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Research Article

PROXIMATE CONTENT AND CHEMICAL COMPOSITION OF *OCIMUM VIRIDIS* LEAF AND *OCIMUM GRATISSIUM* LEAF

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ABSTRACT
This study is aimed at determining the proximate content, concentration of some micro/macro elements and the phytochemistry of *Ocimum viridis* (scent leafs) and *Ocimum gratissimum* (jaw's mallow leafs) with the view of updating its usage in orthodox and herbal medicine by man in the treatment of dysentery, diarrhea and wound healing. The leaves sampled of *Ocimum viridis* and *Ocimum gratissimum* were collected from Zannari, Jere L.G.A, Borno state, Nigeria. The proximate analysis was carried out using methods of Association of Analytical Chemist and the results showed that *Ocimum viridis* has higher dry matter (99.70%), crude protein (12.48%), Nitrogen free extract (2.03%), Ash (6.5%) and fats (7.0%). While carbohydrate (83.40%), crude fibre (45.50%) and moisture content (0.4%) were estimated to be higher in *Ocimum gratissimum*. The levels of eight (8) elements (Ca, F, Cu, Cr, Mn, Zn, Fe, and Pb) were determined using Atomic Absorption Spectrophotometry. The results revealed higher concentration in *Ocimum gratissimum* except for iron and manganese concentration which are higher of the elements understudy in *Ocimum viridis* only the lead was not detected in the samples by the methodology employed for the analysis. The concentration of anions (nitrates, phosphates and sulphates) was estimated using the Smart Spectro Spectrophotometer. It shows that, Ocimum viridis has higher concentration of 11.08 mg/g and 7.04 mg/g in nitrates and sulphates respectively. And *Ocimum gratissimum* has higher concentration of phosphates 6.28 mg/g. The phytochemicals and some heavy and trace elements as well as a few anions were evaluated using standard procedures. The phytochemical screening of both the fresh and dry leafs revealed the presence of very high cardiac glycosides and the flavonoids, terpenoids, saponins, tannins and carbohydrates shows difference in variation of the leafs. And alkaloids were found to be absence in both fresh and dry leafs. The elemental content obtained shows that these sampled leaves could serve as a good dietary source for essential micronutrient because these levels are within the safety baseline content for human consumption, based on World Health Organization acceptable limits.

KEYWORDS: Proximate analysis, micro and macronutrients and phytochemistry of *O.*viridis and *O.*gratissimum.

INTRODUCTION

Vegetables are essential components of the human diet which contains a number of nutritional important such as vitamin A, B, & C which can also be from fruits. Most vegetables from leaves, roots or stems of plants are good sources of vitamins, proteins, fibers and minerals element for human nutrition[1]. A great variety of nutrients are found in vegetables consumed everyday through leaves, spinach, cabbage, carrot, onions, tomatoes and so on. Nutrients are chemicals that an organism needs to live and grow from its environments because they enrich the body system, build and repair tissues, which gives heat and energy to regulate the body processes[2]. The nutrients needed in large quantities are "Macros" and those required in small quantities are "Micros". Basically, any of these nutrients are needed in moderate quantities for the body's mechanism, where there are deficiencies in them; it can lead to ill-health[3] . Most of these micro element play a key role in the human body when consumed because they contain a highly soluble minerals like Calcium, Iron, Manganese, Copper, Potassium, Phosphorus which maintains the acid-base balance of the hydrogen concentration of the body tissue that helps in the complete adsorption of vitamins, proteins, fats and carbohydrates of the food[4] .

The both plants are normally taken as food (spices) which may not really threaten the efficacy of some conventional antibiotics that may have been taken concomitantly with it as been believed in the practices of trado-medicine. The leafs are used in traditional medicine for the treatment of several ailments such as urinary tract, healing of wound, skin and gastro intestinal infections which was evaluated for its antibacterial activities against some clinical bacteria.

Many vegetable contain a substance known as *β-carotene* which is being converted to Vitamin A in the body. Generally, any deep green, yellow and orange colored vegetable are rich source of *β-carotene* like Pawpaw, Carrot, Tomatoes, Pumpkin etc. vitamins B is essential for growth and health eye, skin, nail etc. while the deficiency of it can lead to premature wrinkle, eczema and so on. While vitamin C helps to maintain body tissue, especially bones, teeth, glum and protection against infections. The deficiency can lead to tooth decay, bleeding from gum, premature aging and anemia[5] .

MATERIALS AND METHODS

Plant Collection and Identification

The plant material (Scent leaf and Jew's melon) used in this study was collected in November, 2009 from Zannari ward, Jere LGA, Borno state, Nigeria. The plant specimen was identified by a plant taxonomist, Prof. S. S. Sanusi, Department of Biological Science, while the voucher specimen No. 09-733B was deposited at the Post-Graduate Research Laboratory, Department of Chemistry, and University of Maiduguri.

Elemental content analysis of the plant material

The macro and micro element were determined using Perkin-Elmer Analyst 300 single beam Atomic Absorption Spectrophotometery and the data was obtained in parts per million (ppm) which was then converted to mg/g. The laboratory procedure for the preparation and determination of macro and micro elements were used as outlined by Radojevic and Bashkin[7]for plant samples.

Ashing, digestion and analysis of samples

The air-dried plant samples were pulverized manually in wooden mortar and pestle into coarse powder. 5.0g of each sample was independently packed into an acid-wash porcelain crucible and then placed in a muffle furnace for three (3) hours at 550^0c. The crucible were removed from the furnace and cooled. 10ml of 6 M HCl were added and covered; this content was heated on a steam bath for

15minutes. 1ml of HNO_3 was later added for an hour so as to dehydrate Silica and completely digest organic substances. Lastly 5ml of 6 M HCl and 10ml of water were added and the mixture was heated on a steam bath to complete dissolution. The mixture was cooled and filtered through a Whatman No.1 filter paper into a 100ml volumetric flask and then made up to the mark with distilled water[7].

Preparation and Determination of Proximate Composition

The air-dried leaves were and ground into fine powder. About 10.0g of the grounded leaves was exhaustively processed for various parameters according to the Association of Official Analytical Chemists methods[8and 9].

The proximate analysis (carbohydrates, fats, crude protein, moisture, dry matter, crude fiber, nitrogen free extract and ash) of the leaves were determined using AOAC methods. Using weight difference, moisture and ash were obtained. The fiber content was estimated from the loss in weight of crucible and its content on ignition. Carbohydrate was determined when the sum of the percentage of moisture, ash, crude protein and fats were subtracted from 100. The nitrogen value, which is the precursor for protein of a substance, was determined by micro kjeldahi method, involving digestion, distillation and finally titration of the sample[8]. The nitrogen value was converted to protein by multiplying with a factor of 6.25. The determination of crude lipids content of the samples was done using soxhlet type of direct solvent extraction method. The solvent used was petroleum ether (boiling range 40 -60^{0}c). While the nitrogen free extract was calculated indirectly by difference as the sum of crude protein, fibre, fats and ash subtracted from 100. The result of proximate value was all estimated as percentage[8, 9] .

Anions analysis in plant samples

Determination of nitrate

The concentration of nitrate in the leaves *ocimum viridis* and *ocimum gratissimum* were carried out by standard cadmium reduction method using Smart spectro Spectrophotometer[10] . Plant samples solution were prepared by chopping each sample into smaller sizes. About 0.5g of the samples was transferred into 100ml flask and soaked with distilled water. The flask was corked and shaken for 30min, then filtered into another 100ml volumetric flask and the volume made to the mark with distilled water[7] .

Determination of phosphates

The air-dried and fine powdered samples of *ocimum viridis* and *ocimum gratissimum* were used. About 0.5g was weighed into crucibles; this was then underplayed wit 5 ml of (20%) (w/v) magnesium acetate and thereafter evaporated to dryness. The content of the crucible was then transferred into the muffler furnace and heated to 500^{o}C. Furthermore, the crucible contents were ashed at 500^{o}C for 4 hours and then removed and cooled in desiccators. 10ml of 6 M HCl were then added to each of the crucible and covered and then heated on a water bath for 15minutes. The contents of the crucible were completely transferred into different evaporating basins and 1ml of concentrated HNO_3 was added. The heating continued for an hour to dehydrate silica. 1ml of 6 M HCl further added, swirled and then followed by addition of 10ml distilled water and again heated on the water bath for complete dissolution. The contents of the evaporating basins were cooled and then filtered through a Whatman No. 1 filter paper into 100 ml volumetric flasks and the volumes made up to the marks with distilled water[7] The phosphate was determined using batch direct reading 2000 spectrophotometer.

Determination of sulphate

Sulphate was determined using smart spectrophotometer[10]. The samples were prepared as follows: 5 ml of magnesium nitrate solutions were added to each of the grounded samples in the crucibles which were then heated to 180^{o}C on a hot plate. The heating process was allowed to continue until the color of the samples changed from brown to yellow[8] . The samples were then transferred to the furnace at a temperature of 500^{o}C for 4 hours. Magnesium nitrate was added to prevent loss of sulphur. The contents of each crucible were carefully transferred to different evaporating basins; 10ml of concentrated HCl were added to each and covered with watch glass. On cooling, 10 ml of distilled water were added to each of the basins and the contents filtered into 100ml volumetric flask and the volume made up to the mark with distilled water[7].

RESULTS AND DISCUSSIONS

Proximate Composition

The proximate analysis shows variant proportions of nutrients and contents in their results, and the data are presented on **Figure 1**. The observed value for carbohydrate in *O. viridis* was 73.72 less than that in *O. gratissimum* 83.40. Certain plants like Croton tiglium that can yield carbohydrates up to a low amount of 15.5%[11]. With this comparison, it shows that *O viridis* and *O gratissimum* are good source of carbohydrate. The crude protein in *O viridis* is 12.48 while that of *O gratissimum* is 11.20, and is almost 2.5 fold less than results reported on Croton tiglium[11] . The moisture content of *O viridis* and *O gratissimum* was valued as 0.3 and 0.4 respectively; it's higher than O. limbata with lower moisture content of 1% [11]. Due to the lipophilic nature in leaves, the fat content result obtained was in *O viridis* 7.30 and *O gratissimum* 3.0. The crude fiber was lower in *O viridis* 29.5 than *O gratissimum* 45.5. While the nitrogen free extract was more in *O viridis*, 2.03 than *O gartissimum* 1.79. Ash content of *O viridis* 6.5 and *O gratissimum* 2.0 was in line with the standard recommended range of 1.5 – 2.5% of nuts and seeds suitable for animal feed[12]. The highest proximate values were presented by percentage dry matter in both *O viridis* and *O gratissimum* 99.7 and 99.6 respectively.

From these results there is an order of increasing nutrient among the plant leaves; Dry matter > Carbohydrate > Fibre > Protein > Fat > Ash > Nitrogen > Moisture content.

Anions

The levels of anions in the leaves of O. viridis and O. gratissimum are presented in **Figure 2**: The results shows that the concentration (mg/g) of nitrates and sulphates were higher in the *O. viridis* leaves than the *O. gratissimum* leaves. While, the O. gratissimum leaves are higher in phosphates. Thus, the contents of nitrates tend to varies in order of their increasing pattern of plants, 12. However, our finding does not agreed with the principles of increasing order.

Elemental Analysis

The results of macro and micro elements concentration reported in mg/g are presented in **Table 1**

Macro elements

According to WHO recommendation, medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals and regulates the limit of toxic metal like Lead (Pb), Cadmium (Cd) and Arsenic (As) that amount to 10.00, 0.30 and 1.0mg/g respectively[14] . Although, macro and micro elements could however produce harmful effect in excessive amount[1]. The results of the two macro elements analyzed (Calcium and Fluoride) shows that, the concentration of calcium was higher

than that of fluoride in both species. This shows that most of the leaves store more of macro elements for their photosynthesis.

Micro elements

This study reports the concentration of six (6) micro elements. Chromium (Cr), Copper (Cu), Iron (Fe), Lead (Pb), Manganese (Mn) and Zinc (Zn). The result shows that iron (Fe) has the highest concentration in both leafs species of 14.8348 and 10.3241 mg/g respectively. The lowest concentration is in Copper (Cu) of the both leaves of 0.1644 and 0.3148 mg/g respectively. And the concentration of lead (Pb) was not detected. The other micro elements like Iron (Fe), Copper (Cu) and Manganese (Mn) are considered as essential for normal life processes. Whereas, the function of Zinc(Zn) in the human being is well documented as essential for the normal functioning of the cell which include protein synthesis, carbohydrate metabolism, cell growth and cell division. And the deficiency syndrome manifests itself to retardation of growth.

The concentration of iron plays an importance role in the production of hemoglobin with protein and oxygenation of red blood cells which improves the function of enzymes in protein metabolism and it's absorbed in the small intestine that is stored in the liver, bone marrow and blood[15] .A comparison of the levels of the essential elements calcium, magnesium and zinc with the WHO dietary requirement show that these elements occur in levels below the maximum permissible level allowed by WHO which are 500.00ppm, 615.00ppm and 15.00ppm respectively and hence occur within safe limit[2] . The concentration of manganese in the leaves studied was found to be within the range of daily dietary intake of 2.5-5.0mg well being[2] . Thus, the concentration of chromium does not apparently pose a health threat because it is found within the range of 1.6-1.7mg, safe for human consumption[5] . Some micro elements are essential while some are not for it will have as well defined evidence in human metabolism.

Furthermore, the level of micronutrient obtained does not appear to pose any serious health hazard problem of concern yet. The study has showed that the samples could serve as a good dietary source for essential micronutrients. Since their deficiency or toxicity in humans may result in severe consequence.

Phytochemical composition of *ocimum viridis* and *ocimum gratissimum* leaves

From **Table 2.** shows that, there is a very high concentration of cardiac glycosides of the both leafs. The terpenoids has a low concentration in ocimum gratissimum. While, Carbohydrates, Tannins, Saponins and Flavonoids are found to be moderate in both leafs and alkaloids are absence in both leafs as well.

From **Table 3.** shows that ocimum gratissimum is higher in concentration of Flavonoids, Saponins, Carbohydrates and Cardiac glycosides. While, the Tannis and Terpeoids are of moderate concentration both leafs and there is also an absence of Alkaloids in both leafs

The phytochemical screening of the both leafs showed the presence of high cardiac glycoside. Although, they are known to exert pronounced physiological action this may be poisonous to animals and man. Inspite of its toxicity, its drugs are choice for the treatment of congestive heart. Saponins are also glycoside in nature having expectorant action and cardiotonic activity[12] .

The important active chemical constituents which are products of secondary metabolism in plants are alkaloids, glycosides, tannins, flavonoids, terpenoids and primary which are amino acids, enzymes, peptides, vitamins and so on. May not have therapeutic effect, but may possibly increase the efficiency of the therapeutically important principles. Hence, better therapeutic effects are obtained by combination of active principles in each leafs than by single isolated substance.

CONCLUSION

In conclusion, the proximate and chemical contents of leaf were mostly found to be within the permissible region set by World Health Organization (WHO). The phytochemistry reveals the presence of secondary metabolites which are rich in volatile oils with 75% of thymol and some parts of Northern Nigeria used it in the treating of diarrhea and other diseases. The elemental anaylsis also showed a safe level of all the elements determined in both leafs.

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Table 1: **Elemental analysis of O. *viridis* and O. *gratissimum* leaves**.

Elements		Concentration (mg/g) *Ocimum viridis*	*Ocimum gratissimum*
Macro elements	Calcium (Ca)	0.6235	0.6858
Microelements	Fluoride (F^-)	0.2100	0.260
	Chromium(Cr)	1.6000	1.6800
	Copper (Cu)	0.1644	0.3869
	Iron (Fe)	14.835	10.324
	Lead (Pb)	ND	ND
	Manganese (Mn)	0.4211	0.3148
	Zinc (Zn)	2.1430	2.9004

ND = Not detected

Table 2. Phytochemical Screening of O. *viridis* and O. *gratissimum* leaves

Fresh analysis of *o. viridis* and *o. gratissimum*

Constituents	***Ocimum viridis***	***Ocimum gratissimum***
Carbohydrate	++	+++
Cardiac glycosides	+++	+++
Flavonoids	+	+
Saponins	++	++
Tannins	+	+
Terpenes	++	+
Alkaloids	-	-

Table 3 Dry analysis of *o. viridis* and *o. gratissimum*

Constituents	***Ocimum viridis***	***Ocimum gratissimum***
Carbohydrate	+	++
Cardiac glycosides	+	++
Flavonoids	+	++
Saponins	+	++
Tannins	+	+
Terpenes	+	+
Alkaloids	-	-

Note: (+++) Highly present, (++) Moderate present, (+) Present and (–) Absence

Figure 1:

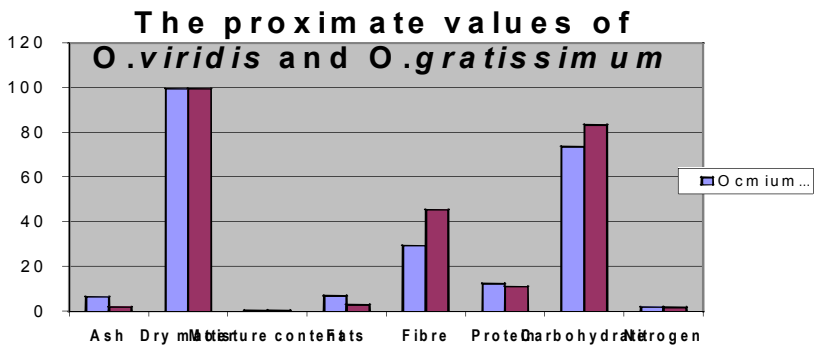
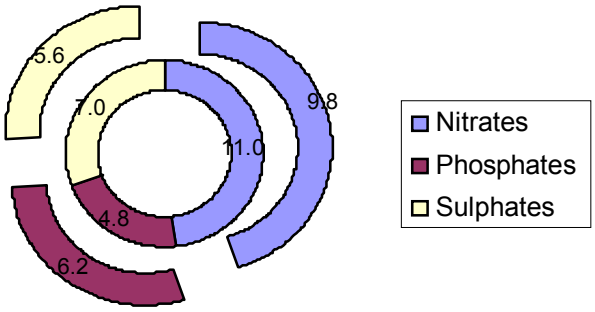


Figure 2

Concentration of anions (mg/g) in *O. viridis* and *O. gratissimum* leaves



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