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Evaluation of nutritive and anti-nutritive properties of two fresh leafy vegetables (*Vigna unguiculata* and *Ficus exasperata*) consumed in North of Côte d'Ivoire

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Abstract
Leafy vegetables are vitally important foods because they provide micronutrients essential for human health. *Vigna unguiculata* and *Ficus exasperata* are species used in the preparation of traditional dishes food, particularly in the North of Côte d'Ivoire. However, their nutritional importance is not well known by consumers. This study aims to evaluate the nutritive value of these two leafy vegetables. Powders of *V. unguiculata* and *F. exasperata* were prepared for biochemical analyzes. Results revealed significant differences at the 5% level. *Ficus exasperata* powder has a higher content of ash (16.79±0.17%) and carbohydrates (51.47±0.20%), compared to *V. unguiculata* which is richer in protein (30.71±0.03%), fibre (17.59±0.01%) and vitamin C (31.67±1.44 mg/100 g). *Ficus exasperata* leaves contain more polyphenols (845.28±0.71 mg/100 g), while *V. unguiculata* leaves contain the highest level of oxalate (62.33±3.67 mg/100 g). The nutritional quality of these two leafy vegetables could improve the nutritional status of the Ivorian people.

Keywords: *Ficus exasperata, Vigna unguiculata,* leafy vegetables, phytochemistry, anti-nutritive

Introduction

Malnutrition is defined as a nutritional state in which a deficit or excess of energy, protein and micronutrients results in measurable adverse effects on the affected person [1]. Malnutrition includes under- and over-nutrition and indiscriminately affects individuals across various stages in life, right from infants and children to adolescents and older adults [2]. In most developing countries, malnutrition is one of the main causes of mortality and morbidity in young children [1].

Leafy vegetables are vitally important foods, providing essential micronutrients for human health [3]. They could improve the nutrition and health of consuming populations [4]. Indeed, tropical leafy vegetables provide 10 to 100 times more minerals and vitamins than some exotic vegetables like lettuce [5]. Therefore, they represent an opportunity for developing countries like Côte d'Ivoire, where traditional vegetables can help solve many public health problems [4, 6].

In Côte d'Ivoire, 26 plant species have been inventoried as traditional vegetables grown for their leaves. These species are divided into 15 botanical families, 19 local names and 20 common names [7]. Many studies have shown that their consumption is mainly related to the region and food practices [8]. Thus, in the West of Côte d'Ivoire, the leaves of okra (*Abelmoschus esculentus*), soko (*Celosia argentea*), potato (*Ipomea batatas*), cassava (*Manihot esculenta*) and tikliti (*Myrianthus arboreus*) are mainly consumed, while in the Center of the country, jute mallow (*Corchorus olitorius*) is the most consumed [9]. In the Northern part of Côte d'Ivoire, the leaves of *Amaranthus hybridus* (boronbrou), *Andasonia digitata* (baobab), *Ceiba patendra* (fromager), *Hibiscus sabdariffa* (dah) and *Vigna unguiculata* (haricot) are the most appreciated by the population [10, 11].

Previously, biochemical and nutritional characteristics have been determined for some leafy vegetables from Cameroon, South Africa and Côte d'Ivoire [6, 11]. However, very few scientific reports are known about the nutritional potential of leafy vegetable species consumed in soups or used for soup preparation.

In Côte d'Ivoire, 10 species of spontaneous leafy vegetables consumed have been identified [12]. In addition, recently, a local investigation revealed that *Ficus exasperata* and *Vigna unguiculata* are among the most leaves widely used in the preparation of diets in Northern Côte d'Ivoire and seem to have biological properties. However, their nutritional importance is not well known by consumers [13].
Therefore, this study investigates some physico-chemical, phytochemical and anti-nutritional composition of two indigenous leafy vegetables (*Ficus exasperata* and *Vigna unguiculata*) mainly consumed by rural people in Northern Côte d'Ivoire, in order to promote their potential use and thus contribute to food security.

Materials and Methods
Plant material
Two leafy vegetables included *Vigna unguiculata* (L) *Walp* (Fabaceae) and *Ficus exasperata Vahl* (Moraceae) were harvested fresh and at maturity during November 2020 in a field located in the city of Katiola (North of Côte d'Ivoire) (Figure 1). The leaves were identified and authenticated by National Floristic Center of University Felix Houphouet Boigny (Abidjan, Côte d'Ivoire).

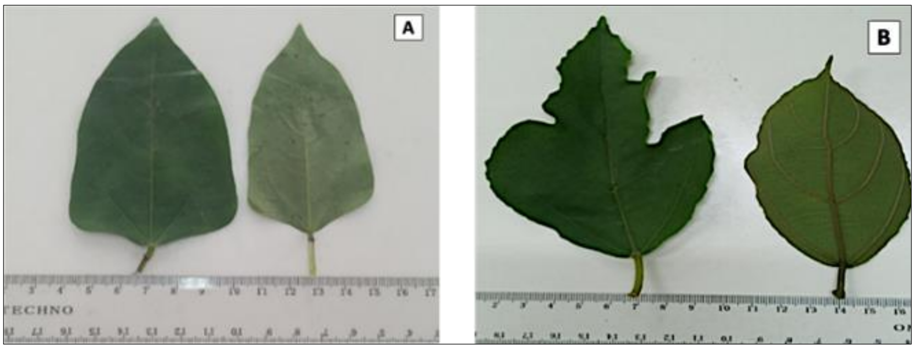

Fig 1: *Vigna unguiculata* leaves (A) and *Ficus exasperata* leaves (B)

Sample preparation
The fresh leaves collected were sorted, cleaned with distilled water to remove dirt and oven-dried (Memmert, Germany) at 45 °C for 72 h. The dried samples obtained were ground to fine powder using a blender (NASCO model), then sieved with a 250 µm mesh. The dried powders were stored at room temperature in different bottles before analysis.

Proximate analysis
Proximate analysis of samples was performed in triplicate according to standard methods of AOAC [14]. Moisture content was obtained by drying samples in an oven at 105 °C for a constant weight. Ash content was determined by incinerating dried samples in a muffle furnace at 550 °C for 4 h. Crude fibre content was estimated by weighting the insoluble residue obtained with the acid (H_2SO_4, 0.25 M) and the alkaline (NaOH, 0.3 M). Crude protein content was calculated by multiplying the estimated nitrogen content by 6.25, using Kjeldahl method. Crude fat level was carried out using Soxhlet extraction method. Carbohydrate content was calculated by using weight difference method. All the proximate were expressed in percentage based on the dry matter.
The energy value was estimated using the Atwater's conversion factor according to Food and Agricultural Organization [3]. Titratable acidity expressed as percentage (%) of lactic acid and pH value were determined by AOAC method [14].

Phytochemical analysis
Determination of vitamin C
Vitamin C content was determined by the titration method using 2, 6-dichlorophenol indophenol (DCPIP) described by Pongracz [15], with minor modification. Leaf sample (10 g) were soaked for 10 min in 20 mL of metaphosphoric acid (3%, w/v) / acetic acid (8%, w/v). The mixture was centrifuged at 4000 rpm for 20 min. One (1) mL of the obtained supernatant was titrated with 0.5 g/L DCPIP to a persistent rosy pink color. Similarly, 1 mL of 1 mg/mL ascorbic acid used as standard was titrated against 0.5 g/L DCPIP.

Determination of total phenolic compounds
Extraction of phenolic compounds
Phenolic compounds were extracted using 1 g of leaf sample was soaked in 10 mL of methanol 70% (v/v) [16]. The resulting mixture was homogenized by manual shaking for 2 min at room temperature, and then centrifuged at 4200 rpm for 5 min. The pellet was collected in 10 mL of methanol 70% (v/v) and centrifuged again. A third extraction was carried out under the same conditions. The three supernatants were pooled in a vial and the volume was adjusted to 50 mL with distilled water. The mixture constituted the total methanolic extract.

Polyphenol content
The content of total phenolic compounds was measured using the Folin-Ciocalteu method [16]. A sample of 1 mL of the methanolic extract was oxidized with 1 mL of Folin-Ciocalteu's reagent and neutralized by 1 mL of Na_2CO_3 (20%, w/v). The reaction mixture was incubated in the dark for 30 min and absorbance was read at 765 nm using a spectrophotometer (BK_UV 1000). The polyphenol content was estimated using a calibration curve of gallic acid (1 mg/mL) as standard.

Flavonoid content
The flavonoid content was determined using the aluminum chloride colorimetric method [17]. A sample of the methanolic extract (0.5 mL) was mixed with 0.5 mL of methanol, 0.5 mL of aluminum chloride (10%, w/v), 0.5 mL of potassium acetate (1M) and 2 mL of distilled water. After incubated the mixture in the dark for 30 min, the absorbance was read at 415 nm using a spectrophotometer (BK-UV 1000). The flavonoid content of the samples was determined using a calibration curve of quercetin (0.1 mg/mL) as standard.

Tannin content
The tannin content was carried out according to the method using vanillin reagent [18]. A sample of the methanolic extract (1 mL) was mixed with 5 mL of vanillin reagent and incubated for 30 min in the dark. Then the absorbance was measured at 500 nm (spectrophotometer BK - UV 1000) and the tannin content was estimated using a calibration curve of tannic acid (2 mg/mL) as standard.

Determination of oxalate content
The oxalate content was performed by the titrimetric method using potassium permanganate [19]. A quantity (1 g) of oven-dried and ground sample was mixed with 75 mL of H_2SO_4 (3 M). The mixture was magnetically stirred for about 1 h and then filtered using Whatman n°1 filter paper. An aliquot (25 mL) of the filtrate was collected and hot-titrated with 0.05 M $KMnO_4$ solution to a persistent pink color.

Determination of phytate content
Phytate content was determined according to the method using Wade's reagent [20]. About 1 g of the dried and ground sample was homogenized in 20 mL of HCl (0.65 N). The resulting mixture was stirred continuously for 12 h at room temperature and then centrifuged at 3000 rpm for 40 min. An aliquot (0.5 mL) of the supernatant obtained was mixed with 3 mL of Wade's reagent and incubated for 20 min in the dark. After incubation, the absorbance was read at 490 nm using a spectrophotometer (BK_UV 1000). Finally, the phytate content of the samples was estimated using a calibration curve of phytic acid (10 mg/mL) as standard.

Mineral analysis
The leaf powders were dry calcined in a muffle furnace and then dissolved in a mixture of HCl/HNO_3, before analysis. The mineral content of samples obtained was determined using a flame Atomic Absorption Spectrophotometer VARIAN, model AA-20 [14].

Statistical analysis
The results obtained were expressed as mean±SE and analyzed using Statistica 7.1 software. Differences between means of the parameters studied were carried out using Duncan's test. Statistical differences with $p<0.05$ were considered significant.

Results
Physicochemical and nutritional characteristics of the two fresh leafy vegetables
The physicochemical characteristics of the leafy vegetables studied are shown in Table 1. *Ficus exasperata* has a neutral pH (7.01±0.02), which reflects its low acidity rate titratable (0.02±0.01 meq/100 g) compared to *Vigna unguiculata*. *Ficus exasperata* exhibited high levels of ash (16.79±0.17%), crude fat (3.62±0.02%) and carbohydrate (51.47±0.20%) than *V. unguiculata* (9.27±0.03, 1.29±0.05 and 41.14±0.03%, respectively). Furthermore, low levels of moisture (80.37±3.55%), crude fibre (15.52±0.02%), proteins (12.60±0.01%) and energy values (288.88±0.64 Kcal/100 g) were observed in *F. exasperata* compared to *V. unguiculata*. Results also show that the vitamin C content of *V. unguiculata* (31.67±1.44 mg/100 g) is higher than that of *F. exasperata* (25.83±2.89 mg/100 g). All observed values for the two leaf powders are significantly different at the 5% level.

Table 1: Physicochemical characteristics of the two fresh leafy vegetables

Parameters	Leafy vegetables	
	Vigna unguiculata	*Ficus exasperata*
pH	6.78±0.05^a	7.01±0.02^b
Titratable acidity (meq/100 g)	0.06±0.01^b	0.02±0.01^a
Moisture (%)	87.69±0.33^b	80.37±3.55^a
Ash (%)	9.27±0.03^a	16.79±0.17^b
Crude fibre (%)	17.59±0.01^b	15.52±0.02^a
Crude proteins (%)	30.71±0.03^b	12.60±0.01^a
Crude fat (%)	1.29±0.05^a	3.62±0.02^b
Carbohydrates (%)	41.14±0.03^a	51.47±0.20^b
Energy value (Kcal/100 g)	299.01±0.32^b	288.88±0.64^a
Vitamin C (mg/100 g)	31.67±1.44^b	25.83±2.89^a

Values obtained are means ± SD for three determinations. On the lines of each parameter, the means assigned different letters are significantly different at $p<0.05$ according to Duncan's test.

Phytochemical composition of the two fresh leafy vegetables
Figure 2 shows significant differences ($p<0.05$) in the phytochemical composition of the methanolic extracts of the leaves studied. The total polyphenol content is higher in *F. exasperata* (845.28±0.71 mg/100 g DM) than *V. unguiculata* (735.56±0.83 mg/100 g DM). Analysis of tannins and flavonoids in leafy vegetables revealed lower contents in *F. exasperata* (50.38±2.28 mg EAT/100 g DM and 116.54±0.77 mg EQ/100 g DM, respectively) than in *V. unguiculata* (112.01±0,43 mg EAT/100 g DM and 141.75±0.45 mg EQ/100 g DM, respectively).

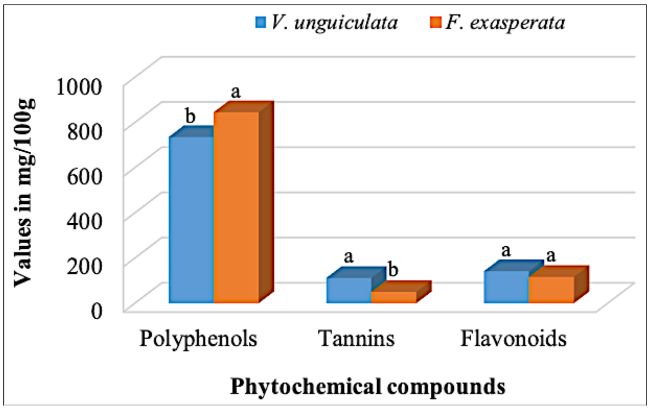

Fig 2: Phytochemical composition of the two fresh leafy vegetables

Anti-nutritional compounds of the two fresh leafy vegetables
In order to assess the bioavailability of the constituent elements of the two leafy vegetables, the content of anti-nutritional compounds (phytates and oxalates) in these leaves was determined. Figure 3 shows that *F. exasperata* has a phytates rate (7.45±0.24 mg/100 g DM) significantly ($p<0.05$) higher than *V. unguiculata* (6.13±0.08 mg/100 g DM). Regarding the oxalate contents, the rate recorded for *F. exasperata* (36.33±3.18 mg/100 g DM) is relatively lower than that for *V. unguiculata* (62.33±3.67 mg/100 g DM) at $p<0.05$.

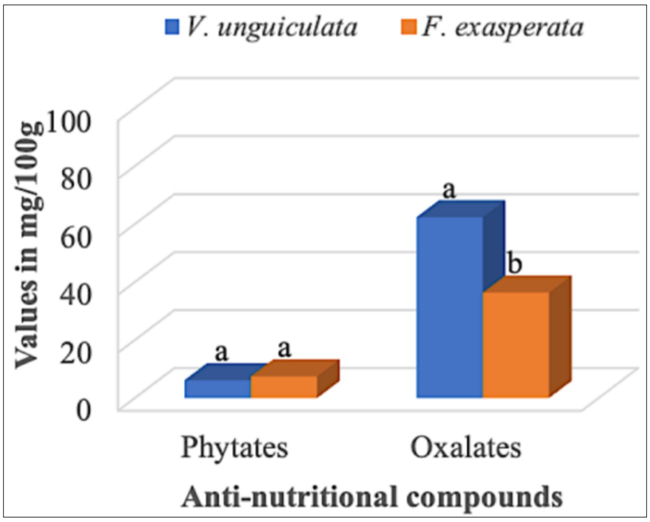

Fig 3: Anti-nutritional compounds in the two fresh leafy vegetables

Mineral composition of the two leafy vegetables

Minerals such as calcium (Ca), iron (Fe), potassium (K), magnesium (Mg) and sodium (Na) were determined in *V. unguiculata* and *F. exasperata* leaves studied (Table 2). The most important minerals in the 2 leaves are Ca (260.31-280.70 mg/100 g) and K (210.71-242.80 mg/100 g), but the highest values were found in *F. exasperata* species. The leaves of *V. unguiculata* also contained 201.02 mg/100 g Na, 162.40 mg/100 g Mg and 20.50 mg/100 g Fe, while those of *F. exasperata* contained 144.01, 175.50 and 12.21 mg/100 g of these minerals, respectively.

Table 2: Mineral composition of the two fresh leafy vegetables

Minerals (mg/100 g)	Leafy vegetables	
	Vigna unguiculata	*Ficus exasperata*
Ca	260.31	280.70
Fe	20.50	12.21
K	210.71	242.80
Mg	162.40	175.50
Na	201.02	144.01
Na/K	0.95	0.59
Oxalates/Ca	0.24	0.13
Oxalates/Fe	3.04	2.97
Phytates/Ca	0.02	0.02
Phytates/Fe	0.29	0.61

Discussion

Leafy vegetables play an important role in the diets of people around the world, particularly in Africa where they provide the bulk of nutritional and medicinal needs [21]. Indeed, from a nutritional point of view, most leafy vegetables are consumed for their richness in micronutrients, fibre, proteins and vitamins.

This study has highlighted the nutritional potential of the leafy vegetables *Vigna unguiculata* and *Ficus exasperata*. It appears that the leave powders of *V. unguiculata* are slightly more acidic than those of *F. exasperata*. Samples with acidic pH could be better preserved and preserved against the proliferation of pathogenic microorganisms. Thus, the *V. unguiculata* leaf powders could be stored for a long time. However, its high moisture content would make its leaves more perishable than those of *F. exasperata*.

The ash content is an indication allowing to know the mineral content in a food [22]. The ash contents obtained for the raw leaves of *F. exasperata* (16.79±0.17%) are higher than those of *V. unguiculata* (9.27±0.03%), which could reflect its richness in minerals. Indeed, the *F. exasperata* leaves used in this study contain a higher ash content than some *Ficus* species consumed in Nigeria with contents between 10 and 13% [23, 24] and then those reported for some leafy vegetables consumed in Cameroun (11.86-14.20%) [25]. Therefore, *F. exasperata* species from Côte d'Ivoire could be a good source of minerals for human and animal nutrition.

However, it should be noted that the two leaf species studied have appreciable mineral contents, particularly Ca, K, Mg and Na. These levels are lower than those of some species of leafy vegetables consumed in northern part of Côte d'Ivoire [11]. The Ca contents (260.3 - 280.7 mg/100 g) of the two leafy vegetables studied are higher than those of different species of leafy vegetables most consumed in western part of Côte d'Ivoire (111.02 - 246.28 mg/100 g) [26], but they remain lower than other *Ficus* species (1130 - 4680 mg/100 g) [23, 27]. Calcium plays several essential roles as a cofactor in the catalysis of several enzymes, its helps muscle contraction, bones and teeth formation, the heartbeat and nerve cell communication [23, 24]. The high level of Ca in *F. exasperata* shows that it could be a good dietary source of Ca than *V. unguiculata*. Moreover, in this study, the levels of Mg (175.50 mg/100 g) and K (242.80 mg/100 g) for *F. exasperata* leaves are higher than those of *V. unguiculata* (162.40 mg/100 g Mg; 210.71 mg/100 g K). These levels are however below those of some *Ficus* species (720 - 3360 mg/100 g) from Nigeria [23, 27]. In the study of some common wild leafy vegetables from Nigeria, low levels of Mg (13.10 to 64.11 mg/100 g) were also recorded, although Mg is the most abundant mineral [28]. Magnesium plays an essential role in the structural stability of nucleic acids and in intestinal absorption. Despite the low Mg content recorded in our study, *F. exasperata* and *V. unguiculata* leaves could prevent the occurrence of severe diarrhea, migraines, hypertension, muscle degeneration, cardiomyopathy, atherosclerosis, growth retardation, stroke and congenital malformations in humans [11, 27, 29]. Regarding Na content, *V. unguiculata* leaves contain a highest concentration (201.02 mg/100 g) than those of *F. exasperata* (144.01 mg/100 g) of this study and *F. capensis* from Nigeria (170 mg/100 g) [27]. Sodium and potassium are the main cations of intracellular and extracellular fluids, which are involved in the electrolyte balance, the regulation of plasma volume, nerve and muscle contraction in the body [11, 27]. The Na/K ratio in the body is very important for prevention of high blood pressure and a Na/K ratio less than 1 is recommended [11]. The calculated Na/K ratio revealed values of 0.59 and 0.95 for *F. exasperata* and *V. unguiculata*, respectively. Hence, consumption of these leaves would probably reduce high blood pressure diseases.

Furthermore, the *F. exasperata* leaf powders contain less fibre (15.52±0.02%) than those of *V. unguiculata* (17.59±0.00%). These values are higher than those (8 - 10%) for three fresh leaves (*Solanum macrocarpon*, *Amaranthus hybridus* and *Ocimum gratissimum*) consumed in Benin [30]. The values obtained in this study are also close to those found in some *Ficus* species from Nigeria which are around 17.20% [23, 24]. Low-fibre diets have been linked to heart disease, colon cancer, hypertension, obesity, serum cholesterol, appendicitis, diabetes and constipation [31]. This is because fibre helps maintain human health and lowers cholesterol levels in the body. Therefore, the leaves of *F. exasperata* and *V. unguiculata* could be recommended to preserve people from these metabolic diseases.

The results also show a low-fat content in *V. unguiculata* leaf powders (1.29±0.05%) compared to *F. exasperata*

(3.62±0.02%). Both values are lower than those (4.2 - 27.0%) reported for some leafy vegetables consumed in West Africa [23, 24, 32]. This low-fat content obtained shows that *V. unguiculata* and *F. exasperata* have a low energy value like most leafy vegetables and may be recommended for people suffering from obesity or following a low-fat diet [24, 30, 32]. Therefore, the consumption of diet based on these leafy vegetables would naturally reduce fat intake in the body, which is a precursor for cardiovascular diseases.

As regard protein contents, the levels recorded in this study for *F. exasperata* (12.60%) is found to be less than 15.01% [24], but higher than 6.91% [23] for the same species consumed in Nigeria. On the other hand, the protein contents obtained in *V. unguiculata* leaf powders (30.71%) are higher than values recorded in *Ficus* species (6 - 16%) and some leaves (0.03-1.53%) consumed in Nigeria [23, 24, 28]. However, the protein contents of *V. unguiculata* are in the range of those (28 - 31%) reported for the same *Moringa* leaves grown in Central Namibia [33]. The leaves of *F. exasperata* and *V. unguiculata* could be a good source of protein as they provide more than 12% of their calorific value in protein [34].

The leafy vegetables used in this study also contained appreciable levels of vitamin C (25.83 - 31.67 mg/100 g) and polyphenols (735.56-845.28 mg/100 g). An increased consumption of leafy vegetables would provide additional vitamin C, which would help prevent the rare disease of scurvy. Vitamin C also promotes the assimilation of iron.

In the current study, tannins were found in *F. exasperat*a and *V. unguiculata* leaves with respective contents of 50.38±2.28 and 112.01±0.43 mg/100 g. These contents are lower than those reported in the leafy vegetables of *Ocimum gratissium* (80 mg/100 g) and *Vernonia amygdalina* (220 mg/100 g) [35]. However, when the tannins are higher than the recommended limit values (760 to 900 mg/100 g), they can act as anti-nutritional factors by affecting the nutritional potential of the food. Furthermore, the flavonoid content of *V. unguiculata* (141.75±0.45 mg/100 g) is higher than that of *F. exasperata* (116.54±0.77 mg/100 g). However, these values are found to be higher than those (16.40 - 27.58 mg/100 g) reported in some leafy vegetables consumed in Northern Côte d'Ivoire [11]. Flavonoids in plants possess medicinal benefits which includes antioxidant and anti-inflammatory activities [27]. Consumption of such flavonoid-rich foods protects humans against diseases associated with oxidative stress [36]. Therefore, the leaves of *F. exasperata* and *V. unguiculata* would be useful for the organism.

Regarding anti-nutritional compounds, *F. exasperata* powder has a high level of phytates (7.45±0.24 mg/100 g), while *V. unguiculata* contains more oxalates (62.33±3.67 mg/100 g). The oxalate contents (36.33 - 62.33 mg/100 g) of the leafy vegetables studied are higher than those of phytates (6.13 - 7.45 mg/100 g). These oxalate values are higher than that of the lethal dose of 2-5 mg/kg, but the phytate values are within the lethal dose of 50-60 mg/kg [37]. However, these values are lower than those reported for some leafy vegetables consumed of Côte d'Ivoire with contents between 780 to 1310 mg/100 g for oxalates and 17.25 to 86.45 mg/100 g for phytates [11]. Oxalates and phytates are chelating agents which causes the bioavailability of essential minerals to decrease and turn into insoluble compounds whose absorption and digestion is less in the small intestine [38].

The phytate/calcium ratios (0.02) and phytate/iron ratios (0.29 - 0.61) found in this study are globally quite low. As for the oxalate/calcium ratio, it is 0.13 for *F. exasperata* and 0.24 for *V. unguiculata*. These ratios are comparable to those (0.26 - 0.43) of some leafy vegetable species in Côte d'Ivoire [26] and far below that (2.25) determined in Cameroon [25]. Thus, these low levels recorded in the leafy vegetables studied would reflect a low risk of interference with calcium. On the other hand, iron bioavailability could be affected because the oxalate/iron ratios (2.97 to 3.04) are high for the two leafy vegetable species studied. There is a potential for interference with iron in the plant, making it less available in the body compared to calcium. However, several processing methods (soaking, boiling or frying) applied to the leaves before consumption, would easily remove these anti-nutrients from the plants.

Conclusion

The present study reveals that the leaves of *V. unguiculata* are certainly reference foods but have the same nutrient compounds as *F. exasperata*. The *F. exasperata* leaves from Côte d'Ivoire represents an important source of carbohydrates, minerals and polyphenols, whereas *V. unguiculata* is rich in protein, vitamin C and flavonoids. These species could be used as a good source of food to improve the nutritional status and health of the Ivorian population. However, further studies are needed to evaluate the effect of cooking on the nutrient composition of these leaves, as they are widely consumed in soups.

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