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Quantification of the nutritional content, phenolic and flavonoids of some selected indigenous vegetables of south-western Nigeria

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Abstract- Some uncommon (indigenous) vegetables consumed in Osun State, Nigeria were analysed to determine their nutritional and phytochemical contents using standard analytical procedures. The total phenolic and flavonoids were determined using the crude aqueous extracts of these vegetables by *Folin-Ciocalteau* assay and appropriate standard analytical methods respectively. The results showed variations in the total phenolic contents (TPC) and total flavonoid contents (TFC) among the different uncommon vegetables. Malabar spinach (Amunututu) has the highest amount of TPC (57.51g/100g ± 3.98) and while the lowest value (28.38g/100g ±0.15) was recorded for Wild lettuce (Yarin). The TFC was found to be highest in extract of Spinach leaves (Wororwo) with 60.88± 9.14% and lowest amount was recorded for Black night shade (Odu) 37.43± 4.58 %. The results of the study showed the significance of the uncommon vegetables in the treatments and prevention of various ailments and diseases. They also indicate their nutritional importance in human beings.

***Keywords:* *Flavonoids, Folin-Ciocalteau assay, Phenolic, Proximate compositions, Vegetables*.**

1. Introduction

About 1.02 billion people in the World do not have enough food to eat. The number of undernourished people increased by 75 million in 2007 and by 40 million in 2008, largely as a result of higher food prices. Most of the undernourished people of the World are found in Africa and 907 million people in developing countries are hungry [1]. Malnutrition is said to be responsible for about 50% of the estimated 10.6 million deaths of under five years old children globally [2]. Micronutrient malnutrition also known as 'Hidden Hunger' now afflicts over two billion people worldwide resulting in poor health, low workers' productivity, high rate of mortality and morbidity, increases rate of coronary heart diseases, cancers, stoke and diabetes and permanent impairment of the cognitive abilities in infants born to the malnourished mothers [3].

Food based approaches are recommended for the alleviation of micronutrient malnutrition. The approaches include the use of locally available foods such as vegetables [4]. Foods of plant origin may contain many phytochemicals in addition to nutrients such as proteins, fats carbohydrates, vitamins and minerals. Phytochemicals have been identified in foods and there may be more than 100 different phytochemicals in just one vegetable [5]. Epidemiological studies have indicated relationships between vegetable consumption and chronic diseases such as hypertension, cancer and heart diseases. Vegetables consumption has been strongly recognized for providing health protecting properties [6]. Some of the potential health benefits of phytochemical have been attributed to their action as antioxidants and inhibitors of lipid peroxidation [7].

Phytochemical compounds such as phenolic are strong antioxidants against free radicals and reactive oxygen species which have been implicated as the major cause of chronic human diseases such as cardiovascular diseases, diabetes and degenerative diseases [7] [8] [9]. In recent years, consumption of vegetables in the diet has been highlighted for its contribution towards lowering all the risks of life threatening diseases. They are valuable in maintaining alkaline reserve in the body and are valued mainly for their high vitamins, dietary fibre and mineral contents. Vegetables are home for many antioxidants. It helps to protect the human body from oxidant stress, diseases and cancers and also helps the body to develop the capacity to fight against these diseases by boosting human body's immunity [10].

Phytochemicals are naturally occurring components in fruits, vegetables, legumes and grains. They give plants its colour, flavour and are parts of the plant's natural defence system. Hence we need to discover the potentials of our local vegetables possessing these phyto-nutrients and providing vital data for food processors, nutrition workers as well as the consumers of the selected green leafy-vegetables. Vegetables are parked with soluble as well as insoluble dietary fibre known as Non- Starch Polysaccharides (NSP) such as cellulose, gums, pectin etc. These substances absorb excess water in the colon, retain a good amount of moisture in the faecal matter and help its smooth passage out of the body [10]. These natural protective effects have contributed to various components such as carotenoid, vitamin C and E and phenolic and thiol (SH) compounds.

Phenols and flavonoids are phytochemicals synthesized by plants as secondary products and defence mechanisms for their survival. An enormous variety of plants have been studied for new sources of phenolic compounds but there is paucity of report about phenolic, flavonoids and antioxidant activity of indigenous vegetable from Osun state South-west of Nigeria. Thus, a need for the investigation of antioxidative activity in leafy indigenous vegetables. The present study therefore aimed at determining the proximate parameters as well as total phenolic and flavonoids compounds in some selected indigenous leafy vegetables consumed in Osun state of Nigeria and evaluates the potentials of these vegetables as healthy diets.

2. Materials and Methods

2.1 Samples collection and preparation

Most of the selected indigenous vegetables were recognized by the villagers in situ during short field walks and were collected for scientific identification by Botanists. Identification of some of the plants was done and Voucher samples were prepared and deposited in our laboratory in the department of Biochemistry unit of the Science Laboratory Department of the Federal Polytechnic, Ede, Nigeria.

Information on the plants seasonal availability, part used as food and the methods of preparation and preservation was obtained during a survey of the three senatorial district of Osun state, Nigeria. A questionnaire was administered to 120 respondents. The vegetables were identified by the villagers and authenticated by Botanists while virtual herbariums of the samples were kept. The leaves of these plant materials were properly washed in tap water and then rinsed in distilled water. The rinsed leaves were air dried, grinded into fine powder using an electric blender and was packed in air tight containers and stored for further analysis [11].

Extracts of each of the samples was prepared by soaking 10 grams each of the samples into 100mL each of methanol for 48 hours. The extracts are then filtered using *Whatman* filter paper. Rotary evaporator was used to evaporate the extracts to dryness.

2.2 Phytochemical analysis

2.2.1 Total phenol

The total phenolic concentration was determined by spectroscopic method. Methanolic solution of the extract 1 mg/mL was used for the analysis. The reaction mixture was prepared by mixing 0.5 mL methanolic extract solution, 2.5 ml of 10 % *Folin-Ciocalteu* reagent and 2.5 ml of 7.5 % sodium hydrogen carbonate [12]. Methanol (0.5 ml), 2.5 mL of 10 % *Folin-Ciocalteu* reagent was dissolved on 100 mL of distilled water and 2.5 mLof 7.5 % sodium hydrogen carbonate was also dissolved in 100mL of distilled water. The samples were incubated in a water bath at 45 ^{0}C for 45 minutes.

The absorbance was determined using visible spectrophotometer at 710 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of Gallic acid for the dilution of 0.05 mL, 0.10 mL, 0.15 mL, 0.20 mL, 0.25 mL, 0.30 mL, 0.35 mL and 0.40 mL. The calibration curve was constructed based on the measured absorbance and the concentration of phenolics was read (mg/mL) for the calibration line. The content of phenolics in the extracts was expressed in terms of Gallic acid equivalent (grams of Gallic acid/grams of extract). The Gallic acid was used as the standard stock solution and was prepared by dissolving 5 grams of Gallic acid in 1000 mL of distilled water.

2.2.2 Total flavonoids

The total flavonoids were determined by the reported method [13]. 5 grams of the sample was boiled in 2M hydrochloric acid solution for 30 minutes and filtered using *Whatman No 42* filter paper. 5 mL of each of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoid precipitate was recovered by filtration using filter paper. The resulting weight difference gave the weight of the flavonoid in each sample.

2.3 Proximate composition

The moisture content was determined by gravimetery using oven dry method. The crude protein, crude fat and crude fibre were determined by the official methods of analysis [14]. The carbohydrate content was determined by difference.

2.4 Mineral elements analysis

Phosphorus was determined photometrically using the *molybdate-vanadate* method [14]. Potassium, calcium, magnesium, iron, zinc and copper were determined in the ash from incineration of the vegetable leaves and the metal contents were calculated on dry weight basis using a Perkin-Elmer 2280 atomic absorption spectrophotometer.

2.5 Data analysis

The Students t-test in the Statistical Package for Social Sciences (SPSS) for Windows Standard Version 8.0 was used for the statistical evaluation with $P < 0.05$ considered statistically significant.

3. Results and discussion

Table 1: Nutrient composition on dry weight basis of the indigenous vegetables as determined by AOAC methods (g/100g)

	African spinach (*Teteabalaye*)	Malabar spinach (*Amunututu*)	Wild lettuce (*Yanrin*)	Spinach leaves (*Worowo*)	Black night shade (*Odu*)	Soft vegetable (*Ebolo*)
Nutrients						
Protein	6.34±2.58	5.21± 1.96	6.23±2.69	5.45±2.41	6.34±3.77	5.48±2.92
Fibre	9.02± 0.02	10.41± 0.04	1.90 ±1.36	14.15± 2.22	12.25 ± 2.26	1.90 ±0.43
CHO	9.23± 0.47	8.42±1.55	8.51±2.60	10.31±4.55	12.21± 3.23	9.12±2.65
Fat	0.34± 0.24	0.41±0.69	0.51±0.45	0.42±0.24	0.45±0.30	0.55±0.69
Ash (%)	4.70± 0.58	4.62± 1.46	1.9 ±1.35	5.4±2.24	2.7± 0.39	1.9 ±0.65

Values are means of triplicate determinations.

Table 2: Mineral elements composition of some indigenous vegetable in Osun state, South-western Nigeria

	African spinach (*Teteabalaye*)	Malabar spinach (*Amunututu*)	Wild lettuce (*Yanrin*)	Spinach leaves (*Worowo*)	Black night shade (*Odu*)	Soft Vegetable (*Ebolo*)
Mineral (mg/100g)						
Iron	13.12±4.37	11.43± 0.82	17.54±3.02	8.75±1.82	23.20± 3.7	14.13±2.23
Zinc	2.91± 0.91	5.83± 1.0	22.40± 3.13	4.57 ±1.33	23.12± 2.15	20.23±3.32
Cu	10.23±4.81.	7.61± 1.38	11.63±3.12	1.83 ± 0.48	21.21±3.35	22.43±4.12
A/acid	18.10± 6.67	62.12± 8.21	70.40±6.53	78.0± 14.65	55.43± 8.23	46.34±5.11
K	129± 49	650 ± 146	600±135	384±98	1090± 215	634±202
Ca	125± 86	679± 215	370 ± 144	380±150	400± 215	478±169
Mg	99±68	440± 191	600±271	290± 236	370±215	412± 89
P	66±11	578 ±133	500± 121	626±111	66±47	56±32

Values are means of triplicate determinations.

3.1 Proximate analysis

The insoluble dietary fibre in the vegetables was in the range of 1.90 (g/100g) in water lettuce and soft vegetable (Ebolo) to 14.15 (g/100g) in Spinach leaves (*Worowo*) as shown in Table 1. The previous study reported values at variant with values recorded in the present study. [15] The observed variation may be due to differences in the maturity stages of the plants, the seasonal variation, fertilizers application, plant variety used for the study, geographical location and the analytical method used for the study [16]. Also, cooking and processing methods alter the physical and chemical properties of plant cell wall and this in turn affects the vegetable performance as dietary fibre and as a result, the dietary fibre of the plant extract may be underestimated [17].

3.2 Total phenolic

The total content of phenolic compounds varied from 28.28 GAE mg/g in wild lettuce (*Yarin*) to 57.51 GAE mg/g in *M. Spinach* (*Amunututu*) is shown in Fig.1. There is significant difference ($p < 0.05$) between the total phenolic compounds present in indigenous vegetable and exotic ones. All the indigenous vegetables had higher total content of phenolic compounds than the common vegetables. Lower values were reported for indigenous vegetables of Botswana origin [18].

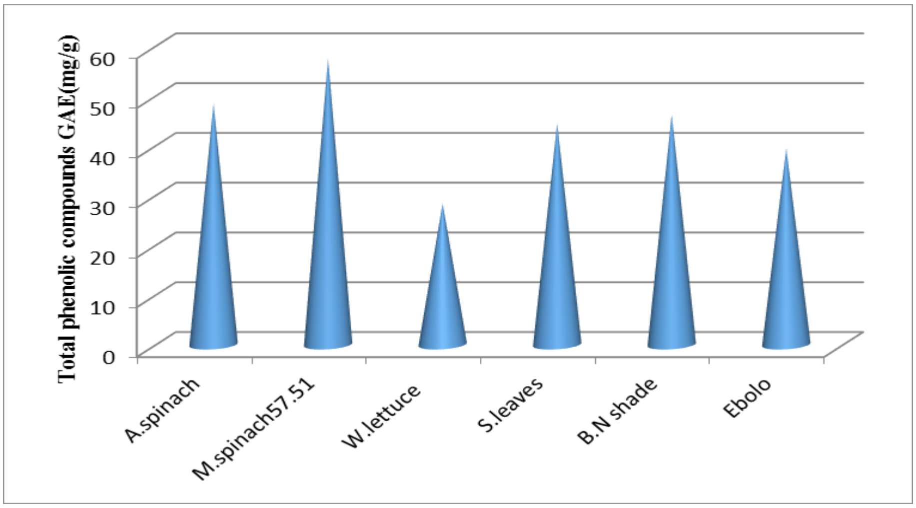

Figure 1: Total phenolic compounds in indigenous leafy vegetable

3.3 Total flavonoids

The contents of flavonoids of the studied vegetables were higher compared the contents of common vegetables exotic vegetables previously analysed [19]. The total flavonoids in the indigenous leafy vegetables analyzed were higher than those of common vegetables in the area of study. The total flavonoids ranged from 37.43 mg/g in *Black night shade* (Odu) to 60.80mg/g in spinach leaves (*Worowo*). Okro, *C.olitorus* bitter leaves and pupkin leaves (Uguw) which are commonly consumed in the areas under study had 51.25, 14.35, 51.14, and 43.17 mg/g respectively as shown in Tig.2.. Variation in the composition of flavonoids might be attributed to genetic factors as the plants belonged to different generic. The environment effects were perceptible because the plants were collected from different ecological regions characterized by diverse weather patterns [20].

3.4 Mineral element characterization

The iron content of the indigenous vegetables analyzed range from 8.75 mg/100g in Spinach leaves (Worowo) to 23.20 mg/100g in black night shade (Odu). Higher value was reported for Amaranthus spp [21]. Relatively higher values were from previous study compared to this work. The observe difference might have been influenced by factors such as pH and soil type, water availability to the plant, plant variety, climatic conditions, Plant age [21] and the use of fertilizers [22]. The zinc content ranged from 2.91 mg/100g in Spinach leaves to 17.54 mg/100g in wild lettuce (*Yarin*). The copper content ranged from 7.61 mg/100g in *M. spinach* (*Amunututu*) to 22.43 mg/100g in (*Ebolo*). Ascorbic acid (A/acid) varied from18.10 mg/100g in A. Spinach (Tete-abalaye) to 78.0 mg/100g in Spinach leaves (worowo). All the indigenous vegetables were highly rich in macro-element of potassium, calcium and magnesium as presented in Table 2.

Figure 2; Total flavonoids concentration in indigenous vegetables

The values reported for these elements were similar to those reported in the previous studies. [1] [14] [20]. Significant amount of phosphorous were present in the indigenous vegetable compared to the common vegetables. *Worowo (Spinach leaves)* (626 mg/100), 578 mg/100g (*Amunututu*) (*M.spinach*) and 500 mg/100g in *Yarin (*wild lettuce) respectively. The level of phosphorous in the indigenous vegetables may contribute significantly to phosphorous requirements of especially the elderly who are likely to suffer from osteoporosis. The micro-nutrient content of the indigenous vegetables was higher than that of most exotic vegetables.

4. Conclusion

The results in this study revealed that the indigenous (not so popular among young generations) and common vegetables assayed are poor sources of macro-nutrients such as protein, fat, fibre and carbohydrate. However, compared to some common vegetables (Okro, *Chocorus olitorus*, bitter leaves and pumpkin leaves), the indigenous vegetables had higher macro-nutrient content. The micro-nutrient content of the indigenous vegetables was higher than that of commonly consumed vegetable amongst the younger generations in the area under study. Phenolic compounds were present in all vegetables assayed though in different proportions. All the Indigenous vegetables had higher total content of phenolic compounds than the common vegetables in the areas under study.

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