

Determination of proximate Composition and phytochemical constituents of *Corchorus Olitorious* (Ewudu) Harvested in Ugep, Cross River State".

ABSTRACT

Aims: To Determine the proximate composition and phytochemicals constituents of *Corchorus olitorious* leaves harvested in ugep, Cross River State. The leaves were purchased locally.

Methodology: The leaves were washed to remove sand and other particles like pieces of wood which may act as contaminants, thereafter the leaves were dried to a certain temperature. The dried leaves were blended into powder using a standard method of AOAC (2005) (Association of analytical chemist). Evaluation of *Corchorus Olitorous* for proximate and phytochemical composition were conducted using standard methods. **Results:** The result obtained on the proximate composition of *Corchorus Olitorious* revealed the following; Moisture (84.40 ± 0.10 mg/100g), Ash (2.90 ± 0.10 mg/100g), Protein (4.66 ± 0.02 mg/100g), Fibre (2.70 ± 0.10 mg/100g), Fat (4.30 ± 0.10 mg/100g) and Carbohydrate (85.10 ± 0.02 mg/100g). The phytochemical analysis of the leaf extracts of *Corchorus olitorious* which was done both

Qualitatively and quantitatively revealed the presence of alkaloids (2.40 ± 0.10 mg/100g), glycoside (2.53 ± 0.01 mg/100g), saponins (3.32 ± 0.02 mg/100g), Tannins (0.47 ± 0.01 mg/100g), flavonoid (6.30 ± 0.10 mg/100g), reducing compounds (4.60 ± 0.02 mg/100g) and polyphenols (6.50 ± 0.02 mg/100g). **conclusion:** the leafy vegetables of this plant if consume in sufficient amount would contribute greatly to the nutritional requirement for human health.

Key: Proximate; phytochemical; polyphenols; flavonoid; saponins; alkaloids; Tannins.

Introduction

Less utilized green leafy vegetables are plants consumed in relatively small quantities as a side dish or relish with the staple food. Usually these vegetables are the leaves, roots or stems of herbaceous plant. Their flower, calyces and immature seeds or fruits may also be consumed. They are also used as boiled vegetables or added to soups and stews and their consumption is considered inferior in taste and nutritional value when compared to their cultivated counterparts [1]

However, with persistence of malnutrition in the developing countries in spite of increased basic food production, micronutrient deficiencies and other nutrition related diseases still afflict over 2 billion people worldwide [2]. These had become a source of interest to nutritionist and other concerned scientific researchers. In fact, several studies [3] showed that many species of wild green leafy vegetables are rich sources of nutrients.

Leafy vegetable is also known to add taste and flavor as well as substantial amount of protein, fiber, minerals and vitamins to the diet. In addition, some plants with promising bioactive properties also contain useful minerals and food value for human and animal consumption [4].

Moreover, leafy vegetables are the cheapest and most available sources of substantial amount of vitamins A and C to the most vulnerable group, viz: rural populace. Although, in tropical Africa millions of people still suffer from vitamin A and C deficiency despite the increased consumption of leafy vegetables. These leafy vegetables are relatively inexpensive, easily and quickly cooked and rich in several nutrients especially -carotene and vitamin C which are essential for human health [5].

Several vegetable species in Nigeria and West African countries are used partly as condiments or spices in human diets or as supplementary feeds for livestock such as rabbits,

poultry, swine and cattle [6]. These vegetables are harvested at all stages of growth and fed either as processed, semi-processed or fresh to man while they are usually offered fresh to livestock. It is established that green vegetable leaves are also source of proteins because of their ability to synthesize amino acids from a wide range of virtually every available primary material such as water, carbon dioxide, and atmospheric nitrogen (as in legumes).

Vegetables also contain phytochemicals including carotenoids, flavonoids and ascorbic acid that help protect the body from long-term degenerative diseases [7]. According to Sofowola [8], phytochemicals are plant metabolites which act as natural defense systems for host plants, and also provide characteristic colour, aroma and flavour in specific plant parts. They are a group of non-nutrient compounds that are biologically active when consumed by human. Many phytochemicals are health-promoting and are of many diseases preventive [9]. Both epidemiological and clinical studies have proven that phytochemicals present in cereals, fruits and vegetables are mainly responsible for reduced incidence of chronic and degenerative diseases among populations whose diets are high in these foods [10].

Vegetables have been reported to have a high concentration of antioxidant components [11], and have anti-inflammatory properties [12]. Some anti-nutritional phytochemicals in *Corchorus olitorius* (jute) leaves such as tannins and phytic acid also exhibit protective effects, thus making them to serve a dual purpose of reducing some essential nutrients and protecting the body against a number of biochemical, physiological and metabolic disorders.

Hence the study is therefore designed to evaluate proximate composition and phytochemical analysis of *Corchorus olitorius* (jute) leaves.

MATERIALS AND METHODS

Collection and identification of plant materials

The plant material was harvested in ugep, yakurr local government area of Cross River State. It was kept in a dry polythene bag to reduce decomposition and transported to University of Calabar. The leaves were authenticated at the Department of Botany, University of Calabar and Voucher specimen deposited in the Departments herbarium number 3435 was kept for future reference.

Preparation of plant materials

The leaves of *Corchorus olitorius* (jute) was washed with clean water to remove sand and other particles like pieces of wood which may act as contaminants. The dried samples were then pulverized using electric blender to obtain fine powder. The pulverized sample with characteristic dark green colour was sieved (0.2mm) and stored in airtight containers until required for the proximate and phytochemical tests

Chemical reagents

The following chemicals was used. Ethanol, 1% aqueous HCl, chloroform, Glacial acetic, chemicals that was used include; concentrated hydrochloric acid, benzene, 10% ammonia and 3 % ammonia solution. Reagents that was used include cyanide solution and Fehlings solution. Petroleum ether was also used as the solvent for the non-aqueous extract while ethanol was used for the alcoholic extract. Pieces of aluminum metal was used as well as Dregendoff's reagent.

Proximate analysis

Proximate analysis was carried out on the samples of *Corchorus olitorius* (jute) leaves according to the methods of the “Association of Official Analytical Chemists [13] (AOAC, 2005) to determine the moisture content of the samples, crude protein, crude fat, ash, total dietary fiber and available carbohydrate of the dry samples. The proximate analysis of the samples was done in triplicates.

Estimation of moisture content

Five grams (5 g) of each sample was weighed into a crucible W_1 , weights of the crucible and sample before oven drying, W_2 , and after drying, W_3 , was noted. The samples were then removed at intervals, cooled and weighed until a constant weight was achieved. Percentage moisture content was calculated as follows:

$$\frac{W_2 - W_3}{W_2 - W_1} \times 100$$

W_1 = weight of crucible

W_2 = weight of crucible + sample before drying,

W_3 = weight of crucible + sample after drying

Crude protein

Two grams (2g) of each sample was weighed into a clean and dry Kjeldahl flask and digested with concentrated sulphuric acid in the presence of kjeldahl catalyst copper sulphate and sodium tetraoxosulphate (VI). The flask was heated by inclining it over a hot plate after which five milliliters (5ml) of each of the digested samples was pipetted into the distillation unit and 5ml of NaOH solution was added. This was done to make the solution alkaline and to enable ammonia (NH_3) to be liberated out of the solution. The ammonia liberated was distilled directly into a 5ml of boric acid indicator which changed the color to green. This solution was then titrated with 0.1M HCl, to get the amount of acid needed to regenerate the blue color of the

boric acid indicator and produce ammonium chloride. Titration continued until the green color turned blue. The crude protein was gotten from nitrogen content by multiplying the value by 6.25.

Crude fat

Two grams (2g) of each of the samples was weighed into a pre-weighed filter paper (W_1) and then the weight of the filter paper and the sample was noted before extraction (W_2). The filter papers containing the samples were properly tied and then put into the Soxhlet extractor and suspended above an already weighed receiving flask containing petroleum ether (40-60°C) below the condenser. The flask was heated for 6 hours to extract the crude fat. After the extraction, the flask containing the crude fat was disconnected from the Soxhlet extractor and oven dried at 100°C for 24 hours. Afterwards, it was cooled in a desiccator and weighed until constant weight was obtained (W_3). The difference in weight was expressed as percentage crude fat content.

$$\% \text{ ether extract} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times \frac{100}{1}$$

Total dietary fiber

Five gram (5 g) each of ground triplicate samples was transferred into a filter paper for defatting by a soxhlet extractor using petroleum ether. The defatted sample was centrifuged and the petroleum ether portion discarded. The defatting step was repeated twice and weight loss due to fat removal was recorded for later use in correction of final dietary fiber content. The residual petroleum ether was allowed to evaporate from the defatted sample under fume hood and the residues was suspended in MES-TRIS buffer and gelatinized in the presence of heat-stable α -amylase at 95–100 °C and then enzymatically digested sequentially with protease and amyl glucosidase at 60 °C to remove digestible protein and starch. The enzyme digestants were

then filtered through tarred fritted glass crucibles. Crucibles containing insoluble dietary fiber was rinsed with ethanol and acetone, and dried overnight in an oven at 105 °C while the filtrates were mixed with 95% ethanol to precipitate soluble dietary fiber. After about an hour, precipitates were filtered through tarred fritted glass crucibles. Each set of duplicate insoluble and soluble fiber residues will be ashed in a muffle furnace at 525 °C for 5 hours and then weighed and determined gravimetrically.”

Ash content

Two gram (2g) of each sample was weighed into small dry crucibles of known weight. The samples in the crucibles was placed in a furnace and ashed at 550 °C, which was kept constant for 3 hours. The ashed samples was removed from the furnace, cooled and kept in a desiccator until constant weights was obtained.

Available carbohydrate

The carbohydrate determination was by getting the difference between the summation of values of crude protein, crude fat, ash, dietary fiber and moisture contents of the samples and expressed in percentage.

% available carbohydrate =

100 – (Weight in grams [protein + fat + moisture + ash + dietary fiber] in 100g of food.

Calorific value

The calorific value will be calculated using the Atwater system, by multiplying the percentage of available carbohydrate and crude protein with 16.8 kJ and crude lipid with 37.8 kJ. The values will be then obtained in kilojoules per 100 g of the sample.

Screening Test for alkaloids

Two ml of each was stirred with 5 ml of 1 % aqueous HCL in water bath. 1 ml of the filtrate of each sample was treated with few drops of Dragendoff's reagent and a second 1 ml with Mayer's reagent. Turbidity and precipitation with either of those reagents was taken as evidence for the presence of alkaloid [14].

A few drops of Wagne's reagent was added to 1 ml of the sample. Orange coloration of precipitation indicate the presence of alkaloids.

Test for glycosides (Sofowara, 1984) salkowski test

Two ml of each extract was dissolved in 2 ml of chloroform sulphuric acid and carefully added to form, a lower layer. A reddish brown colour at the interface indicated the presence of glycoside.

Two ml of each extract was dissolved in 2 ml of glacial acetic acid containing 2 ml of concentrated H_2SO_4 . A violet ring appearing below the brown ring was acetic acid layer, gradually spread through the layer showed the presence of glycosides.

Test for saponins (Sofowara, 1984) frothing test

Two ml of the alcoholic extract was diluted with 10 ml of distilled water and heated in the water bath. After heating, this was shaken vigorously, stable foam indicated the presence of saponins.

Test for tannins (Trease and Evans, 1989)

Two ml of alcoholic plant extract was stirred with 10 ml of distilled water and heated in the water bath. 1 ml of 1 % $FeCl_3$ was added to indicate blue-black, green or blue-green precipitation or colouration indicated the presence of tannins.

Test for reducing compounds (Trease and Evans, 1989) Fehlings test

Two ml of plant extract was put in test tubes and 5 ml of Fehlings solution was added to it and heated in the water bath for 5 min. The formation of brick-red precipitation or colouration indicated the presence of reducing compounds.

Test for flavonoids (Sofowora, 1984)

Two ml of the alcoholic extract was added. A few pieces of aluminum metal and concentrated HCl was also added. The formation of orange, red, crimson or magnetic indicated the presence of flavonoids.

Test for polyphenol (Sofowora,1984)

Two ml of alcoholic plant extract was treated with 5 ml of distilled water and heated for 30 min in a water bath. 1 ml of 1 % FeCl_3 was added to the mixture and thereafter followed by adding 1 ml of 1 % potassium ferrocyanide solution. The formation of green-blue colouration indicated the presence of polyphenol.

Quantitative analysis

After screening the different extracts for the presence of the various phytochemical substances, the processed sample was subjected to quantitative analysis to determine the amount of each of the phytochemicals present in the test sample according to the methods described by [15] Ekwenye and Okorie (2010).

Determination of alkaloids

The Alkaline precipitation, gravimetric method [16]. was used. A measured weight of the processed sample was dispersed in 100 ml of 10% acetic acid in ethanol solution. The mixture was shaken well and allowed to stand for 4 hours at room

temperature being shaken every 30 minutes. At the end of this period, the mixture was filtered through whatman No 42 grade of filter paper.

The filtrate (Extract) concentrated by evaporation, to quarter of its original volume the extract was treated with dropwise addition of concentrated NH_3 solution to precipitate the alkaloid. The dilution was done until the NH_3 was in excess.

The alkaloid precipitate was removed by filtration using weighed whatman No 42 filter paper. After washing with 1% NH_4OH solution, the precipitate in the filter paper was dried at 60°C and weighed after cooling in a desiccator. The alkaloid content was calculated as shown below:

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{Wt. of sample}} \times 100$$

W_1 = Weight of filter paper alone.

W_2 = Weight of filter paper x alkaloids precipitate.

Determination of glycosides

This was carried out following the methods described by AOAC . Using this method, 1.0g of the ground dried sample was weighed and dissolved in 200 ml of distilled water contained in a 250 ml flask and allowed to stand for 2 hours. Full distillation was then carried out and 150 – 170 ml of distillate was collected in a 250 ml conical flask containing 20 ml of 2.5 % NaOH. An antifoaming agent (tanic acid) was added before distillation. Then, 100 ml of the distillate was measured into a fresh 200 ml flask and 8.0 ml of 6 N NH_4OH and 2.0 ml of 5 % KI was added, mixed and titrated with 0.02 N silver nitrate (Ag NO_3) using a micro-burette against a black background. Permanent turbidity indicated end points. The process was repeated and the average titre volume was calculated using the formula below, as described by AOAC (2005):

$$\text{Glycoside (mg/g)} = \frac{\text{Titre volume (ml)} \times 1.08 \text{ (g)}}{\text{Weight of sample (g)}}$$

Determination of saponins

This was done by the double solvent extraction gravimetric method. 5.0g of the processed sample was mixed with 50 ml of 20% aqueous ethanol solution and incubated for 12 hrs. at a temperature of 55⁰C with constant agitation. After that, the mixture was filtered through whatman No. 42 grades of filter paper. The residue was re-extracted with 50 ml of the ethanol solution for 30 minutes and the extracts was weighed together.

The combine extract was reduced to about 40ml by evaporation and then transferred to a separating funnel and equal volume (40 ml) of diethyl ether was added to it. After mixing well, there was partition and the other layer was discarded while the aqueous layer was reserved. This aqueous layer was re-extracted with the ether after which its pH was reduced to 4.5 with dropwise addition of dilute NaOH solution.

Saponin in the extract was taken up in successive extraction with 60 ml and 30 ml portion of named butanol. The combine entrant (ppt) was washed with 5 % NaCl solution and evaporated to dryness in a previously weighed evaporation dish. The saponin was then dried in the oven at 60⁰C (to remove any residual solvent) cooled in a desiccator and re-weighed. The saponin content was calculated as shown below:

$$\% \text{ Saponin} = \frac{W_2 - W_1}{W}$$

W

W = Weight of sample used

W_1 = Weight of empty evaporation dish

W_2 = Weight of dish + saponin extract

Determination of tannins

Tannin content of the sample was determined by Folin Denis colometric method [17]. A measured weight of the processed sample 5g was mixed with distilled water in the ratio of 1:10 (w/v). The mixture was shaken for 30 minutes at room temperature and filtered to obtain the extract.

A standard tannic acid solution was prepared, 2ml of the standard solution and equal volume of distilled water; was dispersed into a separate 50ml volumetric flask to serve as standard and reagent blank respectively. Then 2 ml of each of the sample extracts was put in their respective labelled flask.

The content of each flask was mixed with 35ml distilled water and 1 ml of the Folin Denis reagent was added to each. This was followed by 2.5 ml of saturated Na_2CO_3 solution. Thereafter each flask was diluted to the 50 ml mark with distilled water and incubated for 90minutes at room temperature. Their absorbance, was measured at 760nm in a spectrophotometer with the reagent blank at zero. The Tannin content was calculated as shown below:

$$\% \text{ Tannin} = \frac{100 \times \text{au} \times C \times \text{Vt}}{w \quad \text{as} \quad \text{Va}}$$

W = weight of sample

au = absorbance of test sample

as = absorbance of standard tannin solution

C = Concentration of standard tannin solution

V_t = Total volume of extract

V_a = Volume of extract analyzed

Determination of reducing sugar

The quantitative estimation of sugar was carried out using Benedict's quantitative test. Precisely 10 ml of the plant extract was diluted in 90 ml of distilled water. This solution was then transferred to a burette and titrated against 20 ml of standard Benedict's reagent in a 100 ml conical flask placed on electric hot plate with anti-bump chips placed inside the conical flask. Titration continued until the blue colour of the Benedict's reagent was completely changed and the end point recorded. The process was repeated three times and the average volume of titre calculated. Result obtained was then computed against that of a glucose standard and using the formula described by AOAC, (2005) as presented below:

18.9 mg standard glucose x average value of titre

10 ml Benedicts reagent

Determination of flavonoids

Flavonoid was determined using the method described. A measured weight of the processed sample 5g was boiled in 100 ml of 2MHCl solution under reflux for 40 minutes. It was allowed to cool before being filtered. The filtrate was treated with equal volume of ethyl acetate and the moisture was transferred to a separation funnel. The flavonoid extract (contained in the ethyl acetate portion) was received by filtration using weighed filter paper. The weight was obtained after drying in the oven and cooled in a desiccator. The weight was expressed as a percentage of the weight analyzed. It was calculated as shown below: -

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{W} \times 100$$

Wt of sample 1

W_1 = Weight of filter paper alone.

W_2 = Weight of filter paper x flavonoid precipitate.

Determination of polyphenol content

Polyphenol content of the sample was determined using the spectrophotometric method as described. One gram of the sample was extracted in 10 ml of pure methanol and filtered with Whatman No. 1 filter paper and 1.0 ml of the filtrate was then mixed with equal volume of Folin- ciocaltean reagent in a test tube, which was followed by the addition of 1.0 ml of standard solution which was also treated in the same way. Thereafter, 1ml of sodium bicarbonate solution was added to both tubes. Absorbance of both mixtures was read and their respective content was calculated using the formula below:

$$\% \text{ Polyphenols} = \frac{AE \times C \times VE}{AP \times Ws} \times \frac{100}{1}$$

Where:

AE = Absorbance of extract

C = Concentration (mg/ml), of standard phenolic acid

VE = Total volume of extract

AP = Absorbance of standard phenolic acid solution

Ws =Weight of sample analyzed.

Statistical Analysis

The Determination of proximate composition and phytochemical constituents of the samples were carried out with specific standard analytical procedures, Data were presented as mean and standard deviation one-way analysis of variances ANOVA and Duncan multiple range test were carried with SPSS version 16 software significance was accepted $p < 0.005$.

RESULT

This research demonstrates the amounts of proximate (moisture, ash, proteins, fat, fibre and carbohydrates) and phytochemical composition (alkaloids, glycosides, saponins, tannins, flavonoids, reducing compound and polyphenol) in, *Corchorus olitorius*.

Proximate composition of *Corchorus olitorius*

The result of the proximate analyses shown in Table 1 revealed the composition of *Corchorus olitorius*: Moisture (84.40 ± 0.10 mg/100g), Ash (2.90 ± 0.10 mg/100g), Protein (4.66 ± 0.02 mg/100g), Fibre (2.70 ± 0.10 mg/100g), Fat (4.30 ± 0.10 mg/100g) and Carbohydrate (85.10 ± 0.02 mg/100g).

3.2 Phytochemical composition of *Corchorus olitorius*

The result of the qualitative phytochemical analyses (Table 2) showed that *Corchorus olitorius* contained the following bioactive compounds: alkaloids, glycoside, saponins, Tannins, flavonoid, reducing compounds and polyphenols.

Result of the quantitative phytochemical analyses of *Corchorus olitorius* (Table 3) revealed abundance of bioactive compounds as follows; alkaloids (2.40 ± 0.10 mg/100g), glycoside (2.53 ± 0.01 mg/100g), saponins (3.32 ± 0.02 mg/100g), Tannins (0.47 ± 0.01 mg/100g), flavonoid (6.30 ± 0.10 mg/100g), reducing compounds (4.60 ± 0.02 mg/100g) and polyphenols (6.50 ± 0.02 mg/100g).

Table 1

Proximate composition of *Corchorus olitorius*

Proximate	Concentration (mg/100g D.M)
Moisture	84.40±0.10
Ash	2.90±0.10
Protein	4.66±0.02
Fat	4.30±0.10
Fibre	2.70±0.10
Carbohydrate	85.10±0.02

Values are expressed as mean \pm SD, n =3.

Table 2

Qualitative phytochemical composition of the ethanolic leaf extracts of *Corchorus olitorius*

S/N	Chemical constituent	Ethanol extract
1	Alkaloids	++
2	Glycosides	++
3	Saponins	++
4	Tannins	+
5	Flavonoids	++
6	Reducing compound	++
7	Polyphenol	+++

Table 3 Quantitative phytochemical composition of the ethanolic leaf extract of *Corchorus olitorius*

Number of analysis	Concentration (mg/100g D.M)
Alkaloids.	2.40±0.10
Glycosides.	2.53±0.01
Saponins.	3.32±0.02
Tannins.	0.47±0.01
Flavonoids.	6.30±0.10
Polyphenol.	6.50±0.02
Reducing compounds.	4.60±0.02

Values are expressed as means \pm SD, n =3

Discussion

Vegetable cells contain important quantities of water. It plays a vital role in the evolution and reproductive cycle and in physiological process. It has effects on the storage period length and on the consumption of tissue reserve substances.

Vegetables may be edible roots, stem, leaves, fruits or seeds. Each group contributes to diet in its own way [18]. Vegetables also act as buffering agents for acidic substances produced during the digestion process [19]. Vegetables contain both essential and toxic elements over a

wide range of concentrations [20]. The concentration of these elements is a function of the concentration in the soil on which the vegetable is planted [21]. Table 1 showed the levels obtained in the analyzed plant *C. olerius* leaves ($84.40 \pm 0.10\text{mg}/100\text{g}$) were very high. The value obtained for *C. olerius* ($84.40 \pm 0.10\text{mg}/100\text{g}$) is very much similar to the work done by [22]. who obtained $85.78 \pm 0.11\text{mg}/100\text{g}$. got lower value of $30.90 \pm 0.05\text{mg}/100\text{g}$. Water in vegetables is in the following forms:

Bound water or dilution water which is present in the cell and forms true solutions with minerals or organic substances;

Colloidal bound water which is present in the membrane, cytoplasm and nucleus and acts as a swelling agent for these colloidal structure substances during drying/dehydration processes;

Constitution water directly bound on the chemical component molecules and which is removed with difficulty;

These forms of water that is determined as moisture content generally constitute about 90.96 % of vegetables weight [23]. Moisture content is one of the most commonly measured properties of food materials. It is important to food scientists for a number of reasons:

Legal and labeling requirements: There are legal limits to the maximum or minimum amount of water that must be present in certain types of food.

Economic. The cost of many foods depends on the amount of water they contain - water is an inexpensive ingredient, and manufacturers often try to incorporate as much as possible in a food, without exceeding some maximum legal requirement.

Microbial stability: The propensity of microorganisms to grow in foods depends on their water content. For this reason, many foods are dried below some critical moisture content.

Food quality: The texture, taste, appearance and stability of foods depend on the amount of water they contain.

Food processing operations: Knowledge of the moisture content is often necessary to predict the behavior of foods during processing, e.g. mixing, drying, flow through a pipe or packaging.

Aids digestion and lowers shelf life: Moisture content of vegetables makes them easy to digest and reduce shelf life because moisture content facilitates bacterial action resulting in spoilage [24]. It is therefore important for food scientists to be able to reliably measure moisture contents of plants foods especially vegetables.

When organic compounds are heated at about 500 – 600 °C they decompose and the remaining residue is the ash. It consists of oxides and salts containing anions such as phosphates, chlorides, sulfates and other halides and cations such as sodium, potassium, calcium, magnesium, iron and manganese. During the ashing process organic salts decompose, losing the carbon-containing moiety. The metal from such salts forms oxides or reacts with other ions of the matrix. Some metals (e.g. cadmium and lead) may be volatilized during ashing. Therefore, measurement of ash contents suggests the amounts of inorganic matter present in the vegetable which is converted to metal oxides, water and carbon dioxide from the organic components of the vegetables. Table 1 indicates the amounts of ash in the analyzed sample, with *C. olitorius* (2.90±0.10 mg/100g). Values higher than that obtained in this work

(2.90±0.10 mg/100g) includes Adebayo, 2010 (3.27 ±0.20mg/100g), [25]. (10.5 ±0.00mg/100g) and Onwordi (2009) (20.20 ±0.15mg/100g) while those lower are Islam *et al.* (2004) (1.80 ±0.18g/100g) and Adeniyi (2011) (0.64 ±0.10mg/100g). These variations of ash content in the analyzed plant may not be unconnected to the different sources of the samples because different locations contain varied minerals and in different concentration.

Table 1 above indicates the protein content of the analyzed plant. The protein content of *C. olitorius* determined in this work is 4.66±0.02 mg/100g which is lower than that determined by Onwordi (2009) (11.20 ± 0.00mg/100g) and Adeniyi (2012) (6.21 ± 0.13mg/100g) and higher than that determined by Adebayo (2010) (1.82 ±0.04mg/100g), Islam *et al.* (2004) (2.56 ±0.10mg/100g), Ndlovu and Afolayan (2008) (1.63 ±0.02mg/100g). These differences observed might be due to variation in the environmental factors and agronomic practices at the various locations that the analyzed plant leaves were harvested [26].

Lipid content is used to denote a mixture of both oil and fats occurring widely in organic tissue especially in the adipose tissue of animals and in the seeds, nuts, fruits and leaves of plants. The quantity of lipid determined in *C. olitorius* (4.30±0.10 mg/100g) in this work is depicted in Table 1. This amount is much higher than that determined by Ndlovu and Afolayan (2008) (1.72 ±0.09mg/100g), Onwordi (2009) (0.32 %) and Adeniyi *et al.* (2011) (5.07 ±0.10mg/100g) but lower than value obtained by Islam *et al.* (2002) (5.07 ±0.17mg/100).

Table 1 above depicts the amounts of fiber determined in the analyzed plant. In *C. olitorius*, the fiber is at 2.70±0.10 mg/100 g which is lower than the amount obtained by [27] Yekeen *et al.* (2013) (7.40 ±0.10mg/100g) and higher than that obtained by Ndlovu and Afolayan, 2008 (1.30 ±0.16mg/100g) in the same plant. For *C. tridens* Linn, the crude fiber

content is $4.70 \pm 0.11 \text{ mg/100g}$ which is lower than what was obtained by [28]. ($8.00 \pm 0.07 \text{ mg/100g}$).

There are several types of fiber in plant materials that function differently and provide distinctive health benefits. They may either be soluble or insoluble. Soluble fibers bind with fatty acids and slow digestion so blood sugars are released more slowly into the body. These fibers help lower cholesterol and help regulate blood sugar levels for people with diabetes. Insoluble fibers help to hydrate and move waste through the intestines and control the pH levels in the intestines. These fibers help prevent constipation and keep one regular. However, there is evidence indicating that these complex carbohydrates (fibers) directly interact with the food antioxidants and interfere with the adequate assimilation of these compounds [29].

Table 1 above also indicates the amount of carbohydrate in the plant analyzed in this work. For *C. olitorius* the amount determined $85.10 \pm 0.02 \text{ mg/100g}$ was higher than that found by Yekeen et al., 2013 ($48.17 \pm 0.12 \text{ mg/100g}$), [30]. ($19.58 \pm 0.05 \text{ mg/100g}$) and Ndlovu and Afolayan, 2008 ($69.5 \pm 0.02 \text{ mg/100g}$). This contradicts the statement made by [31], stating that: “vegetables are never good sources of carbohydrate, as they contain appreciable quantities which ranged from $5.69 - 10.38 \text{ mg/100g}$ but they could also serve as good sources since the RDA value for this category of food is $7.0 - 100 \text{ mg/g}$ ”.

Medicinal plants are of great importance to the health of individuals and communities and their medicinal values lie in some chemical substances that produce definite physiological actions on the human body [32].

Phytochemicals also known as phytonutrients are naturally occurring substances found in plants. The result of the preliminary phytochemical analysis of the ethanolic leaf extract of *C.*

olitorius showed presence of alkaloids, glycoside, saponins, Tannins, flavonoid, reducing compounds and polyphenols (Table 2). These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals. Most alkaloids have been reported to have a very strong bitter taste and are very toxic and for these reasons they are used by plant to defend themselves against herbivore, and attacks by microbial pathogens and invertebrate pests [33]. Glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure [34]. Saponins as a class of natural products are involved in complexation with cholesterol to form pores in cell membrane bilayers [35] and as such may be used as anti-cholesterol agents or cholesterol lowering agent, anti-microbial, as well as many physiological activities such as stimulation of phagocytic cells, host-mediated tumor activity and a wide range of anti-infective actions have been attributed to tannins [36]. Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues [37]. Flavonoids have been reported to interfere with the activities of the enzymes involved in ROS generation, quenching of free radicals, chelating transition metals and rendering them redox inactive in the Fenton reaction [38].

The quantitative phytochemical analysis indicated that leaves of *C. olitorius* contained appreciable amount of phytochemicals. The result showed that alkaloid level in *C. olitorius* (2.40 ± 0.10 mg/100g) was higher than the values reported for alkaloid in the leafy vegetable like *V. amygdalina* (1.78 ± 0.02 mg/100g) [39].

Amount of glycoside determined in this work 2.53 ± 0.01 mg/100g is lower than the values determined for glycosides in leafy vegetables like *Corchorus tridens* Linn (1.11 ± 0.02 mg/100g) and *Vernonia amygdalina* Del (0.54 ± 0.05 mg/100g).

The saponin content, although high, was still lower than that reported for some commonly consumed vegetables such as onion [40]. Saponins are bitter and reduce the palatability of food. They have increased potential to lower cholesterol levels in humans due to the hypocholesterolemic effect of saponins [41]. These phytochemicals, either alone or in combination with others, are powerful tools to combat various ailments, especially cancers.

Tannin level of *C. olerius* (0.47 ± 0.01 mg/100g) was higher than the safe level of tannins (0.15 - 0.20%) as recommended by [42].

Flavonoids was detected in *C. olerius* (6.30 ± 0.10 mg/100g), which is higher than the report of Nnam *et al.* (2012) for some leafy vegetables, *V. amygdalina*, *O. gratissimum*, *G. latifolium* and *G. africanum*. Flavonoids have been reported to function as pigments and antioxidants [43]. Flavonoids lower high blood pressure as well as cholesterol in animal studies and have strong anti-inflammatory. Flavonoids and alkaloids are known to protect the body by decreasing the risk of heart diseases, stroke and some types of cancer.

The amount of polyphenol in *C. olerius* is 6.50 ± 0.02 mg/100g and is the highest among the phytochemicals analyzed in this work. This amount is higher than the values determined for polyphenols in *S. nigrum* (4.58 ± 91.40 mg/100g) and lower than the values obtained in *C. album* (8.61 ± 90.76 mg/100g) [44]. Polyphenols are secondary metabolites in plants. These compounds are one of the most widely occurring groups of phytochemicals that exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, antithrombotic, cardio-protective and vasodilatory effects [45].

Conclusion

Many local vegetable materials are underutilized because of inadequate scientific knowledge of their nutritional potentials. The results of this study showed that the plant

contain appreciable amounts of Bioactive components with potentials of improving the nutritional status and promoting the health of people especially in developing countries like Nigeria, where nutrient deficiencies are prevalent.

UNDER PEER REVIEW

REFERENCES

- 1 Vainio-Matilla, K. (2000): Wild vegetables used by the samba in the Usambara mountains, NE Tanzania. *Annual Botany Science*, 37, 57 – 67.
- 2 Ejoh, A. R., Tanya, A.N.; Djuikwo, N.V. & Mbofung, C.M. (2005): Effect of processing and preservation methods on vitamin C and total carotenoid levels of some *Vernonia* (Bitter leaf) species. *African Journal of Food and Nutrition Development*, 5, 105 – 117.
- 3 Ekop, A.S. (2007). Determination of chemical composition of *Gnetum africanum* (AFANG) seeds. *Pakistan Journal of Nutrition*, 6(1), 40 – 43.
- 4 Alabi, D.A., Onibudo, M.Z. & Amusa, N.A. (2005): Chemicals and nutritional composition of four botanicals with fungitoxic properties. *World Journal of Agricultural Science*, 1(1), 84 – 88.
- 5 Tomori, W.B. & Obijole, O. A. (2000). Mineral Composition of some less utilized vegetable in Nigeria. *African Journal of Science and Technology*, 1(2) 153 – 157.
- 6 Aletor, V.A. & Adeogun, O.A. (1995): Nutrient anti-nutrient components of some tropical leafy vegetables. *Food Chemistry*, 53, 375 – 379.
- 7 Sofowora, E. A. (1984). Medicinal plants and traditional medicine in Africa. John Wiley and Sons, New York, Pp 142-147.
- 8 Sofowora, L. A. (1993). *Medicinal plants and traditional medicine in Africa*. Spectrum Book Ltd, Ibadan, pp. 55 – 71.
- 9 Birt, D. A. (2006). Phytochemicals and cancer prevention: from epidemiology to mechanism of action. *Journal of the American Dietetic Association*, 106, 20 – 24.
- 10 Shahidi, E. (1996). *Natural antioxidants chemistry, health effects and applications*. Champmaign 111; AOAC Press. 241p.

- 11 Hunter K.J. & Fletcher J.M., (2002). The antioxidant activity and composition of fresh, frozen, jarred and canned vegetables. *Innovative Food Science and Emerging Technology*, 3, 399–406.
- 12 Olumayokun, A., Olajid, R., Ogunleya, T.O. & Erinle, Y. (2004). Anti-inflammatory properties of *Amaranthus spinosus*. *Pharmaceutical Biology*, 4(6), 521-525.
- 13 AOAC. (2005). *Official Method of Analysis* (18th ed.). Association of Official Analytical Chemists Washington D.C. p. 106.
- 14 Trease, G.E. & Evans, W.C. (1989). *Pharmacognosy*. 11th edn. Brailliar Tiridel Can. Macmillan publishers.
- 15 Ekwenye, U.N., Okorie, C.F. (2010). Antibacterial activity of Tetrapleura tetraptera Taub. Pod Extract. *International Journal of Pharmaceutical and Biosciences*, 1(4):734-741.
- 16 Harborne, J. B. (1973). *Phytochemical methods* (p. 278). London: Chapman and Hall.
- 17 Kirk, I. & Sawyer, J. (1998). Pearson's Food Composition and Analysis. *Food Composition*, 19:15-50.
- 18 Robinson, D.S. (1990). *Food biochemistry and nutritional value*. Longman Scientific and technical publisher, New York. USA.
- 19 Onwordi, C.T., Anuoluwa, M.O. & Adedoja, D.W. (2009). The proximate and mineral compositions of three leafy vegetables commonly consumed in Lagos, Nigeria. *African Journal of Pure and Applied Chemistry*, 3(6):102-107
- 20 Ajewole, K. (1999). Analysis of the nutritive elements in some Nigerian leafy vegetables. Proceedings 23rd Annual NIFST Conference, Abuja. 25-27.

- 21 Adeniyi, S.A., Ehiagbonare, J.E. & Nwagwu, S.C.O. (2011). Nutritional evaluation of some staple leafy vegetables in Southern Nigeria. *International Journal of Agricultural Food Science*, 2(2), 37 – 43.
- 22 Adebayo, A.O. (2010). Effect of processing methods on chemical and consumer acceptability of Kenaf and Corchorus vegetables. *Journal of American Science*, 6 (2), 165 – 170.
- 23 FAO corporate document (2013). Fruit and vegetables processing.....www.fao.org/docrep/v5030E/v5030E06.htm. Accessed on 19/02/2013.
- 24 Olaiya, C.O. & Adigun, A.A. (2010): Chemical manipulation of tomato growth on flavonoids, lycopene and mineral content. *African Journal of Plant Science*, 4(6), 167 – 171.
- 25 Ndlovu, J. & Afolayan, A.J. (2008). Nutritional analysis of the South African Wild vegetable (*Corchorus olitorius* L.). *Asian Journal of Plant Science*, 7(6): 61 – 618.
- 26 Shokombi, O.S., Anionwu, O.A., Sonuga, O.S. & Tayo, G.O. (2011). Effect of post harvest processing on the nutrient and anti-nutrient composition of *Vernonia amygdalina* leaf. *African Journal of Biotechnology*, 10(53): 10980 – 10985.
- 27 Yekeen, T.A., Akintaro, O.I., Akinboro, A. & Azeez, M.A. (2013): Evaluation of cytotoxic and nutrient composition of three commonly consumed vegetables in South-Western Nigeria. *African Journal of Food Agricultural Development*, 13(2): 7452 – 7466.
- 28 Asibey-Berko, E. & Tayie, F.A.K. (1999): Proximate analysis of some underutilized Ghanaian vegetables. *Ghana Journal of Science*, 39: 91 – 92.

- 29 Palafox-Carlos, H., Ayala-Zavala, J.F. and Gonzalez-Aguiler, G.A. (2011): The role of dietary fiber in the bio accessibility and bioavailability of fruit and vegetable antioxidants. *Journal of Food Science*, 76(1): R6 – R15.
- 30 Idris, S., Yisa, J. & Ndamitso (2009): Nutritional composition of *Corchorus olitorius* leaves. *Nigerian Journal of Clinical Medicine*, 5(2), 187 – 192.
- 31 Ijeomah, A.U., Ugwona, F.U. & Ibrahim, Y. (2012): Nutrient composition of three commonly consumed indigenous vegetables of North-Central Nigeria. *Nigerian Journal Agricultural Environment*, 8(1), 17 – 21.
- 32 Edeoga, H.O., Okwu, D.E. & Mbaeble, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4 (7), 685-688.
- 33 Harbone, J.B. (1998). Methods of extraction and isolation. In: *Phytochemical methods*. Chapman and Hall, London. Pp60-66.
- 34 Yukari, I., Youichi, F., Ikuko, N. & Itsuru, Y. (1995). Quantitative HPLC analysis of cardiac glycosides in *Digitalis purpurea* leaves. *Journal National Productivity*, 58(60), 897-901.
- 35 Francis, C., George, G., Zohar, K., Harinder, P.S., Makhar, L.M. & Klaus, B. (2002). The biological action of saponins in animal system: a review. *British Journal of Nutrition*, 88(6), 587- 6051.
- 36 Haslam, E. (1996). Natural Polyphenols (vegetable tannins) as drugs: possible modes of action. *Journal of National Productivity*, 59, 205- 215.
- 37 Okwu, D.E. & Emenike, I.N. (2006). Evaluation of the phytonutrients and vitamin contents of Citrus fruits. *International Journal of Molecular Medicine and Advance Science*, 2, 1–6.

- 38 Aiyegoro O.A. & Okoh, A.I. (2009). Phytochemical screening and polyphenolic antioxidant activity of aqueous crude leaf extract of *Helichrysum pedunculatum*. *International Journal of Molecular Science*, 10, 4990–5001.
- 39 Nnam, N.M., Onyebuchi, J. C. & Madukwe, E. A. (2012). Nutrient and phytochemical composition of some leafy vegetables with medicinal significance. *Nigerian Journal of Nutritional Science*. 33, 2-6
- 40 Nwinuka, N.M., Ibeh, N.M. & Ekeke, G.I. (2005). Proximate composition and levels of some toxicants in four commonly consumed spices. *Journal of Applied Science of Environmental Management*, 9(1), 150155.
- 41 Nnam, N.M., Udofia, U. S. & Atafit, U. A. (2009) Nutrient and Phytochemical Composition of onion (*Allium Cepa*) and ginger (*Zingiber Officinale*). *Nigerian Journal of Nutrition Science*. 30(2), 50-53.
- 42 Schiavone, A., Guo, K. & Tassone, S. (2008). Effects of a Natural Extract of Chestnut Wood on Digestibility, Performance Traits and nitrogen Balance of Broiler Chicks. *Poultry Science* 87 (3), 521- 527.
- 43 Prior, R.L., Gul, W.U.X, Jacob, R. A., Sotouden, G., Kudedr, A. A. & Cook, R.A. (2007). Plasma Antioxidant Capacity Changes Following a Meal as a Measure of the Ability of a Food to Alter in View Antioxidant Status. *Journal of American College of Nutrition*. Apr; 26(2), 170-81.
- 44 Afolayan, A. J. & Jimoh, F. O. (2009). Nutritional quality of some wild leafy vegetables in South Africa. *International Journal of Food Sciences and Nutrition*, 60(5), 424431
- 45 Manach, C., Mazur, A. & Scalbert, A. (2005). Polyphenols and prevention of cardiovascular diseases. *Current Opinion Lipidology*, 16, 77-84.

UNDER PEER REVIEW