International Journal of Scientific World, *5 (1) (2017) 23-27*

International Journal of Scientific World

Website: www.sciencepubco.com/index.php/IJSW
doi: 10.14419/ijsw.v5i1.6969
Research paper

Proximate composition, mineral content and amino acid profile of *Irvingia gabonensis* O'Rorke baill leaf

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Abstract

Several plants are utilized for medicinal and nutritional purposes. *Irvingia gabonensis* O'Rorke Baill leaf is used in herbal medicine for treatment of a number of ailments. This study was therefore carried out to investigate the proximate composition, antinutritional factors, mineral composition and amino acid profile of *Irvingia gabonensis* O'Rorke Baill leaf. The proximate and antinutritional factors analyses were done using standard procedures. The mineral analyses were done using flame photometry, titrimetic method, molybdo vanadate method and atomic absorption spectrophotometry and the amino acid profile was done with the aid of Applied Biosystems PTH amino acid analyzer. Results of proximate analyses were carbohydrates (75.15±1.29 %), protein (11.43±1.07 %), fat (1.99±0.74 %), fibre (4.89±0.61 %), ash (6.71±0.28 %), moisture (5.12±0.03 %) and caloric value (364.30±5.95 Kcal). Antinutrients (phytate, oxalate and cyanide) levels in the leaf were also very low. Results from mineral analyses obtained revealed that the leaf is also a very rich source of calcium, potassium, sodium, magnesium and so on. Compared with the World health organization (WHO) standards, results of the amino acid profile showed that the leaf is very rich in isoleucine, leucine, lysine, phenylalanine, threonine, valine and tyrosine which are nutritionally essential amino acids. Furthermore, extraction of the leaf using ethanol reduced the levels of these amino acids but not below the recommended WHO standard levels for most of the essential amino acids. *Irvingia gabonensis* O'Rorke Baill leaf is therefore a potential source of key nutrients.

Keywords: *Amino Acid; Irvingia gabonensis O'Rorke Baill; Minerals; Nutritional; Proximate.*

1. Introduction

Currently, there is an upsurge in the utilization of plants believed to possess high nutritional and medicinal value by most locals especially those in developing countries like Nigeria. This has necessitated an urgent need for scientific investigation of the various plant parts utilized in order to provide scientific information on such plants that could either validate or invalidate the claims of the users. *Irvingia gabonensis* O'Rorke Baill is an indigenous forest tree belonging to the group of plants classified as non-timber forest products. It is a mostly found in Southern and Eastern Nigeria, Sierra Leone and Equatorial Africa and has been listed as one of the most important trees for domestication in the humid lowlands of West Africa [1], [2]. It is commonly called bush mango or wild mango and grows to 15-40m with a slightly buttressed hole. It occurs in the wild low land forest [3], the dark green foliage is dense and the leaves are elliptical. The yellow/white flowers occur in bundles or clusters between February and March, and the fruit appears during the rainy season between July and September. The tree matures and flowers at 10 to 15 years of age, while flowering and fruiting times are dependent on the geographical location. The ripe fruit is green while the edible mesocarp is soft, juicy, and bright orange in colour. The mesocarp has a turpentine flavor and may taste sweet to slightly bitter. The seeds or kernels of the tree are classified as oilseeds [4], [5].
In Cameroon, the stem bark is used to treat hunch back and infections [6]. The aqueous decoction of the leaves is also used as antidote for some poisonous substances. The Senegalese use the decoction of the stem bark in treating gonorrhea, hepatic and gastrointestinal disorders [3]. The root bark is also prescribed in poultice form to treat wounds. The analgesic effects of the stem bark of this plant has also been demonstrated [7]. Ngondi et al., [8] have also reported the cholesterol and triglycerides lowering effect of the seed extract in obese people, thus proving its efficacy in weight reduction. The antibacterial and antifungal activities of the stem bark have also been documented [9]. It is also known that the seed extract and the stem bark have antioxidant activity [10], [3]. The hematological properties and prophylactic effect of the ethanolic stem bark extract of this plant on cadmium-induced toxicity in Wistar rats was reported by Ojo et al., [11], [12]. Ethanol extract of the leaf has been reported to possess a very high antioxidant activity [13]. The hepatoprotective and nephroprotective effects of the ethanol leaf extract against cadmium-induced hepatotoxicity and nephrotoxicity in Wistar rats has also been documented [14].
As this plant has been recommended for domestication, and the leaf extracts in form of decoctions are consumed for medicinal purposes, it is therefore very crucial to also investigate the nutritional and antinutritional properties of the leaf. This study was therefore carried out to evaluate the proximate composition, antinutritional factors, mineral content and amino acid profile of *Irvingia gabonensis* O'Rorke Baill leaf.

2. Materials and methods

2.1. Collection, identification and processing of plant sample

Fresh and matured leaves of the plant were harvested from Ammanagwu village in Arochukwu local government area of Abia State, Nigeria and identified by Mr. Daniel Etefia of the department of pharmacognosy, University of Uyo, Akwa Ibom State, Nigeria and a voucher specimen was deposited in the herbarium. The leaves were washed with normal water and then rinsed with distilled water to remove any contaminating dust particles and air-dried at room temperature for about one week. The dried leaves were pulverized and a portion of it was macerated in 80% ethanol for 72 hours. It was then filtered and concentrated with a water bath at 45ºC into a paste. The dried raw leaf sample and the extract respectively were used for the analyses.

2.2. Proximate analyses

The moisture, ash, crude fibre, crude protein, crude fat and carbohydrate contents of the dried leaf sample were investigated according to the methods prescribed by AOAC [15]. The moisture and ash content were determined using weight difference method. Fiber content was estimated from the loss in weight of the crucible and its content on ignition. The protein content was estimated using the Kjeldahl method. The carbohydrate content was determined as the difference obtained after subtracting crude protein, fat, ash and fibre content from the total dry matter. The results were reported in percentage.

2.3. Determination of antinutritional factors

Antinutritional factors, phytate, oxalate and cyanide content were estimated in the dried raw leaf sample. Oxalate in the sample was estimated according to the method of Harbone, 1998 [16]. Phytate in the sample was estimated according to the method of Sofowora, 2006 [17]. Cyanide content was estimated according to the method of Anon, 1990 [18].

2.4. Mineral analyses

The concentrations of different minerals in the dried raw leaf sample were estimated. Sodium and potassium concentrations were estimated using flame photometry method. Calcium and magnesium concentrations were estimated using the versanate EDTA titrimetric method. The molybdo vanadate method was used to estimate phosphorus concentration in the leaf sample. All other mineral analyses (zinc, iron, copper, lead, mercury, selenium, arsenic and nickel) were done using atomic absorption spectrophotometry.

2.5. Sample preparation for amino acid analyses

Amino acid analyses were carried out on both the dried raw leaf sample and the ethanol leaf extract of the plant. The method of Sparkman et al., 1958 [19] was adopted. Each sample was dried to constant weight, defatted, hydrolyzed, evaporated and loaded into the Applied Biosystems PHT Amino Acid Analyzer as follows: 2 g of the dried sample was weighed into extraction thimble and the fat extracted with chloroform : methanol (in the ratio of 2:1) mixture using soxhlet extraction apparatus. 1g of the defatted coagulated leaf concentrate was then weighed into ampoule. 7mls of 6N HCl was added sequel to the passage of nitrogen into the ampoule to remove any trapped oxygen and to avoid oxidation of some amino acids during hydrolysis. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105ºC for 22hrs. The ampoule was allowed to cool before broken opening the tip and the content was filtered to remove the humans. The filtrate was then evaporated to dryness at 40ºC under vacuum using a rotary evaporator. The residue was dissolved with 5mls of acetate buffer (pH 5.0), and stored in plastic specimen bottles. 10 μl was then dispensed into the cartridge of the automatic amino acid analyser which is designed to separate and analyse free, acidic, neutral and basic amino acids of the hydrolysate. The amount of each amino acid present in the sample was calculated in g/100 g protein from the chromatogram produced.

2.6. Statistical analysis

Each experiment was done in triplicates with the exception of amino acid analyses which were done in duplicates. The results were presented as mean ± standard deviation using Microsoft Excel.

3. Results

3.1. Proximate composition of *Irvingia gabonensis* O'Rorke baill leaf

Results from proximate analyses of the dried raw leaf sample revealed the carbohydrate content to be (75.15±1.29 %), protein (11.43±1.07 %), fat (1.99±0.74 %), fibre (4.89±0.61 %), ash (6.71±0.28 %), moisture (5.12±0.03 %) and caloric value (364.30±5.95 Kcal) as shown in Table 1 below.

Table 1: Proximate Composition of *Irvingia gabonensis* O'Rorke Baill Leaf

Parameter	% Composition
Carbohydrate	75.15±1.29
Protein	11.43±1.07
Fat	1.99±0.74
Fibre	4.89±0.61
Ash	6.71±0.28
Moisture	5.12±0.03
Energy Value (Kcal)	364.30±5.95

Values are expressed as mean± standard deviation. n=3

3.2. Antinutritional factors content of *Irvingia gabonensis* O'Rorke baill leaf

The levels of phytate, oxalate and cyanide in the leaf sample were investigated. Results revealed the levels to be: Phytate 3.45±0.02mg/100g, oxalate 1.87±0.03mg/100g and cyanide 0.81±0.002 (mg/100g) as shown in table 2 below.

Table 2: Antinutritional Factors Content of *Irvingia gabonensis* O'Rorke Baill Leaf

Antinutritional Factor	Concentration (mg/100g)
Phytate	3.45±0.02
Oxalate	1.87±0.03
Cyanide	0.81±0.002

Values are expressed as mean± standard deviation. n=3

3.3. Mineral content of *Irvingia gabonensis* O'Rorke baill leaf

The results for the mineral analyses obtained were: calcium (Ca) 708.13±0.15, potassium (K) 671.09±0.04, sodium (Na) 93.11±0.04, magnesium (Mg) 43.72±0.02, manganese (Mn) 16.12±0.02, phosphorus (P) 11.07±0.01, zinc (Zn) 6.06±0.03, iron (Fe) 3.03±0.03 all in mg/100g. Others were nickel (Ni) 0.46±0.006, lead (Pb) 0.32±0.02, cobalt (Co) 0.07±0.006, copper (Cu) 0.02±0.006, arsenic (As) 0.04±0.00 all in mg/kg as shown in table 3 below. Selenium (Se) and mercury (Hg) were not detected.

Table 3: Mineral Composition of *Irvingia gabonensis* O'Rorke Baill Leaf

Elements	Concentration (mg/100g)
Ca	708.13±0.15
K	671.09±0.04
Na	93.11±0.04
Mg	43.72±0.02
Mn	16.12±0.02
P	11.07±0.01
Zn	6.06±0.03
Fe	3.03±0.03
	Concentration (mg/kg)
Ni	0.32±0.02
Pb	0.32±0.02
Co	0.07±0.006
Cu	0.02±0.006
As	0.04±0.00
Se	ND
Hg	ND

Values are expressed as mean± standard deviation. n=3. ND= Not detected

3.4. Amino acid content of *Irvingia gabonensis* O'Rorke baill leaf and ethanol leaf extract

Results showed that the *Irvingia gabonensis* O'Rorke Baill leaf is very rich in essential amino acids as compared with WHO/FAO standards as shown in table 4 below.

Table 4: Amino Acid Content of *Irvingia gabonensis* O'Rorke Baill Leaf and Ethanol Leaf Extract

Amino acid	Conc.(g/100g Protein) Raw leaf	Conc.(g/100g Protein)Ethanol Leaf Extract	WHO/FAO [20] Standards (g/kg/day Protein)
Leucine*	8.57±0.01	6.28±0.03	0.039
Lysine*	6.26±0.03	4.28±0.01	0.03
Valine*	4.98±0.02	4.10±0.01	0.026
Phenylalanine*	4.79±0.00	4.07±0.01	0.025
Isoleucine*	4.61±0.007	3.78±0.01	0.02
Threonine*	3.88±0.00	3.34±0.04	0.015
Tyrosine*	3.08±0.01	3.43±0.02	0.025
Methionine*	1.53±0.01	0.93±0.00	0.0104
Glutamic acid	13.57±0.72	12.12±0.01	
Aspartic acid	11.11±0.01	10.83±0.03	
Arginine	6.02±0.007	4.98±0.01	
Serine	4.64±0.00	3.68±0.04	
Alanine	4.59±0.00	4.47±0.00	
Proline	4.17±0.01	2.24±0.01	
Glycine	3.72±0.74	4.07±0.01	
Histidine	2.48±0.01	2.01±0.007	
Tryptophan	1.49±0.02	0.87±0.01	0.004
Cystine	1.33±0.00	1.10±0.01	

Values are expressed as mean± standard deviation. n=2. *= Essential Amino acids

4. Discussion

Proximate analysis of vegetables is very vital in the assessment of their nutritional value [21]. Carbohydrates are a major class of food nutrients that serve as a principal source of energy alongside lipids and proteins. The carbohydrate content of *Irvingia gabonensis* O'Rorke Baill leaf (75.15±1.29 %), indicates that it is high when compared to *Talinum triangulare* leaves (10.87±3.99) [22], *Moringa oleifera* leaves [23], [24], and *Annona muricata* leaves [25]. This indicates that the leaves are a very rich source of carbohydrates and thus a valuable energy source as carbohydrates are a major source of energy required for the maintenance of life. Proteins are a very important class of biomolecules needed for maintenance and repair of body tissues, synthesis of vital hormones and an alternative source of energy when there are deficiencies of other rich sources. The protein content of *Irvingia gabonensis* O'Rorke Baill leaf from this study (11.43±1.07 %), is high when compared to that of *Talinum triangulare* leaves (3.52±0.32) [22], *Moringa oleifera* leaves (10.74 ± 1.3) [26], but low when compared to *Annona muricata* leaves (25.00 ± 0.06) [25]. *Irvingia gabonensis* O'Rorke Baill leaf may therefore be considered a good source of protein.

Dietary fat is the main source of energy but it is not expected to exceed the daily recommended value of not more than 30 calories in order to avoid obesity and related diseases [27]. The fat content of *Irvingia gabonensis* O'Rorke Baill leaf from this study was relatively low. Dietary fibre helps in the reduction of serum cholesterol level, risk of coronary heart disease, colon and breast cancer and hypertension. The fibre content of *Irvingia gabonensis* O'Rorke Baill leaf from this study (4.89±0.61 %), is low when compared to *Annona muricata* leaves [25], but high compared to *Amaranthus hybridus* (1.6%) [28]. Ash in food is the residue left after the removal of moisture and incineration of the organic materials at a temperature of about 500°C [29]. The ash content of *Irvingia gabonensis* O'Rorke Baill leaf from this study (6.71±0.28 %), is high when compared with 1.8% reported for sweet potatoe leaves of [30] and compares favourably with that reported for *Moringa oleifera* leaves [24] but low compared with *Annona muricata* leaves [25]. This indicates that *Irvingia gabonensis* O'Rorke Baill leaf is a rich source of mineral elements as the ash content in food is a measure of its mineral content. The moisture content of *Irvingia gabonensis* O'Rorke Baill leaf obtained from this study was low indicating its high shelf life and hence resistance to microbial attack with long storage [31]. The results of the antinutrients indicate that *Irvingia gabonensis* O'Rorke Baill leaf is very low in the antinutrients analysed.

Minerals are inorganic elements that are structural components of body tissues, involved in the maintenance of acid-base balance, involved in the regulation of body fluids, important in the transport of gases and in muscle contractions [32], [33]. Every living organism requires these minerals for their normal life processes [34]. Calcium is an important constituent of bones and teeth, functions in regulation of nerve and muscle function, helps in converting prothrombin to thrombin during blood coagulation, functions in enzyme activation such as adenosine triphosphatase (ATPase), succinate dehydrogenase, and lipase. It is also important for membrane permeability, involved in muscle contraction, normal transmission of nerve impulses and in neuromuscular excitability [35]. Calcium content of *Irvingia gabonensis* O'Rorke Baill leaf obtained from this study (708.13±0.15 mg/100g), indicates that the leaf is rich in calcium. The daily required intake for calcium is 600mg/kg/day in childhood and 1000 mg/kg/day in adulthood [36]. The leaf of this plant can therefore adequately supply the recommended calcium intake in childhood and a very high percentage of the required adult calcium intake per day. Potassium is the major cation in intracellular fluid and it is involved in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction, cell membrane function and Na^+/K^+-ATPase. Potassium is also required during glycogen synthesis [35]. The potassium content of *Irvingia gabonensis* O'Rorke Baill leaf obtained from this study (671.09±0.04 mg/100g), is high compared with 96.91±1.05 mg/100g of *Chromolaena odorata* leaf [37], *Annona muricata* leaves (36.31mg/100g) [25], and *Vernonia amygdalina* leaves (62.79mg/100g) [38] but low when compared with *Moringa oleifera* leaves [26]. The result however shows that *Irvingia gabonensis* O'Rorke Baill leaf is rich in potassium. Sodium is the major cation in extracellular fluids. It helps in regulating plasma volume and acid-base balance, vital in the maintenance of osmotic pressure of the body fluids, helps in preserving normal irritability of muscles and cell permeability, activates nerve and muscle function and involved in Na^+/K^+-ATPase, maintains membrane potentials, helps in transmission of nerve impulses and the absorptive processes of monosaccharides, amino acids, pyrimidines, and bile salts [35]. The sodium content of *Irvingia gabonensis* O'Rorke Baill leaf obtained from this study (93.11±0.04 mg/100g), is high compared with *Chromolaena odorata* leaf [37], *Annona muricata* leaves (69.49mg/100g) [25], and *Vernonia amygdalina* leaves (48.31mg/100g) [38] and Moringa oleifera leaves [26]. With respect to FAO/WHO recommended daily intakes, the results obtained from this study indicates that *Irvingia gabonensis* O'Rorke

Baill leaf is rich in zinc and iron but low in magnesium [36]. Zinc functions as a cofactor and is a constituent of a number of enzymes such as lactate dehydrogenase, alcohol dehydrogenase, glutamate dehydrogenase, alkaline phosphatase, carbonic anhydrase, carboxypeptidase, superoxide dismutase, retinene reductase, DNA and RNA polymerases [35]. Iron helps in the transportation of oxygen being part of hemoglobin. It also aids the synthesis and packaging of neurotransmitters, their uptake and degradation into other iron-containing proteins which may directly or indirectly alter brain function [39]. Manganese content of *Irvingia gabonensis* O'Rorke Baill leaf obtained from the present study (16.12±0.02 mg/100g) is high compared with 8.25±1.25mg/100g of *Annona muricata* leaves [25], and 0.81±0.10 mg/100g of *Chromolaena odorata* leaves [37]. Manganese is a cofactor of a number of enzymes such as hydrolase, decarboxylase, and transferase enzymes [33]. It also functions in glycoprotein and proteoglycan synthesis and it is a component of mitochondrial superoxide dismutase; a potent antioxidant [35]. Phosphorus is a constituent of bones, teeth, phosphorylated metabolic intermediates and nucleic acids. It functions in the formation of high energy compounds, like adenosine triphosphate (ATP) and it is also involved in the synthesis of phospholipids and phosphoproteins antioxidant [35]. The phosphorus content of *Irvingia gabonensis* O'Rorke Baill leaf obtained from this study (11.07±0.01 mg/100g), is high compared with 5.00±0.12 mg/100g of *Moringa oleifera* leaf protein concentrates [40]. Nickel, lead, cobalt, copper and arsenic contents of *Irvingia gabonensis* O'Rorke Baill leaf obtained from this study were very low. Selenium and mercury were not detected.

Amino acids are the building blocks of proteins. These amino acids could be classified as nutritionally essential amino acids or nutritionally non-essential amino acids. The nutritionally essential amino acids are those that must be supplied through the diet because they cannot be synthesized by the body. Some of these amino acids are also classified as semi-essential because the body may not be able to synthesize them under certain conditions. In the present study, amino acid analyses of *Irvingia gabonensis* O'Rorke Baill leaf and ethanol leaf extracts revealed that both the dried leaf and ethanol leaf extract are a very rich source of essential amino acids when compared with WHO/FAO recommended intakes of these amino acids [20] as indicated in table 4. However, extraction of the leaf with ethanol reduced the concentrations of these amino acids but not below the recommended standards.

5. Conclusion

The present study has unraveled the very high nutritional potential of *Irvingia gabonensis* O'Rorke Baill leaf. The leaf is very rich source of essential minerals and amino acids that are needed for proper function of the body system. Also, it is a rich source of energy and relatively safe for consumption owing to its very low concentrations of antinutrients and toxic elements.

Acknowledgement

Special thanks to National Root Crop Research Institute (NRCRI), Nigeria for allowing us access to their laboratories and equipments and aiding the analyses carried out.

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