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## Nutritional composition and bioavailability prediction calcium, iron, zinc and magnesium in *Justicia galeopsis* leaves in Côte d'Ivoire

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### ABSTRACT

Wild edible plants are known to make important contributions to the livelihoods of local communities of sub-Saharan Africa countries including Côte d'Ivoire. Some have an important nutritional intake. The aim of this study was to assess the physicochemical and nutritive properties of *Justicia galeopsis* leaves to ascertain its nutritional suitability as well as health benefits. *J. galeopsis* leaves were purchased at market in Abengougou Division. Physicochemical composition, micronutrients and antinutritional factors were determined. All results, based on dry matter, had indicated that *J. galeopsis* leaves were very rich in fiber (33.85%), protein (21.11%), vitamin B12 (4173.09 mg/kg) and vitamin C (892.17 mg/kg of fresh leaf) but low in fat (4.06%). The mineral elements contents were high (17.76%) with remarkable amount of potassium (103.08 g/kg), phosphorus (77.66 g/kg), calcium (59.87 g/kg), sodium (28.30 g/kg), iron (373.01 mg/kg), zinc (177.84 mg/kg) and manganese (128.57 mg/kg). The presence of phytate (33.83 mg/100g), oxalate (740.67 mg/100g) and tannin (66.33 mg/100g) were revealed. The molar ratios of [Phytate]/[Ca] (0.00), [Phytate]/[Zn] (0.15), [Phytate]/[Fe] (0.11) and [Oxalate]/[(Ca+Mg)] (0.07) indicate the high bioavailability for these minerals. This work revealed that *J. galeopsis* leaves are very nutritive. They could help cover people's nutritional needs and contribute to food security.

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**Keywords:** Wild edible plants, *Justicia galeopsis* leaves, Abengourou Division, nutritional composition, antinutritional factors, mineral bioavailability.

### INTRODUCTION

Côte d'Ivoire has a genetical diversity of plant and this flora contains many wilds food plants (Djaha and Gnahoua, 2014). These wild food plants are real sources of dietary supplements and incomes in rural areas (Kouamé et al., 2008). According to Djaha and Gnahoua (2014), these plants

contribute effectively to nutrition as an integral part of the diet and / or asset of family food security. They are the subject of flourishing trade whose main actors are women (Betti et al. 2016). Indeed, these plants are valuable sources of nutrients, especially, in rural areas where they contribute substantially to the intake of proteins,

minerals, vitamins, fiber and other nutrients, which are usually rare in daily diet (Idoko et al., 2014).

Despite their importance in some local food systems, wild food plants are increasingly neglected because of the adoption of Western eating habits. In Côte d'Ivoire, for some populations, consuming these plants is a sign of poverty. In contrary, the consumption of the manufactured products is a sign of ease and social success (Kouakou, 2015). The valorization of wild food plants becomes essential to remove all prejudices.

Studies of the valorization of wild food plants were initiated to inventory them through ethnobotanical surveys and then to evaluate and show the nutritional potential of some of them. So, *Justicia galeopsis* T. Anderson ex C.B. Clarke leaves has been discovered by Yao et al. (2014). For these authors, this plant seems endemic to Abengougou Division in the East of Côte d'Ivoire and is very little known in other parts of the country. They have also exhibited mainly its medicinal purpose and its high amount of total phenolics. A consumption survey realized later by Loukou et al. (2018) in this Division has shown that all the population of this region had already consumed *Justicia galeopsis* leaves for their flavor. The consumption of its leaves in sauce was also mentioned by Yao et al. (2014). However, the literature does not mention nutritional value of the leaves of this species which is necessary to increase the levels of consumption already too low.

The aim of this work was to assess the physicochemical and nutritive properties of *Justicia galeopsis* leaves in order to ascertain its nutritional suitability as well as health benefits.

## MATERIALS AND METHODS

### Sampling

*Justicia galeopsis* leaves, commonly known in the local language "Agni" as *Assiaploua* were purchased at market in Abengougou Division. Random selection of leaves was done by purchasing from randomly selected sellers in the market. Abengougou is a Division of Côte d'Ivoire located in the eastern part of this country, between 5°45 N and 7°10 N latitudes and 3°10 W and 3°50 W longitudes (Aka et al., 2013). Plants were identified and authenticated by National Floristic Center (University Felix Houphouët-Boigny, Abidjan-Côte d'Ivoire). The collected plants (300 g) were destalked, washed with distilled water, drained at ambient temperature and oven-dried (Memmert, Germany) at 60 °C for 72 h (Chinma and Igyor, 2007). The dried materials obtained were ground with a laboratory crusher (Culatti, France) equipped with a 10 µm mesh sieve. The dried powdered samples obtained were stored in polythene bags at 4 °C until further analyses.

### Analysis

#### *Physicochemical composition*

Moisture, matter dry, ash, pH, proteins, lipids and titratable acidity were determined by AOAC method (AOAC, 1990). Total fiber content was determined using Weende method (Wolf, 1968). The amount of carbohydrates was determined by difference as follows:

% Carbohydrates = 100 - (% moisture + % proteins + % lipids + % ash + % fiber). Total sugar was determined by Dubois et al. (1956). The amount of reducing sugars was estimated by the method of Bernfeld (1955).

#### *Minerals*

Mineral content was estimated by dry ashing of dried powdered sample (5 g) in a

muffle furnace (Pyrolabo, France). Ash obtained was dissolved in 5 mL of HCl/HNO<sub>3</sub> and analyzed using the atomic absorption spectrophotometer (AAS model, SP9).

The studied mineral were calcium (424.7 nm), magnesium (285.2 nm), phosphorus (178.2 nm), potassium (766.5 nm), sodium (589.6 nm), iron (248.3 nm), copper (324.8 nm), zinc (213.9 nm), manganese (279.5 nm), cobalt (240.7 nm).

Selenium content was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES Optima 2100DV, Perkin Elmer) at 196.1 nm. A mass of 0.1452 g was weighed and digested with 20 ml of nitric acid (7M) in an autoclave at 102 °C for 3 hours. After digestion, the sample was filtered and filled to 100 ml before analysis.

#### ***Vitamins and Carotenoids***

Quantitative determination of B-group vitamins (B<sub>1</sub>; B<sub>2</sub>; B<sub>6</sub>; B<sub>9</sub> and B<sub>12</sub>) was performed by reversed-phase high-performance liquid chromatography (RP-HPLC). In brief, *Justicia galeopsis* powder (1 g) was placed into a 100 mL volumetric flask, dissolved in 50 mL of dilution solvent (mixture of 25 mL of sodium carbonate solvent + 475 mL of mobile phase) and kept in an ultrasonic bath during 10 min. Then, the contents were cooled and taken to volume with the same dilution solvent. The mixture was through a micropore filter (0.45 µm). Ten milliliters were withdrawn in a 20 mL volumetric flask and diluted to volume with mobile phase. Twenty microliters of the filtrate were injected into the HPLC system. Quantification of vitamin B content was accomplished by comparison to vitamin B standards.

Standard stock solutions for thiamine, riboflavin, pyridoxine, folate and cobalamin were prepared as reported previously

(Ringling and Rychlik, 2013). The method used a Hypersil C8 (250 x 4.6 mm, 5 µm) column, under the following chromatographic conditions: gradient elution, using a mobile phase composed by 880 mL of potassium phosphate buffer (pH 2.65) and 120 mL of methanol as an organic modifier. The mobile phase was delivered at a flow rate of 1 mL/min and the detection was made at 280 nm. The amount of vitamin C in analyzed samples was determined by titration using the method described by Pongracz et al. (1995). About 10 g of ground fresh leaves were soaked for 10 min in 40 mL metaphosphoric acid-acetic acid (2 %, w/v). The mixture was centrifuged at 3000 rpm for 20 min and the supernatant obtained was diluted and adjusted with 50 mL of bi-distilled water. Ten (10) mL of this mixture was titrated with dichlorophenol-indophenol (DCPIP) 0.5 g/L. Carotenoids content was carried out according to Rodriguez-Amaya (2001). Two (2) g of ground fresh leaves were mixed three times with 50 mL of acetone until loss of pigmentation. The mixture obtained was filtered through Whatman No. 4 filter paper and total carotenoids were extracted with 100 mL of petroleum ether. Absorbance of extracted fraction was then read at 450 nm by using a spectrophotometer (PG Instruments, England). Total carotenoids content was subsequently estimated using a calibration curve of β-carotene (1 mg/mL) as standard.

#### ***Oxalates, phytates and tannins quantification***

Oxalates content was performed using a titration method (Day and Underwood, 1986). One (1) g of dried powdered sample was weighed into 100 mL conical flask. A quantity of 75 mL of sulphuric acid (3 M) was added and stirred for 1 h with a magnetic stirrer. The mixture was filtered and 25 mL of

the filtrate was titrated while hot with  $\text{KMnO}_4$  solution (0.05 M) until an extremely faint pale pink color persisted for 15-30 sec. Phytates content was determined using the Wade's reagent colorimetric method (Latta and Eskin, 1980). A quantity (1g) of dried powdered sample was mixed with 20 mL of hydrochloric acid (0.65 N) and stirred for 12 h with a magnetic Stirrer. The mixture was centrifuged at 12000 rpm for 40 min. An aliquot (0.5 mL) of supernatant was added with 3 mL of Wade's reagent. The reaction mixture was incubated for 15 min and absorbance was measured at 490 nm by using a spectrophotometer (PG Instruments, England). Phytates content was estimated using a calibration curve of sodium phytate (10 mg/mL) as standard. Tannin content was determined using the method described by Broadhurst and Jones (1978) and was expressed as the equivalent of tannic acid.

#### **Determination of molar ratio of antinutrients to minerals**

The molar ratio between antinutrient and mineral was obtained using the formula (1) describes by Woldegiorgis et al. (2015).

Molar ratio = [Moles of antinutrient] / [Moles of mineral] = [mg antinutrient/MW of antinutrient] / [mg mineral/MW of mineral].

Where MW = atomic weight. (1)

#### **Data analysis**

All the analyses were performed in triplicate and data were analyzed using EXCELL and STATISTICA 7.1 (StatSoft). The values were expressed in the form of the mean  $\pm$  standard deviation (SD).

## **RESULTS**

### **Physicochemical properties**

The physicochemical properties of *Justicia galeopsis* leaves examined in this study were presented in Table 1 and based on

dry matter. The leaves of *J. galeopsis* had a pH of  $7.33 \pm 0.08$  and a titratable acidity of  $5.66 \pm 0.57$  meq/100 g. The leaves' ash content was  $17.76 \pm 1.18\%$ . Lipid and protein contents were respectively  $4.06 \pm 0.70\%$  and  $21.11 \pm 0.03\%$ . Reducing sugars and total sugars, their rates were respectively  $0.14 \pm 0.00$  and  $0.90 \pm 0.00\%$ . The crude fibers were about  $33.86 \pm 0.43\%$ .

### **Minerals composition**

The mineral contents of the leaves of *Justicia galeopsis* on dry matter basis were shown in Table 2. They contained very high levels of macroelements including potassium ( $103.08 \pm 0.64$  g/kg), phosphorus ( $77.66 \pm 0.46$  g/kg), calcium ( $59.87 \pm 0.32$  g/kg), and sodium ( $28.30 \pm 0.46$  g/kg). The lowest macronutrient content was magnesium (Mg) with  $3.52 \pm 0.02$  g/kg. The leaves were also rich in trace elements including iron ( $373.01 \pm 10.83$  mg/kg), zinc ( $177.84 \pm 15.81$  mg/kg), manganese ( $128.57 \pm 8.36$  mg/kg) and copper ( $16.32 \pm 1.40$  mg/kg). The results also showed low levels of cobalt ( $0.23 \pm 0.03$  mg/kg) and selenium ( $<0.01 \pm 0.01$  mg/kg).

### **Vitamins composition and carotenoids**

Vitamins and carotenoids compositions were indicated in Table 3. The leaves of *Justicia galeopsis* had a vitamin B12 rate of  $4173.09 \pm 15.27$  mg/kg DM. Vitamin C and carotenoid contents were respectively  $892.17 \pm 1.13$  mg/kg and  $38.1 \pm 0.08$  mg/kg of fresh matter. The rate of vitamin B9 in the leaves was  $8.77 \pm 0.18$  mg/kg DM. Vitamin B2 rate was  $5.57 \pm 0.47$  mg/kg DM while vitamin B1 rate was  $1.94 \pm 0.06$  mg/kg DM.

### **Antinutrients quantification and molar ratios of antinutrients and minerals**

The results of oxalates, phytates and tannin quantification of *Justicia galeopsis*

leaves based on dry matter were presented in Table 4. Oxalate was the most abundant antinutritive factor ( $740.67 \pm 12.70$  mg/100g) in the leaves followed by tannin ( $66.33 \pm 2.31$  mg/100g) and then Phytate ( $33.83 \pm 2.45$  mg/100g). The molar ratios between antinutrients and minerals of *J. galeopsis* leaves were showed in Table 5. The results

indicated that molar ratio of [Phytate]/[Fe], [Phytate]/[Zn], [Phytate]/[Ca], [Ca][Phytate]/[Zn], [Oxalate]/[Ca] and [Oxalate]/[(Ca + Mg)] for *J. galeopsis* leaves were 0.11, 0.15, 0.00, 0.28, 0.05 and 0.07 respectively. These molar ratios were low compared with their corresponding critical value.

**Table 1:** Physicochemical properties of *Justicia galeopsis* leaves (% of dry matters).

Parameters	<i>Justicia galeopsis</i> leaves
Moisture (%)	$18.05 \pm 0.07$
pH	$7.33 \pm 0.08$
Titrateable acidity (meq/100 g)	$5.66 \pm 0.57$
Ash (%)	$17.76 \pm 1.18$
Crude fibers (%)	$33.86 \pm 0.43$
Lipids (%)	$4.06 \pm 0.70$
Proteins (%)	$21.11 \pm 0.03$
Carbohydrates (%)	$23.21 \pm 0.00$
Total sugar (%)	$0.90 \pm 0.00$
Reducing sugars (%)	$0.14 \pm 0.00$

Data are represented as means  $\pm$  SD (n=3).

**Table 2:** Mineral composition of *Justicia galeopsis* leaves.

Minerals	<i>Justicia galeopsis</i> leaves
<b>Macro elements (g/kg)</b>	
Calcium (Ca)	$59.87 \pm 0.32$
Magnesium (Mg)	$3.52 \pm 0.02$
Phosphorus (P)	$77.66 \pm 0.46$
Potassium (K)	$103.08 \pm 0.64$
Sodium (Na)	$28.30 \pm 0.46$
<b>Oligoelements (mg/kg)</b>	
Cobalt (Co)	$0.23 \pm 0.03$
Copper (Cu)	$16.32 \pm 1.40$
iron (Fe)	$373.01 \pm 10.83$
Manganese (Mn)	$128.57 \pm 8.36$
Selenium (Se)	$<0.01 \pm 0.01$
Zinc (Zn)	$177.84 \pm 15.81$

Data are represented as means  $\pm$  SD (n=3).

**Table 3:** Vitamins and carotenoids composition of *Justicia galeopsis* leaves.

Parameters (mg/kg of DM)	<i>Justicia galeopsis</i> leaves
Carotenoids (mg/kg FM)	38.1 ± 0.08
Vitamin B <sub>1</sub>	1.94 ± 0.06
Vitamin B <sub>12</sub>	4173.09 ± 15.27
Vitamin B <sub>2</sub>	5.57 ± 0.47
Vitamin B <sub>6</sub>	ND
Vitamin B <sub>9</sub>	8.77 ± 0.18
Vitamin C (mg/kg of FM)	892.17 ± 1.13

Data are represented as means ± SD (n=3). DM: Dry Matter; FM: Fresh matter; ND: Not detected

**Table 4:** Antinutritional factors content of *Justicia galeopsis* leaves.

Parameters (mg/100 g of DM)	<i>Justicia galeopsis</i> leaves
Phytate	33.83 ± 2.45
Oxalate	740.67 ± 12.70
Tannin (mg EC/100 g)	66.33 ± 2.31

Data are represented as means ± SD (n=3). DM: Dry Matter

**Table 5:** Molar ratio between phytate and minerals, Oxalate and minerals.

Antinutrient/mineral ratio	Value	Critical value*
[Phytate]/[Fe]	0.11	0.4
[Phytate]/[Zn]	0.15	<1.5
[Phytate]/[Ca]	0.00	0.5
[Ca][Phytate]/[Zn]	0.28	<0.5
[Oxalate]/[Ca]	0.05	2.5
[Oxalate]/[(Ca + Mg)]	0.07	2.5

Values are expressed as mean ± standard deviation, SD (n=3). \*Source: Hassan et al. (2007), Frontela et al. (2008), Obah and Amusan (2009).

## DISCUSSION

Physicochemical properties of *Justicia galeopsis* leaves have showed high content of crude fiber, followed by protein and ash content. The amount of fiber (33.86%) in the studied leaves was greater than those of *S. monostachyus*, a green leafy vegetable in Nigeria (12.90%) mentioned by Afolabi et al. (2012). Its consumption could therefore help the proper functioning of the digestive system, reduce the risk of

hypertension, constipation, diabetes, colon cancer. *J. galeopsis* leaves are a good source of protein (21.11%). They could therefore contribute to ensuring food security for the population. This value of protein was higher than that found by Obichi et al. (2015) for *S. monostachyus* leaves (10.11%). The lipid content of *J. galeopsis* leaves was relatively low (4.06%). The low value of fat recorded showed that *J. galeopsis* can be recommended as weight reducing diet since low fat food

reduces the level of cholesterol and obesity (Gordon and Kessel, 2002). They can therefore be consumed in abundance and safely without risk of cardiovascular disease, obesity and other related diseases. This value is like fat percentage reported in *S. monostachyus* (Afolabi et al., 2012) which are about 4.64%. According to Afolabi et al. (2012), these leaves would be a good source of fat-soluble vitamins.

Mineral analysis revealed that leaves of *J. galeopsis* contained a high rate of potassium phosphorus, calcium, sodium, iron, zinc and manganese. They are a good source of minerals. They can therefore be recommended for infants, pregnant women and the elderly, to cover mineral needs. In fact, the consumption of 16.73 g, 114.29 g, 21.45 g and 33.75 g of these fresh leaves could cover respectively the recommended daily doses for an adult in calcium (1000 mg/day), magnesium (400 mg/day), iron (8 mg/day) and zinc (6 mg/day) mentioned by Huskisson et al. (2007). The values found for potassium and calcium are high compared with those reported by Acho et al. (2014) for *Basella alba* leaves which were, respectively 28.93 g/kg and 41.37 g/kg and Anin-Atchibri et al. (2012) for *Solanum nigrum* leaves (49.85 mg/kg, potassium; 15.090 mg/kg, calcium). The consumption of *J. galeopsis* leaves would provide much more potassium and calcium than *S. nigrum* and *B. alba*.

Vitamin B12 content of *Justicia galeopsis* leaves was high, followed by vitamin C, carotenoids, vitamin B9 and vitamin B2. Vitamin B1 content was low. The consumption of only 134.5 g of leaves could significantly help pregnant women cover their needs which are 120 mg/day for vitamin C and 2.6 µg/day for vitamin B12 (Schlienger, 2014) and protect them. *J. galeopsis* vitamin C levels is greater than that of *S.*

*monostachyus* leaves (420 mg/kg) mentioned by Obichi et al. (2015), *Basella alba* (700 mg/kg) and *Corchorus olitorius* (700 mg/kg) studied by Acho et al. (2014). The consumption of *J. galeopsis* leaves could reduce iron deficiency which is the most common nutritional disorder. Indeed, thanks to its high content in vitamin C, iron present in *J. galeopsis* leaves may be available. According to Thompson (2011), a meal containing many inhibitors of iron absorption should preferably contain at least 25 mg of ascorbic acid and possibly more to significantly boost absorption of non-heme iron.

Carotenoids are considered sources of provitamin A in plants. Their daily dose is between 3.6 and 4.8 mg/day. The carotenoid contents of *J. galeopsis* leaves were 46.49 mg/kg. Therefore, a consumption of about 105 g of leaves would cover the needs and fight against vitamin deficiency. *J. galeopsis* leaves are very nutritious. Indeed, consumption of their leaves brings important and sufficient rate of vitamins and minerals necessary for the human body.

The phytate content of *J. galeopsis* (740.67 ±12.70 mg/100g dry weight) was similar to that reported for *Melocia corchorifolia* leaves which is 788.57±18.63 mg/100g DW (Hassan et al., 2011). Acho et al. (2014) reported low rate of phytate in some leafy vegetables such as *Basella alba*, *Colocasia esculenta* and *Corchorus olitorius* as respectively 19,78 mg/100 g, 26,27 mg/100 g and 38,75 mg/100 g wet weight. These contents were equivalent to 194.30 mg/100 g, 148.84 mg/100 g and 246.50 mg/100 g dry weight. Phytate content in the leaves of *J. galeopsis* was less than 0.04 % reported in food items (Adeduntan and Oyeride, 2009). Phytate is known to decrease the bioavailability of minerals, especially

calcium, magnesium, iron and zinc (Bhandari and Kawabata, 2004). Hurrell (2004) reported that a phytic acid intake of 4-9 mg/100 g dry matter decreases iron absorption by 4-5 folds in human. To predict the effect of phytate on the bioavailability of iron, zinc and calcium, phytate to nutrients ratios were calculated. The calculated molar ratios of phytate to iron, zinc and calcium of *J. galeopsis* were below the critical level of 0.4, 1.5 and 0.5 respectively as outline by Frontela et al. (2008) and Hassan et al. (2011). This result means that absorption of iron, zinc and calcium was not adversely affected by phytate. It's not the case of *C. olitorius* and *S. melongena* whose [phytates]/ [Fe] molar ratios were above the critical level of 0.4 (Acho et al. 2014) indicating that the phytates of these leafy vegetables may hinder iron bioavailability (Umar et al., 2007).

The analyzed *J. galeopsis* leaves have total oxalate content of 740.67 ±12.70 mg/100g dry matter which is low compared with those reported by Acho et al. (2014) in the leaves of *B. alba* (650 mg/100g), *C. esculenta* (580 mg/100g) and *C. olitorius* (780 mg/100g) when there are expressed in dry weight (6385 mg/100g, 3286 mg/100g and 4961 mg/100g, respectively). From the result, it was observed that in *J. galeopsis* [oxalate]/[Ca] ratio (0.05) and [oxalate]/[(Ca+Mg)] ratio (0.07) are below the critical level of 2.5 known to impair calcium bioavailability (Umar et al., 2007). The present of oxalate in *J. galeopsis* leaves don't affect the bioavailability of calcium.

## Conclusion

This study has revealed that *Justicia galeopsis* leaves consumed in Côte d'Ivoire could help to cover the nutritional needs of the populations. They are very rich in fibers, proteins, minerals and vitamins. The minerals

analysis of *J. galeopsis* leaves indicated that it is rich in most mineral elements with high predicted bioavailability for iron, calcium, zinc and magnesium. Mineral and vitamin contents of these leaves are enough to satisfy the recommended dietary allowances of these micronutrients. The leaves of *J. galeopsis* can be considered as an alternative source of energy and nutrients that are important to prevent malnutrition and deficiency diseases in developing countries. The consumption of this plant could therefore provide several health benefits. As a spontaneous food plant, this plant deserves to be popularized. Thus, it could contribute to populations' food security.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

ALL is the main investigator of the research; she wrote the experimental protocol and the first draft of the manuscript. KYBA contributed to write the experimental protocol and the first draft. KHK developed the protocol, collected the data. KB has improved the versions of the manuscript and he monitored the group for all research. All authors have read and approved the final version of the manuscript.

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