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Phytochemical, proximate and anti-nutrient compositions of four leafy vegetables used in South Eastern Nigeria

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Chemical constituents of plants are influenced by environmental factors and fluctuations just as many other polygenic traits. Four different green leafy vegetables commonly used in the diets of South Eastern Nigeria were analyzed with a view to determine the phytochemicals, proximate and anti-nutrient compositions of these ecotypes. The vegetables, of which three are spices, are scent leaf (*Ocimum gratissimum* L.), fluted pumpkin (*Telfairia occidentalis*), amaranth globe (*Gongronema latifolium* Benth.) and ashanti pepper (*Piper guineense* Schumach. and Thonn.) leaves. The preliminary phytochemical analysis indicates the presence of the phytochemicals from trace amounts to strongly present. The quantitative tests showed that *O. gratissimum* had significantly ($P \leq 0.05$) the highest tannin content of 1074.94±0.009 mg/100 g. Significant variations were observed in all the other phytochemicals except in alkaloid and phenol content. Proximate and antinutrient compositions showed significant variation in the different vegetables. *T. occidentalis* had highest values in ash (13.51%), crude fibre (33.52%), protein (25.49%) and phytate (8.58 mg/100 g) contents. The results obtained in this study clearly indicate that the four leafy vegetables are readily available sources of nutrients and prove the extensive use of these vegetables in ethnomedicine; and their potential in drug formulation.

Key words: Alkaloid, ethnomedicine, proximate, spice, vegetables.

INTRODUCTION

Vegetables are the edible parts of herbaceous plants that are consumed wholly or in parts, raw or cooked as part of main dish or salad; they may be aromatic, bitter or tasteless (Dhellot et al., 2006). They include leaves, stems, roots, flowers, seeds, fruits, and bulbs. Leafy vegetables however, are source of macro and micro-nutrients that play major role in maintaining healthy living. They are regular ingredients in the diet of the average Nigerian and provide appreciable amounts of nutritive minerals (Ajewole, 1999). Even though the bulk of their weight is water, leafy vegetables represent a veritable natural pharmacy of minerals, vitamins and phyto-chemicals (George, 2003). Leafy vegetables investigated in this experiment were *Ocimum gratissimum*, *Piper guineense*, *Gongronema latifolium* and *Telfaria occidentalis.*

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T. occidentalis commonly called fluted pumpkin is a tropical vine grown in West Africa as a leafy vegetable. They belong to the family Cucurbitaceae (Akoroda, 1990). The fluted gourd grows in many nations of West Africa but is mainly cultivated in Nigeria, used primarily in soups and herbal medicines (Akoroda, 1990). Fluted pumpkin leaves have a lot of nutritive value. This made the leaves and tender stems potentially useful as food supplements (Nkang et al., 2002). The leaves contain a high amount of vitamins A and C, antioxidants, hepatoprotective and antimicrobial properties (Oboh et al., 2006). The leaf extract is useful in the management of cholesterolemia, liver problems and impaired defense immune systems (Eseyin et al., 2005). The leaves are rich in iron and play a key role in the cure of anaemia; they are also noted for lactating properties and are in high demand for nursing mothers (Okoli and Mgbeogwu, 1983). In Nigeria the fresh leaves are ground and the juice used as tonic by women that have just given birth; its high iron content assists in the replenishment of lost blood, used for treatment of anaemia, chronic fatigue and diabetes (Aderibigbe et al., 1999; Dina et al., 2006; Alada, 2010). Longe et al. (1983) reported that minerals such as calcium, potassium, magnesium, iron, sodium and phosphorus, antioxidants and vitamins such as thiamine, riboflavin, nicotinamide and ascorbic acid were present in fluted pumpkin. Phytochemicals such as phenols were concentrated in the testa, pulp and husk and amino acids such as alanine, aspartate, glycine, leucine and many others (Fasuyi and Nonyerem, 2007).

P. guineense (also called African pepper, Ashanti pepper, Benin pepper, false cubeb, Guinea cubeb, uziza pepper and Guinea pepper) belonging to the family Piperaceae are semi-cultivated in countries such as Nigeria where the leaves and seeds are used as flavouring agent (Asawalam, et al., 2007). Studies have shown that apart from the use of these plants as spices and condiments, they have several other wide applications in the local treatment and management of many diseases. It provides oil used as aromatic in the drink industry and also medicinally (Sumathykutty et al., 1999). Okwu (2001) reported that phytochemical analyses of *P. guineense* showed the presence of alkaloids, flavonoids, saponins, tannins, resins and essential oils. These phytochemicals have a lot of pharmacological properties as proved by earlier studies (Okwu, 2001). He also reported that the presence of alkaloids signified the possession of medicinal properties within the leaves. The flavonoids possessed antioxidant activity, anti-inflammatory and antiviral activities (Okwu, 2001).

O. gratissimum is a herbaceous plant which belongs to the family Lamiaceae (Calixto, 2000). It is widely distributed in tropical and warm temperature regions (Okigbo and Ogbonnaya, 2006). *O. gratissimum*, also called scent leaf is grown in gardens and used as a tea leaf for fevers (Okigbo and Ogbonnaya, 2006). It is germicidal and has found wide use in toothpastes and mouth washes as well as topical ointments (Pessoa et al., 2003; Holets et al., 2003). It is used as an excellent gargle for some throats and tonsillitis. It is also used as an expectorant and a cough suppressant. The plant extract is used against gastrointestinal helminths of animals and man (Chitwood, 2003). Phytochemical studies show that both aqueous and methanolic extracts of *O. gratissimum* are rich in tannins, steroids, terpenoids, flavoniods and hydrogen-cyanide and equally has a good antioxidant activity (Afolabi et al., 2007).

G. latifolium (amaranth globe) belongs to the family Asclepiadaceae and is a tropical rainforest plant primarily used as spice and vegetable in food (Nwosu and Malize, 2006). It is listed among the twenty-eight medicinally important vegetables of South West Nigeria (Ayodele, 2008) and also as one of the aromatic plants of medicinal importance from Nigeria (Ogunwande et al., 2007). The leaves are sharp-bitter and sweet and widely used as a leafy vegetable and as a spice for sauces, soups and salads. *G. latifolium* is also widely used in West Africa for medicinal and nutritional purposes. Ugochukwu et al. (2003) reported that aqueous and ethanolic extracts of Amaranth globe had hypoglycemic, hypolipidemic and antioxidative properties while Morebise et al. (2002) showed that it has anti-inflammatory properties. They also have industrial uses in brewing beer. Ogundipe et al. (2003) discovered that leaves of Amaranth globe were possible hop substitute for brewing beer and that water extracts of the powdered leaves gave low bittering values, but extraction of the powdered leaves with organic solvents significantly increased analytical bitterness to levels comparable with hops.

It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect (Afolabi et al., 2007). The medicinal value and multiple biological functionalities of several plants are defined by their phytochemical constituents (Fallah et al., 2005). The medical values of leafy vegetables are dictated by their phytochemical and other chemical constituents (Fallah et al., 2005), However, these chemical constituents including the phytochemicals are influenced by environmental factors just as many other polygenic traits. Therefore, there is need to determine the chemical compositions of the eco-types grown at Enugu, South Easthern, Nigeria. The aim of this study was to determine the chemical compositions, proximate, mineral and phytochemical constituents, of four leafy vegetables, out of which three also serve as spices, and to relate the above to their nutritional and the ethno medicinal uses by the indigenous population of South Eastern Nigeria.

MATERIALS AND METHODS

Collection and identification of plant material

The leafy vegetables, *O. gratissimum*, *P. guineense*, *G. latifolium* and *T. occidentalis*, were collected from Enugu, in Enugu state, South Eastern Nigeria. The specimens were properly identified and

Table 1. Qualitative phytochemical screening of the leaves of *O. gratissimum*, *P. guineense*, *G. latifolium* and *T. occidentalis.*

Phytochemical	*O. gratissimum*	*P. guineense*	*G.latifolium*	*T. occidentalis*
Alkaloid	++	++	++	+
Glycoside	++	+	+	++
Saponin	+	+	+	+
Tannin	+++	++	++	++
Flavonoid	++	++	++	++
Steroid	++	++	+	+
Terpenoid	++	++	++	+
Phenol	++	+++	+++	++++
HNC	++	+	+	++

++++ = Very strongly present; +++ = strongly present; ++ = present; + = Trace; HNC, hydrogen cyanide.

authenticated in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Preparation of plant extracts

Drying process

The samples were thoroughly washed, air dried under shed for seven days at room temperature, to avoid loss of active compounds. The dried leaves were ground to powder using electric grinder. The powdered samples were then stored in appropriately labeled air-tight bottles for further use (Trease and Evans, 2002).

Extraction process

Aqueous extracts were used for the phytochemical analysis. The process was hot water method following the procedure of Handa (2008) and Handa et al. (2008).

Phytochemical, proximate and anti-nutrient analyses

Both the proximate, antinutrients, qualitative and quantitative phytochemical analyses of the leafy samples of *O. gratissimum*, P. *guineense*, G*. latifolium* and *T. occidentalis* were carried out to detect the presence of some secondary metabolites using the standard methods by the Association of Official Analytical Chemistry (AOAC, 2002). The phytochemicals tested for were alkaloids, saponins, glycosides, tannins, steroid, terpenoid, flavonoids, Hydrogen cyanide and Phenols.

RESULTS

The qualitative analysis showed a trace amount of alkaloid in *T. occidentalis* and the presence of alkaloid in the other three samples. Glycoside was present in *O. gratissimum* and *T. occidentalis* but showed trace amount in *P. guineense* and *G. latifolium*. Saponin was in trace amount in all the four leaf samples. Phenol was present in *O. gratissimum,* strongly present in *P. guineense* and *G. latifolium* but very strongly present in *T. occidentalis.* Similar variations were observed in the test for Tannin, hydrogen cyanide, terpenoid and steroid however, flavonoid was present in all the samples (Table 1). The quantitative assessment of the phytochemical constituents showed significant variations in the actual compositions of the different phytochemicals in the vegetables (Table 2). All the phytochemical components were significantly different from each other at $P \leq 0.05$. The tannin content of the vegetables ranged from 716.6 mg/100 g in *P. guineense* to 1074.94 mg/100 g in *O. gratissimum*.The values for flavonoid, saponin, glycoside and steroid also ranged from 229.6 mg/100 g to 235.8 mg/100 g, 0.86 mg/g to 0.97 mg/g, 5.45 mg/g to 6.48 mg/g and 5.51 mg/g to 9.67 mg/g, respectively (Table 2). *G. latifolium* had the highest values in alkaloid and terpenoid while the least was recorded in *T. occidentalis*

The result of the proximate composition on the leaves of *O. gratissimum, P. guineense, G. latifolium* and *T. occidentalis* showed that the moisture content ranged from 2.58% in *T. occidentalis* to 9.14% in *O. gratissimum* (Table 3). Percentage protein ranged from 17.16 to 25.49% among the vegetables. Variations in the percentage compositions of ash content, crude fibre, fat and carbohydrate were also observed in the different leafy vegetables. The Anti- nutrient composition showed the presence of Phytate and oxalate (Table 4). The value of phytate in *O. gratissimum* (1.26 mg/100 g) differed significantly from *P. guineense* (1.57 mg/100 g) but there was no significant difference between the values in *T. occidentalis* and *G. latifolium*. The values for oxalate across the vegetables ranged from 3.89 - 8.58%.

DISCUSSION

The study revealed that the extracts of these vegetables are rich in phytochemicals such as saponins, alkaloids, flavonoids, terpenoids, steroids, glycosides, tannins, hydrogen cyanide and phenols. Most of these

Table 2. Quantitative phytochemical compositions of *O. gratissimum, T. occidentalis, G. latifolium* and *P. guineense* leaves.

Sample	Tannin (mg/100 g)	Flavonoid (mg/100 g)	Glycoside (mg/g)	Saponin (mg/g)	Steroid (mg/g)	Terpenoid (mg/100 g)	Phenol (mg/100 g)	HNC (mg/g)	Alkaloid (mg/100 g)
O.gratissimum	1074.94^{a**} ± 0.009*	2320.09^{c} ± 0.008	6.39^{b} ± 0.004	.91^{b} ± 0.006	7.19^{b}±0.004	11.78^{b} ±0.006	983.88 ±0.006	.65^{a} ± 0.005	1406.51 ± 0.006
T. occidentalis	859.96^{c} ± 0.008	233.34^{b} ± 0.006	6.4^{a} ± 0.006	.89^{c} ± 0.004	5.51^{d} ±0.003	10.64^{d} ±0.005	1403.23 ±0.005	.38^{c} ± 0.002	677.51 ± 0.005
G. latifolium	906.91^{b} ± 0.006	229.63^{d} ± 0.004	5.84^{c} ± 0.004	.86^{d} ± 0.006	9.67^{a} ±0.005	12.37^{a} ±0.004	1291.26 ±0.005	.45^{b} ± 0.005	1577.24 ± 0.005
P. guineense	716.63^{d} ± 0.006	235.81^{a} ± 0.008	5.45^{d} ± 0.007	.97^{a} ± 0.009	5.62^{c} ±0.004	11.32^{c} ±0.005	1290.33 ±0.006	.65^{a} ± 0.006	1132.79 ± 0.006
L.S.D (P ≤ 00.05)	00.014	0.012	0.009	0.013	0.007	0.009	NS	0.008	NS

HNC, Hydrogen cyanide. * Values are mean ± SD; NS, not significant. ** Values followed by different letters are significantly different from each other.

Table 3. Proximate Composition (%) of the leaves of *O. gratissimum, P. guineense, G. latifolium* and *T. occidentalis.*

Sample	Moisture	Protein	Ash	Fat	Fibre	Carbohydrate
O. gratissimum	9.14^{a} ± 0.003	19.88^{b} ± 0.004	10.58^{c} ± 0.007	10.25^{c} ± 0.006	12.57^{d} ± 0.006	37.58^{b} ± 0.018
T. occidentalis	2.58^{c} ± 0.003	25.49^{a} ± 0.004	13.52^{a} ± 0.005	12.62^{b} ± 0.004	33.52^{a} ± 0.008	12.28^{d} ± 0.020
G. latifolium	4.63^{b} ± 0.005	17.16^{d} ± 0.003	10.13^{d} ± 0.003	10.14^{d} ± 0.004	16.58^{c} ± 0.005	41.36^{a} ± 0.007
P. guineense	4.63^{b} ± 0.004	19.36^{c} ± 0.007	11.39^{b} ± 0.004	14.63^{a} ± 0.004	21.37^{b} ± 0.004	28.62^{c} ± 0.013
L.S.D (P ≤ 00.05)	0.007	0.009	0.009	0.008	0.011	0.029

Table 4. Anti-nutrient composition of *O. gratissimum, T. occidentalis, P. guineense,* and *G. latifolium* leaves.

Sample	Oxalate (mg/100 g)	Phytate (mg/100 g)
O. gratissimum	1.26^{b} ± 0.008	3.9^{c} ± 0.005
T. occidentalis	0.94^{c} ± 0.003	8.58^{a} ± 0.003
G. latifolium	0.94^{c} ± 0.006	7.8^{b} ± 0.002
P. guineense	1.57^{a} ± 0.003	7.8^{b} ± 0.007
L.S.D (P ≤ 00.05)	0.009	0.01

phytochemical constituents are potent bioactive compounds found in medicinal plant parts which are precursors for the synthesis of useful drugs. These phytochemicals generally have a wide range of pharmacological activities or actions (Louio et al., 2002). Alkaloids, the most revered of all phytochemicals are said to be pharmacologically active and their action are felt in the automatic nervous system, blood vessels, respiratory system and gastrointestinal tract (Louio et al, 2002). The observed presence and quantity of tannins in all the vegetables could be of great medicinal importance since tannins serve as good antioxidant (Okwu, 2001).

O. gratissimum had high values in all the phytochemicals tested in this study; this makes this vegetable and spice very important in the claimed pharmacological properties and uses in

ethnomedicine. Koleva et al. (2000) reported that the medicinal values of *O. gratissimum* lie in the phytochemical component which produces definite physiological actions. These components are associated with bioactivities having health impacts. From recent findings, *O. gratissimum* has proved to be useful in medication for people living with HIV and AIDS (Nwinyi et al., 2009). *P. guineense* extracts are, equally, good sources of antioxidants, which are widely believed to be important line defense against oxidative stress leading to a lot of diseases like insomnia, and diabetes.

Flavonoids were abundant in the four leafy vegetables but had significantly the highest value in *P. guineense*. They are recognised for their anti- oxidant, anti-carcinogenic, anti-microbial and anti- tumour properties (Del-Rio et al., 1997). Linus (1991) reported that flavonoids which are naturally occurring low molecular weight polyphenolic compounds located in fruits and vegetables are known to inhibit formation of plaques and streaks in arteries and as such hinder hypertension and other cardiovascular diseases. Del-Rio et al. (1997) noted that flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity. Phenols which are abundant in the four leafy vegetables have been reported to have potential as antioxidants due to their being effective hydrogen donors (Oboh and Akindahunsi, 2004) while Osuagwu and Nwosu (2006) noted that saponins were involved in ulcer protection and certain antimicrobial activity. Steroid having the highest value in *G. latifolium* was reported as being effective in regulating carbohydrate and protein metabolism and possesses anti-inflammatory properties (Omotayo and Borokini, 2012). In recent years, the prevention of cancer and cardiovascular diseases has been associated with the ingestion of spices and vegetables rich in natural antioxidants (Chu et al., 2002).

All the vegetables studied contain appreciable amount of protein which indicates that the vegetables can be used for building and repairing of body tissues, regulation of body processes and formation of enzymes and hormones. The fibre content was highest in *T. occidentalis*, followed by *P. guineense*, the substantial amount of fibre in all the vegetables showed that they can help in keeping the digestive system healthy and functioning properly. Fibre aids and speeds up the excretion of waste and toxins from the body, preventing them from sitting in the intestine or bowel for too long, which could cause a build-up and lead to several diseases (Hunt et al., 1980). The low percentage carbohydrate and fat contents in the vegetable could be an advantage in the diets of people based on age and body mass. Lipids among other things had been reported to help the human body to absorb fat-soluble vitamins such as vitamins A and E (Osborne and Voogt, 1978). That means that the low lipid content in these vegetables could be an advantage by helping uptake of water soluble vitamins. Low amounts of phytate and oxalate antinutrients were detected in the vegetables. The beneficial aspects of antinutrients and other bioactives have been discussed when used in low levels (Gemede and Ratta, 2014)

In conclusion these vegetables contain low calories and negligible quantities of utilizable energy; hence, they are ideal for obese people who can satisfy their appetite without consuming much carbohydrate. All the plants studied have proved to be very important because of their chemical constituents, this explains their wide usage by the people of South Eastern Nigeria in diets and in ethnomedicine. Even though they have anti-nutrients, their values are too small to be harmful, therefore, consumption of these vegetables is encouraged both as food for nutrients and as medicine in ethnomedecine. The authors, therefore, suggest intensified research that would be directed towards harnessing the potentials of these vegetables in drug formulation and development in addition to their daily use in diets.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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