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(RESEARCH ARTICLE)

Nutrient compositions of leaves of *Bombax buonopozense*

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Abstract

There is high prevalence of micronutrients deficiency in low income countries resulting to malnutrition. This stude was designed to evaluate the nutrient compositions of leaves of *Bombax Buonopozense*. Minerals, amino acids and vitamins constituents were investigated using AAS, amino acid analyzer, spectrophotometer respectively. Result of dietary minerals constituent in the leaves of *B. buonopozense* revealed a total of eight minerals- manganese (0.02±0.00), zinc (0.02±0.00), copper (0.03±0.00), cobalt (0.04±0.00), iron (0.06±0.00), sodium (3.13±0.20), calcium (93.72±0.20) and potassium (669.22±0.20) in ascending order of concentrations. Total of seventeen amino acids consisting of eight essential and nine non-essential amino acids were identified in the leaves- arginine, valine, methionine, leucine, threonine, phenylalanine, histidine, isoleucine, lysine, proline, serine, tyrosine, alanine, glycine, glutamate, aspartate and cysteine. Five water and two fat soluble vitamins totaling seven in all were present, they are vitaminB6 (34.08± 0.20), vitaminB1 (22.74± 0.20), vitamineB2 (22.29± 0.20), vitamin A (15.91 ± 0.20), vitaminB12 (10.31± 0.20), vitaminE (9.33± 0.20µ/l), vitaminB3 (0.95± 0.02) vitamin C (0.42± 0.02) in decending order. *Bombax Buonopozense* leaves are rich in micro nutrients hence could as supplement in micronutrients deficient diet to prevent micronutrients deficiency.

Keywords: *Bombax buonopozense*; Micronutrients; Vitamins; Amino acids; Minerals

1 Introduction

Vegetables are parts of a plant that can be eaten in whole or in part, in a raw form or processed by cooking it, as a main dish or a side dish as salad. They can be stems, tubers, roots, bulbs, leaves, flowers, seeds, fruits and fungi[1].They are very rich sources of vitamins and minerals, They also help the body to resist cancers as can be found in the case of stomach cancer. They are abundant in nature, and exist in varieties of colours, textures and shapes and serve as food for man [2].

Due to increased information and awareness of the benefits of consuming foods with high nutritional quality among the Nigerian populace, this has resulted to an increase in the demand for knowledge of the nutritional composition of vegetable foods. With the ever growing population, the basic vitamins required by man, is becoming increasingly difficult to meet up with. Hence it becomes imperative to evaluate the nutrient compositions of *Bombax buonopozense (B. buonopozense* vegetable

The dried calyx, mixed with bark or spines were formerly used in cleaning the teeth, or chewed as a substitute for kola-nut. In Sierra Leone, the young fruits along with it's attached calyx is used as salad toppings. The flowers are eaten by honeybees as food. The fresh leaves are eaten by goats as fodder. The seeds are used to produce edible oils. In Nigeria, the Ebonyians in the southeastern part of the country consume a large amount of its leaves. It is usually eaten raw or fresh as a local dish with palm oil in pepper sauce and also serves as a component of local salad which is consumed with

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rice among the natives. It is sometimes cooked in native soups and stew, and also in the preparation of potatoes, yam and plantain porridge.

Despite all the numerous uses of this plant. There is insufficient information as regards to the nutritional composition of the leaves of*Bombax buonopozense* which has limited the exploitation of the plant like other vegetables.

2 Material and methods

2.1 Methods

2.1.1 Collection and identification of sample

Fresh leaves of *Bombax buonopozense* were collected from the wild in Ubgoayim (II), in Ezzeagu Local Government Area of Ebonyi State, in Nigeria and was taken to the University of Port Harcourt herbarium where it was identified by Dr. Ekeke and given the reference number, UPH/P/076.

2.1.2 Preparation of pulverised sample

Bombax buonopozense leave ample was washed to remove dirt after which it was allowed to dry at room temperature for 4 weeks. The dried sample was then pulverized using a mortar and pestle to get a finely ground powdered form. It was then sieved to remove the unground particles before taken to the laboratory for analysis.

2.1.3 Analysis of Mineral Content Bombax buonopozense

A 1.0g of finely ground sample was weighed into porcelain crucible, and ignited in a furnace for 6-8hours at 4500 ^{o}C until grayish ash was obtained. It was allowed to cool on top of asbestos sheet and 5ml of 1M $HNO_3$was added and evaporated on a hot plate. It was returned to the furnace and heated at 4000^{o}C for 10-15 minutes until a perfect white ash was obtained. The sample was allowed to cool on top of asbestos sheet and 10ml 1N HCl was added and the solution was filtered into 50ml volumetric flask. The crucible and the filter paper were washed with additional 10ml portion of 0.1NHCl solution. The prepared sample was used for the determination of the various minerals analyzed in an AAS as the sample solution was aspirated into the flame spectrophotometer and absorbance was recorded for each element. Calibration curve for absorbance of standard solution for each metal was plotted. A plot of absorbance against concentration gave linear graph. The slope of the graph is used to calculate the concentration of each metal in a given sample using absorbance values.

2.2 Analysis of Vitamin Content *Bombax buonopozense*

2.2.1 Determination of vitamin C & Bs

For the determination of vitamin C and the B-vitamins, 1 ml of the analyzed liquid was measured into the centrifugal test-tube along with 1 ml of the PR and mixed thoroughly at room temperature for 30 minutes. The mixture was centrifuged in the tube (7000rpm), and the whole of the separated supernatant was collected with a pipette (the supernatant was the test sample for spectrophotometric measurements). The sample was run and the absorbance of the sample was measured in a spectrophotometer at wavelengths of 269 nm, 266 nm, 261 nm, 324 nm, 530 nm and 478.5 nm for Vit B_1, Vit B_2, Vit B_6, Vit $B_{12}$and Vit C respectively. The concentrations were extrapolated automatically from the calibration curve.

2.2.2 Determination of vitamin E concentration Bombax buonopozense

For the determination of vitamin E concentration, 0.5 ml of the analyzed fluid was measured into the test-tube I (centrifugal) with a tight stopper, along with 0.5 ml of anhydrous ethanol and the mixture shaken vigorously in the plugged test tube for 1 minute. About 3 ml of xylene was added and the test tube plugged and shaken vigorously for another 1 minute. The tube was centrifuged to separate the extract (1500×*g*, 10 minutes); simultaneously 0.25 ml solution of batophenanthroline was measured into another test-tube II. After which 1.5 ml of the extract (upper layer), was transferred to the test-tube II and the content mixed together. 0.25 ml of $FeCl_3$ solution was then measured into test tube II, the contents of the test tube was mixed, and 0.25 ml of H_3PO_4 solution was added and mixed again – this way a test sample obtained for spectrophotometric measurements. Sample was put in a spectrophotometer and concentration was extrapolated automatically from the calibration curve.

2.2.3 Determination of vitamin A content in Bombax buonopozense

For the determination of vitamin A concentration, about 1 ml of the sample liquid was measured into test-tube I (centrifugal) with a tight stopper and 1 ml of the KOH solution was added the tube was plugged and shaken vigorously for 1 minute. It was then heated in a water bath at 60^{o}C for 20 minutes and allowed to cool in cold water. 1 ml of xylene was added and the tube plugged and shaken vigorously again for 1 minute. It was centrifuged (1500×*g*, 10 minutes), the whole of the separated extract (upper layer) was collected and transferred into the test tube II made of "soft" (sodium) glass

Sample was put in a spectrophotometer and the absorbance of the sample was measured in a spectrophotometer at a wavelength of 325nm. Concentration was extrapolated automatically from the calibration curve [3].

2.2.4 Determination of Amino Acid Profile of Bombax buonopozens

The sample was dried until a stable weight was obtained, it was then defatted, and went through hydrolysis, after which it went through evaporation in a rotary evaporator it was then loaded into the Technicon sequential Multi-Sample Amino Acid Analyzer (TSM).

2.3 Defatting Sample

The sample was defatted using a mixture of chloroform/methanol in the of ratio 2:1. 4g of the sample was loaded into the extraction thimble and extraction took place for 15 hours in soxhlet extraction apparatus [4].

2.3.1 Determination of Nitrogen Content of Bombax buonopozense

A little quantity (200mg) of pulverized sample was weighed, wrapped in Whatmann filter paper (No.1) and placed in the Kjeldhal digestion flask. 10ml of concentrated sulphuric acid (10ml) was added. 0.5g mixture of sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 respectively was added into the flask as catalyst to enhance digestion. Four pieces of anti-bumping granules were added. The flask was then put in Kjeldhal digestion apparatus for a duration of about 3 hours until the liquid became light green. The digested sample was allowed to cool and then distilled water was used to dilute it to 100ml in a standard volumetric flask. Aliquot (10mL) of the diluted solution with 10ml of 45% sodium hydroxide was placed in the Markham distillation apparatus and allowed to distill into 10ml of 2% boric acid consisting of 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected.

The distillate went through titration, usng standardized 0.01 N hydrochloric acid to obtain a grey colouration.

$$\text{Percentage Nitrogen} = (a-b) \times 0.01 \times 14 \times V \times 100W \times C$$

Where:

a. = Titre value of the digested sample
b. = Titre value of blank sample
V. = Volume after dilution (100ml)
W. = Weight of dried sample (mg)
C. = Aliquot of the sample used (10ml)
14. = Nitrogen constant in mg.

2.3.2 Hydrolysis of the sample

A known weight of the defatted sample was weighed into a glass ampoule. 7ml of 6NHCl was introduced and oxygen was released by passing nitrogen into the ampoule (this is to prevent oxidation of some amino acids which could possibly occur during hydrolysis e.g methionine and cysteine). A bunsen burner flame was then used to seal up the glass ampoule, before placing it in an oven preset at 105°C ± 5^{0}C for a duration of 22 hours. The ampoule was cooled before being broken open at the tip and the content was filtered.

The filtrate went through evaporation to dryness at a temperature of 40°C under vacuum in a rotary evaporator. The residue was dissolved using 5ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

2.3.3 Loading of the hydrolysate into TSM analyzer

The quantity loaded was between 5 to 10 microlitre. This was distributed into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. This analysis took a duration of about 76 minutes.

2.4 Statistical analysis

Data were analyzed in M±SD using descriptive statistic of SPSS for window 16 USA

3 Results

3.1 Result of Minerals Composition of the Leaves of *B. buonopozense*

A total of eight dietary mineral comprising of five micro and three mineral were found present in the leaves of *Bombax buonopozense* as shown in Table 1. They are manganese, zinc, copper, cobalt, iron, sodium, calcium and potassium in ascending order of concentrations.

Table 1 Mineral Composition of the Leaves of *B. buonopozense*

Parameter	Composition (ppm)
Sodium	3.13±0.20
Calcium	93.72±0.20
Cobalt	0.04±0.00
Copper	0.03±0.00
Zinc	0.02±0.00
Iron	0.06±0.00
Manganese	0.02±0.00
Potassium	669.22±0.20

Data were analyzed in M±SD in triplicate determination

3.2 Result Amino Acid Profile of the Leaves of *B. buonopozense*

Table 2 Amino acid profile of the leaves of *B. buonopozense*

Parameter	Composition (ppm)
Lysine	3.68±0.20
Histidine	1.90± 0.20
Arginine	5.27± 0.20
Aspartate	8.65± 0.20
Threonine	3.01± 0.20
Serine	1.82± 0.20
Glutamate	11.01± 0.20
Proline	3.13± 0.20
Glycine	3.63± 0.20
Alanine	3.98± 0.20
Cysteine	0.76± 0.02
Valine	4.01± 0.20

Metheonine	0.86± 0.02
Isoleucine	3.33± 0.20
Leucine	8.22± 0.20
Tyrosine	2.98± 0.20
Phenylalanine	3.87± 0.20

Data were analyzed in M±SD in triplicate determination

A total of seventeen amino acids consisting of eight essential and nine non-essential amino acids were identified and quantified in the leaves of *B. buonopozense* as shown in Table 2**.** They are arginine, valine, methionine, leucine, threonine, phenylalanine, histidine, isoleucine, lysine, proline, serine, tyrosine, alanine, glycine, glutamate, aspartate and cysteine.

3.3 Result of Vitamin Profile of the Leaves of *B. buonopozense.*

Result from Table 3 showed the presence of five water and two fat soluble vitamins totaling seven in all. Among the vitamins that were present, vitamin B_6 was found to have the highest concentration. They are vitaminB_6 (34.08± 0.20), vitaminB_1 (22.74± 0.20), vitamineB_2 (22.29± 0.20), vitamin A (15.91 ± 0.20), vitaminB_{12} (10.31± 0.20), vitaminE (9.33± 0.20µ/l), vitaminB_3 (0.95± 0.02) vitamin C (0.42± 0.02) in decending order.

Table 3 Vitamin Profile of the Leaves of *B. buonopozense*

Parameter	**Composition (ppm)**
Vitamin A	15.91 ± 0.20
Vitamin B_1	22.74± 0.20
Vitamin B_2	22.29± 0.20
Vitamin B_3	0.95± 0.02
Vitamin B_6	34.08± 0.20
Vitamin B_{12}	10.31± 0.20
Vitamin C	0.42± 0.02
Vitamin E	9.33± 0.20µ/l

Data were analyzed in M±SD in triplicate determination

4 Discussion

The leaves of *Bombax buonopozense* contains various important minerals as shown in Table 1 below. Of those analyzed, potassium, calcium and sodium were found to be at higher concentrations with potassium having the highest concentration among those analyzed. The result of this study is consistent with the report from research by [5] on mineral constituents of Bombax buonopozense leaves in which the three most abundant minerals were potassium, calcium and sodium in descending order. However results of mineral concentrations in research works reported by [6] in *Bombax buonopozense sepals*, [7] in *Bombax buonopozense Cclyx*, and [8] in leaves of *Bombax buonopozense P. Beauv* did not follow the pattern in the present study. Potassium is an extracellular cation which as an electrolyte helps in healthy balance of body fluid [9], also crucial for heart functioning and contraction of skeletal and smooth muscles. Calcium is a major constituent of bones and teeth. It aids normal functioning of cardiac muscles, blood coagulation, regulates cell permeability, nerve-impulse transmission and mechanism of neuromuscular system [10, 11]. Sodium is an intracellular cation which regulate plasma volume, acid-base balance, and nerve and muscle contraction [9].

A total of seventeen amino acids consisting of eight essential and nine non-essential amino acids were identified and quantified in the leaves of B.*buonopozense* as shown in Table 2**.** Among the amino acids identified, glutamic acid had the highest concentration. The work of [12] on "amino acid profile of *Bombax Buonopozense* (West African Bombax) leaves supported the findings of this study". "Functional amino acids"(FAA) such as arginine, cysteine, glutamine, glutamate, glycine, leucine, proline, and tryptophan are amino acids that regulate key metabolic pathways of cells necessary for survival, growth, development, and reproduction of animals [13,14]. Hence amino acid constituents of *Bombax Buonopozense* leave fall short of the composition of FAA by lacking tryptophan and glutamine. General functions of

amino acids include synthesis of tissue proteins in animals, regulate mRNA translation as signaling molecules, regulation of immune function, and acts as important precursors for the synthesis of neurotransmitters, and certain hormones [15, 16, 13, 17]. Glutamate participate in the transamination, regulate mRNA translation as signaling molecules, act as precursor for glutamine synthesis source of ATP and up-regulating ammonia detoxification, arginine synthesis via N-acetylglutamate synthesis, synthesis of glucosamine-6-phopshae and functions of monocytes, macrophages, lymphocytes, and neutrophils [13,17,18,19,20,21(Wu, 2009.

Result from Table 3 showed the presence of five water and two fat soluble vitamins totaling seven in all. Among the vitamins that were present, vitamin B_6 was found to have the highest concentration. [8] Reported the presence of three water and one fat soluble vitamins in the leaves of *Bombax buonopozense P. Beauv* of which vitamin C was the highest.. VitB_6 is involved in biosynthesis of amino acid, neurotransmitters [22] and degradation of cellular storage compounds [23-24].

5 Conclusion

Bombax buonopozense P. Beauv leaves revealed the presence of micronutrients of which potassium, vitamin B_6 and glutamate for mineral, vitamin and amino acid respectively were highest hence *Bombax buonopozense P. Beauv* leaves could serve as supplements in micronutrients deficient diets to prevent micronutrients deficiency.

Compliance with ethical standards

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Disclosure of conflict of interest

We declare that there is no conflict of interest.

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