

Proximate, vitamin assays and anti-oxidant properties of an underutilised indigenous vegetable *Heliotropium indicum* L. (Lamiales: Boraginaceae) in West Africa in enhancing diet diversification

Tomilayo Amusan, Muibat Bello, Oluwole Oladeji*

Department of Pure and Applied Chemistry. Ladoké Akintola University of Technology. P. M. B. 4000. Ogbomoso. Nigeria. *Email: oladejioluwole@gmail.com.

Abstract. Green leafy vegetables are important component of human diets, providing fibre, minerals and vitamins. Recently, non-conventional food plants are incorporated in diet to provide not only nutrient but also traditional treatment for various ailments. In a way to combat the devastating effect of malnutrition, there should be a long term intervention such as dietary diversification which can be achieved by increasing the production of locally produced foods and non-conventional vegetables. This study analysed the nutritional composition and anti-oxidant potential of *Heliotropium indicum* L. (Lamiales: Boraginaceae). The proximate analyses were carried out using the methods described by the Association of Official Analytical Chemist (AOAC). Vitamins were quantified by high performance liquid chromatography (HPLC). Mineral content were determined by Atomic Absorption Spectrophotometric (AAS) technique. The anti-oxidant activity was tested spectrophotometrically using ascorbic and gallic acid as standards. The nutrient constituents revealed that the fat content ranged between $0.67\% \pm 0.05\%$ and ash content ranged between $15.7\% \pm 0.04\%$. Varying levels of vitamins like ascorbic acid, retinol, tocopherols, riboflavin, thiamine and niacin was quantified in the samples. Ascorbic acid content ranged between 622.6 mg/100 g. The aqueous extracts of the samples significantly ($P < 0.05$) inhibited DPPH radical with an IC_{50} value of 38 $\mu\text{g/mL}$. It is evident from this study that these indigenous leafy vegetable which are not widely known and consumed are of high nutritional quality and anti-oxidant potential. This vegetable can contribute significantly to the nutrient requirement of man and could complement the conventional ones in enhancing food security and sustainable livelihood. Hence, their cultivation and consumption should be encouraged.

Keywords: Malnutrition; Dietary diversification; Proximate analysis; DPPH radical; Food security.

Resumo. *Composição centesimal, vitaminas e propriedades anti-oxidantes de um vegetal nativo subutilizado Heliotropium indicum L. (Lamiales: Boraginaceae) na África Ocidental na melhoria da diversificação da dieta.* Os vegetais de folhas verdes são componentes importantes das dietas humanas, fornecendo fibra, minerais e vitaminas. Recentemente, plantas não convencionais são

Recebido:
25/10/2016

Aceito:
21/12/2016

Publicado:
31/12/2016



Acesso Aberto
Artigo completo



ORCID

- 0000-0002-8973-3758
Tomilayo Amusan
- 0000-0002-1949-2418
Muibat Bello
- 0000-0003-3355-2488
Oluwole Oladeji

incorporadas na dieta para fornecer não só nutrientes, mas também o tratamento tradicional para várias doenças. De forma a combater o efeito devastador da desnutrição, deve haver uma intervenção a longo prazo, como a diversificação da dieta, que pode ser alcançada através do aumento da produção de alimentos produzidos localmente e vegetais não convencionais. Este estudo analisou a composição centesimal e o potencial antioxidante de *Heliotropium indicum* L. (Lamiales: Boraginaceae). A composição centesimal foi realizada utilizando os métodos descritos pela Associação de Químicos Analíticos Oficiais (AOAC). As vitaminas foram quantificadas por cromatografia líquida de alto desempenho (HPLC). O teor de minerais foi determinado pela técnica de espectrofotometria por absorção atômica (AAS). A atividade anti-oxidante foi testada espectrofotometricamente utilizando ácido ascórbico e ácido gálico como padrões. Os constituintes nutrientes revelaram que o teor de gordura variou entre $0,67 \pm 0,05\%$ e o conteúdo de cinzas variou entre $15,7 \pm 0,04\%$. Os níveis variáveis de vitaminas, como ácido ascórbico, retinol, tocoferóis, riboflavina, tiamina e niacina foram quantificados nas amostras. O teor de ácido ascórbico variou entre 622,6 mg/100 g. Os extratos aquosos das amostras significativamente ($P < 0,05$) inibiram o radical DPPH com um valor IC_{50} de 38 $\mu\text{g/mL}$. É evidente, a partir deste estudo, que este vegetal nativo, que não é amplamente conhecido e consumido, é de alta qualidade nutricional e potencial antioxidante. Este vegetal pode contribuir significativamente para suprir a necessidade de nutrientes do homem e poderia complementar os nutrientes convencionais no reforço para a segurança alimentar e para os meios de subsistência sustentáveis. Portanto, seu cultivo e consumo devem ser encorajados.

Palavras-chave: Má-nutrição; Diversificação dietética; Composição centesimal; Radicais DPPH; Segurança alimentar.

Introduction

Heliotropium indicum belongs to Boraginaceae Family. The generic name is derived from the Greek Helios; for sun and trope, for turning, suggesting that the leaves and flowers turn towards the sun, however, this is not the habit of this species. The epithet species name *indicum* relates to India where it is supposed to be a native plant species, however Waterhouse (1993) considers *H. indicum* to be of Tropical American origin. Its closest relative is *H. elongatum*, a species of South-Eastern South America (Johnston, 1998). It is locally called 'Ogbe-ori akuko' by the Yoruba in Nigeria. It is distributed widely throughout the world's tropical regions and it has proved difficult to establish its precise origin. The name suggests an Asiatic origin,

and other sources (Holm et al., 1977; Kostermans et al., 1987) indicate it originates from the Old World, but Waterhouse (1993) consider it to be of tropical America origin. It occurs as a weed in Africa, the Caribbean region of South America and Central America, tropical regions of North America and southern Asia. Recently it has been introduced to Australia where it is found in Northern Territory and Queensland (Craven, 1996).

This plant has been extensively studied for a variety of bioactive principles and screened for different pharmacological activities such as analgesic activity (Ashutosh et al., 2011), wound healing activity, reproductive activity (Dolui et al., 2012), histo-gastroprotective effect. Leafy green vegetables are an important component of the human diet, providing fibre, minerals, proteins and vitamins and

are low in calories. They are also a very good source of antioxidants (Gupta and Prakash, 2011). The increase in the consumption of African leafy vegetables is proportional to the positive effect on health and economic well-being of both rural and urban populations. Apart from promoting good health when indigenous green leafy vegetables are consumed, it will also help to enhance crop diversity, alleviate poverty and promote food security (Barry, 2008). Green leafy vegetables are known to be low in calorie and also contain low carbohydrate contents. These characteristics make them ideal for promoting and maintaining healthy body weight and coupled with the high fibre content, these vegetables particularly help with reducing type II diabetes.

In recent time, nutritionists, researchers and government organisations worldwide are searching for reliable, cheap and high quality diet from plant origin. (Adebowale et al., 2004). Providing inexpensive plant-based diet supplements has led to the examination of underutilised indigenous vegetables. Some authors (Gropper et al., 2005) suggested the need to consume high vegetable meals to prevent colon and stomach cancers. Others (Ball, 2006) reported on high vitamin, dietary fibre and mineral contents of vegetables and the role they play in maintaining alkalinity in the body. The high dietary fibre in green leafy vegetables helps regulate the digestive system aiding bowel health and for weight management. Several studies have shown that high folate intake from green leafy vegetables may lower the risk of colon polyps by 30% to 40% compared to low intake of this vitamin suggesting that diets low in folate may increase the risk of colon cancer (Panda, 2010). Low folate intake has also been associated with cancers of the breast, cervix and lung. Copious consumption of vegetables treat haemorrhoids, gallstones, obesity and constipation; the antioxidants in vegetables decrease the risk of heart disease and vitamin K contents of dark green leafy vegetables provide a number of health benefits including: protecting bones osteoporosis and helping to fight against

inflammatory diseases (Whitney et al., 2002).

Nigeria faces huge food security challenges. United Nation FAO stated in one of its report that over 870 million people are malnourished or hungry (Woteki, 2013). Human malnutrition is high in populations who depend mainly on starch-based diets as their source of nutrient and energy (Siddhuraju et al., 2002). With a view to provide inexpensive plant-based diet supplements, there is a need to examine underutilised indigenous vegetable. Research studies have revealed that wild or semi-wild plants are nutritionally important because of high vitamins, minerals, essential fatty acids and fibre contents (Tukan et al., 1998). How will increased consumption of African leafy vegetables (ALVs) have a positive effect on nutrition, health and economic wellbeing of both rural and urban populations? Will it also enhance crop diversity, alleviate poverty and promote food security?

Despite the great value of this traditional leafy vegetable, not much research has been carried out on it especially in the area of nutritional and medicinal benefit of many indigenous green leafy vegetables which are under exploited or underutilized, this study is designed to analyse the vegetable which is regarded as weed by many in the Savannah Zone of Nigeria. The main objectives of this research are therefore to explore the nutritional constituent and anti-oxidant activity of *Heliotropium indicum* with the aim of promoting their consumption and thus enhance diet diversification; determine the level of nutrients present in the vegetable; quantify the various classes of vitamins and the anti-oxidant activity of the plant sample; and quantify the nutritive and toxic mineral elements present in the leaf samples.

Materials and methods

Collection and Identification of sample

H. indicum was obtained from a farm located in Osogbo, Osun State South-West geopolitical zone of Nigeria. The

plant was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, with voucher No. FHI 11207 (*Heliotropium indicum*).

Sample treatment and preparation

The leaves were separated from the stalks, washed and air-dried for 25 days, pulverized into fine powder, sieved with a sieve of mesh size of 20 mm and stored in a clean, dry air-tight sampling bottle and kept at 4 °C until required for analysis. All reagents were of analytical grade and of sigma unless otherwise stated.

Proximate analysis

Proximate analysis was carried out following the method described by the Association of Analytical Chemists (AOAC, 1990).

Moisture content. About 2.0 g of the sample was weighed (W_1) into pre-weighed crucible (W_0) and placed into a hot drying oven at 105 °C for 24 h. The crucible was removed, cooled in desiccator and weighed. The process of heating, cooling and weighing were repeated until a constant weight (W_2) was obtained.

Ash content. About 2.0 g of the sample was weighed (W_1) into pre-weighed empty crucibles (W_0) and placed into a Lenton furnace at 550 °C for 3 h. The ash was cooled in desiccators and weighed (W_2). The weight of the ash was determined by the difference between the powdered leaf samples, pre-weighed crucible and the ash in the crucible.

Crude lipids. About 2.0 g was accurately weighed (W_0) into a porous thimble and covered with a clean white cotton wool. Petroleum ether (200 mL) was poured into a 250 mL extraction flask, which was previously dried in the oven at 105 °C and weighed (W_2). The porous thimble was placed into the Soxhlet and the rest of the apparatus was assembled. Extraction was done for 5 h. The thimble was removed carefully and the extraction flask placed in a water bath so as to

evaporate the petroleum ether and then dried in the oven at a temperature of 105 °C to completely free the solvent and moisture. It was then cooled in desiccators and reweighed (W_1). The percentage crude lipid was calculated using the equation below:

$$\% \text{Crude Lipid} = \frac{W_1 - W_2}{W_0} \times 100$$

Where: W_0 = weight of sample (g); W_1 = weight of Flask+ oil (g), W_2 = weight of flask (g).

Crude fibre content. About 2.0 g of the sample was accurately weighed (W_0) into a 1 dm³ conical flask. About 100 mL of distilled water and 20 mL of 20% sulphuric acid were added and boiled gently for 30 min. The content was filtered through Whatman No. 1 filter paper. The residue was scrapped back into the flask with a spatula. Water (100 mL) and 20 mL of 10% sodium hydroxide were added and allowed to boil gently for 30 min. The content was filtered and the residue was washed thoroughly with hot distilled water, it was then rinsed once with 10% Hydrochloric acid and twice with ethanol and finally three times with petroleum ether. It was allowed to dry and scrapped into the crucible and dried overnight at 105 °C in an air oven. It was then removed and cooled in the desiccators. The sample was weighed (W_1) and ashed at 600 °C for 90 min in a Lenton muffle furnace. It was finally cooled in desiccators and weighed again (W_2). The percentage crude fibre was calculated using equation.

$$\% \text{Crude fibre} = \frac{W_1 - W_2}{W_0} \times 100$$

Where: W_0 = weight of sample (g); W_1 = weight of dried sample (g); W_2 = weight of ash sample (g).

Crude protein. About 2.0 g was accurately weighed along with 20 mL of distilled water into a micro-Kjeldahl's digestion flask. It was shaken and allowed

to stand for sometimes. One tablet of selenium catalyst was added followed by the addition of 20 mL concentrated sulphuric acids. The flask was heated on the digestion block at 100 °C for 4 h until the digest became clear. The flask was removed from the block and allowed to cool. The content was transferred into 50 mL volumetric flask and an aliquot of the digest (10 mL) was transferred into another micro-Kjeldahl's flask along with 20 mL of distilled water, and placed in the distilling outlet of the micro-Kjeldahl's distillation unit. A conical flask containing 20 cm³ of boric acid indicator was placed under the condenser outlet. Sodium hydroxide solution (20 mL, 40%) was added to the content in the Kjeldahl's flask by opening the funnel stopcock. The distillation was started and the heat supplied was regulated to avoid sucking back. When all the available distillate was collected in 20 mL of boric acid, the distillation was stopped. The nitrogen in the distillate was determined by titrating with 0.01 M of H₂SO₄; the end point was obtained when the colour of the distillate changed from green to pink. The crude protein was calculated using equation below

$$\% \text{ Crude protein} = \% \text{N} \times 6.60$$

The nitrogen content of the sample is given by the formula below:

$$\% \text{N} = \frac{T_v \text{ Na} \times 0.014 \times V_1 \times 100}{G \times V_2}$$

Where T_v = titre value of acid (cm³); Na = concentration or normality of acid (mol/dm³); V_1 = volume of distilled water used for distilling the digest (50 cm³); V_2 = volume of aliquot used for distillation (10 cm³); G = original weight of sample used (g).

Carbohydrates. The method of James (1995) was adopted where the total proportion of carbohydrate in the leaves sample was estimated by difference. That is by subtracting the % sum of food nutrients: % protein, % crude lipids, % crude fibre

and % ash from 100%. This is done by using the equation below:

$$\% \text{ CHO} = 100\% - (\% \text{ Crude protein} + \% \text{ Crude lipid} + \% \text{ Crude fibre} + \% \text{ ash})$$

Mineral analysis

The mineral elements comprising Sodium, Calcium, Potassium, Magnesium, Iron, Zinc, Copper, Manganese, Nickel and Lead were identified. Atomic absorption spectrophotometer was used to analyze the minerals separately after acid digestion of the sample, as described in the official method of the Association of Official Analytical Chemists.

About 2.0 g was accurately weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550 °C in a muffle furnace. The resultant ash was dissolved in 5.0 mL of HNO₃/HCl/H₂O (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5.0 mL of de-ionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 mL volumetric flask by filtration through Whatman No. 42 filter paper and the volume was made to the mark with de-ionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer. A 10 cm long cell was used and concentration of each element in the sample was calculated on percentage (%) of dry matter, i.e. mg/100 g sample.

Vitamin assays

Preparation of buffer, solutions and samples. For buffer preparation, 1.08 g of hexane sulphonic acid sodium salt and 1.36 g of potassium dihydrogen phosphate (KH₂PO₄) in 940 mL of HPLC water and 5 mL of triethylamine was added to it and the pH was adjusted to 3 mL with orthophosphoric acid. The mobile phase was prepared by mixing of the mixture of buffer and methanol with a ratio of 96:4 and filtered through 0.45 μ membrane filter and degassed by using helium gas. Extraction solution was made by mixing 50 mL of acetonitrile with 10 mL of glacial

acetic acid and the volume was finally made up to 1,000 mL with double distilled water. 10 g of each sample was homogenized, weighed and transferred into conical flasks and 25 mL of extraction solution was added, kept on shaking water bath at 70 °C for 40 min. Thereafter, the sample was cooled down, filtered and finally the volume was made up to 50 mL with extraction solution.

High performance liquid chromatography (HPLC). Calibration curve was made by using mix standards in mobile phase with five point calibrations, analyzed independently by HPLC and a standard curve was plotted between concentration and peak area. The data of peak areas and the used standard vitamin concentration were treated by linear least-square regression and the regression equation thus obtained from standard curve, was used to estimate water-soluble and fat soluble vitamins in different samples. For HPLC analysis, a Waters symmetry C18 column (4.6 x 250 mm 5 µm) was used with a linear gradient of Buffer: methanol (96:4) at a constant flow rate of 1 mL/min with 2,300 pressure by using Waters pump (1,515 isocratic) and a UV (2487) detector was employed for the detection of peaks,

using two channels simultaneously at a wavelength of 265 nm, a bandwidth of 5 nm and another wavelength of 280 nm.

Antioxidant assays

DPPH scavenging activity. The free radical scavenging activity of each sample was measured in-vitro by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described by Brand-Williams et al. (1995) and Bursal et al. (2011). The stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and stored at 20 °C until it is required. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98 ± 0.02 at 517 nm using the spectrophotometer. 3 mL aliquot of this solution was mixed with 100 µL of the sample at various concentrations (25-100 µg/mL). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The control was prepared as above without any sample. Ascorbic acid and gallic acid was used as positive reference standard. The scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation:

$$\text{Scavenging effect (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Reducing power activity. The reducing power was based on Fe (III) to Fe (II) transformation in the presence of the solvent fractions (Fejes et al., 2000). The Fe (II) can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Various concentrations (25-100 µg/mL) of each sample (2 mL) were mixed with 2 mL phosphate buffer (0.2 M, pH 6.6), and 2 mL of potassium ferricyanide (10 mg/mL). The mixture was incubated at 50 °C for 20 min followed by addition 2 mL of trichloroacetic acid (100 mg/L). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution. 2 mL from each of the

mixture earlier mentioned was mixed with 2 mL of distilled water and 0.4 mL of 0.1% (w/v) fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm. Ascorbic acid and gallic acid were used as positive reference standard. The higher the absorbance of the reaction mixture the higher its reducing power.

Nitric oxide scavenging activity assay. Nitric oxide generated from sodium nitroprusside (SNP) was measured according to the method described by Morocci et al. (1994). About 4 mL of the aliquots (plant extract or standard solution), of concentrations (25, 50, 75, 100 µg/mL)

were taken in different test tubes and 1 mL of sodium nitroprusside (5 mM in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 h at 30 °C. 2 mL sample was withdrawn from each mixture and mixed with 1.2 mL of Gries's reagent (1% Sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H₃PO₄). The absorbance of the chromophore formed

during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthylethylene diamine was measured at 550 nm. Also, about 4 mL of ascorbic acid at different concentrations (25, 50, 75, 100 µg/mL) were used as standard. The percentage (%) inhibition activity was calculated from the following equation:

$$(\%) \text{ inhibition activity} = \frac{[A_0 - A_1]}{[A_0]} \times 100$$

Where: A₀ is the absorbance of the control; A₁ is the absorbance of the extract or standard.

Statistical analyses

The data obtained from the analysed samples was summarized into mean and standard deviation using ANOVA for comparison with the aid of Turkey HSD test at < 0.05 significant level.

Results and discussion

Proximate compositions

The results of proximate composition of the three vegetables samples

were reported in Table 1. The moisture content of this leaf was found to be 8.85 ± 0.55 g/100 g in *Heliotropium indicum*. When these results were compared to the moisture content reported for some conventional leafy vegetables with the range of 55.76 ± 0.05 to 91.82 ± 0.04 g/100 g (Kwenin et al., 2011) it was found to be lower. The low moisture content of these leafy vegetables will help to prolong their shelf life.

Table 1. Proximate composition of *Heliotropium indicum* vegetable sample (g/100g) dry weight.

Plant samples	Moisture	Crude protein%	Crude fibre%	Ash%	CHO %	Crude Fat%
<i>Heliotropium indicum</i>	8.85±0.55 ^e	28.71±0.2 ^b	7.165±0.90 ^a	15.71±0.04 ^{ba}	38.91±1.6 ^b	0.675± ^b
<i>Amarathus hybridus</i> *	8.35±0.00	32.95±0.01	19.60±0.01	17.70±0.01	15.40±0.02	1.61±0.02

Mean ± standard deviation of triplicate determinations. Mean with the same superscript in the same column are not significantly different at 5% probability level.

*Amarathus hybridus** used as standard being a conventional vegetable; Values from (Akubugwo, 2007).

The crude protein content on dry weight basis of these samples ranges between 28.71 ± 0.2. Therefore, the protein content of these selected vegetables is considerably higher when compared to conventional vegetables such as *A. hybridus* 19.92 g/100 g DW reported by Akubugwo

(2007), *Leisanthera african* having 13.1-14.9 g/100 g reported by Isong et al. (1999) and *Piper guinese* 29.78 g/100 g by Akindahunsi et al. (2005). This sample can complement other dietary source of protein for the alleviation of protein malnutrition. According to Pearson (1976) as quoted by

(Akubugwo et al., 2007) plant food with calorific value from protein is considered good source of protein.

The crude fat content is between 0.68 ± 0.04 g/100 g and crude fat is low when compared to reported values (8.3-2.7 g/100 g DW) in some vegetables consumed in West Africa (Sena et al., 1998). However it was found to be lower compared with some edible green leafy vegetables reported for *Calchorus africanum* leaves and 1.85%-8.71% DM, leafy vegetables of Southern India and Nigeria (Gupta et al., 2005). Vegetables are poor sources of fat and this is of great benefit for people that requires less fat in their diet because high amount of fat have implication on health related disease and cardiovascular disorder (Anita et al., 2006).

The carbohydrate content ranges between 38.91 ± 1.6 DW was found to be higher when compared to values reported for *Amarathus Incurvatus* (20% DW), *M. balsamina* leaf 23.7% (Hassan, 2006). But lower when compared to *Tribulus terrestris* 55.56%, water spinach leaves 54.20%, (Asibey et al., 1999). The crude ash content ranges from 15.71 ± 0.04 g/100 g for *H. indicum* to 16.97 ± 0.1 for *S. elegans*; this indicates that the leaves are rich in mineral elements. The values obtained was higher compared to 1.8% reported in sweet potatoes leaves (Asibey et al., 1999) thus this indicate that these vegetables are good sources of minerals, but lower when compared to 19.61% in *Amaranthus hybridus* leaves (Nwaogu et al., 2000) and 18.00% balsam apple leaves (Hassan and Umar, 2006).

The crude fibre content between 7.165 ± 0.9 on DW basis is high compared to reported values of *L. batatas* 7.10%, *T. triangulare* 6.20%, (Akindahunsi et al., 2005) and low when compared to 29% balsam apples leaves (Hassan and Umar, 2006). *H. indicum* has its crude fibre value within the required range of most leafy vegetables 0.70%-12.0%. Dietary fibre helps to lower serum, cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetics, colon and breast cancer (Ishida et al., 2000). The estimated calorific value for *H. indicum* is

214.58 kcal/100 g. Thus, the calorific value agrees with the general observation that vegetables have low energy values (Lintas, 1992) due to their low fat content (Sobowale et al., 2011).

Concentration of mineral elements by AAS

The concentration of different mineral elements in the vegetable sample was determined using Atomic absorption spectrophotometric technique (AAS) were reported in Table 2. Minerals are important in our diets, they serve as cofactors for many physiological and metabolic function; they are of interest due to their pro- oxidant activities and health benefit (Alpha et al., 1996).

The vegetables sample was high in calcium with 246.65 ± 0.23 . The level of calcium of this vegetables was found to be high when compared to 17.95 mg /100 g in *Cassia siemea* leaves (Ngaski, 2006) and 44.15 mg/100 g in *Amaranthus hybridus* (Akubugwo et al., 2007) but lower when compared to 941 mg/100 g reported by Hassan and Umar (2006) in *Mormordica balsamma*.

Iron content was found to be high for the three samples 246.65 ± 0.23 *H. indicum* had the highest 272.8 mg/100 g while *S. elegans* had the lowest 245.4 mg/g. The iron content of these vegetables is high when compared to some green leafy vegetables such as *Amaranthus hybridus* 13.58 mg/100 g reported by Akubugwo (2007) *Ipomea batatas* 16.0 mg/100 g by (Anita et al., 2000). Iron is an essential trace element for haemoglobin formation and in the oxidation of carbohydrates, protein and fat (Adeyeye et al., 1999). The level of sodium was quantified in *H. indicum* was found to be 271.0 mg /100 g. The value of sodium content was high compared to vegetables such as *Senna obtusifolia* (45 mg/100 g). Sodium is the principal cation in intracellular fluids. It regulates plasma volume and acid- base balance, involved in maintenance of osmotic pressure of the body fluids and involved in Na^+ / K^+ - ATP-ase, maintenance of membrane potentials transmission of nerve impulses

and adsorptive processes of monosaccharide, amino acid, pyrimidine and bile (Soetan, 2010). The magnesium content of *H. indicum* was found to be 50.15 mg/100 g. This value was high compared to *Amaranthus hybridus*

(23.18 mg/100 g) reported by Nwaogu et al. (2006). The RDA value for magnesium for adults male is 350 mg (NRC, 1989) and *H. Indicum* vegetables contributes to 14.32%, respectively, to the RDA.

Table 2. Level of some mineral nutrients in the leaves of *H. indicum*.

Mineral element	<i>H. indicum</i> (mg/100g)	%rda (male adults)
Na	271.25±0.20	1.5 g/day
K	124.55±0.02	8 mg/day
Zn	102.48±0.50 ^a	1300 µg/day
Ca	246.65±0.23 ^b	4.5 g/day
Mg	50.2±0.30	11 mg/day
Fe	272.25±0.01	420 mg/day
Mn	2.42±0.06 ^p	2.3 mg/day
Cu	1.73±0.02 ^b	900 µg/day
Pb	ND	-
Ni	ND	ND

ND: Not detected; RDA: Recommended daily allowance; source of values: Food and Nutrition Board (2011).

Mean ± standard deviation of triplicate determinations.

Mean on the same rows are compared using different alphabet superscript, i.e. Mean values which have the same alphabet are not significantly different at 5% probability level.

Copper is a constituent of enzymes like cytochrome oxidase, catalase peroxidase, ascorbic acid oxidase and plays an important role in iron absorption. The copper content of *H. indicum* was 1.70 mg/100 g and high when compared to 1.28 mg/100 g in *T. terrestris* leaves (Hassan et al., 2005), 0.90 mg/100 g in *Cassia siemea* leaves (Ngaski, 2000). The RDA values for copper are 1.5-3.0 mg for male adults (NRC, 1989) thus implies that *H. indicum* is a good sources of copper in our diets. Potassium content in *H. indicum* was found to be 124.10 mg/100 g and this is high when compared to 54.20 mg/100 g of *Amaranthus viridis* reported by Akubugwo et al. (2007) and lower when compared to 220.00 mg/100 g in *Cassia siemea* leaves (Ngaski, 2006). Potassium plays an important role in controlling skeletal muscles (Mensah et al., 2008).

Zinc is a co-factor and constitute of many enzymes dehydrogenase, glutamic dehydrogenase etc. Zinc dependent

enzymes are involved in macronutrient metabolism and cell replications (Arinola et al., 2008). It is also needed for tissues repairs such as Zn is an integral constituent of insulin (Murray et al., 2000). The Zn content of 102.45 mg/100 g was found in *H. indicum*. The zinc content was found to be higher when compared to 6.85 mg/100 g in *Cassia siemea* (Ngaski, 2006). Consumption of these vegetables could complement other food sources in effecting positive impact of Zn supplementation on the growth of stunted children and on the prevalence of selected childhood disease such as diarrhoea (Hassan et al., 2009). Manganese is another microelement essential for human nutrition. *H. indicum* has manganese of 2.40 mg/100 g and falls within the range 0.98-38.0 mg/100 g of some locally green leafy vegetables reported by Hassan and Umar (2006). The RDA value for Manganese is 2-5 mg/100 g for male adults (NRC, 1989). These vegetables can be a good source of

manganese. Nickel was among the suggested essential minerals with no established RDA (Eastmond et al., 2008). Nickel and lead was not detected in the vegetable sample.

Vitamins composition

Vitamins are organic compounds occurring in natural foods especially in vegetables either as such or as utilizable "precursors". Vitamins are needed for

maintenance of skin, mucus, membranes, bones, teeth and hair, vision and reproduction. They help body to absorb calcium and phosphorus; needed for bone growth and maintenance (Rumeza et al., 2006). Vitamins are involved in blood clotting, normal functioning of nervous system and endocrine glands. Some water soluble vitamins (Vit B₁, B₂, B₃, C,) and some of the fat soluble vitamins (A, D, E) compositions were reported in the Table 3.

Table 3. Some vitamin composition of *Heliotropium indicum* (mg/100g).

Vitamins	<i>H. indicum</i>	%RDA (male adult)
A (Retinol)	152.96	700 µg/day
B1 (Thiamine)	31.90	1.1 mg/day
B2 (Riboflavin)	3.50	1.1 mg/day
B3 (Niacin)	51.73	12 mg/day
C (Ascorbic acid)	622.60	75 mg/day
D (Cholecalciferol)	4.73	10 µg/day
E (Tocopherols)	11.63	12 µg/day

RDA: Recommended Daily Allowance; Source of values: Food and Nutrition Board (2011).

Vitamin C (ascorbic acid) is a water soluble vitamin required in high amount. Vitamin C prevents scurvy disease and also aids in the formation of folic acid derivatives which are essential for DNA synthesis (Chatterjee and Shinde, 1998). From Table 3, *H. indicum* has the value of Vitamin C of 622.60 mg/100 g. These results suggest the plant is a good source of vitamin C when compared to the daily recommended intake of ascorbic acid which is 40 mg as reported by Chinma and Igyor (2007). Vitamin B₁, B₂, B₃ (Thiamine, Riboflavin and Niacin), respectively, are members of Vitamin B Complex. These vitamins act as co-enzymes in various oxidative reactions. Thiamine is necessary for the normal metabolism of carbohydrate; its deficiency causes anorexia, fatigue, constipation and retarded growth (Rumeza et al., 2006). From Table 3, *H. indicum* was found to have thiamine content of 31.9 mg. Riboflavin helps to release energy from foods, promotes good vision and healthy

skin. It also helps to convert the amino acids tryptophan into Niacin. Deficiency of riboflavin in human produces lesion in the corner of mouth, inflammation of tongue (glossitis) and lesion on the lips and around the eyes and nose (Khalil, 2004). The amount of riboflavin in *H. indicum* is 3.50 mg. Niacin was found to be 51.73 mg. Niacin deficiency results in pellagra (rough skin).

Fat soluble vitamins determined were A, D, E. Vitamin A (Retinol) was found to be 152.96 mg in *H. indicum*. Vitamin A plays an important role in vision, bone growth, reproduction and immunity. Vitamin D (Cholecalciferol) was found to be 4.73 mg in *H. indicum*. Vitamin D is responsible for enhancing intestinal absorption of calcium, iron, magnesium, phosphate and zinc. Vitamin E (Tocopherols) acts as an anti-oxidant, enzymatic activity regulator, neurological function and also protect lipids and prevent oxidation of polyunsaturated fatty acids

(Whitney, 2011) and was found to be 11.63 mg.

Anti-oxidant activities

The term 'antioxidant' refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by reactive oxygen species (Khilifi et al., 2006). Reactive Oxygen Species (ROS), sometimes called Active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions (O_2^-) and hydroxyl radicals (OH) as well as non-free radical species such as hydrogen peroxide (H_2O_2). These ROS play an important role in degenerative or pathological processes, such as aging, cancers, coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammations (Huang et al., 2005). Antioxidants interfere with the oxidative processes by scavenging free radicals,

chelating free catalytic metals and by acting as electron donors (Gulcin et al., 2005).

DPPH radical scavenging

The DPPH scavenging activities of the extracts were recorded in terms of % inhibition as shown in Table 4. It was observed from the table that as concentration increases there is also increase in % inhibitions. At 25 $\mu\text{g/mL}$, ethanolic extract of *H. indicum* has the lowest % inhibition of 28.03 while at 100 $\mu\text{g/mL}$, the ethanolic extract also had lowest % inhibition at 70.57%. Higher % inhibition indicates better scavenging activity or anti-oxidant potentials. IC_{50} value was determined from the plotted graph of scavenging activity against various concentrations of extracts, which is defined as the efficient concentration of antioxidant necessary to decrease the initial DPPH radical's concentration by 50%. The lowest IC_{50} indicates the strongest ability of the extracts to act as DPPH radicals scavengers (Figure 1).

Table 4. DPPH scavenging activity of aqueous and ethanolic extract of *H. indicum*.

Concentrations ($\mu\text{g/mL}$)	Inhibitory activities (%)			
	<i>H. indicum</i> (aqueous)	<i>H. indicum</i> (ethanolic)	Ascorbic acid	Gallic acid
25	30.83	28.03	42.29	46.71
50	50.43	45.35	51.33	75.29
75	67.64	50.19	71.32	83.76
100	75.41	70.57	83.78	89.43
IC_{50}	38.60	62.50	38.00	20.50

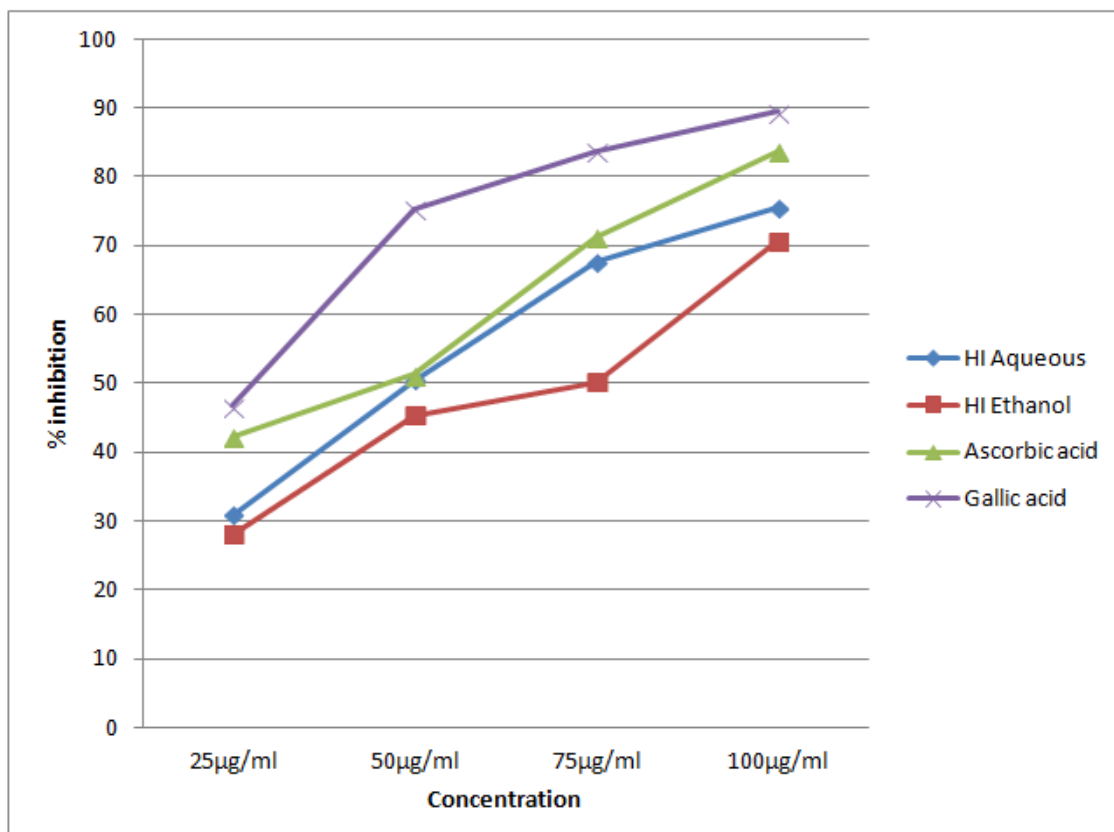


Figure 1. DPPH Dose inhibition curve and IC_{50} values of *H. indicium* (ethanolic and aqueous extract).

Reducing power activity

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron (Yildirim et al., 2000). In this assay, the ability of extracts to reduce Fe^{3+} to Fe^{2+} was determined. Table 5 shows the reducing activities of the extracts in comparison with ascorbic acid and Gallic acid as standard. The higher the absorbance of the reaction mixture, the higher would be the reducing power. At 25 $\mu\text{g/mL}$ concentration, ethanolic extract of *H. indicium* had the maximum reducing power with absorbance of 0.146 nm which compares favourably with the ascorbic acid standard of 0.152 absorbance. At 100 $\mu\text{g/mL}$ concentration,

aqueous extract of *H. indicium* has the lowest reducing power with absorbance 0.352 when compared with the standard it compares favourably with Gallic acid which has 0.556 as the absorbance value. The reducing power of the reference compounds (ascorbic acid and Gallic acid) was found to be higher than all the tested extract. From the results it can be deduced that ethanolic extract might therefore contain high amount of reductones than the aqueous extract. Hence, the ethanolic extract of the vegetables may act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reaction.

Table 5. Reducing power activities of aqueous and ethanolic extract of *H. indicum*.

Concentrations ($\mu\text{g/mL}$)	Inhibitory activities (%)			
	<i>H. indicum</i> (aqueous)	<i>H. indicum</i> (ethanolic)	Ascorbic acid	Gallic acid
25	0.146	0.137	0.152	0.211
50	0.183	0.201	0.375	0.252
75	0.235	0.252	0.401	0.462
100	0.352	0.384	0.632	0.556

Nitric oxide scavenging activity

Nitric Oxide (NO) is an important bio regulatory molecule, which has a number of physiological effects including control of blood pressure, neural signal transduction, platelet function, antimicrobial and antitumor activity (Jagatia et al., 2004). Nitric oxide also shows toxic property after reaction with oxygen and superoxide radicals. Nitric oxide (NO^*) released from sodium nitroprusside has a strong NO^+ character which can alter the structure and function of many cellular components. The NO scavenging capacity was concentration dependent with 100 $\mu\text{g/mL}$ scavenging most efficient, the aqueous extract of

H. indicum had the low inhibition activity 64.05% leading to the reduction of the nitrite concentration in the assay medium. The scavenging power of the reference compounds (ascorbic acid and gallic acid) was found to be higher than all the tested extract with activity of 81.11 and 87.65% at 100 $\mu\text{g/mL}$. The toxicity of nitric oxide increases when it reacts with superoxide to form the peroxynitrite anion (ONOO^-), which is a potential strong oxidant that can decompose to produce OH and NO_2 (Pacher et al., 2007). The present study shows that aqueous extract of *M. jalapa* has a potent nitric oxide scavenging activity (Table 6).

Table 6. Nitric oxides scavenging activity of aqueous and ethanolic extract of *Heliotropium indicum*.

Concentrations ($\mu\text{g/mL}$)	Inhibitory activities (%)			
	<i>H. indicum</i> (aqueous)	<i>H. indicum</i> (ethanolic)	Ascorbic acid	Gallic acid
25	39.51	37.53	42.27	44.58
50	44.29	48.1	51.33	56.82
75	57.91	56.44	73.58	78.24
100	64.05	66.75	81.11	87.65

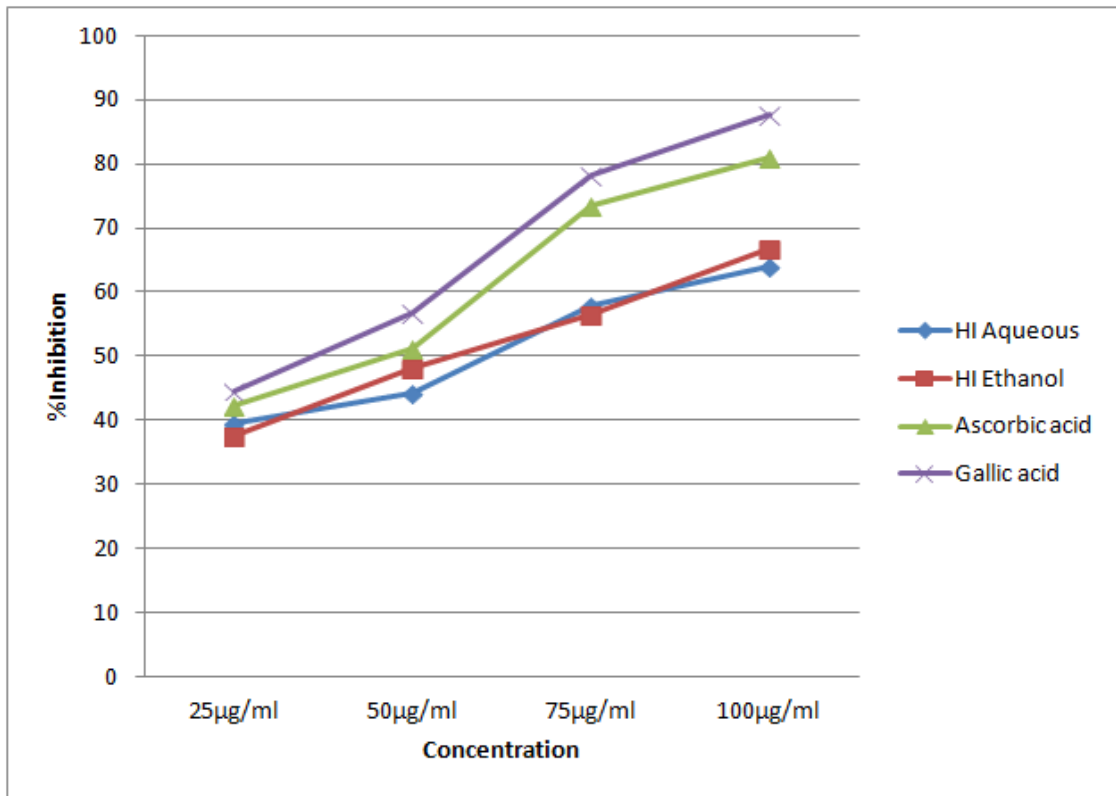


Figure 2. Nitric oxide scavenging curve and IC_{50} values of *H. indicium* (ethanolic and aqueous extract).

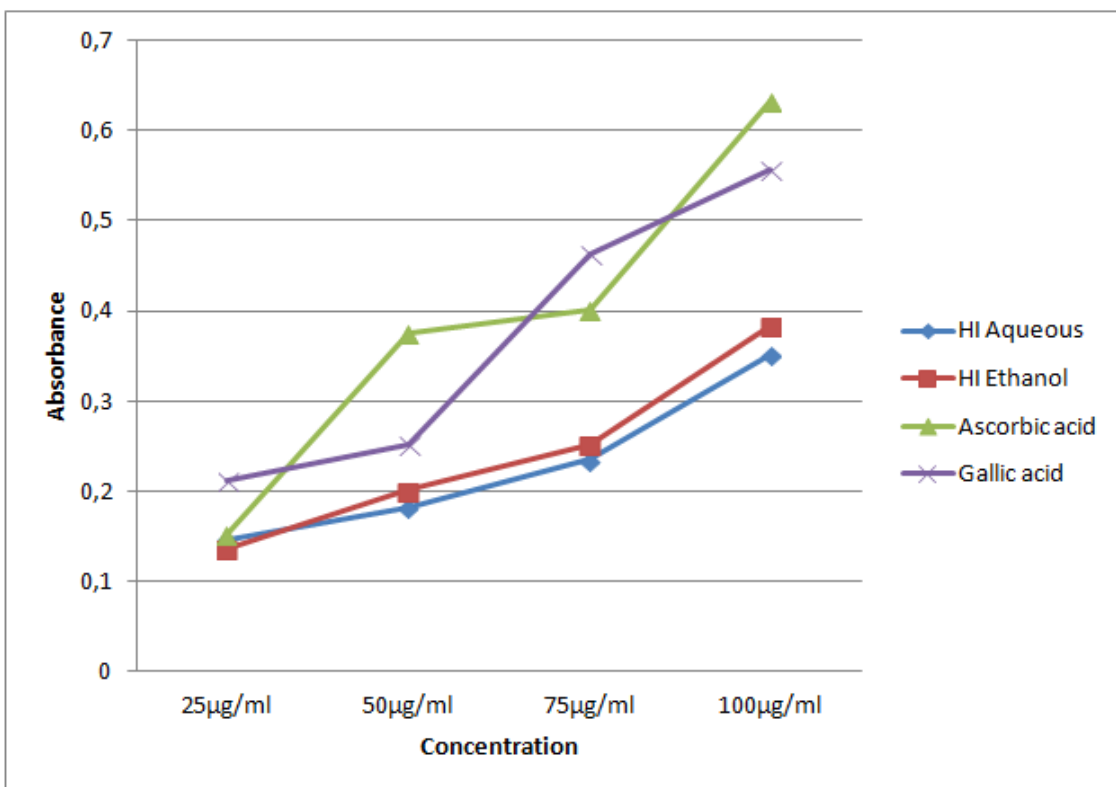


Figure 3. Reducing power activities curve of *H. indicium* (ethanolic and aqueous extract).

Conclusion

Leafy green vegetables are an important component of the human diet, providing fibre, minerals and vitamins and are low in calories. This study revealed the nutrient supplying potentials of the vegetables to be reliable, from the low moisture content compared to the conventional ones which indicates that these vegetables can have a prolonged shelf-life, thus can be preserved and exported. The low fat content which compares favourably with some edible green leafy vegetables which is of great benefit to people who require less fat in their diets. These vegetables contain sufficient amount of mineral nutrients; calcium, Iron, magnesium zinc, etc. which are important in our diets, they serve as co-factors for many physiological and metabolic functions. The anti-oxidant properties of these vegetables were active and potent when the extracts were tested using various parameters; it compares favourably with the reference compounds (ascorbic acids). The antioxidants present in these plant extracts may function by combining with oxygen or preventing oxygen from reacting with components of the food. These vegetables can contribute significantly to the nutrient requirements of man and could complement the conventional ones in enhancing food security and sustainable livelihood. Hence, their cultivation and consumption should be encouraged.

Acknowledgement

The authors want to appreciate the efforts of Doctor M. A. Ogundiran of the Department of Pure and Applied Biology, LAUTECH, Ogbomoso, Nigeria for his contributions during the course of the study.

Conflict of interest statement

Authors declare that they have no conflict of interests.

References

- Adebowale, K. O.; Lawal, O. S. Comparative study of the functional properties of bambarra groundnut (*Voandzeia subterranean*), jack bean (*Canavalia ensiformis*) and mucuna bean (*Mucuna pruriens*) flours. **Food Research International**, v. 376: p. 355-365, 2004.
- Adeyeye, E. I.; Otokiti, M. K. Proximate composition and some nutritionally valuable minerals of two varieties of *Capsicum annum* (Bell and Cherry peppers). **Discovery and Innovation**, v. 11, p. 75-81, 1999.
- Akubugwo, I. E.; Obasi, N. A.; Chinyere, G.C.; Ugbo, A. E. Nutritional and chemical value of *Amaranthus hybridus* L. leaves from Afikpo. **Nigeria African Journal of Biotechnology**, v. 6, no. 24, p. 2833-2839, 2007.
- Alpha, E.; Bala M.; Ackurt, F.; Yilmaz, T. Nutritional composition of hazenuts and its effects on glucose and lipid metabolism. **Journal of International Society for Horticultural Science**, v. 445, p. 305-310, 1996.
- Antia, B. S.; Akpan, E. J.; Okon, P. A.; Umoren, I. U. Nutritive and anti-nutritive evaluation of sweet potatoes (*Ipomoea batatas*) leaves. **Pakistan Journal of Nutrition**, v. 5, no. 2, p. 166-168, 2006.
- AOAC - Association of Official Analytical Chemists. **Official methods of analysis**. 15. ed. Washington, D. C.: Association of Official Analytical Chemists, 1990.
- Ashutosh, M.; Bikash, B. N.; Anuj, K.; Agrahari, A. R. A study on the anagelsic activity of *Heliotropium indicum*. **International Journal of Pharmaceutical Research and Development**, v. 3, no. 10, p. 1-4, 2011.
- Akindahunsi, A. A.; Salawu, S. O. Photochemical screening and nutrient-anti-nutrient composition of selected tropical green vegetables. **African Journal of Biotechnology**, v. 4, p. 497-501, 2005.
- Asibey-Berko, E.; Tayie, F. A. Proximate analysis of some underutilized Ghanaian vegetables. **Ghana Journal of Science**, v. 39, p. 91-92, 1999.
- Ball, G. F. Vitamins in Foods Analysis, bioavailability and stability. **Australian Journal of Marine and Fresh Water**, v. 36, p. 785-787, 2006.

- Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of free radical method to evaluate antioxidant activity. **LWT - Journal of the Swiss Society of Food and Science Technology**, v. 28, p. 25-30, 1995.
- Bursal, E.; Gulcin, I. Polyphenol contents and *in vitro* antioxidant activities of lyophilized aqueous extract of kiwi fruit (*Actinidia deliciosa*). **Food Research International**, v. 44, p. 1482-1489, 2011.
- Chatterjea, M. N.; Shinde, R. **Textbook of Medical Biochemistry**. 3. ed. New Delhi: Jaypee Brothers Medical Publishers, 1998.
- Chinma, C. E.; Igyor, M. A. Micronutrients and anti-nutritional contents of selected tropical vegetables grown in Southeast Nigeria. **Nigeria Food Journal**, v. 25, no. 1, p. 111-116, 2007.
- Dolui, A. K.; Atul, K.; Manabendra, D. Reproductive activities of *Heliotropium indicum* isolate against *Helopeltis theivora* and toxicity evaluation in mice. **Journal Environmental Biology**. v. 33, p. 603-607, 2012.
- Fejes, S.; Blazovics, A.; Lugasi, A.; Lemberkovics, E.; Petri, G.; Kery, A. *In vitro* antioxidant activity of *Anthriscus cerefolium* L. (Hoffm.) extracts. **Journal of Ethnopharmacology**, v. 69, p. 259-265, 2000.
- Gulcin, I.; Alici H. A.; Cesur, M. Determination of *in-vitro* antioxidant and radical scavenging activities of propofol. **Pharmacology Bulletin**, v. 53, p. 281-285, 2005.
- Gupta, S.; Lakshmi, A. J.; Majunath, M. N.; Prakash, J. Analysis of nutrient and antinutrient content of underutilized green leafy vegetables. **LWT - Food Science. Technology**, v. 38, p. 339-345, 2005.
- Gropper, S. S.; Smith, J. I. Antioxidant nutrients, reactive oxygen species and disease. **Journal of Advanced Nutrition and Human Metabolism**, v. 4, p. 368-377, 2005.
- Hassan, L. G.; Umar, K. J. Nutritional value of Balsam Apple (*Momordica balsamina* L.) leaves. **Pakistan Journal of Nutrition**, v. 5, no. 6, p. 522-529, 2006.
- Holm, L. G.; Plucknett, D. L.; Pancho, J. V.; Herberger, J. P. **The world's worst weeds: distribution and biology**. Honolulu, Hawaii: University Press of Hawaii, 1977.
- Ishida, H.; Suzuno, H.; Sugiyama, N.; Innami S.; Todokoro, T.; Maekawa, A. Nutritional evaluation of chemical component of leaves, stalks and stems of sweet potatoes (*Ipomoea batatas*). **Journal of Food Chemistry**, v. 68, p. 359-367, 2000.
- Isong, E. U.; Adewusi, S. A. R.; Nkanga E. U.; Umoh, E. E.; Offiong, E. E. Nutritional and phytochemical studies of three variation of *Gnetum africanum* ("afang"). **Journal of Food Chemistry**, v. 64, p. 489-493, 1999.
- Jagetia, G. C.; Rao, S. K.; Baliga, M. S.; Babu, K. S. The evaluation of nitric oxide scavenging activity of certain herbal formulations *in vitro*: a preliminary study. **Journal of Biochemistry**, v. 18, p. 561-565, 2004.
- Johnston, I. M. Studies in the Boraginaceae, VII. The South American species of *Heliotropium*. **Contributions from the Gray Herbarium of Harvard University**, v. 81, p. 3-73, 1998.
- Kenneth, R. evaluation of five leafy green vegetables crop. **Journal of Agriculture**, v. 16, p. 345-351, 2012.
- Khali, I. A.; Saleemulla, A. Chemistry One. Bio-Analytical Chemistry. Pakistan: T. S. Printers, 2004.
- Khilfi, S.; Hachimi, E.; Khalil, A.; Es-Safi, A.; Belahyam, A.; Tellal, A.; Abbouyi, E. L. A. *In-vitro* antioxidant properties of *Salvia verbenaca* L. hydromethanolic extract. **Indian Journal of Pharmacology**, v. 38, p. 276-280, 2006.
- Kostermans, A. J., Wirjhardja, S.; Dekker, R. J. The weeds: description, ecology and control. Weeds of rice in Indonesia. **American Journal of Human Genetics**, v. 24, p. 5655, 1987.
- Lintas, C. Nutritional aspects of fruits and vegetables consumption. **Journal of Nutrition**, v. 19, p. 79-87, 1992.
- Mensah, J. K.; Okoli, R. I.; Ohaju-Obodo, J. O.; Eifediyi K. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo People of Nigeria. **African Journal of Biotechnology**, v. 7, no. 14, p. 2304-2309, 2008.
- Moyo, B.; Masika, P. J.; Hugo A.; Muchenje, V. Nutritional characterization of Moringa (*Moringa oleifera* Lam.) leaves. **African Journal of Biotechnology**, v. 10, no. 60, p. 12925-12933, 2011.
- Nepka, C.; Asproдини, E.; Kouretas, D. Tannins, xenobiotic metabolism and cancer chemo prevention in experimental animal. **European Journal of Drug Metabolism and Pharmacokinetics**, v. 24, no. 2, p. 183-189, 1999. <http://dx.doi.org/10.1007/BF03190367>
- Nnamani, C. V.; Oselebe, H. O.; Agbatutu A. Assessment of nutritional values of three underutilized indigenous leafy vegetables of Ebonyi State, Nigeria. **Nigeria Journal of**

- Agriculture**, v. 8, no. 9, p. 2321-2324, 2009. Available from: <<http://www.ajol.info/index.php/ajb/article/view/60582/48824>>. Accessed on: Sept. 22, 2016.
- Panda, P. Effects of green leafy vegetables and fruits on health. **Journal of the Science of Food and Agriculture**, v. 7, no. 4, p. 145-151, 2010.
- Perry, L.; Metzger, J. **Medicinal plants of East and Southeast Asia**: attributed properties and uses. Cambridge: The MIT Press, 1980.
- Rumeza, H.; Zafar I.; Mudassar, I.; Shaheena, H.; Masooma, R. Use of vegetables as nutritional food: role in human health. **Journal of Agricultural and Biological Science**, v. 1, no. 1, p. 40-46, 2006.
- Siddhuraju, P.; Becker K.; Makkar, H. P. Chemical composition, protein fractionation, essential amino acid potential and anti-metabolic constituents of an unconventional legume, Gila bean (*Entada phaseoloides*) seed kernel. **Journal of the Science of Food and Agriculture**, v. 82, p. 192-202, 2001.
- Sobowale, S. S.; Olatidoye, O. P.; Olorode, O. O.; Akinlotan, J. V. Nutritional potentials and chemical value of some tropical leafy vegetables consumed in South West Nigeria **J. Sci. Multidisciplinary Res.**, v. 3, p. 55-65, 2011.
- Turan, M.; Kordis, S.; Zeyin, H.; Dursan, A.; Sezen, Y. Macro and micro minerals content of some wild edible leaves consumed in eastern Anatolia. **Tailors and Francis Journal**, v. 8, p. 129-130, 2003.
- Waterhouse, D. F. The major arthropod pests and weeds of agriculture in Southeast Asia. **Journal of Australian Centre for International Agricultural Research**, v. 56, 141, 1993.
- Whitney, E. N.; Rolfes, S. R. **Understanding nutrition**. 12. ed. Boston: Cengage Learning, 2011.
- Wolf, G. The discovery of vitamin D: the contribution of Adolf Windaus. **Journal of Nutrition**, v. 134, no. 6, p. 1299-1302, 2011. Available from: <<http://jn.nutrition.org/content/134/6/1299.full.pdf+html>>. Accessed on: Sept. 22, 2016.