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Proximate and Phytochemical Composition of Some Lesser Known Leafy Vegetables Consumed In Northern Senatorial District of Cross River State, Nigeria

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Abstract The proximate and phytochemical composition of seven leafy vegetables (*Albizia zygia, Basella alba, Ficus glumosa, Hibiscus cannabinus, Pterocarpus santalinoides, Solanum nigrum and Vitex doniana*) consumed in the Northern Senatorial District of Cross River State, Nigeria was determined using standard methods. The proximate composition showed that moisture content of the fresh samples ranged from 68.20 to 86.43 % while the dry samples had moisture content ranging from 12.53 to 27.80%. Fresh *Basella alba* had the highest moisture and crude fat (86.43±0.18% and 6.43±0.15%DM respectively). On dry matter basis, *Vitex doniana* was found to contain the highest amount of crude protein (55.60±0.12%) and energy (871.33±8.65Kcal). Dietary fibre analysis indicated that *Ficus glumosa* had the highest value (1.79±0.02%).*Hibiscus cannabinus* was found to be significantly ($p<0.05$) high in carbohydrate (18.30±0.12%) compared with other vegetables analyzed whereasash was found to be significantlyhigh in *Albizia zygia* (13.47±0.09%). The quantitative phytochemical analysis showed that *Hibiscus cannabinus* contained the highest amount of *alkaloid* (2.12±0.01mg/100g) while *Solanum nigrum*had the highest amount of tannin (0.69±0.02mg/100g) and steroids (8.33±0.02mg/100g). Oxalate and cyanate content was low in *Vitex doniana* (1.31±0.00mg/100g and 0.07±0.01mg/100g respectively). The amount of flavonoids contained in the vegetables was generally low, with *Ficus glumosa* and *Pterocarpus santalinoides* containingequal and highest amount (0.05±0.00mg/100g), while *Pterocarpus santalinoides* had the highest content of saponin (6.46±0.03mg/100g) and glycoside (6.13±0.01mg). *Ficus glumosa* had thehighest phytatecontent ((7.30±0.01mg/100g). The results obtained in this study indicate that the seven leafy vegetables are good sources of nutrients as well as may possess ethno-medicinal potentials for drug formulations.

Keywords: *leafy vegetables, seven, nutrient, proximate composition, phytochemical analysis*

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1. Introduction

In developing countries such as Nigeria, vegetables are the cheapest and most readily available sources of valuable nutrients such as proteins, vitamins and minerals [1]. They contain important food constituents that can be used for body building, as sources of energy, regulatory and protective materials as well as for the maintenance of overall good health and prevention of diseases [2]. Vegetables may be defined as the fresh portion of herbaceous plants that can be eaten either in raw or cooked forms [3]. They may be edible leaves, stems, roots, fruits or seeds, with each group contributing to the diet in its unique way. They are sometimes accompanied by tender petioles and shoots. According to Misra and Misra [4] and Igile *et al.*, [5] leafy vegetables are a valuable part of the dietary regimen of Africans, providing essential minerals and vitamins needed for growth, development and maintenance of optimal health. The fresh young leaves of the seven leafy vegetables in this study are used to prepare various types of soup, vegetable sauce and porridge after undergoing treatments such as washing, boiling and drying. The bark of *Ficus glumosa* produces a gum-like liquid which is usually chewed as chewing-gum [6]. Traditionally, *Hibiscus cannabinus* is produced primarily for its fibre content and the immature plant is used as fodder for animals due to its high protein content at the early stage of growth [7]. *Pterocarpus santalinoides* is commonly found around riverine forests. *Basella alba* is a fast growing perennial vine that is extremely heat tolerant and is considered to be one of the finest tropical spinach throughout the tropics due to its easy adaptation to various soil types [8]. *Albizia zygia* possesses antioxidant activity which may be due to the presence of a variety of plant phenolics and terpenoids that act as free radical scavengers and regulators of metabolism respectively [9].

Solanum nigrum grows mainly as a weed in moist habitats and on various soil types such as stony, shallow or dry soils [10]. Apart from the dietary use of the leaves [11], Ramya *et al*. [10] has reported that; the traditional herbalists in Algeria and most parts of Asia (especially India) use the whole plant (decoction), leaf (cooked), leaf paste (applied directly), whole plant (taken as food), roots (boiled with little sugar), roots (extracted juice) to treat burns and dermal infections, stomach ache and stomach ulcer, rabies and wounds, cough, indigestion, female infertility, asthma and whooping cough respectively. It has also been used for the treatment of sexually transmitted diseases [12]. *Vitex doniana* is found around the South and eastern parts of Nigeria, where the young leaves are prepared into vegetable sauce and porridge after undergoing treatments such as washing, boiling and drying [9]. The fruits have been reported to be a good source of essential vitamins and minerals, high sugar content used in preparation of a good number of syrups and beverages; the leaves are used by traditional medical practitioners in the management of some diet related non-communicable diseases such as heart disease, cancer and inflammatory diseases [9,13]. t is possible that the phytochemical content of these vegetables could be reason for their efficacy as traditional herbs used for the treatment of myriads of diseases.

In spite of the traditional uses of these vegetables, inadequate information on the nutritional composition, production and consumption of indigenous green leafy vegetables is causing gradual neglect of some of the useful ones that have been used for food over the years. The available information is dispersed and making comparisons with such data often difficult, due to the gaps in coverage as well as differences in the methodologies used. Creation of awareness regarding the nutritional potentials of these local vegetables through chemical analysis of the vegetable samples is therefore essential. Information from the chemical, nutrient and anti-nutrient analysis of these vegetables would provide nutritionists with the means to formulate adequate healthy diets and provide government with the necessary food database for policy making [14]. The present study seeks to assess seven leafy vegetables consumed in the Northern senatorial district of Cross River State for their nutrient and phytochemical composition, with the view to providing nutritional information that will bridge the information gap that exists, encourage cultivation of these vegetables and increase their demand, thereby enhancing the social and economic status of the peasant farmers and inhabitants of communities where these vegetables are domicile.

2. Material and Methods

2.1. Sample Collection and Treatment

The seven leafy vegetables (*Albizia zygia, Vitex doniana, Solanum nigrum, Ficus glumosa, Hibiscus cannabinus, Pterocarpus santalinoides, Basella alba*) were collectedfrom local farms in Obudu, Obanliku and BekwarraLocal Governmentareas of the Northern senatorial district of Cross River State, Nigeria and voucher specimens were deposited in the Herbarium of the Department of Botany, University of Calabar, Nigeria.The vegetable samples were properly washed under a running tapwater in the laboratory; wet weight of each vegetable was determined and the remaining samples were oven-dried, ground into powderusing a manual grinderand preserved in tightly sealed plastic containers forchemical and phytochemical analyses.

2.2. Proximate Analysis

2.2.1. Determination of %moisture, Moisture, Ash, Crude Protein and Crude Fat

The analyses of the percentage moisture, ash, crude protein and crude fat (ether extract) of the seven leafy vegetables were carried out according to the standard methods of analysis of the Association of Official Analytical Chemists [15].

2.2.2. Determination of Dietary Fibre

Dietary fibre was determined by sequential enzymatic hydrolysis and further determined gravimetrically using the AOAC-Prosky (991.43) methods [16,17]. One gram (1g) of dry sample was weighed, grounded and transferred into a 400 ml beaker. A soxhlet extractor was used to de-fat sample by stirring with 25 ml petroleum ether. Defatted sample was centrifuged and the petroleum ether portion discarded. The de-fatting step was repeated twice and weight loss due to fat removal was recorded for later use in correction of final dietary fibre content. The residual petroleum ether was allowed to evaporate from the defatted sample under fume hood. Residues were suspended in MES-TRIS buffer and digested sequentially with heat-stable α-amylase at 95–100°C, protease at 60°C, and amyloglucosidase at 60°C. Enzyme digestants were filtered through tarred fritted glass crucibles. Crucibles containing insoluble dietary fiber were rinsed with dilute alcohol followed by acetone, and dried overnight in an oven at 105 °C. Filtrates were mixed with 95% ethanol to precipitate soluble dietary fibre. After about an hour, precipitates were filtered through tarred fritted glass crucibles. One of each set of triplicate insoluble and soluble fibre residues were ashed in a muffle furnace at 525°C for 5 hours and then weighed and determined gravimetrically. The percentage dietary fibre was calculated as follows:

a) Soluble or insoluble fibre residues = % original sample weight - % ash.

b) Total fibre = (sample residue - sample protein residue - sample ash residue - blank)/Weight of sample.

2.2.3. Determination of Total Carbohydrate Content

The percentage total carbohydrate content of each vegetable sample was determined by summing up the percentages of moisture, ash, crude protein, fat (ether extract) and subtracting from 100. The difference in value was taken as the percentage total carbohydrate content of each sample.

2.2.4. Energy Content

This was calculated in (Kcal/100g) using the equation: [(37 x fat) + (17 x carbohydrate) + (17 x protein)].

2.3. Phytochemical Analysis

Tannin content was determined using the ferric chloride test method described by [18]. Ferric chloride and potassium Ferro-cyanide were used to develop the colour of tannin extracted from the sample, and absorbance read at 710nm.Oxalate content was determined using the method described by [19]. Oxalic acid was extracted from the sample and precipitated as calcium salt. The oxalate was dissolved in Sulphuric acid and concentrations of oxalate in the solution determined by titration with $KMnO_4$ for a faint pink end point.1ml 0.05 $KMn0_4$ = 2.2mg oxalate. Cyanate content was determined using the alkaline picrate colorimetric method described by [20]. Samples were weighed and then dissolved in 50ml distilled water and the solution allowed to stand for about 18hours and then filtered. The filtrate was then mixed with 4ml alkaline picrate and allowed to stand for 4min after which absorbance was measured at 490nm. Alkaloid content was determined using the alkaloid precipitation gravimetric method described by [18]. Sample were mixed with 200ml of 10% acetic acid in ethanol and allowed to stand for 4 hours. Concentrated ammonium hydroxide was added drop wise until full turbidity was observed. Alkaloid precipitates were recovered by filtration using weighed filter paper, 1% NaOH was used to wash the residue and the filter paper dried in the oven for 30mins and weighed after cooling in a desiccator and result expressed as percentage of sample analyzed.

Flavonoid content was determined using ethyl acetate precipitation gravimetric method described by [18]. The sample was mixed with 200ml 2M hydrochloric acid solution and boiled for thirty minutes until hydrolyzed. Cooled at room temperature and 20ml ethyl acetate added to the filtrate to precipitate the flavonoids which was recovered by filtration using a previously weighed filter paper. The filter paper was dried in the oven at 100°C for one hour. The weight of the flavonoid was determined by difference and then expressed as a percentage of the weight of sample analyzed.Phytate content was determined using the spectrophotometric method described by [21]. 0.5g of sample was weighed out and transferred into a 500ml flat bottom flask. The flask with its content was then placed in a shaker and extracted with 100ml of 2.4% HCl for one hour at a temperature of 25°C. After this, the mixture was decanted and filtered. Distilled water was then use to dilute 5ml of the filtrate to 25ml, and 10ml of the diluted filtrate was then collected into a flask, after which 15ml of 0.1M sodium chloride was added to it. The mixture was then passed through a filter paper (Whatman No. 1), eluting inorganic phosphorus in the process, with 15ml of 0.7M sodium chloride also eluting phytate. The absorbance was measured at 520nm. Saponin content was determined using froth and emulsion test described by [18]. Samples were mixed with 200ml of 20% ethanol and boiled for 4 hours to reduce it by half. The sample was treated with 40ml diethyl ether, 60ml N-butanol and two portions of 10ml each of 5% aqueous sodium chloride. The extract was collected in a previously weighed petri-plate, evaporated to dryness and reweighed after cooling in a desiccator by weight difference. Saponin content was then expressed as a percentage of the weight of the sample analyzed.

Glycoside content was determined using the spectrophotometric method described by [22]. Samples were purified and 10ml of the purified filtrate was transferred into a clean dry test tube and 10ml of Baljet's reagent was added. A blank sample was prepared using 10ml of distilled water instead of the 10ml purified filtrate and 10ml Baljet's reagent. The setup was left for one hour (to allow for maximum colour development). The solution was later diluted with 20ml distilled H_2O and mixed thoroughly. The absorbance was then measured at 495nm using a spectrophotometer.Steroid content was determined using the method described by [23]. 1ml of extract of solution was transferred into 10 ml volumetric flasks. 2ml Sulphuric acid (4N) and 2ml iron (III) chloride (0.5% w/v) were added followed by 0.5ml potassium hexacyanoferrate (III) solution (0.5% w/v). The mixture was then heated in a water-bath maintained at 70±20 °C for 30 minutes with occasional shaking and diluted with distilled water. The absorbance was then measured at 780 nm against the reagent blank

2.4. Statistical Analysis

SPSS software was used for computation and analysis of different parameters. All determinations were done in triplicates and data were reported as mean and standard error of mean. These data were subjected to analysis of variance (ANOVA) for significant difference at $p<0.05$.

3. Results and Discussion

3.1. Proximate Analysis

Proximate analysis was carried out on selected vegetables: *A. zygia, B. alba, F.s glumosa, H. cannabinus, P. santalinoides, S. nigrum and V. doniana.*The data obtained is presented in Table 1.

Moisture content of the fresh samples ranged from 68.20±0.26% to 86.43±0.18% which is similar to results reported by Agbaire [24], Adepoju and Oyewole [25] for some leafy vegetables. *Ficus glumosa* (68.20±0.26%) had the least moisture content while *Basella alba* (86.43±0.18%) had the highest moisture content. The result of this study is consistent with the study of Udo *et al.* [26] who reported that leafy vegetables have a moisture content ranging from 72 to 93%. On dry matter basis, moisture content of samples in this study ranged from 12.53±0.18% to 24.77±0.15%. The high moisture content of vegetables indicates freshness and perishability, as well as indicating that they may play a key role in aiding the digestion of food [25].

Total carbohydrate was most abundant (18.30±0.12%) in *Hibiscus cannabinus* compared to *Pterocarpus santalinoides* which had the least total carbohydrate content (13.40±0.17%). Compared with other vegetables reported by Adnan *et al.* [27] and Nkafamiya *et al.* [28], the carbohydrate content of the vegetables in this study is relatively low, with values ranging from 13.40 ± 0.17 to18.30 ± 0.12%. Since the recommended dietary allowance (RDA) for carbohydrate for both normal healthy male and female adults is 130g/100g, the results from this study implied that the vegetables may be poor sources of dietary carbohydrates and so may not be recommended to vegetarians as the sole source of dietary

carbohydrate and energy. Results from this study showed that the leafy vegetables analyzed had high protein content, with *Vitex doniana* (55.60±0.12%) having the highest content. This is inconsistent with the study of Adepoju and Oyewole [25] that reported the protein content of *Adenia cissampeloides* and *Ceiba pentandra* were 23.0% and 18.5% respectively.

There was similarity in total fat content of *Pterocarpus santalinoides* and *Hibiscus cannabinus* (3.33±0.15%) while other vegetables such as *Basella alba* (6.43±0.15%) and *Ficus glumosa (*5.23±0.13%) had significantly ($p<0.05$) higher amount of crude fat. The result obtained in this study is relatively low compared to values obtained by Uhegbu *et al.* [29] for .*Lavandula angustifolia* (6.52%) and *Valeriana officinalis* (14.35%). These vegetables may therefore be recommended as part of weight reducing diets due to their low fat content.

Results showed low dietary fibre content in the vegetables analyzed with similar contents of dietary fibre seen in *Pterocarpus santalinoides* and *Hibiscus cannabinus* (1.62±0.07%). However, these values are relatively high when compared with data obtained by Blessing *et al.* [2] for soybean (0.2%) and Berlandier nettle spurge seed (1.6%). Dietary fibre is essential in aiding digestion and decreasing waste transit time in the gastrointestinal tract and increasing stool bulk, softening it and preventing constipation [2]. These vegetables may, therefore, be very useful in the control of body weight, blood cholesterol and protection against colon cancer.

3.2. Phytochemical Analysis

The results of the quantitative analysis of phytochemicals are presented in Table 2. Saponins are glycosides containing polycyclic aglycone moiety of either C27 steroid or C30 triterpenoids attached to a carbohydrate sugar [5]. They have the capacity to affect the digestibility of proteins and inhibit a handful of enzymes involved in the digestion of proteins such as trypsin and chymotrypsin resulting in the reduction of bioavailability of proteins and other nutrients [30]. However, they may also produce hypocholesterolemic, anti-cancer, anti-infertility and anti-inflammatory effects, which are healthy. The low level of saponin content in this study (in the range of 3.99± 0.02 to 6.46± 0.03mg/100g) suggests that on consumption, the probability of these vegetables to cause reduction in nutrient uptake could be low. This view is supported by the findings made by Igile *et al.* [5] which reported that saponins were safe and non-toxic at low levels of < 10%.

Alkaloids were present in appreciable amounts (1.12± 0.01 - 2.12± 0.01mg/100g). Alkaloids are small organic molecules that belong to a large group of chemical compounds synthesized by vascular plants [31]. They are one of the most therapeutically efficient bioactive substances known in plants. Purely isolated natural alkaloids and their synthetic derivatives are used as basic medicinal agents due to their anti-spasmodic, analgesic, anti-spasmodic and bactericidal properties [32].

Table 1. Proximate composition and energy value of some selected lesser known vegetables (% DM)

Sample	Moisture	Moisture (Wet wt.)	Ash	Protein	Fibre	Fat	Carbohydrate	Energy (Kcal/100g)
P. Santalinoides	24.77 ± 0.15^{a}	72.33 ± 0.21^{a}	6.60 ± 0.12^{a}	51.87 ± 0.09^{a}	1.61 ± 0.03^{a}	3.33 ± 0.09^{a}	13.40 ± 0.17^{a}	836.33±13.48^{a}
V. doniana	20.67 ± 0.15^{b}	75.20 ± 0.20^{b}	6.20 ± 0.12$^{a, b}$	55.60 ± 0.12^{b}	0.94 ± 0.41^{b}	4.33 ± 0.03^{b}	14.47 ± 0.23^{b}	871.33 ± 8.65^{a}
B. alba	12.53 ± 0.18^{c}	86.43±0.18^{c}	4.73±0.35^{b}	49.93±0.71^{c}	1.68± 0.01^{a}	6.43± 0.15^{c}	16.70 ± 0.12^{c}	711.00± 5.13^{b}
A. zygia	20.53 ± 0.20^{b}	75.90±0.23^{b}	13.47± 0.09^{c}	43.87± 0.12^{e}	1.42± 0.08$^{a, b}$	4.47± 0.12^{b}	15.30 ± 0.12^{d}	799.00± 11.59^{c}
F. glumosa	27.8 ± 0.15^{d}	68.20±0.26^{d}	6.23± 0.47$^{a, b}$	51.67± 0.32^{f}	1.79± 0.02^{b}	5.23± 0.13^{d}	14.40 ± 0.12^{b}	765.00± 23.58^{b}
S. nigrum	17.67 ± 0.15^{e}	80.50± 0.17^{e}	7.20± 0.10^{d}	49.43± 1.03^{c}	1.67± 0.01^{b}	4.40± 0.12^{b}	14.33 ± 0.15^{b}	662.33±4.91^{d}
H. cannabinus	20.73 ± 0.15^{b}	80.50± 0.18^{e}	8.27± 0.09^{e}	47.23± 0.15^{g}	1.62± 0.07^{b}	3.33± 0.15^{a}	18.30 ± 0.12^{c}	693.67± 13.59^{d}

Values are expressed as the mean ± SEM of triplicate experiments. Furthermore, mean values carrying different superscripts vary ($P<0.05$) at 95% confidence.

Table 2. Phytochemical composition of some lesser known vegetables (mg/100g)

Sample	Flavonoid	Tannin	Saponin	Glycoside	Alkaloid	Oxalate	Phytate	Cyanate
P. Santalinoides	0.05± 0.00^{a}	N. D	6.46± 0.03^{a}	6.13± 0.01^{a}	1.12± 0.01^{a}	0.88± 0.01^{a}	7.27± 0.09^{a}	0.05± 0.00^{a}
V. doniana	0.03± 0.00$^{a, b}$	0.30± 0.00$^{a, b}$	5.12± 0.01^{b}	5.82± 0.03^{a}	1.85± 0.01^{b}	1.31± 0.00^{b}	5.37± 0.15^{b}	0.07± 0.01^{a}
B. alba	0.03± 0.00$^{a, b}$	0.21±0.03$^{a, b}$	4.44±0.02^{c}	4.16±0.01^{b}	2.01±0.02^{b}	0.87± 0.01^{a}	6.70±0.10^{c}	0.05±0.01^{a}
A. zygia	0.02± 0.00^{b}	0.22± 0.02$^{a, b}$	5.37± 0.04^{b}	4.35± 0.03^{b}	1.13± 0.01^{a}	0.82± 0.01^{a}	5.43± 0.13^{b}	0.04± 0.00^{a}
F. glumosa	0.05 ± 0.00^{a}	N. D	4.87± 0.02^{c}	5.52± 0.01$^{a, c}$	1.14± 0.01^{a}	0.85± 0.01^{a}	7.30± 0.10^{a}	0.04± 0.01^{a}
S. nigrum	0.04± 0.00^{a}	0.69± 0.02^{a}	3.99± 0.02^{d}	5.21± 0.01^{c}	1.55± 0.01^{a}	0.91± 0.00^{a}	6.40± 0.21^{c}	0.05± 0.01^{a}
H. cannabinus	N. D	0.57± 0.01^{a}	5.55± 0.02$^{a, b}$	5.34± 0.01^{c}	2.12± 0.01^{c}	0.83± 0.00^{a}	5.23± 0.07^{b}	0.03± 0.00^{a}

Values are expressed as the mean ± SEM of triplicate experiments. Furthermore, mean values carrying different superscripts vary ($P<0.05$) at 95% confidence.

Tannin content in this study ranged from not detected (N.D) to 0.69 ±0.02mg/100g. This range is similar to the value obtained by Nsor *et al.* [33], [5] for some leafy vegetables. Tannins are phenolic compounds that are water soluble with the ability to precipitate proteins from aqueous solutions. They are capable of binding to proteins, making them bio-unavailable. However, the values obtained in this study are lower than values from other plants [34,35,28].

The flavonoid content of the vegetables in this study was relatively low compared with the values reported by Akubugwo *et al.* [36]. However, the fact that these vegetables contained relatively low flavonoid levels did not deny the fact that they may yet possess some bio-active functions such as anti-oxidative, anti-allergy, anti-viral, anti-tumorigenic, anti-inflammatory, anti-microbial as well as protection against free radical damage, platelet aggregation and hepatoxins [37,38]. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage, and have strong anti-cancer and anti-ulcer activity and protection against the different levels of carcinogenesis [37].

The result of anti-nutrients (oxalate, phytate and cyanate) showed that they were present at varying levels, but mostly low. At levels of about 6%, these anti-nutrients have been reported to be deleterious in their effects [5]. For instance, oxalate is capable of binding to calcium ions (and other divalent elements), forming complexes (calcium-oxalate crystals) and rendering it bio-unavailable as the body does not absorb nor utilize it in this form [28]. The calcium-oxalate crystals so formed may also form precipitates around the renal tubules, causing renal stones as a result [38]. Similarly, cyanate content was low (in the range of 0.03± 0.00 - 0.07± 0.01mg/100 g) and non-toxic to both humans and animals at these amounts. According to Igile *et al.* [5], it has been established that diets containing high amounts of cyanide could cause cerebral damage and lethargy in both humans and animals. It could also lead to inhibition of cytochrome oxidase activity, thereby stopping the formation of ATP and the release of inorganic phosphate to body tissues. As a result, the body is deprived of needed energy, leading to death.

Phytate content was observed to be in the range of 5.23± 0.07 - 7.30± 0.10mg/100g. According to Igile *et al.* [5], a diet containing phytate in the range of 1-6% for a long period of time tends to decrease the bioavailability of mineral elements in mono-gastric animals. However, Obichi *et al.* [39] reported that phytate had been linked to the prevention of kidney stones, dental decay and calcification of blood vessels. Phytic acid is known to be a very potent chelator, forming protein and mineral-phytic acid complexes thereby decreasing protein and mineral bioavailability [40]. Also, phytate has been associated with some nutrition-related diseases such as rickets in children and osteomalacia in adult humans respectively [40]. Generally, besides the relatively high phytate content, the levels of anti-nutrients in the leafy vegetables were low to significantly interfere with nutrient utilization. They are below established levels of toxicity [28].

4. Conclusion

The results of this study showed that these seven leafy vegetables contain appreciable amounts of nutrients with potentials to improving the nutritional status and promoting the health of people, especially, in developing countries like Nigeria, where nutrient deficiencies are prevalent. The phytochemical composition showed that these vegetables may have potential medicinal uses, especially in drug formulation.

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