

**ANTIOXIDANT NUTRIENT PROPERTIES AND ANTIOXIDANT
ACTIVITIES OF *OBENETETE (CLERODENDRUM VOLUBILE)*, A NON-
CONVENTIONAL LEAFY VEGETABLE CONSUMED IN NIGERIA**

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ABSTRACT

There is an increasing interest in the nutritional and health protecting properties of indigenous and under-utilized food resources particularly leafy vegetables that constitute basic sauce ingredient for rural communities in sub-Saharan Africa. It has been suggested that an inadequate intake of trace minerals and vitamins in the diet may have a negative influence on the effectiveness of these antioxidant defence mechanisms. Leafy vegetables' inclusion in diets has been shown to be protective against incidence of chronic, degenerative and age-related diseases, due to the presence of antioxidants. Leafy vegetables are abundant in most developing countries and grow all year long in the wild. The antioxidant nutrients of the leaves of *Clerodendrum volubile* were evaluated and compared to gallic acid which served as the control. The leaves were air-dried at room temperature and grounded to fine powder. Two grams of the dried powdered plant sample were analysed for antioxidant minerals (Mn, Se, Fe, Cu, Zn) as well as for vitamins C and E. The total flavonoid and phenolic contents were also determined. Dried powdered plant samples were extracted at room temperature by percolation with ethanol. The extracts were used to determine the reducing ability and (2,2'-diphenyl-1-picrylhydrazyl) DPPH scavenging ability. The manganese content (84.35 ± 4.45 mg/g) was the highest; —followed by zinc (24.50 ± 1.56 mg/g) and selenium (2.50 ± 0.21 mg/kg) content was lowest. Vitamin C had a higher content than that of E. The total flavonoid content was observed to be high (124 mg/g RUTIN). There was a corresponding increase in the reducing ability of the leaves with increase in concentration of the extracts in a dose-dependent relationship. A significant difference ($p < 0.05$) was observed between the DPPH scavenging ability of the leafy vegetable extract (74.26 μ g/ml) and gallic acid with highest reducing ability (0.28 μ g/ml) observed at highest dose of the extract (100 μ g/ml). *Clerodendrum volubile* is rich in micronutrients and flavonoids which may be responsible for its high antioxidant activities, and can act as primary and/or secondary antioxidants.

Key words: antioxidant, phenol, flavonoids, micronutrients, 2,2'-diphenyl-1-picrylhydrazyl (DPPH)

INTRODUCTION

Leafy vegetables inclusion in diets has been shown to be protective against incidence of chronic, degenerative and age-related disorder diseases, due to the presence of antioxidants [1]. Antioxidants are compounds which quench molecular oxidation, and play a vital role in guarding the body defence mechanism against free radicals and reactive oxygen species (ROS), which are generated continuously in the body due to both normal metabolism and certain diseases [2, 3]. When an imbalance occurs between oxidants and antioxidants in favour of the oxidants, oxidative stress sets up; hence, this may contribute to the aging process as well as to chronic diseases such as cancer and coronary heart disease [4]. Leafy vegetables have been reported as potential sources of minerals and vitamins and therefore possess strong antioxidant properties due to the presence of these nutrients which possess antioxidant attributes [5, 6]. Leafy vegetables are abundant in most developing countries especially in the rural areas where they grow wild. Over the years, efforts have been made to study the nutritional qualities of the wild leafy vegetables such as *Clerodendrum volubile* (Beauv.).

Clerodendrum volubile is commonly found in southern part of Nigeria, with absence of scientific data base. It is commonly known among the Urhobos and Itsekiris of the Niger-Delta as *Obenetete*. It belongs to the Family *Lamiaceae* (*Verbenaceae*). It is a climbing shrub of 3 m, glabrous except the inflorescences, it is found in the deciduous forest and secondary jungle, across the Region, Dakar to Fernando Po [7]. Its general and traditional uses include: (i) Ornamental, (ii) social use in: religion and, superstitions (iii) Food uses include: sauce ingredients and spice and (iv) Medicinal application in diseases such as: arthritis, rheumatism, dropsy, swellings, oedema, gout and pain-killers [7]. *Clerodendrum volubile* is a common vegetable consumed mostly in southern Nigeria amongst the Itsekiris and Urhobos. It is often regarded as weed in other parts of the country. Knowledge of its nutritional and antioxidant properties will promote the utilization of this under-utilized leafy vegetable in all parts of the country. This study was therefore, set out to evaluate the antioxidant nutrient properties of the leaves of *Clerodendrum volubile*; investigating the free radical scavenging activities; and determining its total phenol and flavonoids contents.

MATERIALS AND METHOD

Plant material

About 250 g of fresh leaves of *Clerodendrum volubile* were purchased from Uselu market in Benin City, Nigeria. They were identified and authenticated at the Forestry Research Institute (FRIN) Ibadan, Nigeria. The leaves were air-dried at room temperature and grounded to fine powder, using a laboratory mill (Perten Lab Mill 3100, <250 μ m) and stored in air-tight containers at room temperatures for laboratory analysis.

Antioxidant nutrient analysis

Dried powdered plant sample (2 g) was 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 elements (Mn, Se, Fe, Cu, Zn) using atomic absorption spectrophotometer (Unicom-Optics WFX-320) and flame photometer (Jenway PFP7) [8]. Vitamin C and E were determined according to AOAC methods [8].

Extraction

Dried powdered samples were extracted at room temperature by percolation with ethanol. All extracts were concentrated over a rotary vacuum evaporator (Buchi R210). The extracts were stored in refrigerator for laboratory analysis.

Determination of total phenol and flavonoid contents

Total phenolic and flavonoid compound contents were determined by the Folin-Ciocalteu and Colorimetric aluminium chloride methods respectively [9].

Determination of reducing property

The reducing property of the leaf ethanolic extract was determined by assessing the ability of the sample extracts to reduce FeCl_3 solution using gallic acid as reference [10].

Free radical scavenging assay

The free radical scavenging ability of the sample extracts against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was evaluated using gallic acid as reference [11].

Determination of total antioxidant capacity

The total antioxidant capacity was determined according to novel method for measuring antioxidant capacity [12].

Statistical Analysis

All data were expressed as mean + SD and test significance was by the analysis of variance (ANOVA) with significance inference set at 5% using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, IL, USA)..

RESULTS

Antioxidant nutrient analysis

Amongst the antioxidant nutrient analysed in the leafy vegetable (Table 1), the manganese content was 84.35 ± 4.45 mg/kg; whereas the contents of zinc and selenium were 24.50 ± 1.56 mg/kg and 2.50 ± 0.21 mg/kg respectively. The vitamin C content was 11.36 ± 2.83 $\mu\text{g}/100\text{g}$, while that of vitamin E was 0.60 ± 0.06 $\mu\text{g}/100\text{g}$.

Total phenol, flavonoid content and antioxidant capacity

The result of the total phenolic, flavonoid content and antioxidant capacity contents are shown in Figure 1. The leafy vegetable showed a high total flavonoid content.

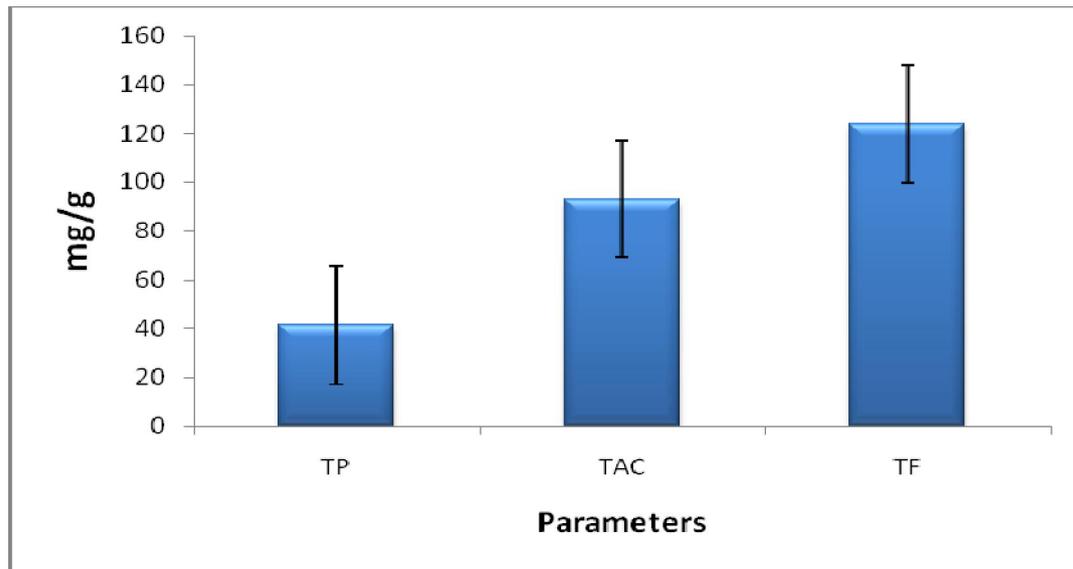


Figure 1: Total phenol, flavonoids contents and antioxidant capacity

TP: Total phenol (mg/g GAE); TAC: Total antioxidant capacity (mg/g GAE); TF: Total flavonoids (mg/g RUTIN)

Antioxidant activities

Reducing ability of the ethanolic extract is as presented in Figure 2. Gallic acid was used as a reference. There was a corresponding increase in the reducing ability with increase in concentration of the extracts, indicating a dose-dependent relationship. A significant difference ($p < 0.05$) was observed between the reducing ability of the leafy vegetable extract and gallic acid with highest reducing ability observed at highest dose of the extract

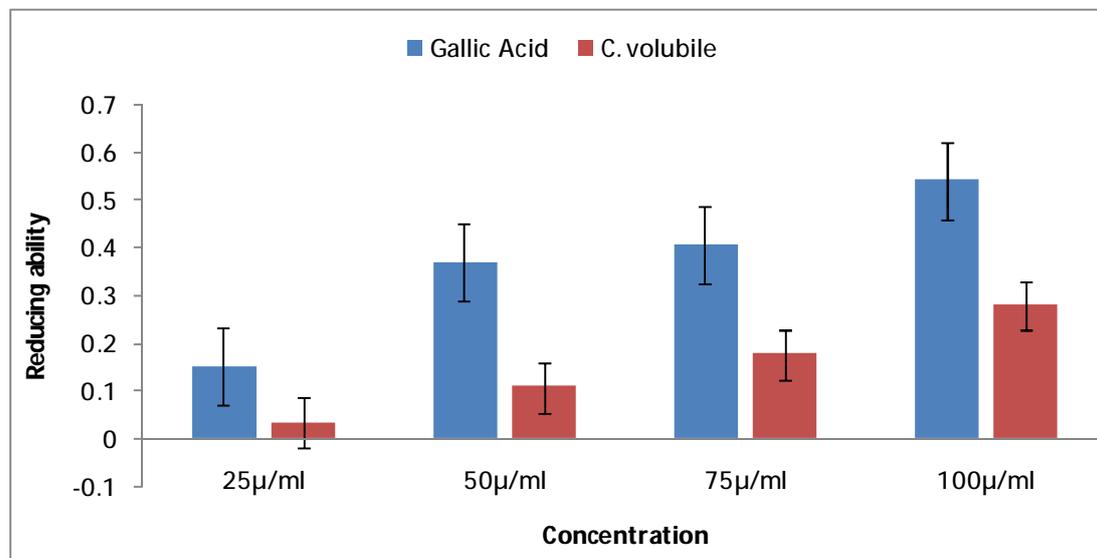


Figure 2: Reducing ability of C. volubile

The scavenging ability of the ethanolic extract against stable DPPH in ethanolic solution is presented in Figure 3, and it is expressed as percentage (%) scavenging ability. Gallic acid was used as a reference. The results for the ethanolic extract followed a dose-dependent pattern. A significant difference ($p < 0.05$) was observed between the scavenging ability of the leafy vegetable extract and gallic acid with the highest scavenging ability observed at highest dose of the extract.

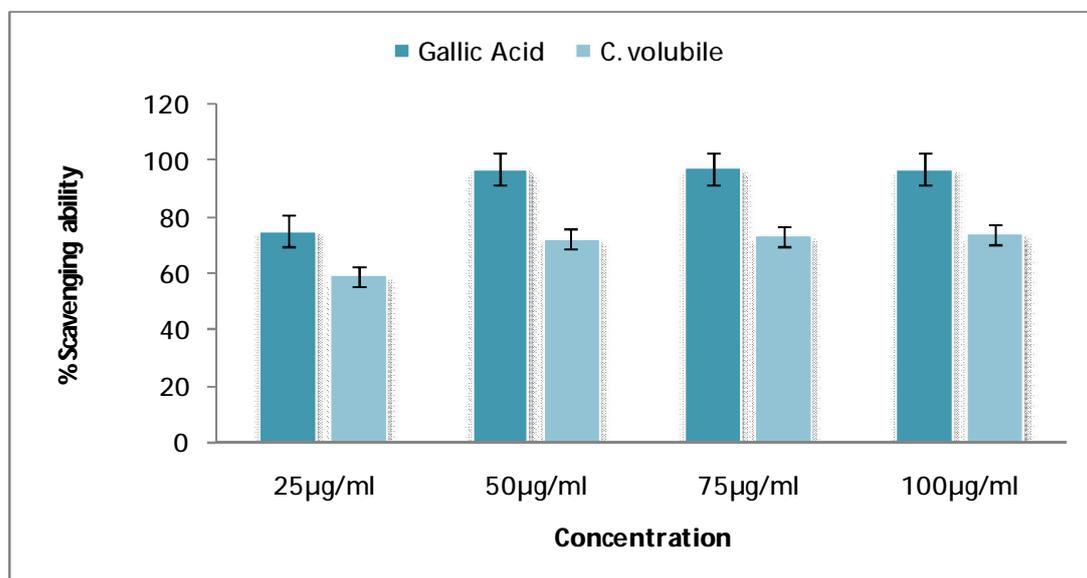


Figure 3: DPPH scavenging activity of *C. volubile*

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 sauce ingredient for rural communities in sub-Saharan Africa. It has been suggested that an inadequate dietary intake of trace minerals and vitamins may compromise the effectiveness of these antioxidant defence mechanisms [13]. This article evaluated the antioxidant nutrient properties of the leaves of *Clerodendrum volubile*.

Antioxidant nutrient

The Manganese content was high compared to *Telfairia occidentalis* leaf meal [14]. Manganese is a cofactor of several enzymes involved in fatty acid and cholesterol biosynthesis as well as mitochondrial Mn- Superoxide Dismutase (SOD) complex, which neutralises the superoxide radical [15]. Manganese also acts as a cofactor for catalase [16]. The high concentration of manganese observed in *C. volubile* makes it a potential dietary supplement of the trace mineral and thus may boost antioxidant activity.

The zinc and copper contents observed were also similar to those of *Telfairia occidentalis* leaf meal [14]. Zinc and copper are components of CuZn-Superoxide

Dismutase (CuZnSOD), an important antioxidant enzyme. Their contents were appreciably high in the vegetable. Deficiencies in copper rather than zinc affect the activity of this enzyme [17]. Zinc has a stabilising effect on membranes possibly by displacing bound transition metal ions and thereby preventing peroxidation of membrane lipids [18]. The Iron content of *C. volubile* is relatively high compared to other studied vegetables like spinach, 2.7mg/kg, pumpkin, 0.8mg/kg and lettuce, 1.2mg/kg [19]. Iron has been reported as a cofactor for the antioxidant, catalase [20]. Although iron overload is generally regarded as examples of diseases involving oxidative stress, its deficiency is linked with increased production of Reactive Oxygen Species (ROS). The moderate concentration observed in the leaves of *C. volubile* may be beneficial, as high concentration of iron has been shown to enhance pro-oxidant activity via the Fenton reaction [21].

Selenium functions as cofactor for reduction of antioxidant enzymes such as glutathione peroxidases and thioredoxin reductase [22]. The low selenium content observed in *C. volubile* can be compensated by consumption of other vegetables and legumes rich in Selenium and the amino acids, selenocysteine and selenomethionine.

Vitamin C has been reported as relatively high in cabbage, 36.6 mg/kg; lettuce, 4 mg/kg; and spinach, 28 mg/kg [19]. It is a well known antioxidant. Compared to these conventional leafy vegetables, the vitamin C content of *C. volubile* is appreciably high and thus a good source.

Vitamin E is more localised and concentrated than the other micronutrients, both in the hydrophobic interior of biological membranes and phospholipid 'coats' of plasma lipoproteins [23]. Biochemical investigations have clearly demonstrated the protective role of Vitamin E against low density lipids (LDL) oxidation. The low vitamin E content observed in *C. volubile* makes it a poor source of the vitamin, and may be compensated by consumption with other vegetables

Total phenol and flavonoid content

The leafy vegetable contained a relatively high total phenolic content compared to values reported in *Viscum album* [24]. Nevertheless the value compared favourably with results observed in *Clerodendrum- reticulata* var. and *Clerodendrum- reticulata* var. [25].

The total flavonoid content of *C. volubile* was very high compared to that observed in *C. reticulata* var. and *C. reticulata* var. [25].

The relationship between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as vegetables [26]. Antioxidant activity of vegetables significantly increases with the presence of high concentration of total phenol and flavonoid contents.

Antioxidant activities

There was a corresponding increase in the reducing ability with increase in concentration of the extracts, indicating a dose-dependent relationship. Reducing ability is a measure of the ability of the ethanolic extracts to reduce Fe^{3+} to Fe^{2+} . Antioxidant was considered as any species that reduces the oxidizing species that would otherwise damage the substrates [27]. They treated the “total antioxidant power” as the “total reducing power”. The scavenging ability of the ethanolic extract against stable DPPH in ethanolic solution also followed a dose-dependent pattern. The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples [28]. The antioxidant nutrient and total flavonoid contents of *C. volubile* may be responsible for the observed high scavenging activity of this leafy vegetable. This free radical scavenging ability of the ethanolic extract is an indication that *C. volubile* is a potential an excellent dietary source of antioxidant that can be used in the prevention and management of various ROS-related ailments such as Parkinson and Alzheimer diseases [24].

CONCLUSION AND RECOMMENDATION

Results of this present study have revealed that *Clerodendrum volubile* is quite rich in antioxidant nutrients and flavonoids which may be responsible for its high antioxidant activity, and can act as primary and/or secondary antioxidants, being free radical scavengers. This leafy vegetable like other non-conventional vegetables grows in the wild, there is need therefore for the domestication of the vegetable. In vivo studies in laboratory animal model is also recommended to further ascertain the antioxidant properties of the leafy vegetable.

Table 1: Antioxidant nutrient properties of *C. Volubile*

Minerals	Values (means \pm SD)
Manganese (mg/kg)	84.35 \pm 4.45
Zinc (mg/kg)	24.50 \pm 1.56
Iron (mg/kg)	9.55 \pm 0.21
Selenium (mg/kg)	2.50 \pm 0.21
Copper (mg/kg)	5.05 \pm 0.21
Vitamin C (μ g/100g)	11.36 \pm 2.83
Vitamin E (μ g/100g)	0.60 \pm 0.06

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