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(RESEARCH ARTICLE)

Phytochemistry, proximate and antioxidant properties of some indigenous leafy vegetables

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Abstract

The use of leafy vegetables in diet is a common practice among the people of the Northern ethnic groups of Ghana. In this study, a survey was conducted in the Bunkpurugu-Yunyoo district and Kanvilli of the Tamale Metropolis of the Northern Region of Ghana, to document the indigenous leafy vegetables routinely consumed as part of their alimentary culture. The phytochemical content, proximate composition and antioxidant properties of the plants cited were assessed. Nine leafy vegetables: *Amaranthus cruentus, Hibiscus sabdariffa, Corchorus olitorius, Vernonia amygdalina, Phaseolus vulgaris, Ipomoea batatas, Adansonia digitata, Moringa oleifera* and *Annona reticulate* were inventoried. These plants contained various groups of phytochemicals and were generally rich in carbohydrates, protein and fibre, but low in fat. They contain an appreciable amount of energy and also demonstrated good antioxidant activities. These characteristics of the vegetables suggested their usefulness in the maintenance of good health, which may also explains why these groups of Ghanaians in the Northern region are regarded as being very strong and healthy.

Keywords: Corchorus; Amygdalina; Moringa; Phaseolus; Adansonia

1. Introduction

Leafy vegetables (LV) are edible leaves often used as components of food [1]. They may consist of young, succulent stems, flowers and very young fruits together with the leaves [2].

Leafy vegetables play vital roles in human nutrition; serving as valuable sources of minerals, vitamins proteins and fibre for the majority of people. In developing countries, they are usually consumed, though, in relatively small amounts as side dishes [3, 4]. LV contain high amounts of dietary fibre, which helps to regulate the digestive system, manage the body's weight and improve health [5]. Aside these benefits, they have been strongly associated with good health and vision, reduced risks for some forms of cancer, stroke, diabetes, anaemia, gastric ulcer and also treat haemorrhoids, gallstones, obesity and constipation [6, 7]. They contribute to the reduction of malnutrition, especially in children, by their content of protein, vitamins, calories and minerals needed in diets [8, 9]. They also contain non-nutrient bioactive phytochemicals that have been reported to offer protection against cardiovascular diseases and other ailments [10]. They are, in addition, good sources of naturally occurring antioxidant compounds that inhibit or delay the oxidation of biomolecules by inhibiting the initiation or propagation of oxidizing chain reactions [11]. They thus protect body cells from damage caused by oxidative stress, which is linked to several chronic diseases such as cancers, diabetes mellitus, cardiovascular diseases and several neurodegenerative disorders in humans [11].

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In Africa, leafy vegetables are increasingly being recognized as possible contributors of both micronutrients and bioactive compounds in diets [12]. However, Ghanaian diet comprises mainly of starchy roots, fruits and cereals [13]. Thus, according to the Food and Agricultural Organization [13], the dietary supply, though meets population energy requirement, is lower in proteins and lipids than recommended. The people from the northern part of the country however have consumed indigenous leafy vegetables (ILV) as a major component of their diet for generations, and this has become part of their culinary culture. This study inventoried ILV consumed in two communities in the Northern Region of Ghana and evaluated their phytochemical constituents, antioxidant properties and proximate composition to assess their nutritional value as well as other health benefits to the people.

2. Material and methods

2.1. Study area

The sites chosen for the survey were two communities: Bunkpurugu of the Bunkpurugu-Yunyoo District and Kanvilli of the Tamale Municipality, both in the Northern Region of Ghana (Fig. 1). This region has a rainfall, humidity, temperature and vegetation typical of the savannah zone.

Figure 1 District map of the Northern Region of Ghana showing the study areas: (A) Bunkpurugu-Yunyoo District and (B) Tamale Municipality. Inset: map of Ghana. Source: adapted from Wikimedia Creative Commons.

2.2. Survey data collection

Information on the vegetables was collected using a semi-structured validated questionnaire. Informants' consent was initially requested by administering informed consent forms following the explanation of the purpose of the study to the participants. The forms were filled and willingly signed or thumb-printed after which the questionnaires were administered. A total of 40 indigenes, 20 from each community, were interviewed using the prepared questionnaire as a guide. The respondents who were mostly farmers and residents within the communities for at least the past 10 years were interviewed. Demographic data of the respondents were first collected on their gender, age, ethnicity, religion, level of formal education, main occupation and duration of residence within the community. Questions related to the vegetables included respondent's knowledge of indigenous vegetables used in preparing food in the areas, purposes for using particular indigenous vegetables as part of their meals if any, frequency of their inclusion in diets, the form in which they are prepared, if the vegetables are also used as ornamental plants, their seasonal availability, whether grown or cultivated among others. Oral and informal interviews were conducted with both closed and open-ended questions. This was followed by collection of plant samples from their respective fields.

2.3. Collection, processing and extraction of plant materials

During the field visits, samples of the plant species were collected to prepare herbarium specimens and for further studies. The identities of the plant species were authenticated in the Departments of Horticulture, Food Science and Pharmacognosy, all of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Specimens with voucher numbers were deposited at the Herbarium of the Department of Herbal Medicine, KNUST.

The taxonomic validity of the plant names were checked using the plant database; www. thePlantList.org (accessed January, 2017). The remaining samples were cleared of all extraneous materials, air-dried at room temperature for about two weeks and milled into a coarse powder. The dried powdered plant materials were accordingly analysed as described below.

2.4. Phytochemical screening

Plant materials were tested for the presence of the various classes of secondary metabolites using standard methods [14, 15].

2.5. Total antioxidant capacity using phosphate molybdenum (PM) assay

The antioxidant assay was based on the reduction of molybdenum, Mo^{6+} to Mo^{5+} by the extracts and subsequent formation of a green phosphate molybdate (Mo^{5+}) complex in an acidic medium [16]. Ammonia molybdate (4 mM), disodium hydrogen phosphate (28 mM) and sulphuric acid (0.6 M) were added together in a beaker to prepare the reagent solution. Test tubes containing 1 ml each of the different concentrations of the extract (31.25 - 500 µg/ml) and 3 ml of the reagent were incubated at 95˚C for 90 minutes. The process was repeated for concentrations of ascorbic acid as a standard (3.125 -100 µg/ml). A blank solution was prepared by adding 1 ml of methanol and the prepared reagent solution without the extract or standard. After the mixture had cooled to room temperature, the absorbance of the solutions were determined at 695 nm using the UV- visible spectrophotometer (Shimadzu, 1201, Japan). The experiment involving ascorbic acid was used to construct a calibration curve. The antioxidant capacity was expressed as µg of ascorbic acid equivalent (AAEq) per g of extract.

2.6. 2, 2 Diphenyl-picryl hydrazl (DPPH) radical scavenging assay

Free radical scavenging activity was determined as described by Govindarajan et al. [17]. About 1 ml aliquot each of the different concentrations of the extract (31.25- 500 µg/ ml) was added to a 3 ml methanol solution of DPPH (20 mg/L) in a test tube. The reaction mixture was kept at room temperature in the dark for 30 minutes. A blank determination was done by adding 1 ml aliquot of methanol and 3 ml of DPPH solution together. The absorbance of the residual DPPH was determined at 517 nm in UV- visible spectrophotometer (Shimadzu, 1201, Japan). Ascorbic acid was used as the standard. Percentage DPPH inhibition was evaluated by comparing the test and blank solutions as follows:

$$\% \, DPPH \; inhibition = 1 - \frac{As}{A} x \, 100$$

Where A is absorbance of the blank and As the absorbance of the test sample. The IC_{50} value (the concentration at 50% inhibition) was determined from the curve of percentage inhibition and log concentration.

2.7. Total phenolic content

The total phenolic content of the extracts was quantified using the Prussian Blue method [18] with some modification. Gallic acid was used as the reference substance. About 0.1 ml of the various concentrations of the extract (31.25- 500 µg ml^{-1}) and gallic acid (3.125 -100 µg ml^{-1}) were transferred into test tubes and diluted with 3 ml deionized water. To each test tube 1 ml each of $K_3Fe(CN)_6$ (0.008 M) and $FeCl_3$ (0.01 M) was added and left in the dark for 15 minutes. About 5 ml of tragacanth was added to each test tube as a stabilizer and the absorbance of the solutions measured at 725 nm wavelength using the UV- visible spectrophotometer (Shimadzu, 1201, Japan). The concentrations of gallic acid were used to construct a calibration curve and the total phenol content in grams was determined as Gallic Acid Equivalent (GAEq mg/g) of the extract.

2.8. Proximate analysis

Proximate analysis of the samples was carried out according to the methods recommended by the Association of Official Analytical Chemists [19]. The analyses covered the seven proximate factors - moisture, protein, fat, ash, crude fibre, total carbohydrate and energy in fresh vegetables. Moisture content was determined by the oven drying method; ash by furnace dry ashing; crude fibre by an AOAC [19] method; crude protein by the Kjeldahl procedure; crude fat by Soxhlet extraction method and carbohydrate by calculation. Energy value of the samples were estimated in kilojoules per kg of sample by multiplying the protein, fat and carbohydrate percentages by the factors 16.7, 37.7 and 16.7, respectively, and then adding the results [20].

2.8.1. Crude fat content

Five grams (5 g) of powdered plant sample was soxhlet extracted with petroleum ether into a flask previously dried at 110˚C for 5 min and weighed. The extraction was carried out for about 4 hours after which the solvent was completely evaporated on water bath. The flask was placed in a desiccator and cooled to room temperature to remove any residual solvent. It was re-weighed and the percentage fat content of the sample calculated [19].

2.8.2. Crude fiber content

Crude fiber was estimated by acid-base digestion with 1.25% H_2SO_4 and 1.25% NaOH solutions [19]. The dried defatted residue was transferred into a dried clean digestion flask. About 200 ml of 1.25% H_2SO_4 solution was added and boiled for 30 min under reflux in the presence of an anti-foaming material. The boiled material was filtered and the residue washed with several portions of hot water until there was no trace of acid. The washed residue was put back into the flask and refluxed with 200 ml of 1.25% NaOH solution for another 30 min. The content was filtered through a weighed Gooch crucible and thoroughly washed with hot water and then with about 15 ml of 95% ethanol. The crucible with the content was dried at 110˚C to constant weight. The material (in the crucible) was incinerated in a muffle furnace at 550˚C for 30 min when the carbonaceous matter was consumed with only ash left. It was cooled in a desiccator and weighed. The loss in weight was recorded as crude fiber. The percentage crude fiber content was then calculated.

$$\% \text{ crude fiber} = \frac{A-B}{C} x\ 100$$

Where A = weight of dry crucible and sample
B = weight of incinerated crucible and ash
C = weight of sample

2.8.3. Crude protein content

Approximately, 2 g of powdered plant sample was weighed into a 500 ml digestion flask. This was hydrolyzed with 20 ml conc. H_2SO_4 containing selenium catalyst tablet and boiling chips under a fume cupboard into a clear solution. The cooled digest was diluted with distilled ammonia-free water to 100 ml in a volumetric flask and used for the analysis. About 10 ml of the hydrolysate was transferred into a clean Kjeldahl distillation flask and 90 ml distilled water followed by 20 ml of the 40% NaOH were added. The mixture was distilled onto 10 ml of boric acid solution laced with a few drops of methyl red/methylene blue indicator. About 150 ml of distillate was collected and titrated against 0.1 N HCl until the first appearance of pink colour was observed. A reagent blank with an equal volume of distilled water was titrated. The nitrogen content and therefore the protein content was calculated using the formulae below:

$$\text{Total Nitrogen } (N_T)\ (\text{g /kg}) = \frac{(Va - Vb)N}{Ws\ x\ 10} x14.01$$

Where Va = titre value of acid
Vb = titre value of blank
N = Normality of acid
Ws = weight of sample in grams
Therefore
% Crude Protein (CP) = Total Nitrogen (N_T) x 6.25 (Protein factor) [19]

2.8.4. Moisture content

Approximately 5.0 g sample was weighed into a previously weighed moisture can. The sample in the can was dried in the oven at 105˚C for 3 h. It was cooled in a desiccator and weighed. It was again returned to the oven for further drying. Drying, cooling and weighing were done repeatedly at hourly interval until constant weight was achieved. The weight of moisture lost was calculated and expressed as a percentage of the weight of sample analysed [19]. It was given by the expression below:

$$\text{Moisture content} = \frac{Wf-Wd}{Wf} x\ 100$$

Wf = Fresh weight of sample
Wd = dried weight of sample

2.8.5. Ash content

About 2 g of sample was weighed into a previously weighed porcelain crucible and heated to ash in a furnace at 550˚C. After complete combustion, the crucible was cooled in a desiccator and reweighed. The percentage ash content was then determined as below [19]:

$$\% \text{ Ash} = \frac{Wi - Wf}{Ws} x\ 100$$

Where Wi = weight of crucible with ash
Wf = weight of crucible only
Ws = weight of sample (2 g)

2.8.6. Carbohydrates content

Carbohydrate content was determined after completing the analysis for ash, crude fibre, ether extract and crude protein. The percentage carbohydrate content was calculated adding the percentage values on dry matter basis of the analysed contents and subtracting them from 100% crude substance on dry matter basis [19].

Calculation
% Carbohydrate = 100% - (% ash + % crude fibre + % crude fat + % protein)

3. Results

3.1. Respondents' demographics

The survey to document leafy vegetables consumed in the Northern Region of Ghana was carried out in 2 communities: Bunkpurugu of the Bunkpurugu-Yunyoo district and Kanvilli of Tamale municipality starting from May to July, 2016. The respondents were adults above 20 years and have been residents of the areas for more than 10 years. They were both females and males with the former constituting 62.5% of the participants. Indeed, in northern Ghana, it is the woman's chore to cook, and they often make culinary decisions on behalf of the whole family.

3.2. Plant species cited as indigenous leafy vegetables

The results showed the wide range of indigenous leafy vegetables which could be obtained either from the wild or cultivated farms. Nine (9) species belonging to 9 genera in 7 families were mentioned (Table 1.0; Fig. 2). The Malvaceae was the predominant family encountered and had 3 species mentioned; all other families had just a species each.

Table 1 Plant species use as indigenous leafy vegetables

Family	Scientific name	Local name (Dagbani/Mamprusi)
Amaranthaceae	*Amaranthus cruentus* L.	Aleefu
Annonaceae	*Annona reticulate* L.	Dasaaluok
Asteraceae	*Vernonia amygdalina* Del.	Shuwaka
Convolvulaceae	*Ipomoea batatas* (L.) Lam	Wulijo vari
Fabaceae	*Phaseolus vulgaris* L.	Bangli
Malvaceae	*Hibiscus sabdariffa* L	Braa
Malvaceae	*Corchorus olitorius* L.	Ayoyo
Malvaceae	*Adansonia digitata* L.	Kuuka
Moringaceae	*Moringa oleifera* Lam	Jangbaduuk

Figure 2 Photographs of indigenous leafy vegetables cited in the study

3.3. Phytochemical constituents of cited plants

The plant materials contained various phytoconstituents, which included tannins, flavonoids, glycosides, alkaloids, steroidal and non-steroidal saponins (Table 2.0). All plant materials tested positive for glycosides, tannins and flavonoids. *V. amygdalina, C. olitorius* and *A. digitata* contained all the phytoconstituents considered. All but *H. sabdariffa* contain saponins. These phytoconstituents may contribute variously to the health benefits of these leafy vegetables.

Table 2 Phytochemical constituents of the leafy vegetables

Scientific name	Tannins	Flavonoids	Reducing sugars	Alkaloids	Saponins	Sterols
A. cruentus	+	+	+	_	+	+
A. reticulata	+	+	+	+	+	-
V. amygdalina	+	+	+	+	+	+
I. batatas	+	+	+	+	+	_
P. vulgaris	+	+	+	_	+	_
H. sabdariffa	+	+	+	+	-	-
C. olitorius	+	+	+	+	+	+
A. digitata	+	+	+	+	+	+
M. oleifera	+	+	+	+	+	-

Key: '+' denotes presence and '-' absence of constituent.

3.4. Total antioxidant assay

The total antioxidant capacity was expressed as Ascorbic acid equivalent of the sample. The value measures the equivalence of ascorbic acid contained in 1 g of the plant extract (Table 3.0) and is assumed to be proportional to the total antioxidant capacity of the sample. All the plants had appreciable antioxidant capacity with A. cruentus having

the highest Ascorbic acid equivalence of 129.3±27.28 µg/g and hence a high antioxidant capacity. A. digitata had the least Ascorbic acid equivalence of 88.80±5.274 µg/g and hence a low antioxidant capacity.

The antioxidant property of these plants indicates their potential usefulness in reducing oxidative stress which contributes to many chronic diseases such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, stroke, septic shock, aging and other degenerative diseases in humans [21].

Table 3 Ascorbic acid equivalence for the various samples

Sample	Ascorbic acid equivalence (µg/g of extract)
A. cruentus	129.30±5.28
A. reticulata	71.95±4.37
V. amygdalina	99.47±4.19
I. batatas	98.06±3.73
P. vulgaris	97.35±1.36
H. sabdariffa	108.50±2.55
C. olitorius	103.40±5.18
A. digitata	88.80±2.27
M. oleifera	62.37±3.82

3.4.1. Radical scavenging assay using 2, 2 diphenyl-picrylhydrazl (DPPH)

The DPPH radical scavenging capacity was expressed as the IC_{50} value (Table 4.0), which is a measure of the amount of the sample needed to scavenge 50% of the free radical, DPPH. All the plants showed free radical scavenging properties with *P. vulgaris* having the lowest IC_{50} of 231.57±2.3 µg ml^{-1} and hence the highest free radical scavenging capacity. *A. digitata* had the highest IC_{50} of 487.06±3.79 µg ml^{-1} and hence the least free radical scavenging capacity. This thus confirms the low antioxidant capacity observed above for *A. digitata*. The free radical scavenging capacity contributes to the antioxidant property of the plants. High radical scavenging capacity may indicate a better antioxidant property of the plant.

Table 4 IC_{50} values of plant extracts and Ascorbic acid

Plant material	IC_{50} (µg/ml)
A. cruentus	408.0±3.1
A. reticulata	261.4±7.74
V. amygdalina	438.74±6.94
I. batatas	233.2±7.19
P. vulgaris	231.57±2.3
H. sabdariffa	438.06±4.65
C. olitorius	288.0±2.76
A. digitata	487.06±3.79
M. oleifera	489.00±2.82
Ascorbic acid	89.34±6.766

3.5. Total phenolic content

The total phenolic content was measured in terms of the Gallic acid equivalent of the sample expressed as the µg of Gallic acid equivalent in 1 g of plant extract as shown below (Table 5.0). The value is an indication of the amount in µg of the Gallic Acid Equivalent (GAEq) in 1 g of the plant extract. All the plants contained phenols and hence showed antioxidant properties. *A. digitata* having the highest GAEq of 438.4±7.91 µg/g and hence a high total phenol content

and *H. sabdariffa* having the least GAEq of 35.0±46.55 µg/g and hence a low total phenol content. The presence of the phenols though may contribute to the antioxidant activity does not translate into significant antioxidant capacity.

Table 5 Gallic Acid Equivalence of samples

Sample	GAEq (µg/g of extract)
A. cruentus	131.2±1.63
A. reticulata	396.5 ± 44.03
V. amygdalina	179.4±3.10
I. batatas	287.1±0.024
P. vulgaris	227.1±23.97
H. sabdariffa	35.0±46.55
C. olitorius	247.5±7.175
A. digitata	438.4±7.91
M. oleifera	163.4±17.12

3.6. Proximate content of the cited vegetables

The samples were analysed for moisture, ash, lipid, protein, carbohydrate and energy contents (Table 6.0). Among the 3 nutrients: carbohydrate, protein and fat, carbohydrate content was the highest and fat the lowest for all samples. The values indicates the nutritional importance of the vegetables to enhance growth, development and maintenance of health. *A. reticulata* and *M. oleifera* had the highest fibre content. This being an important factor for digestive health and regular bowel movements, can improve cholesterol and blood sugar levels and can assist in preventing some diseases such as diabetes, cardiovascular disease and bowel cancer [6, 7]. These 2 vegetables would therefore be useful for digestion and good bowel movement and maintenance of health. The moisture content ranges from 5% in *A. digitata* and *C. olitorius* to 13% in *I. batatas*. Moisture is an important factor in food quality and preservation [22]. *A. reticulata* has the highest total ash content of 46.71 and *V. amygdalina* the lowest of 4.73%. Total Ash/Minerals Ash refers to the inorganic residue remaining after complete oxidation of organic matter in a foodstuff. The ash content is a measure of the mineral content and other inorganic matter in food [23].

Table 6 Proximate composition of samples

Plant	Moisture (dry)	Ash	Fiber	Protein	Carbohydrate	Fat	Energy
A. cruentus	7	22.72	18.05	28	34.28	8	13416.76
A. reticulata	5.7	31.41	46.71	19.63	37.26	6	11762.63
V. amygdalina	10	4.73	18.78	30.13	51.14	4	15080.09
I. batatas	13	7.7	23.51	26.19	48.11	5	14293.10
P. vulgaris	8	11.61	22.05	31.69	47.7	1	13635.13
H. sabdariffa	11	6.25	19.09	32.03	48.69	2	14234.24
C. olitorius	5	21.15	20.77	24.69	45.16	4	13172.95
A. digitata	5	9.5	22.26	13.81	65.69	6	15538.50
M. oleifera	6	15.49	45.40	29.13	38.38	11	15421.17

Energy was measured in KJ/Kg dry matter

4. Discussion

Indigenous leafy vegetables have been a common component of the diet of the people of the northern ethnic groups of Ghana. Various reasons were assigned for the consumption of this plant part in the communities. According to the participants in this study, the consumption of leafy vegetables is a culinary tradition, and some foods are not eaten without them. For example, corn flour meals such as "Tuo zaafi" is often if not always eaten with *C. olitorius* soup. Aside the cultural reason, the people also take leafy vegetable diets for their nutritional, health and therapeutic

benefits. Some people consumed them just to maintain good health. *H. sabdariffa* for example is said to enhance bowel emptying, thus preventing constipation and unnecessary straining. *V. amygdalina* and *M. oleifera* may be routinely added to meals in order to manage chronic health conditions such as diabetes and hypertensive. Indeed, previous reports confirmed the antidiabetic and antihypertensive activities of *V. amygdalina* [24, 25] and *M. oleifera* [26, 27]. *A. digitata* is habitually used in the diet to maintain good oral health or to treat mouth sores. Of course, *A. digitate* is known to contain large amounts of ascorbic acid [28], which is important for good oral health [29]. *A. reticulata* for indigestion and constipation. Indeed other studies confirmed some of these activities [30–32]. The people cook the leafy vegetables in the preparation of soups and stews and these processes may have serious implications for the vitamins in the plant materials.

Due to the rapid urbanization of the northern region of Ghana, it is very necessary and important to document the indigenous leafy vegetables used in these areas and evaluate their nutritional and health properties; this would prevent the loss of such vital heritage with time.

The survey results demonstrated the rich flora of the Northern region of Ghana in terms of the availability of different types of indigenous leafy vegetables, which offer varieties and broaden the food base for the people. Interestingly, these leafy vegetables are obtained both from the wild and from cultivated farms in season. Unfortunately, some of these vegetables are annuals and are only available for a short period (about 3 months) of the year due to the long drought season that characterize the Northern part of Ghana. Leaves from perennial plants are not always available as these are shed during the drought. This condition may account for the high incidence of the reported undernourishment in that part of the country [13, 33] despite the rich dietary culture of the people.

5. Conclusion

The Northern Ghana is endowed with a rich biodiversity which include indigenous plants which serve as leafy vegetables and form part of the diet of the people. The vegetables are rich in antioxidants, the 3 classes of nutrients: carbohydrate, protein and fat. They also contain appreciable amounts of fibre and energy and are useful in the maintenance of good health. The regular consumption of the indigenous leafy vegetable in the diet may explain why these groups of Ghanaians are often regarded as very strong and healthy.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare no conflict of interest

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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