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Bangladesh J. Sci. Ind. Res. **52(3)**, 201-208, 2017

BCSIR

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

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Nutritional and chemical compositions of two underutilized Vegetables in Nigeria

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Abstract

The proximate, mineral and anti-nutritional compositions of *Basella alba* and *Senecio biafrae* were determined. The vegetables were also screened for phytochemicals and the polyphenols present in them analyzed using reverse-phase high performance liquid chromatography. The results of proximate in % were: moisture (11.75, 12.24), crude protein (19.22, 20.17), ash (20.95, 21.48), total dietary fibre (16.57, 22.51), crude fat (10.49, 13.34) and carbohydrate (21.02, 10.26). Dietary minerals were present in appreciable quantities: sodium (660, 300), potassium (7800, 1430), calcium (1050, 1150), magnesium (350, 310), zinc (41, 34), copper (0.10, 1.80), iron (1.40, 2.00), and phosphorus (20.50, 13.40) in mg/100g respectively. Lead was not detected in *Basella alba* but in trace amount in *Senecio biafrae* (0.002). Vitamins in μg/g were: vitamin A (13.21, 9.78), thiamine (315.22, 315.22), ascorbic acid (14.86, 9.85) and tocopherol (49.89, 192.38). Amino acid profile of the two vegetables showed appreciable essential: histidine (2.14, 2.27), isoleucine (3.01, 3.43), leucine (7.75, 8.85), lysine (2.92, 4.62), methionine (0.89, 0.99), phenylalanine (3.88, 4.22), threonine (2.84, 3.15) and non essential amino acids: valine (3.13, 3.71), arginine (3.97, 4.49), aspartic acid (7.32, 8.28), serine (2.09, 2.22), glutamic acid (10.15, 10.83), proline (2.24, 2.65), glycine (3.07, 3.56), alanine (3.49, 4.03), cysteine (0.79, 0.86), tryosine (2.22, 2.70). Antinutrient factors present in mg/g were: tannin (2.34, 1.45), oxalate (1.17, 0.45) and phytate (1.03, 0.08). Caffeic acid was, most aboundant phenolic acid in both vegetables (32.74, 37.51); and their flavonoid compositions were similar except for catechin which was lacking in *Senecio biafrae*. The nutritional and nutraceutical potential of both vegetables are good and could be exploited to promote health.

Keywords: Proximate; Mineral; Anti-nutritional; Phenolic acid; Flavonoid

Introduction

One third of the world population suffer from minerals and vitamins deficiency in their diet; primarily iodine, iron, vitamin A and zinc, which leads to negative health consequences (Kennedy *et al.,* 2003; WHO, 2000). Several studies have shown that the degenerative and chronic diseases, which are the main causes of death in the world today are related to diets and life styles. To increased health challenges, food insecurity and malnutrition in recent years, especially in developing countries due to increased population and increasing effects of environmental changes, emphasis is now on food as functional food which should be able to provide additional health benefits such as preventing or delaying onset of chronic diseases beyond basic nutrition (Oboh and Rocha, 2006). Fruits and vegetables play important roles not only in the prevention but also the fight against the prevailing chronic diseases like heart, cancer, obesity and many more. The place of wild plants for food and medicine can not be underestimated especially the poverty and food insecurity caused by increasing population and environmental changes. *B. alba* and *S. biafrae* are among many underutilized green leafy vegetables and are not known by average young person in Nigeria, though popular only in certain parts of the country.

B. alba L., is commonly called “Amunututu” among the Yoruba tribe in the southwest part of Nigeria. The plant is reported to be of great ethnomedicinal importance (Deshmukh and Gaikwad, 2014). Fresh succulent leaves of *S. biafrae* are used as a leafy vegetable in some part. There are many ethnomedicinal claims such as the use of its leaf extract to stop bleeding from cuts or injury and treatment of smallpox (Adebooye, 2004). Although, *B. alba* is more popular than *S. biafrae,* the two are grossly underutilized and are becoming rare. In the present study, attempt was made to screen vegetables for phytochemicals and evaluate the nutritional and chemical qualities. Although, many studies have established the nutritional values of these vegetables, utilization are very poor and indepth research on their chemical profile are needed and this may increase the interest of more peoeple in cultivation and utilization. In view of this,

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nutritional and chemical profile were determined in the present study.

Materials and methods

Phytochemical analysis

Preparation of samples

The air-dried vegetable samples collected from local markets in Nigeria were ground and sieve to give 40 mm mesh size powder. The powdered plant materials were subjected to cold maceration process for 72 hours with methanol and filtered to get extracts. The extracts were concentrated under vacuum and evaporated using rotary evaporator at low temperature (45^{o}C).

Phytochemical screening of the extracts

Phytochemical screening of the extracts were carried out using standard qualitative phytochemical methods described by Harborne (1984), Trease and Evans (1989) and Sofowora (1993).

Quantification of phytochemicals by HPLC-DAD

Reverse phase chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm x 150 mm) packed with 5µm diameter particles; the mobile phase was water containing 2 % acetic acid (A) and methanol (B), and the composition gradient was: 5 % of B until 2 min and changed to obtain 25 %, 40 %, 50 %, 60 %, 70 % and 100 % B at 10, 20, 30, 40, 50 and 60 min, respectively, using a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software following the method described by Boligon *et al.,* (2012) with slight modifications. The presence of eight compounds were investigated in each vegetable. The flow rate was 0.7 ml/min, injection volume 50 µl and the wavelength were 254 nm for gallic acid, 280 nm catechin, 327 nm for caffeic, ellagic, rosmarinic and chlorogenic acids, and 365 nm for quercetin, isoquercitrin, rutin and kaempferol. The samples and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. The chromatography peaks were confirmed by comparing their retention time with those of reference standards and by DAD spectra (200 to 500 nm). Calibration curve for gallic acid: Y = 11786x + 1245.9 (r = 0.9998); caffeic acid: Y = 13517x + 1097.2 (r = 0.9993); chlorogenic acid: Y = 11965x + 1375.4 (r = 0.9996); ellagic acid: Y = 14082x + 1256.7 (r = 0.9995); rutin: Y = 13482 + 1160.3 (r = 0.9997); quercetin: Y = 12895x + 1342.5 (r = 0.9993); isoquercitrin: Y = 10832x + 1243.1 (r = 0.9994); kaempferol: Y = 12753x + 1408.5 (r = 0.9998); catechin: Y = 12650x + 1407.9 (r = 0.9995) and rosmarinic acid: Y = 11982x + 1309.7 (r = 0.9995); All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Boligon *et al.,* (2013). LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

Determination of nutritional and chemical composition

Chemical Analyses

Proximate composition (fat, crude fibre, and ash) were determined on dry basis by the standard method of Association of Official Analytical Chemist (AOAC, 2006), the protein content was determined using the micro-Kjedahl method (N x 6.25) and the carbohydrate determination was calculated by difference (AOAC, 2006). Total dietary fibre (TDF) was determined on dried, fat-free sample according to Megazyme TDF Assay procedure, K-TDFR 05/12 (MEGAZYME INTERNATIONAL, IRELAND). The mineral elements were determined using Atomic Absorption Spectrophotometer (Pearson, 1976), vitamins by spectrophotometric methods (Biesalski *et al.,* 1986; Benderitter *et al.,* 1998; Okwu and Josiah, 2006), and antinutrients by titrimetry and spectrophotometric methods (Makkar and Goodchild, 1996; Day and Underwood, 1986).

Determination of amino acid profile

Fresh leaves were washed and weighed prior to pulping using posho mill and then pressed with screw press to separate leave juice. The separated leave juice was heated in batches at 80 – 90 ^{0}C for about 10 min to coagulate the leaf protein. The protein coagulum was separated from the fraction by filtering through cloth filter followed by pressing with screw – press as described for garri making (Aletor *et al.,* 2002). The LPC was then washed with water and repressed. The product was pulverized and freeze-dried prior to analysis. The 4.0g protein isolate was hydrolyzed and evaporated in a rotary evaporator. The Amino Acid profile in the samples were determined using Technicon sequential Multi-Sample Amino Acid Analyzer (TSM) and Technicon standard amino acid solutions (Benitez, 1984). Norleucine was used as the internal standard to correct for variations in the samples.

Statistical analysis

The experimental results were expressed as the mean ± S.D. and statistical significance of difference in parameters amongst groups determined by one way ANOVA followed by Duncan's multiple range tests. Significance was accepted at P>0.05.

Results and discussion

Phytochemical screening

The result of phytochemical screening of both vegetables are similar except for terpenoid which was detected in *B. alba* but absent in S. Bifrae (Table I). They both gave positive test to saponnin, tannins and flavonoids but negative to alkaloid, steroid and anthraquinone. Flavonoids are effective free radical scavenger and antioxidants (Salah *et al.,* 1995; Rio *et al.,* 1997). Tannins, terpenoids and saponins have also been linked to various bioactivities such as astringent, analgesic, anti-inflammatory and antimicrobial properties in many studies (Sofowora, 1993; Enzo, 2007; Okwu and Josiah, 2006). Their presence in the vegetables suggest nutraceutical potential.

Table I. Phytochemical screening of the vegetables

	B. alba	*S. biafrae*
Alkaloid	–	–
Saponin	+	+
Tannin	+	+
Flavonoid	+	+
Steroid	–	–
Terpenoid	+	–
Anthraquinone	–	–

- = Absent
+ = Present

Polyphenols and flavonoids

The presence of eight compounds was identified for each of *B. alba* and *S. biafrae* in the chromatographic analyses (Figures 1 and 2). Gallic acid, caffeic acid, rutin, quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) and isoquercitrin, (2-(3, 4-dihydroxyphenyl -3-oxy-chromen-4-one) which is quercetin 3-glucoside were present in the two vegetables at different concentrations, but quercitrin (2-(3,4-Dihydroxyphenyl)-5,7- dihydroxy-3- [(2S, 3R, 4R, 5R, 6S)- 3, 4, 5-trihydroxy-6-methyl-2-tetrahydropyranyl] oxy]-4-chromenone); a quercetin 3-rhamnoside was not detected in any of the vegetables.

The similarity in the phenolic acid composition of the two vegetables was the presence of gallic and caffeic acid; also caffeic acid (32.74, 37.51) was highest in both as revealed in Table II. Chlorogenic acid (26.39) and rosmarinic acid (18.91) which were lacking in S.biafrae but present in *B. alba* while ellagic acid (9.48) in S.biafrae was not detected in *B. alba.* Flavonoid compositions were similar except for catechin which was lacking in *B. alba.* Rutin, quercetin and kaempferol were significantly higher in B.alba (P≤ 0.05), there was no significant difference in the concentrations of Isoquercitrin in the two vegetables. Polyphenols are potent antioxidants and play various preventive and physiological functions in the human health. Several studies have established their anti-inflammatory, anticarcinogenic, antifibrosis, antiplasmodial and chemopreventive activity in vitro and in vivo (Kaur *et al.,* 2009; Rasool *et al.,* 2010; Han *et al.,* 2006; Zhang *et al.,* 2014; Priyadarsini *et al.,* 2002).

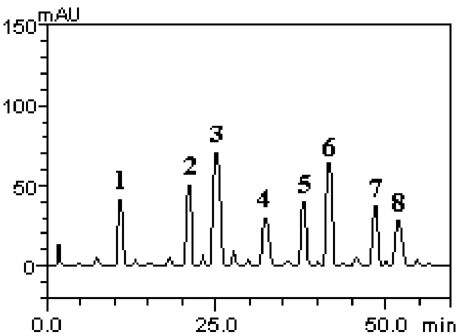

Fig. 1. Reverse-phase high performance liquid chromatography profile of *Basella alba* extract. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), rosmarinic acid (peak 4), rutin (peak 5), isoquercitrin (peak 6), quercetin (peak 7) and kaempferol (peak 8)

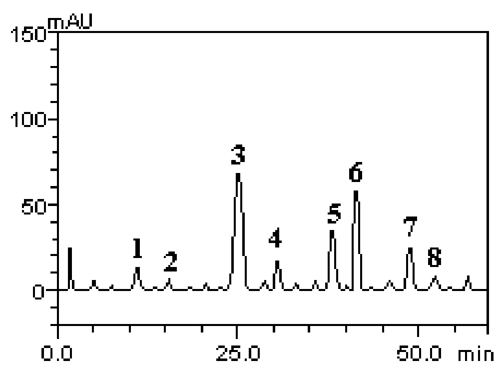

Fig. 2. Reverse-phase high performance liquid chromatography profile of *Senecio biafre* extract. Gallic acid (peak 1), catechin (peak 2), caffeic acid (peak 3), ellagic acid (peak 4), rutin (peak 5), isoquercitrin (peak 6), quercetin (peak 7) and kaempferol (peak 8)

Table II. Phytochemical composition of vegetables *B. alba* and *S. biafrae* (mg/g)

Compounds	*B.alba*	LOD (µg/mL)	LOQ (µg/mL)	*S.biafrae*	LOD (µg/mL)	LOQ (µg/mL)
Gallic acid	21.53 ^{b}±0.01	0.038	0.125	8.15 ^{a}±0.01	0.015	0.049
Chlorogenic acid	26.39 ^{b}±0.01	0.009	0.030	0 ^{a}±0.00	0.00	0.00
Caffeic acid	32.74 ^{a}±0.02	0.015	0.049	37.51 ^{b}±0.01	0.018	0.059
Ellagic acid	0 ^{a} ±0.00	0.00	0.00	9.48 ^{b}±0.03	0.038	0.125
Rosmarinic acid	18.91 ^{b}±0.01	0.042	0.138	0 ^{a}±0.00	0.00	0.00
Catechin	0 ^{a}±0.00	0.00	0.00	3.32 ^{b}±0.02	0.009	0.030
Rutin	21.95 ^{b}±0.03	0.018	0.059	18.63 ^{a}±0.01	0.042	0.138
Isoquercitrin	30.16 ^{a}±0.02	0.021	0.071	30.71 ^{a}±0.01	0.021	0.071
Quercetin	21.10 ^{b}±0.01	0.030	0.098	12.34 ^{a}±0.02	0.007	0.024
Kaempferol	19.32 ^{b}±0.01	0.007	0.024	7.56 ^{a}±0.03	0.032	0.105

Values represent means of triplicate readings ± SD. Values with different superscript along the rows are significantly different ($p \leq 0.05$, n=3).

Nutritional and chemical composition

There were no significant differences in the moisture (11.75, 12.24), ash (20.95, 21.48) and dry matter (88.25, 87.76) of the two vegetables (Table III). Protein, crude fat and total dietary fiber were significantly higher ($P \leq 0.05$) in *S. biafrae* than in *B. Alba,* while carbohydrate was higher in *B. alba*. There was however no difference in the caloric value of both vegetables. Although, high moisture contents in fresh wet vegetables are beneficial in food digestion, excessive moisture favors microbial spoilage and decomposition of active compounds in vegetable commodities (Czech *et al.,* 2001). Crude protein content of both vegetables were quite appreciable and falls within the range of 32.95 ±0.01-19.67 ±0.01 obtained for *Amaranthus hybridus, Curcubita pepo* and *Gnetum africana,* commom leafy vegetables in SE Nigeria (Iheanacho and Udebuani, 2009). The ash (17.2 %), crude fat (14.27 %) and crude fibre (20.17 %) compared favourably as reported for Telfairia occidentalis leaves by Idris (2011).

Most of the mineral analysed in this study were found to be more abundant in *B. alba* than *S. biafrae* except copper (0.10, 1.80 mg/100g) and iron (1.40, 2.00 mg/100g). There was no significant difference in calcium contents (1050, 1150 mg/100g) of the two vegetable leaves. Lead was detected in trace amount in *S. biafrae* (0.002 mg/100g) but not in *B. alba*.The values obtained for potassium (7800, 1430 mg/100g) and sodium (660, 300 mg/100g) in this work were higher than 75.25, 16.85 and 130.24 mg/100g, and 52.76, 15.01 and 42.24 mg/100g reported earlier for bitter leaves (*Veronia amygdalina* L), *Basella alba* (indian spinach) and *Telfaria occidentialis* (Fluted pumpkin) leaves respectively (Asaolu *et al.,* 2012). Iron (1.40, 2.00 mg/100g), much lower than reported (38.4 mg/100 g and 30.6 mg/100 g dw, respectively) for *Amaranthus spinosus* and *Adansonia digitata* leaves, but zinc (41, 34 mg/100g) is higher than (25.5 mg/100 g, 22.4 mg/100 g and 20.9 mg/100 g dw, respectively) reported for *Moringa oleifera, Adansonia digitata* and *Cassia tora leaves* by Barminas *et al.,* (1998). Calcium, magnesium and phosphorus were found in appreciable amounts compared to some common vegetables (Idris, 2011; Iheanacho and Udebuani, 2009).The importance of mineral elements in health maintenance and protection against various diseases is well documented. They function as antioxidants (selenium), biocatalyst (copper and zinc), functions as a co-factor of many enzymes, synthesis of biomolecules, maintenance of electrical potential (magnesium, potassium, sodium), and healthy central nervous system; strong teeth and bone formation, as well as playing crucial function in blood formation and prevention

Table III. Nutritional and Chemical composition of vegetables *B. alba* and *S. biafrae*

	B. alba	*S. biafrae*
Moisture (%)	11.75 ^{a} ±0.07	12.24 ^{a} ±0.06
Crude Protein (%)	19.22 ^{a} ±0.13	20.17 ^{ab} ±0.28
Ash (%)	20.95 ^{a} ±1.45	21.48 ^{a} ±0.32
Total Dietary Fibre (%)	16.57 ^{a} ±0.02	22.51 ^{b} ±0.02
Crude Fat (%)	10.49 ^{a} ±0.33	13.34 ^{b} ±0.49
Carbohydrate (%)	21.02 ^{b} ±1.55	10.26 ^{a} ±0.28
Dry Matter	88.25 ^{a} ±0.07	87.76 ^{a} ±0.06
Caloric Value (Kcal)	299.03 ^{a} ±11.97	296.69 ^{a} ±5.94
Sodium (mg/100g)	660.00 ^{b} ±13.01	300.00 ^{a} ± 10.32
Potassium (mg/100g)	7800.00 ^{b} ± 12.10	1430.00 ^{a} ±13.05
Calcium (mg/100g)	1050.00 ^{a} ±8.87	1150.00 ^{a} ±1..56
Magnesium (mg/100g)	350.00 ^{b} ±.11.41	310.00 ^{a} ±5.98
Zinc (mg/100g)	41.00 ^{b} ±3.01	34.00 ^{a} ±2.45
Copper (mg/100g)	0.10 ^{a} ±0.00	1.80 ^{b} ±0.02
Iron (mg/100g)	1.40 ^{a} ±0.01	2.00 ^{b} ±0.02
Lead (mg/100g)	ND	0.002 ^{a} ±0.00
Manganese (mg/100g)	ND	ND
Phosphorus(mg/100g)	20.50 ^{b} ±0.02	13.40 ^{a} ±0.05
Tannin (mg/g)	2.34 ^{b} ± 0.14	1.45 ^{a} ± 0.05
Oxalate (mg/g)	1.17 ^{b} ± 0.07	0.45 ^{a} ± 0.00
Phytate (mg/g)	1.03 ^{b} ± 0.05	0.08 ^{a} ± 0.00
Vitamin A (β-Carotene Equivalent, μg/g)	13.21 ^{b} ± 0.00	9.78 ^{a} ± 0.00
Thiamine (μg/g)	315.22 ^{b} ± 0.00	315.22 ^{b} ± 0.001
Ascorbic acid (μg/g)	14.86 ^{b} ± 0.02	9.85 ^{a} ± 0.03
Tocopherol (μg/g)	49.89 ^{a} ± 0.01	192.38 ^{b} ± 0.02

Values represent means of triplicate readings ± SD. Values with the same superscript along the column are not significantly different (n=3).

of anemia (Iron), fertility and immune system (zinc and calcium, phosphorus) among other functions (Ayoola *et al.,* 2010; Mohammed and Sharif, 2011). Vitamin A (13.21, 9.78 µg/g) and C (14.86, 9.85 µg/g) were in higher concentrations while vitamin E (49.89, 192.38 µg/g) was lower in *B. alba* than *S. biafrae* but no significant differences in the concentrations of vitamin B1(315.22, 315.22 µg/g) in both vegetables under study.

Vitamin A is important for vision, cell growth and differentiation, normal formation and maintainance of body organs, B complex are needed for building strong immune system, reproductive hormones, metabolism of food and regulating body stress while vitamin C and E are important water and lipid soluble antioxidant vitamins respectively (Ross, 2006; 2010; Solomons, 2006; Mirbagheri, *et al.,* 2008). Phytate (1.03, 0.08 mg/g), oxalate (1.17, 0.45 mg/g) and tannins (2.34, 1.45 mg/g) were detected in higher concentrations in *B. alba* than *S. bifrae* but the values were low compared to the reported values for some common food commodities (Gordan *et al.,* 1984; Macfarlane *et al.,* 1988; USDA, 2011). Raw vegetables like spinach, beet greens, okra, parsley, leeks and collard greens contained 750, 610, 146, 100, 89 and 74 mg/100g serving oxalate respectively (USDA, 2011; Holmes and Kennedy, 2000) while the % composition of phytate per dry samples of some common foods were: coconut (0.36), peanut (0.95-1.76), walnut (0.98), corn (0.75-2.22), oat meal (0.89-2.40), brown rice (0.84-0.99), polished rice (0.14-0.60), wheat (0.39-1.35), wheat flour (0.25-1.37), spinach (0.22) (Phillippy *et al.,* 2002; Macfarlane *et al.,* 1988; Gordan *et al.,* 1984). The nutritional qualities of the leaves cannot be underestimated as indicated by the presence of minerals and vitamins, their regular consumption can play important roles in promoting good health.

Values represent means of triplicate readings ± SD. Values with the same superscript along the column are not significantly different (n=3).

Amino acid composition of vegetables

The result of amino acid analyses of the two green leafy vegetables in Table III indicated that they both contained good quality protein in terms of the protein requirement pattern of their essential amino acids. *S. biafrae* was however richer in quantity; both the essential and nonessential amino acids were higher in *S. bifrae* than in *B. Alba*. The amino acid profile is similar to previously reported for *C. crepidioides* and *S. biafrae* (Dairo *et al.,* 2007). Glutamic acid (10.15, 10.83) was the most abundant in both vegetables which agrees with the findings of Fukushima (1991). While some of the amino acids like arginine and histidine are direct scavengers of free radicals, others play important roles in the synthesis of antioxidant enzymes. For example, cysteine, glycine and glutamic acid are involved in the synthesis of gluthathione reductase (Yun-Zhong *et al.,* 2002). Consumption of these vegetable can therefore help to build up the body's antioxidant status.

Table IV. Amino acid composition of vegetables *B. alba* and *S. biafrae* (g/100g protein)

	B.alba	*S. biafrae*	**RP %*
Histidine	2.14	2.27	1.9
Isoleucine	3.01	3.43	2.8
Leucine	7.75	8.85	6.6
Lysine	2.92	4.62	5.8
Methionine	0.89	0.99	
Phenylalanine	3.88	4.22	6.3[a]
Threonine	2.84	3.15	3.4
Valine	3.13	3.71	3.5
Arginine	3.97	4.49	
Aspartic acid	7.32	8.28	
Serine	2.09	2.22	
Glutamic acid	10.15	10.83	
Proline	2.24	2.65	
Glycine	3.07	3.56	
Alanine	3.49	4.03	
Cysteine	0.79	0.86	2.5[b]
Tryosine	2.22	2.70	

a = Phenylalanin with Tyrosine, b = Cysteine with Methionine, *RP %= Requirement Pattern in % protein (FAO/WHO 1991)

Conclusion

The study indicates that *B. alba and S. biafrae* are both rich in minerals, vitamins amd possess good protein quality. Their phytochemicals and phenolic compound showed good nutraceutical poentials. Their consumption would be of great nutritional benefit. The variation in their nutritional and chemical compositions suggests the importance of varieties in diet, mixed diet is more beneficial as no single food material can supply all the ntritional requirement for a healthy living. It is therefore advisable to include various wild and underutilized vegetables in our diet for maximum health benefit. Increase utilization could encourage cultivation, promote the economy and save the plants from phasing out of existence.

Acknowledgement

We are grateful to Oguntokun MO, Federal University of Technology, Akure, Ondo State, Nigeria, Aline Boligo and Margareth Athayde, Department of Industrial Pharmacy, Federal University of Santa Maria, Brazil for their assistance.

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Received: 09 August 2015; Revised: 01 September 2015; Accepted: 06 April 2017.