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COMPARATIVE PROXIMATE AND PHYTOCHEMICAL ANALYSES OF LEAFY VEGETABLES IN LAGOS STATE

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Abstract

A systematic survey of leafy vegetables in three different markets in Lagos state, Nigeria was carried out to compare their proximate and phytochemical compositions. The vegetable samples were oven dried for two weeks, pulverized into powdery form and soaked in ethanol for extraction of bioactive constituents. The proximate and phytochemical analyses were carried out following standard procedures. A total of sixteen vegetable samples were collected, six are used as spices while others are taken either raw or cooked as vegetables. The phytochemical analysis of the methanolic extracts revealed that the all the leafy vegetables studied are rich in flavonoids, tannins (except in *Laurus nobilis* – bay leaf), saponins (except in *Amaranthus viridis* - slender amaranth) and alkaloids (except *Murraya koenigii* – curry leaf and *Amaranthus hybridus* – green amaranth); with considerable amount of phenols, anthraquinones and phlobatannins while cardiac glycosides were recorded in 75% of the samples studied. Analysis of the proximate composition of the various leafy vegetables examined varied with each species however the ranges recorded across the samples are as follows: moisture content (2.4 – 19.8%), crude fiber (9.8 – 22.4%), total ash (9.0 - 20.3%), crude lipid (0.5 – 13.5%), carbohydrates (0.25 – 9.12%) and total nitrogen (32.5 – 71.1%). This result indicates that the leafy vegetables are valuable reservoir of bioactive compounds of substantial medicinal merit and could be a good source for nutrients supplement and as a source for drug production. Therefore, if consumed in sufficient amount, these vegetables would contribute greatly towards meeting human nutritional requirement for normal growth and adequate protection against diseases arising from malnutrition.

Keywords: Bioactive compounds, Edible leaves, Medicinal plants, Proximate, Spices

Introduction

Leafy vegetables are fresh, edible portion of plant that are either eaten raw or in cooked form (Dhellot *et al.,* 2006). They constitute a highly variable group of crop plants that are grown for their edible leaves, which are rich sources of minerals and vitamin. They are mostly short-lived herbaceous plants available during wet and dry seasons of the year. Vegetables supply some elements in which other food materials are deficient; they are sources of essential minerals elements for proper growth and development (Dhellot *et al.,* 2006). They provide important vitamins for example leafy vegetables such as *Amaranthus* species, *Celosia argentea* (amaranth), *Vernonia amygdalina* (bitter leaf), *Corchorus olitorius* (Jews mallow), *Telfairia occidentalis* (fluted pumpkin) etc are good source of carotene, iron, calcium, vitamins and minerals, ascorbic acid, iodine and phosphorus (Okafor, 1983; Janick, 2011). In Nigeria numerous edible vegetable are used as sources of food while others are used in traditional medicine. They are either gathered from the wild, cultivated or imported. In nutrition, leafy vegetables are ideal for weight management as they are typically low in calories. They are useful in reducing the risk of cancer and heart disease since they are low in fat, high in dietary fiber, and rich in folic acid, vitamin C, potassium and magnesium, as well as containing a host of phytochemicals, such as lutein, beta-cryptoxanthin, zeaxanthin, and beta-carotene (Conde Nast, 2014)

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Green, leafy vegetables provide a great variety of colors from the bluish-green of kale to the bright kelly green of spinach. They run the whole gamut of flavors, from sweet to bitter, from peppery to earthy. Young plants generally have small, tender leaves and a mild flavor (Janick, 2011). In this study, leafy vegetables were collected from markets in Lagos Nigeria in order to compare their proximate and phytochemical compositions.

Materials and Methods

Collection of Samples: Three major markets in Lagos were visited for collection of samples, they are: ' Oyingbo' market in Yaba (N6 48.167, E3 38.312), ' Aiyetoro' market in Epe (N6 67.923, E3 57.546) and ' Laketu' market in Ikorodu (N6 62.121, E3 50.322) Local Government Area respectively. The plants were identified and authenticated at the University of Lagos Herbarium, Lagos, Nigeria and voucher numbers were assigned. The leafy samples collected were weighed and oven dried for two (2) weeks at 40^{o} C and packed in an airtight sampling bag.

Preparation of plant extracts: The dried samples were pulverized, 50 g of each samples were taken and soaked in methanol for 72 hrs, after which the solution was filtered using Whatman' s filter paper. The extraction solvent was evaporated using the vacuum evaporator while the extract was concentrated into paste form. The extracts were weighed and the yield was recorded. The standard proximate and phytochemical tests given for the respective compounds were performed.

Phytochemical Analysis of the Plant Extract: The concentrated extracts were subjected to qualitative test for the identification of various phytochemical constituents following the standard procedures.

Chemical tests were carried out on the methanolic extracts of the leafy vegetables to ascertain the presence of tannin, saponins, phenols, alkaloids, terpenoids, carbohydrate, flavonoids, cardiac glycosides and phlobatanins, using method described by Sofowora (1993) with minor modifications as follows:

Test for Alkaloids: 0.2 g of the extract was boiled with 2 ml of 2% hydrochloric acid, filtered and 2 drops of Dragendorff reagent was added. Orange-red precipitate showed the presence of Alkaloid.

Flavonoids: To 2 ml of the extract, 1 ml of 1 M of NaOH was added. The formation of a yellow coloration indicated the presence of flavonoids.

Saponins (Foam test): To 1 ml of extract, few drops of distilled water were added and the solution was shaken vigorously, observation of persistent foam showed the presence of saponins.

Phenols: About 1 ml of the extract was added to 1 ml of $FeCl_3$ and the resultant solution was shaken. A bluish-green solution gave an indication of the presence of phenols.

Tannins: A portion of the extract was dissolved in water and the solution was filtered. 5% Ferric chloride solution was added to the filtrate. A bluish-green color indicated the presence of tannins.

Phlobatannins: Few drops of 1% HCL was added to a portion of the extract and boiled. The formation of red precipitate indicated the presence of phlobatannins.

Anthraquinones: To 1 ml of extract, 5 ml of concentrated HCL was added. Formation of yellow precipitate indicated the presence of anthraquinones.

Cardiac glycosides: About 1 ml of the extract was dissolved in 2 ml of Acetic acid. Then, few drops of Ferric chloride solution was added, followed by the addition of 2 ml of concentrated sulphuric acid. A brown ring dividing the solution into two layers showed the presence of cardiac glycosides.

Terpenoids: To 1 ml of sample extract, 0.5 ml of acetic anhydride was added; then few drops of concentrated sulphuric acid were added. Slight bluish green coloration indicated the presence of terpenoids.

Proximate Analysis: Moisture content, total ash, crude fiber, crude lipid, were determined using standard methods (AOAC, 1999) while protein was determined by the Kjeldahl method (Pearson, 1976). Carbohydrate was determined by the difference i.e. 100– (others+ Nitrogen).

Crude fiber**:** 5 g of powdered leaves was measured into a beaker and 40 ml of 2 M sodium hydroxide and 40 ml of 2 M hydrochloride acid solution were added. The resultant solution was then filtered, dried and weighed. The dried residue was ashed for 3 hrs at 550^{o} C. The weight of residue was determined as follows:

Percentage Crude fiber

$$= \frac{\text{Initial weight– Final weight}}{\text{Weight of sample}} \times 100$$

Crude Lipid: 5g of each sample was weighed into a separating funnel; 30 ml of diethylether was added to the separating funnel and shaken vigorously for 1 min. The tap was opened to extract into a beaker. This procedure was repeated with 20 ml and 10 ml of diethylether on the sample in the separating funnel. The filtrate was concentrated in the oven at 100^{o} C and then cooled in a desiccator. The weight of residue was determined as follows:

Percentage Crude lipids

$$= \frac{\text{Initial weight – Final weight}}{\text{Weight of sample}} \times 100$$

Moisture Content: 5 g of each sample were measured and then placed in the oven at 150^{o} C for 3 hrs, drying to constant weight. The moisture content was determined thus;

Percentage moisture content

$$= \frac{\text{Fresh weight – Dry weight}}{\text{Weight of sample}} \times 100$$

Total Ash: 5 g of powdered leaves was measured into a crucible and placed in a furnace for 5 h at 550^{o} C until whitish grey ash was obtained from the crucible. The crucible was then reweighed.

$$\% \text{ total ash} = \frac{\text{Fresh weight– Dry weight}}{\text{Weight of sample}} \times 100$$

Total Nitrogen: Powdered leaves samples (12.5 g) was mixed with 100 ml of TCA (7.5% trichloroacetic acid) and homogenized for 1 min and filtered. Then, 20 ml of the filtered sample was transferred to the distillation apparatus. A few drop of antifoaming agent (silicon antifoam) and 2 ml of 20% Boric acid solution and a few drops of indicator (mixed indicator 0.1 g Bromo cresol green + 0.2 g methyl orange) were added. This mixture was distilled, collected and titrated with 0.05 M HCL.

Concentration (c) = (T x 0.7 x 190)/ V

Where T = Volume of HCl used in titration, V = volume of filtered sample used

Results and Discussion

A total of sixteen vegetable samples were collected including members of the family Amaranthaceae (18.75%), Lamiaceae (18.75%) amongst others (Table 1). Of the samples collected, six are used as spices while others are taken as vegetables either raw or cooked.

The phytochemical study revealed that the methanolic extracts of the leafy vegetables are rich in secondary metabolites such as flavonoids, saponins, alkaloids and tannins with considerable amount of phenols, anthraquinones and phlobatannins. The phytochemicals ranges from highly present like Tannins, Saponins, Alkaloids, Flavonoids, to moderately present; cardiac glycoside, phenol, terpenoids to less present; phlobatannins and anthraquinones. All the leafy vegetables studied are rich in flavonoids – a group of polyphenol compounds that are biologically active against liver toxins, microorganisms, inflammation and tumor (Okwu, 2004) as well as cataracts in diabetic patients (Okwu, and Omodamiro, 2005). Tannins, saponins and alkaloids were also recorded in all but one sample respectively.

Table 1: List of Vegetable Samples Collected in Lagos, Nigeria

S/N	**Botanical name**	**Family name**	**Common name**	**Local name**	**Place of collection**	**Voucher numbers**
1.	*Amaranthus hybridus*	Amaranthaceae	Green amaranth	*Tete abalaye*	*Laketu*	LUH 6559
2.	*Amaranthus viridis*	Amaranthaceae	Amaranth	*Soko* green	*Aiyetoro*	LUH 6570
3.	*Celosia argentea*	Amaranthaceae	Red flower cockscomb	*Soko pupa*	*Aiyetoro*	LUH 6558
4.	*Corchorus olitorius*	Tiliaceae	Jew' s mallow	*Ewedu*	*Laketu*	LUH 6562
5.	*Gnetum africanum*	Gnetaceae	African joint_fir	*Okasi*	*Oyingbo*	LUH 6564
6.	*Solanum macrocarpon*	Solanaceae	Egg plant	*Igbo*	*Laketu*	LUH 6557
7.	*Telfairia occidentalis*	Cucurbitaceae	Fluted pumpkin	*Ugwu*	*Oyingbo*	LUH 6561
8.	*Talinum triangulare*	Portulacaceae	Water leaf	*Gbure*	*Aiyetoro*	LUH 6563
9.	*Pterocarpus mildbraedi*	Fabaceae	African padauk	*Oha*	*Oyingbo*	LUH 6556
10.	*Vernonia amygdalina*	Asteraceae	Bitter leaf	*Ewuro*	*Aiyetoro*	LUH 6560
11.	*Gongronema latifolium*	Asclepiadaceae	Bush buck	*Utasi*	*Laketu*	LUH 6582
12.	*Laurus nobilis*	Lauraceae	Bay leaf		*Ikorodu*	LUH 6583
13.	*Murraya koenigii*	Lamiaceae	Curry leaf	*Efinrin oso*	*Laketu*	LUH 6555
14.	*Ocimum gratissimum*	Lamiaceae	Scent leaf	*Efinrin nla'*	*Aiyetoro*	LUH 6565
15.	*Piper guineense*	Piperaceae	Ashanti pepper/ False cubeb leaf	*Uziza*	*Oyingbo*	LUH 6554
16.	*Thymus vulgaris*	Lamiaceae	Thyme	Thyme	*Oyingbo*	LUH 6585

These phytochemical compounds have been reported by several researchers including Addae-Mensah (1992), Okoegwale and Olumese (2001), Okoegwale and Omefezi (2001) as components of traditional herbal preparations used in managing various common ailments. They are precursor for the synthesis of useful drugs and they have a wide range of pharmacological activity on the nervous system, blood vessels, respiratory system and gastrointestinal tract (Louio *et al.*, 2002). In contrast to Mensah *et al.* (2008), tannins were observed to be present in the leaves of *Vernonia amygdalina* and *Gongronema latifolium.* The observed presence and quantity of tannins in all the vegetables with exception to *Laurus nobilis* suggests that they could serve as good antioxidant, anti-diuretic and anti-diarrhea (Okwu, 2004) as well as antibacterial herbs (Banso and Adeyemo, 2007). Saponins are also largely present in the vegetable samples except in *Amaranthus viridis* and they are known to produce inhibitory effects on inflammation and precipitate and coagulate red blood cells (Okwu, 2003). Our findings contradicts Mensah *et al.* (2008), who reported that saponins are absent in *Celosia argentea, Telfairia occidentalis and Gnetum africanum.* Alkaloids are also present in all the vegetables; except *Murraya koenigii* and *Amaranthus hybridus;* these have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Phenols were highly observed in the leaves of *Telfairia occidentalis* suggesting its use in anti-inflammation, cardiovascular protection, anti-aging and cell proliferation. Glycosides are known to lower the blood pressure according to many reports (Yadav and Munin, 2011); these were also recorded in 75% of the samples studied. Also, phlobatannins, anthraquinones were absent in *Vernonia amygdalina, Corchorus olitorius, Celosia argentea, Gnetum africanum* and *Solanum macrocarpon* but present in *Pterocarpus mildbraedi, Amaranthus viridis, Amaranthus hybridus, Talinum triangulare,Ocimum gratissimum, Laurus nobilis* and *Murraya koenigii* (Table 2).

Table 2: Qualitative phytochemical characters of methanolic extracts of leafy vegetables in Lagos

Species	Tannins	Saponins	Alkaloids	Terpenoids	Flavonoids	Phlobatannins	Anthraquinones	Cardiac glycosides	Phenols
Vernonia amygdalina	++	+++	++	++	+	-	++	+	++
Corchorus olitorius	++	++	++	++	++	-	-	+	+
Celosia argentea	+++	++	+	++	++	-	-	++	++
Gnetum africanum	++	++	+++	-	++	-	-	+++	-
Solanum macrocapa	++	++	+++	+	++	-	-	++	+
Pterocarpus mildbraedi	+++	++	+++	-	++	+	+	++	-
Amaranthus viridis	++	-	+++	-	++	+	+	++	+
Amaranthus hybridus	++	++	-	++	+++	+	+	+	+
Talinum triangulare	+	++	++	+++	+++	+	+	-	-
Telfairia occidentalis	+	++	+	-	+	-	-	-	+++
Ocimum gratissimum	++	+	+++	-	+	+	+	++	+
Piper guineense	++	+++	+	++	++	-	+	++	+
Laurus nobilis	-	++	+	+	+	+	+	+	+
Thymus vulgaris	+++	+++	+	-	+++	+	-	-	+
Gongronema latifolium	++	++	+	+++	++	-	-	-	+
Murraya koenigii	++	++	-	+++	++	+	+	+	+

KEY: + (present), - (absent), ++ (moderately present), +++ (highly present)

Analysis of the proximate composition of the various leafy vegetables examined showed that the moisture content, crude fiber, ash, crude lipid, carbohydrates and total nitrogen contents of each species varied however the ranges recorded across the sample studied are as follows: moisture content (2.4 – 19.8%), crude fiber (9.8 – 22.4%), total ash (9.0 - 20.3%), crude lipid (0.5 – 13.5%), carbohydrates (0.25 – 9.12%) and total nitrogen (32.5 – 71.1%). This shows that the vegetable samples generally contain relatively low amount of lipids, carbohydrates and moisture content, moderate amount of crude fiber and total ash and high percentage composition of proteins (Fig. 1). Of all the vegetable samples, *Thymus vulgaris* (71.13%) had the highest nitrogen composition followed by *Ocimum gratissimum* (70.28%) while the least was recorded in *Solanum macrocarpon* (32.47%). This shows that the vegetables are protein rich and are important in building as well as repair of cells in the body.

High fibre content was recorded in *Solanum macrocarpon, Piper guineense, Laurus nobilis* and *Gongronema latifolium* which show that they can help in keeping the digestive system healthy and functioning properly as suggested by Dhingra *et al.* (2012). Total ash content was highest in *Ocimum gratissimum* followed by *Solanum macrocarpon,* this is a reflection of the amount of mineral stored up in the leaves of the plants (Ilodibia *et al.,* 2014). Moisture content was highest in *Solanum macrocarpon* followed by *Talinum triangulare,* this shows that they are the most prone to deterioration since perishability of food increases with high moisture content (Ilodibia *et al.,* 2014). Lipids among other things had been reported to help the human body to absorb fat-soluble vitamins such as vitamins A and E (Meydani and Martin, 2001). Low concentration of lipid was recorded across all the samples with the highest percentage composition in *Vernonia amygdalina* (13.5%) hence these vegetables could aid in the uptake of water soluble vitamins.

Carbohydrate composition in all the vegetables studied was generally low with the highest being 9.12% observed in *Laurus nobilis* therefore they can be seen as important food substitute for people living with high blood sugar.

Conclusion

All the plants studied have proved to be very important because of their chemical constituents; this explains their wide usage by the people of Nigeria in diets and in ethnomedicine. The result suggests that the vegetables if consumed in sufficient amount, would contribute greatly towards meeting human nutritional requirement for normal growth and adequate protection against diseases arising from malnutrition.

Conflict of interest

Authors declare no conflict of interest.

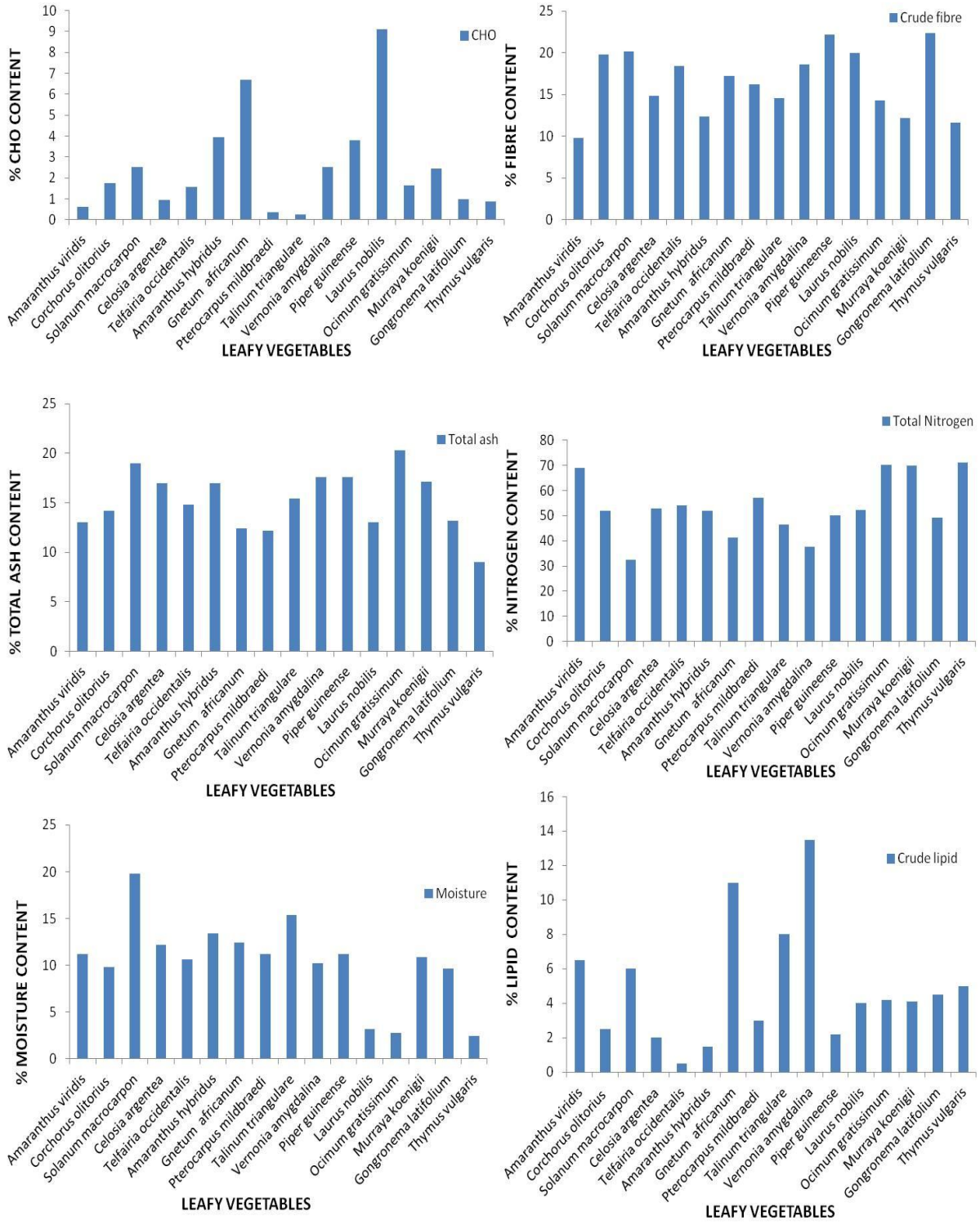

Figure 1: Proximate composition (in percentage) of the leafy Vegetables

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