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Physicochemical, nutritional and functional properties, rate of polyphenols and flavonoids of the *Stenochlaena tenuifolia* (BLECHNACEAE) leaves

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ABSTRACT

The general objective of this study is to contribute to the promotion of edible wild vegetables available in Madagascar. Its specific objective is to determine the physicochemical, nutritional and functional properties and the polyphenols and flavonoids contents of the *Stenochlaena tenuifolia* (BLECHNACEAE) leaves. Physical, physicochemical and biochemical methods have been used to characterize the powder from the leaves of this plant. The results obtained showed that the powder of the *Stenochlaena tenuifolia* leaves has a pH equal to 5.25±0.04 and an acidity equal to 2.57±0.23%. Its water and dry matter contents are respectively equal to 3.35±0.03% and 95.65±0.01%. Its macronutrient content, such as proteins, crude fat and carbohydrates, expressed in grams per 100 grams of dry matter, are respectively 32.30±0.17; 4.27±0.06 and 49.71±0.01. The metabolizable energy provided by this powder (in kcal per 100g) is 292.02±0.01. Its crude ash content is 10.35±0.04%. Its mineral elements content, such as calcium, magnesium, potassium, iron, phosphorus, zinc and copper (in mg per 100 g) of this powder are respectively 81.78±0.29; 287.15±6.60; 3140.59±24.64; 15.64±0.16; 540.90±7.71; 4.91±0.13 and 2.85±0.03. The potential renal acid load of this plant is –52.88. The functional properties, such as water and oil retention capacities, the hydrophilic-lipophilic ratio and the solubility index of the powder produced are respectively 486.29±8.44%; 170.00±8.16%; 2.86 and 20.36±0.20%. Its total polyphenols and total flavonoids contents are respectively 457.01±0.15mg pyrogallol equivalent per gram of dry matter and 15.12±0.23mg quercetin equivalent per gram of dry matter. Therefore, this plant has important nutritional, functional and phytochemical properties. Thus, this wild leaf vegetable can be used to fight against malnutrition and especially protein-energy malnutrition which affects almost the entire population in Madagascar.

Keywords: *Stenochlaena tenuifolia*; leaf vegetable; physicochemical property; nutritional property; functional property; protein-energy malnutrition; Madagascar

INTRODUCTION

Stenochlaena tenuifolia is a species of fern belonging to the family of BLECHNACEAE. It is found in the coastal regions of East and Southern Africa, from Tanzania to the east coast of South Africa, and also in several localities in Madagascar [1-2].

For a long time, plants have been a major source of food for humans thanks to their richness in nutrients. Daily consumption of vegetables of the right quality and in sufficient quantity would ensure good health and prevent various chronic diseases. Their richness in certain macronutrients, trace elements, vitamins and certain phytochemicals [3] are a boon in developing countries like Madagascar where these traditional wild vegetables can help solve many public health problems [4-5].

In Madagascar, protein-energy malnutrition is the major nutritional problem and the backdrop for mortality and morbidity in children under five [6]. It results from a deficit in macronutrients and not in micronutrients [7]. The most common forms of this nutritional disease are marasmus and kwashiorkor [7]. Therefore, the consumption of plant resources rich in macronutrients and micronutrients can be popularized in Madagascar to effectively fight against these nutritional diseases.

Stenochlaena tenuifolia is a leaf vegetable much appreciated by rural populations in Madagascar. Besides its use in food, this plant is also used to treat stomach aches and gout. Its importance and nutritional characteristics are hardly documented. It is for this reason that this study was carried out. Its general objective is to contribute to the promotion of edible wild vegetables available in Madagascar. Its specific objective is to determine the physicochemical, nutritional and functional properties and the polyphenols and flavonoids contents of the leaves of this leaf vegetable.

MATERIALS AND METHODS

i. Plant materials

The plant materials used during this study are the *Stenochlaena tenuifolia* (BLECHNACEAE) leaves.

ii. Harvest of the leaves

The *Stenochlaena tenuifolia* leaves were harvested in January 2019 in the District of Vohemar, Region of SAVA(Sambava-Antalaha-Vohemar-Andapa), Madagascar. Only the leaves most used in human food, called young fronds, were harvested.

iii. Drying of the leaves

The harvested leaves were sorted, then cleaned. After the abduction of their main ribs, the leaves were spread on a clean braid to be dried to the shade (out of the sun), in the open air and at the ambient temperature. Drying in the shade was chosen to avoid degradation of nutrients by the sun's ultraviolet rays.

iv. Transformation of the dry leaves into powder

The dry leaves were pounded by hand in a mortar with a pestle until the powder was obtained. The latter was then sieved through a sieve to obtain a powder with a uniform particle size.

v. Determination of the physicochemical and nutritional properties of the powder produced

Moisture, dry matter, crude ash, crude protein, crude fat and total carbohydrate contents were determined using the method of AOAC [8]. The reducing sugars were determined according to the method of BIPEA [9]. The pH and total acidity were determined according to the method of Oyewole [10] and Vasconcelos *et al.* [11]. The mineral element contents were determined using spectrophotometric atomic absorption (Calcium, Magnesium, Potassium, Iron, Zinc and Copper) and colorimetric (Phosphorus) methods. The metabolizable energy (expressed in kcal/100 g of product) was calculated according to the method of AOAC [8] with some modifications on the specific calorific coefficients used (specific calorific coefficients of Atwater, in kcal/g of proteins, lipids and carbohydrates). The value of PRAL (Potential Renal Acid Load) was calculated according to the formula given by Remer and Manz [12] and Pamplona-Roger [13]. This formula is based on the quantity of magnesium, phosphor, calcium, potassium and proteins that the food contains.

vi. Determination of the functional properties of the powder produced

The water absorption capacity and the water solubility index were determined according to the methods employed by Philips *et al.* [14], Sosulski [15] and Anderson *et al.* [16]. The oil absorption capacity was determined according to the method of Sosulski [15]. The hydrophilic-lipophilic ratio, as defined by Njintang *et al.* [17], was calculated by relating the water absorption capacity to the oil absorption capacity. It is a report that permits to value affinity compared of flours for water and for oil.

vii. Determination of total polyphenols and total flavonoids contents in the powder produced

The dosage of the total polyphenols was carried out using the Folin-Ciocalteu reagent according to the method described by Singleton and Rossi [18]. The level of total flavonoids was determined according to the method described by Dehpeur *et al.* [19]. In this study, 70/30 (V/V) hydro-ethanolic extract was used.

RESULT

The results obtained concerning the physicochemical and nutritional properties of the powder of the *Stenochlaena tenuifolia* leaves are presented in TABLE 1. These results show that this powder has a pH less than 7 and its acidity is low. They also show that the *Stenochlaena tenuifolia* leaves provide energy. The powder produced from these leaves are rich in dry matter and poor in water. The proteins content of these leaves is high. On the other hand, their fat content is low. The carbohydrate and crude ash contents of the powder produced are not negligible. The potassium, phosphorus, magnesium and calcium contents of these leaves are high. The other mineral elements contents (Iron, Zinc and Copper) are low. The PRAL index of the powder produced from these leaves is negative.

The functional properties of the powder produced are given in TABLE 2. According to this table, the water absorption capacity is very high. The oil absorption capacity is less than the water absorption capacity. The hydrophilic-lipophilic ratio for this powder is greater than 1. Its solubility is 20.36 ± 0.20%.

TABLE 3 gives the levels of total polyphenols and total flavonoids. This table indicates that the leaves of *Stenochlaena tenuifolia* contain phytochemicals.

TABLE 1: Physicochemical and nutritional properties of the powder of the *Stenochlaena tenuifolia* leaves

Parameters	Values
Humidity (%)	3.35±0.03
Dry mater (%)	95.65±0.01
pH	5.25±0.04
Total acidity (g of lactic acid/100 g; DMB)	2.57±0.23
Metabolizable energy (kcal/100 g; DMB)	292.02±0.01
Proteins (g/100 g; DMB)	32.30±0.17
Crude fat (g/100 g; DMB)	4.27±0.06
Reducing sugar (g/100 g; DMB)	1.79±0.01
Total carbohydrates (g/100 g; DMB)	49.71±0.01
Crude ash (%; DMB)	10,35±0,04
Calcium (mg/100 g; DMB)	81.78±0.29
Magnesium (mg/100 g; DMB)	287.15±6.60
Potassium (mg/100 g; DMB)	3140.59±24.54
Iron (mg/100 g; DMB)	15.64±0.16
Phosphorus (mg/100 g; DMB)	540.90±7.71
Zinc (mg/100 g; DMB)	4.91±0.13
Copper (mg/100 g; DMB)	2.85±0.03
PRAL (meq/100 g)	–52.88

Each result represents the mean ± standard deviation of 3 independent determinations (n = 3). The difference between the means is significant (p≤0.05). DMB: Dry mater basis.

TABLE 2: Functional properties of the powder of the *Stenochlaena tenuifolia* leaves

Parameters	Values
Water retention capacity (%)	486.29±8.44
Oil retention capacity (%)	170.00±8.16
Hydrophilic-lipophilic ratio	2.86
Solubility (%)	20.36±0.20

Each result represents the mean ± standard deviation of 3 independent determinations (n = 3). The difference between the means is significant (p≤0.05)

TABLE 3: Levels of total polyphenols and total flavonoids of the powder of the *Stenochlaena tenuifolia* leaves

Parameters	Values
Total polyphenols (mg pyrogallol eq/g; DMB)	457.01±0.15
Total flavonoids (mg quercetin eq/g; DMB)	15.12±0.23

Each result represents the mean ± standard deviation of 3 independent determinations (n = 3). The difference between the means is significant (p≤0.05). eq: equivalent.

DISCUSSION

The *Stenochlaena tenuifolia* leaves contain organic acids, because the pH of the powder of these leaves is less than 7, but very close to 7 (Table I). However, the amount of these organic acids is low, since the acidity of the powder produced is low (Table I). Therefore, the powder of these leaves can be considered as a slightly acidic product.

The leaves of this plant can be considered as energy sources, because the energies supplied by the powder of these leaves (292.02±0.01kcal/100g) are not negligible. These energies are mainly supplied by two energy nutrients, such as proteins and carbohydrates, because the level of fat, another energy nutrient, is low (4.27±0.06%). While, the protein and carbohydrate contents of the powder produced are high (Table I).

The protein content of the *Stenochlaena tenuifolia* leaves (32.30±0.17%) is higher than the protein content (29.70g/100g of DM) of the *Moringa oleifera* (MORINGACEAE) leaves harvested in the Ex-Province of Antsiranana (Madagascar) and analyzed by Ramaroson Rakotosamimanana [20]. It is also higher than the value found by Manzo [21] on the *Moringa oleifera* leaves in Niger (24.80%). It is also higher than the value obtained from black nightshade (*Solanum nigrum*) (Family SOLANACEAE) (29.90%) by Ocho-Anin Atchibri *et al.* [22]. The difference in these values can be explained by the varietal difference. In fact, Sheetal *et al.* [23] noted that some tropical leafy vegetables are very rich in proteins.

The crude fat content of the powder produced is close to that found by Ramaroson Rakotosamimanana [20] in the *Moringa oleifera* leaves harvested in the Ex-Province of Antsiranana (Madagascar) which is equal to 5.70g/100g of DM. In general, leaf vegetables are almost poor in fat.

The total carbohydrate content of the *Stenochlaena tenuifolia* leaves is higher than the result found by Fuglie [24] in the powder of the *Moringa oleifera* leaves which is equal to 38.20g/100g of DM. This difference can be explained by the varietal difference.

The crude ash content (10.35±0.04%) is close to the result obtained by Moussa *et al.* [25] on the powder of the *Moringa oleifera* leaves collected in Senegal which is equal to 11.39±0.66g /100g of DM. It should be noted that the crude ash content represents the quantity of mineral salts contained in a sample studied. This high value represents the large amount of mineral salts in this sample. Some literatures indicate that the rate of ashes depends a lot of the variety of the samples, of the nature of soil and the period of harvest [26-27].

The iron and magnesium levels of our sample (Table I) are higher than those obtained by Ramaroson Rakotosamimanana [20] in the *Moringa oleifera* leaves collected in the Ex-Province of Antsiranana (Madagascar). According to this author, the levels of these two mineral elements in the *Moringa oleifera* leaves are respectively 8.30mg/100 g of DM and 271.50mg/100g of DM. The *Moringa oleifera* leaves are richer in calcium (1156.70mg/100 g of DM) [20] than the *Stenochlaena tenuifolia* leaves. This difference can be explained by the inequality of species, harvest season and soil characteristics of harvest locations. The *Stenochlaena tenuifolia* leaves are rich in potassium. The potassium level in the leaves of this plant is higher than the result found by [24, 28] in the powder of the *Moringa oleifera* leaves (1324.00mg/100g). The *Stenochlaena tenuifolia* leaves are richer in phosphorus, zinc and copper than the powder of the *Moringa oleifera* leaves, because, according to Fuglie [24, 28], the levels of these mineral elements are respectively 204.00mg/100g of DM; 3.29mg/100g of DM and 0.57mg/100g of DM. These differences can be explained by the inequality of the samples studied.

The *Stenochlaena tenuifolia* leaves have an alkalizing power and generate more bases than acids, because the PRAL index of the powder of these leaves is negative. This indicates that, according to Piquet [29] and Pamplona-Roger [13], these leaves help maintain the necessary balance between acids and alkalis in our body. Thus, they alleviate the work of the kidneys to eliminate the excess of acid and promote a good health. The substances contained in the leaves of this plant species are therefore able to rebalance the biochemical reaction within our body, especially in the kidney.

The functional properties provide some information on the future applications of the food formulations [30]. During this study, the functional properties of the powder produced, which were evaluated, are water retention capacity, oil retention capacity, hydrophilic-lipophilic ratio and solubility (Table II). The water retention capacity is one of the criteria that determine the sensory quality, in particular, the texture of food products [31]. Food products with a high-water retention capacity retain their friability during storage [32]. The retention of water is due to the presence of hygroscopic constituents having hydrophilic groups. For the powder of the *Stenochlaena tenuifolia* leaves, these hygroscopic constituents are essentially carbohydrates (having free hydroxyl groups: –OH) and proteins (having hydrophilic groups: =CO, –NH–), because this powder is rich into these compounds (Table I). By the presence of these hydrophilic groups, these compounds can, therefore, bind with water molecules via hydrogen bonds. This powder can, therefore, be hold the sensory quality and their friability is retained during storage.

The powder of the *Stenochlaena tenuifolia* leaves is also capable to keep oil. It indicates that this powder closes substances having non-polar chains such as lipids. Moreover, this powder contains lipids, although their level is low (Table I). The oil retention capacity is an important property in feed formulation. It would act as a flavor retainer and mouth feel enhancer; it gives an indication of the retention capacity of flour flavor [33-34].

The hydrophilic-lipophilic ratio is a ratio which makes it possible to evaluate the comparative affinity of flours for water and for oil [35]. During this study, the value found (2.86), which is greater than 1, indicates that *Stenochlaena tenuifolia* powder has more affinity with water than with oil.

The powder of the *Stenochlaena tenuifolia* leaves has particles and/or substances which are soluble in water, since its solubility is not negligible (20.36±0.20%). In fact, it contains carbohydrates and minerals, all of which are water-soluble compounds.

The powder of the *Stenochlaena tenuifolia* leaves contains phytochemicals, which are real natural medicines belonging to plants [36].

Among the phytochemicals present in this powder, there are polyphenols and flavonoids (Table III). These compounds are phytochemicals exerting an antioxidant action [36-38]. They are, therefore, natural antioxidants. It should be noted that antioxidants are substances that have the ability to prevent the oxidation of various types of molecules in our body such as: lipoproteins, nucleic acids and free radicals [38]. Free radicals are compounds that are continuously generated by the human body due to its own metabolic activity or formed as a result of infections, stress and pollution [39-40]. These free radicals can donate electrons; they are thus endowed with pro-oxidant action [39]. These are then the most harmful oxidizing agents and they are aggressive molecules capable of damaging our own molecules [38]. Then, our body needs antioxidants in order to neutralize these free radicals. Thus, they have been shown to be useful in combating many diseases such as: arteriosclerosis, cancer, myocardial infarction, immunosuppression, cellular aging and intoxication by chemicals or unhealthy foods [36, 38]. Thanks to the presence of polyphenols and flavonoids, which are powerful antioxidants, the consumption of the *Stenochlaena tenuifolia* leaves prevents the oxidation of various types of molecules in our body and, therefore, improves our health or ensures the proper functioning of our organization.

The *Stenochlaena tenuifolia* leaves are richer in polyphenols than the *Moringa oleifera* leaves because, according to Belhi *et al.* [41], the total polyphenol content of the *Moringa oleifera* leaves is 236.50mg gallic acid equivalent per gram of dry matter. On the other hand, the *Moringa oleifera* leaves are richer in flavonoids than those of *Stenochlaena tenuifolia*, since, according to Belhi *et al.* [41], the level of flavonoids in the *Moringa oleifera* leaves is 77.33mg quercetin equivalent per gram of dry matter. These differences may be due to the varietal difference, the difference in the control used during the assay and the uneven season of sample collection.

CONCLUSION

The results of this study showed that the *Stenochlaena tenuifolia* leaves are sources of protein, mineral salts and polyphenols. Thus, they can be used for the prevention and correction of malnutrition due to their exceptional nutritional qualities. Then, the powder of the leaves of this plant can be used to model a high-quality infant food. The consumption of this leaf vegetable and its derived products can be popularized. Therefore, this will ensure the proper functioning of the human body thanks to the presence of phytochemicals, such as polyphenols and flavonoids, which are all powerful natural antioxidants.

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