International Food Research Journal 18(4): 1393-1399 (2011)

Nutritional qualities and phytochemical constituents of *Clerodendrum volubile*, a tropical non-conventional vegetable

[1*]Erukainure, O. L., [1]Oke, O. V., [2]Ajiboye, A. J., and [1]Okafor, O. Y.

[1]*Food Technology Division, Federal Institute of Industrial Research, Lagos, Nigeria*
[2]*College of Natural and Applied Sciences, Bells University of Technology, Ota, Nigeria*

Abstract: The nutritional qualities and phytochemical properties of *Clerodendrum volubile*, an under-utilized leafy vegetable in Nigeria were studied. Proximate analysis showed a high percentage of crude protein and ash contents (12.14%), Nitrogen Free Extract (NFE) had a higher percent (11.2%), while the highest percentage was observed in Dry matter (DM) content (93.3%). Mineral analysis revealed high contents of sulphur, chlorine, manganese, iodine, and zinc, with sulphur content being the highest (131.45 mg/kg). A very high content of vitamin A was observed in the leafy vegetable, high contents of ascorbic acid and cyanocobalamin were also observed while Niacin and Pyridoxine contents were moderately high. Phytochemical analysis showed a high concentration of phenol (4.13%), this was followed by saponin (2.54%). A low concentration was observed in alkaloids (0.79%), while tannin had the lowest concentration (0.15%). Results from this study indicate that the leafy vegetable can serve as a good nutritional source in combating malnutrition. The presence of bioactive compounds is an affirmation of the use of this leafy vegetable in the management of various ailments, and thus may serve as a source of ingredient to the pharmaceutical industries.

Keywords: ***Clerodendrum volubile*, proximate analysis, vitamin, mineral, phytochemicals**

Introduction

Malnutrition is a general term for a medical condition caused by an improper or insufficient diet. It most often refers to undernutrition resulting from inadequate consumption, poor absorption, or excessive loss of nutrients, but the term can also encompass overnutrition, resulting from overeating or excessive intake of specific nutrients. Malnutrition is the lack of sufficient nutrients to maintain healthy bodily functions and is typically associated with extreme poverty in economically developing countries. It is a common cause of reduced intelligence in parts of the world affected by famine (Wines, 2006). Malnutrition can be tremendously reduced with an increased use of foods rich in energy, proteins, iron and vitamin A most especially those from the rural environment. The lack of nutritional information and inadequate development of nutritionally improved products from local raw materials have direct bearing on nutrition. Much effort has been concentrated on seeds while leafy vegetables have to a large extent been ignored. Leaves are reportedly inexpensive and easy to cook. They are known as potential sources of minerals and vitamins (Ejoh *et al*., 1996). They are rich especially in carotenoids as well as in iron, calcium, ascorbic acid, riboflavin and folic acid and appreciable amounts of other minerals (Devadas and Saroja, 1980). Nigeria has abundance of leafy vegetables, most of which grow all year long. Most of the species are found in the rural areas and grow in the wild. Over the years, efforts have been made to domesticate these species and to study their nutritional qualities. Among such vegetables is *Clerodendrum volubile*.

Clerodendrum volubile is commonly known among other leafy vegetables in south-south Nigeria, with dearth of nutritional data base. It is commonly known among the Urhobo and Itsekiri tribes of the Niger-Delta as *Obenetete*. It belongs to the Family *Lamiaceae* (*Verbenaceae*). It is a climbing shrub to 3 m high, glabrous except the inflorescences, flowers numerous, 1½ cm long, greenish-white, yellow in throat, in subcorymbose terminal inflorescences; of deciduous forest and secondary jungle, across the Region, Senegal to Fernando Po (Burkill, 1985). Its general uses include (i) Agri-horticulture: ornamental, cultivated or partially tended; (ii) Social: religion, superstitions, magic Leaf; (iii) Food: general; and (iv) Medicines: arthritis, rheumatism, dropsy, swellings, oedema, gout, general healing, pain-killers, pregnancy, antiaborifacients, sedatives, etc (Burkill, 1985). Locally, the leaves are often dried and used as spices in cooking; sometimes the fresh leaves are blended and used for cooking.

In this study, the nutritional qualities of this under-utilized leafy vegetable consumed in south-south Nigeria were determined. The aim of the present study is to ascertain the chemical properties, nutritional composition and phytochemical properties of the leaves of *Clerodendrum volubile*, in order

*Corresponding author.
Email: *loreks@yahoo.co.uk*

to provide data, either for practical use or for basic research needs. This study will contribute to the knowledge of the non-conventional leafy vegetable that could improve its uses.

Materials and Methods

Plant materials

Fresh leaves of *Clerodendrum volubile* were purchased from a local market in Benin-City, Nigeria. They were identified and authenticated at Forestry Research Institute (FRIN) Ibadan, Nigeria. The leaves were air-dried at room temperature and grounded to fine powder, using a laboratory mill and stored in air-tight containers for laboratory analysis. All analysis was carried out in triplicates.

Chemical (proximate) analysis

Proximate analysis was carried out using the standard procedures of the Association of Official Analytical Chemists (AOAC, 1997). Dry matter (DM) content was determined by drying the sample in a vacuum oven at 100°C and dried to a constant weight. Ash content was determined by incineration of 2 g of the sample in a muffle furnace at 600^0C for 8 h. The percentage residue weight was expressed as ash content. Crude fat was determined by soxhlet extraction method using petroleum ether as solvent. Nitrogen was determined using the kjeidahl method and crude protein was calculated by multiplying the percentage nitrogen content by the conversion factor 6.25. Nitrogen Free Extract was determined by the formula: 100 – (%Moisture + %Crude protein + %Crude fat + %Ash). The total energy was determined by Gallenkamp Ballistic Bomb Calorimeter method.

Mineral analysis

Ashes obtained from the proximate analysis were digested with concentrated nitric acid. The resulting solution was evaporated to dryness and dissolved in 100 ml deionised water. The solution was analysed for minerals using atomic absorption spectrophotometer and flame photometer. Sulphur was determined by the Turbidimetric method; while Gravimetric method was used for chlorine determination (AOAC, 1997).

Vitamin analysis

Vitamin C was determined according to the method described by Adeboye (2008). Ascorbic acid was measured by titration with phenolindo-2, 6-dichlorophenol (DPIP). The powdered sample (0.2 g) was mixed with 4 m L^{-1} of a buffer solution made up of 1 g L^{-1} oxalic acid and 4 g L^{-1} sodium acetate anhydrous. This was titrated against a solution containing 295 mg L^{-1} DPIP and 100 mg L^{-1} sodium bicarbonate. The results were expressed as mg/100 g DM. Vitamin A was determined using the method described by Zhang and Hamauzu (2004), where 15 g of powdered sample was homogenized with 10 mL acetone at -20°C. The homogenate was filtered with four layers of cheesecloth. The residue was treated with acetone (-20°C) for three successive extractions until the green colour could no longer be visually detected in the extract and residue. The filtrate was combined and centrifuged at 4000 rpm for 10 min. The supernatant was collected and filtered through a 0.45 µm Advantec filter pore for HPLC analysis. Samples were separated on a Luna 5 µ C18 column at 40°C by an HPLC. The mobile phase consisted of acetonitrile: water and ethyl acetate. The flow rate was 1.0 mL min^{-1}. Samples were detected at 450 nm. The carotenoids were expressed as International Units (IU). Vitamins B1, B2, B3, B5, B6, B9, B12, E and K were determined spectrophotometrically as stated by AOAC (1997).

Phytochemical analysis

The phytochemical properties of the dried powdered plant sample were determined using standard methods described by Harborne (1993); Obadoni and Ochuko (2001); Boham and Kocipai, (1994); Ebrahimzaded *et al*. (2008); and Nabavi *et al*. (2008).

Alkaloids Determination

Five grams of the dried sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide solution and then filtered. The residue which is the crude alkaloid was weighed (Harborne, 1993).

Flavonoid determination

Ten grams of sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Nabavi *et al*., 2008).

Saponin determination

Twenty grams of the powdered sample was placed in 200 ml of 20% ethanol. The suspension was heated over water bath for 4 h with continuous stirring at 55^{0}C. The mixture was filtered and the residue re-extracted with 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at 90^{0}C. The concentrate was transferred into a 250 ml separator funnel and 20 ml diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evapoaration, the sample was dried in the oven to a constant weight. The Saponin content was calculated in percentage (Obadoni and Ochuko, 2001).

Determination of total phenols

The powdered sample was boiled with 50 ml ether for 14 min. 5 ml of the extract was pipetted into a 50 ml flask, 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The sample was made to mark and left to react for 30 min for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelength (Ebrahimzaded *et al*., 2008).

Tannin determination

Fifty grams of the sample was weighed into 100 ml plastics bottles, 50 ml of distilled water was added and shaken for 1 h in a mechanic shaker. This was filtered into a 50 ml volumetric flask and made to mark. 5 ml of filtrate was pipetted into a tube and mixed with 3 ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelengths within 10 min. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured (Boham and Kocipai, 1994).

Statistical analysis

Statistical analyses were carried out using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, IL.USA). Data were expressed as mean + SD.

Results

Table 1 shows the result of the chemical analysis. The result showed a high percentage of crude protein and ash contents (11.2 and 12.14%), NFE had a higher percent (59.78%), while the highest percentage was observed in the Dry matter (DM) content (93.3%). The percentage crude protein content was low, while the percentage gross energy value was the lowest. Result of the mineral analysis is shown in Table 2. The result showed high contents of sulphur, chlorine, manganese, iodine, and zinc, with sulphur content being the highest (131.45 mg/kg). Molybdenum content was the lowest (0.12 mg/kg).

Table 1. Proximate composition of *Clerodendrum volubile*

Vitamins	Values (%)
Crude Protein	11.2 ± 0.36
Crude fibre	5.31 ± 0.30
Crude fat	4.87 ± 0.20
Ash	12.14 ± 0.23
Dry matter	93.3 ± 0.28
NFE	59.78 ± 0.20
Gross Energy	3.98 ± 0.12

Values are mean + SD; n = 3.

Table 2. Mineral composition of *Clerodendrum volubile*

Minerals	Values (mg/kg)
Sodium	2.1 ± 0.21
Potassium	6.7 ± 0.22
Calcium	8.4 ± 0.20
Phosphorus	4.1 ± 0.20
Magnesium	2.3 ± 0.31
Manganese	84.35 ± 0.18
Molybdenum	0.12 ± 0.03
Zinc	24.50 ± 0.40
Iron	9.55 ± 0.28
Nickel	0.30 ± 0.14
Chlorine	94.27 ± 0.13
Iodine	54.75 ± 0.20
Sulphur	131.45 ± 0.23
Chromium	0.76 ± 0.08

Values are means + SD; n = 3.

Table 3 shows the result of fat soluble vitamin analysis. The vitamin A content was very high (3672.54 IU). The contents of vitamins D, E and K were relatively low. Result of the water soluble vitamin analysis (table 4) showed high contents of ascorbic acid and cyanocobalamin. Niacin and Pyridoxine contents were moderately high, while thiamine was the lowest (0.23 µg/100 g). The phytochemical analysis (Table 5) showed a high concentration of phenol (4.13%), this was followed by saponin (2.54%). A low concentration was observed in alkaloids (0.79%), while tannin had the lowest concentration (0.15%).

Table 3. Fat soluble vitamins composition of *Clerodendrum volubile*

Vitamins	Values
Vitamin A (IU)	3672.54 ± 1.80
Vitamin D (µg/100 g)	0.41 ± 0.05
Vitamin E (µg/100 g)	0.60 ± 0.19
Vitamin K (µg/100 g)	0.14 ± 0.03

Values are means + SD; n = 3

Table 4. Water soluble vitamins of *Clerodendrum volubile*

Vitamins	Values (µg/100g)
Thiamine	0.23 ± 0.04
Riboflavin	0.57 ± 0.04
Niacin	1.71 ± 0.04
Pantothenic acid	0.40 ± 0.05
Pyridoxine	2.25 ± 0.23
Vitamin B9	1.40 ± 0.11
Cyanocobalamin	9.50 ± 0.36
Ascorbic acid	11.36 ± 0.15

Values are mean + SD; n = 3

Table 5. Phytochemical properties of *Clerodendrum volubile*

Vitamins	Values (%)
Alkaloids	0.79 ± 0.04
Saponin	2.54 ± 0.07
Flavonoid	1.24 ± 0.05
Phenol	4.13 ± 0.05
Tanin	0.15 ± 0.02

Values = mean + SD; n = 3

Discussion

There is increasing interest in the nutrition and health protecting properties of indigenous and under-utilized food resources particularly leafy vegetables that constitute basic source of ingredient for rural communities in sub-Saharan Africa. In this study, the nutritional qualities of this non-conventional leafy vegetable were determined.

The Crude protein value for *Clerodendrum volubile* is relatively low as observed in conventional vegetables like cabbage, 12.8%; and lettuce, 14% (USDA, 2005). This justifies the non use of this leafy vegetable in diets as a sole source of protein for the alleviation of Protein Energy Malnutrition (PEM). Fevrier and Viroben, (1996) reported that plant foods, when rightly combined with other foods can be of high biological value and satisfactorily meet the protein needs of adults. The crude protein in the leafy vegetable would require dietary supplementation with proteins from cereals and legumes (Ejoh *et al*., 2007). The Nitrogen Free Extract (NFE) level of this leafy vegetable is similar to results observed by Nworgu *et al*. (2007) in fluted pumpkin (*Telfaria occidentalis*) leaves. It is appreciably high compared to spinach and lettuce (USDA, 2005). Singhal and Kulkarni (1987) affirmed that this may be due to the physiological state of the plant before harvesting as well as the time for harvest. The vegetable had an appreciable level of dietary fibre similar to that of *Telfaria occidentalis* (Nworgu *et al*., 2007). Tanya *et al*. (1997) affirmed that leafy vegetables are particularly rich in dietary fibre. Eun-Hee *et al.* (1993) found the average levels of dietary fibre in leafy vegetables of Asian countries to be 33% dry weight. The present study reports a value that is rather lower. High levels of dietary fibre in leafy vegetables are advantageous for their active role in the regulation of intestinal transit, increasing dietary bulk and increasing faeces consistency due to their ability to absorb water (Jenkin *et al*., 1986). The total lipid level of the vegetable was low. Generally this low value corroborates the findings of many authors which showed that leafy vegetables are poor sources of lipids (Ejoh *et al*., 1996). Due to the general low level of crude fat in vegetable leaves and its high level of total unsaturated fatty acid, their consumption in large amounts would be beneficial to individuals suffering from overweight or obesity, and this would constitute a good dietary habit. The high values of ash observed in the vegetable is a good indicator that this food sample is a good source of minerals when compared to values obtained for cereals and tubers (FAO, 1968). The Dry Matter value was high. This will reduce the cost of handling and ensure long term storage, thus will be of great benefits to rural communities which lack access to basic storage facilities and electricity.

The mineral composition of leaves of *Clerodendrum volubile* was high with Sulphur being the highest. Relatively large quantities of sulphur are required, but there is no RDA (Zhao, 1999). Sulphur is known as an essential component of the amino acids, cysteine and methionine, and participates as an enzyme cofactor. The chlorine level was also high. The RDA for chlorine is 2300 mg (USNRC, 2000). Consumption with dietary supplements like table salt can compensate for the low RDA level. Chlorine has been reported for its requirement for production of hydrochloric acid in the stomach and in cellular pump functions. The Manganese content was high compared to reports by Fasuyi and Noyerem (2005) on *Telfairia occidentalis* leaf meal. The RDA for manganese is 2.3 mg (USNRC, 2000), this study reports a value that is far higher. This is an indication that the vegetable is a potential supplement of the trace mineral. Manganese which has antioxidant properties is important for proper food digestion and for normal bone structure. It is a cofactor in function of antioxidant enzymes such as superoxide dismutase. *Clerodendrum volubile* can thus serve as a potential antioxidant. This study reports a rather high level of Iodine contents owing to the fact that the RDA for iodine is 150 µg (USNRC, 2000). Iodine is required for the biosynthesis of thyroxin, thus making the plant a potential source for the prevention and treatment of goiter. The Zinc content was appreciably high compared to that of cabbage, 0.18 mg (USDA, 2005). The RDA for zinc is 11 mg, which indicates Clerodendrum volubile as a good source. Zinc deficiency is associated with dermatitis, poor wound healing, retarded growth and sexual development, and reduced taste acuity. Apoptosis or programmed cell death is potentiated by zinc deficiency. Zinc also functions as an antioxidant and can stabilize membranes (Milbury *et al*., 2008). The Iron content of *C. volubile* is relatively high compared to other studied vegetables like spinach, 2.7 mg and lettuce, 1.2 mg (USDA, 2005). Iron has been shown to be a cofactor for the antioxidant, Catalase. Although iron overload diseases are generally regarded as examples of diseases involving oxidative stress (Young *et al*., 1994), its deficiency is linked with increased production of ROS. The RDA for iron is 8 mg. The moderate level observed in the leaves of *C. volubile* is beneficial, as high concentration of iron has been shown to enhance pro-oxidant activity via the Fenton reaction. The macro minerals: sodium, potassium, calcium, phosphorus and magnesium were relatively low compared to spinach (USDA, 2005). Fasuyi and Noyerem (2005) reported high contents of these macro minerals on *Telfairia occidentalis* leaf meal. Trace levels of Chromium and Nickel were observed in *C. volubile*. Chromium and Nickel are among suggested essential minerals with no established RDA (Anke *et al*., 1984; Eastmond *et al*., 2008). Chromium is implicated in sugar metabolism in humans, but definitive biochemical evidence for a physiological function is lacking (Stearns, 2000).

Total carotenoid level was high compared to that of spinach and lettuce (USDA, 2005). Vitamin A deficiency remains a major problem in Nigeria and indeed Africa. Deficiency of vitamin A, particularly in the third world countries accounts for blindness in 250,000 to 500,000 children a year according to estimates by the World Health Organization (Minsante/UNICEF, 2001). More recently, protective effects of carotenoids against serious disorders such as heart disease, cancer and degenerative eye disease have been recognized. Its role in vision and growth regulation has made the public health officials to look for urgent and rapid methods of combating the problem (Ejoh *et al.*, 2007). This leafy vegetable therefore can serve as a potential source of pro vitamin A (carotenoids). The Vitamin C value was relatively high compared to lettuce, 4 mg but considerably low compared to cabbage, 36.6 mg and 28 mg (USDA, 2005). Deficiency in vitamin C leads to the disease scurvy. The primary cause of vitamin C deficiency is poor diet and/or an increased requirement (King, 2008). Thus the vegetable leaves can be used as a supplement for Vitamin C. The folate content was relatively low compared to spinach, cabbage and lettuce (USDA, 2005). Folate (Vitamin B9) is essential to numerous bodily functions ranging from nucleotide biosynthesis to the remethylation of homocysteine. Both children and adults require folic acid to produce healthy red blood cells and prevent anemia (NIH, 2008). Combination with other folate-rich vegetables will supplement for the low folate content. The cyanocobalamin (vitamin B12) level was reasonably high. Vegetables have been reported to be a poor source of cyanocobalamin (NIH, 2008). Cyanocobalamin is essential for the maintenance of healthy body. It plays a key role in the normal functioning of the brain and nervous system, and for the formation of blood. The observed high content will be of benefit to vegetarians, and can also be used as supplement in combination with other food.

Phytochemicals are natural bioactive compounds from plants with general benefits to human health. *Clerodendrum volubile* showed relatively high total phenol and flavonoid content compared to results obtained by Ademiluyi and Oboh (2008) in African mistletoe leaves (*Viscum album*). The relationship between total phenol and flavonoids contents and antioxidant activity has been widely studied in different foodstuffs such as vegetables (Jayaprakasha *et al*., 2008). Antioxidant activities of vegetables significantly increase with the presence of high concentration of total phenol and flavonoid contents. The saponin and tannin contents were higher than results observed by Nworgu *et al*. (2007) in fluted pumpkin (*Telfaria occidentalis*) leaves. Tannin has been described as an anti-nutrient. It is associated with lower nutritive value of protein foods (Akwaowo *et al*., 2000). Nworgu *et al*. (2007) reported the reduction of tannin content of *Telfaria occidentalis* on soaking. Proper handling before consumption could reduce its content. Alkaloids are used in the pharmaceutical industries in the production of analgesics, owing to its analgesic properties (Okwu and Ndu, 2006). The presence of alkaloid may be responsible for the analgesic use of this leafy vegetable by rural communities in south-south Nigerian. The presence of these phytochemicals may be responsible for the tradomedical use of this leafy vegetable in the management of the various ailments mentioned in the literature.

Conclusion

Traditional leafy vegetables have an essential role in the success within sub-Saharan Africa, of the World Health Organization's (WHO) global initiative on increased consumption of fruit and vegetables. Results from this study indicate that the Clerodendrum volubile can serve as a good nutritional source in combating malnutrition. The presence of bioactive compounds is an affirmation of the use of this leafy vegetable in the management of various ailments, and thus may serve as a source of ingredient to the pharmaceutical industries. The results obtained serves as a nutritional data base for local consumers, as well as for further research purposes. Combination with other foodstuffs is recommended to satisfactorily meet nutritional needs. Further research needs to be carried out on domesticating this under-utilized vegetable, as it is often harvested from the wild, as well as carrying out animal trials to authenticate its tradomedical uses by rural communities.

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