These results are not different from those obtained by [19], who conducted a comparative study on the nutrient content and phytochemical screening of three species of *Ensete ventricosum*, a local variety used in the traditional treatment of various diseases in Ethiopia. Indeed, these authors obtained results showing that there is a significant difference ($p < 0.05$) for calcium, magnesium, iron, zinc, copper and zinc Ca (97 and 123 mg/100g), Mg (11.5 and 15.60 mg/100g), Fe (2.05 and 2.82 mg/100g), Zn (4.38 and 8.51 mg/100g), while for copper, phosphorus, potassium, sodium and manganese there was no statistically significant difference ($p > 0.05$). Cu (0.54 and 0.79 mg/100g), P (93.98 and 126.48 mg/100g), K (897.7 and 1598.3 mg/100g), Na (15.57 and 21.79 mg/100g) and Mn (0.68 and 1.07 mg/100g).

As for the phytochemical analysis of the leaves of these samples (see **Table 3**), the results obtained revealed the presence of all the secondary metabolites measured, except for the absence of tannin, which was noted in the leaves of *Myrianthus holstii*. Our various investigations show that phenol was recorded as the secondary metabolite that tested strongly positive in all the samples analysed, followed by steroids, terpenoids, alkaloids, flavonoids, glucosides, saponins, lipoids, quinones and finally tannins. such as the regulation of artificial blood pressure through the ingestion of aqueous extract from *Ensete ventricosum* leaves which was proven by a strongly positive test for phenol in this species, as it has been scientifically proven that phenol-rich substances play a key role in regulating blood pressure [7] [19].

5. Conclusion and Recommendation

In this study assessing the nutritional value and phytochemical screening of several plant species, the objective was to compare the nutrient content and phytochemical screening results of organs from four plant species to confirm the community's belief in their use as functional foods. The results obtained show a sig-

nificant difference in the carbohydrate, lipid and protein content among the organs of the plant species tested. The mineral composition showed a significant difference for calcium, magnesium, iron and zinc, while for copper, phosphorus, sodium and manganese, there was no significant difference in terms of content. Almost all of the secondary metabolites analyzed were present in all organs. We noticed the presence of phenols, alkaloids and flavonoids. The high protein and lipid content in *Myrianthus holstii* leaves are commonly used by the local community to treat skin diseases and, more specifically, to heal wounds. These results confirm the belief of the local communities of the South Kivu mountains regarding the role of these four wild plant species as functional foods. We recommend further studies be conducted on the same organs of these four wild plants to confirm our findings.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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