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Comparative Analysis of the Phytochemicals, Proximate and Mineral Compositions of Scent Leaf (*Ocimum gratissimum*) and Bitter Leaf (*Vernonia amygdalina*) Leaves

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Authors' contributions

This work was carried out in collaboration between both authors. Author NMM designed the study, wrote the literature, performed the statistical analysis and wrote the first draft of the manuscript. Author NFA wrote the other protocols and managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Ocimum gratissimum (fam.Lamiaceae) 'Scent leaf' and *Vernonia amygdalina* (fam.Asteraceae) 'Bitter leaf' are commonly consumed vegetables in Southern Nigeria. *O. gratissimum* is used by the 'Igbos' of South East Nigeria to flavor soups and stews while it is popular with the 'Yorubas' of South West Nigeria for treatment of stomach disorders. *V. amygdalina* is a well known vegetable in West Africa used as both food and for its pharmaceutical properties. The enormous use of these vegetables in the diet motivated the present study whereby the food compositions, mineral constituents and the phytochemicals of the two vegetables were determined. Qualitative analysis, of the aqueous extracts, revealed the presence of saponins, terpenoids, tannins, flavonoids, steroids, alkaloids, phenolics and anthraquinones in both vegetables. Generally *V. amygdalina* contained higher quantities of secondary metabolites, saponin which was reported as the highest amount was 5.71±0.12 mg/g and 3.52±0.01 mg/g followed by terpenoids 5.64±0.11 mg/g and

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3.40±0.11 mg/g in *V. amygdalina* and *O. gratissimum* respectively. There is however a minute amount of anthraquinone in the two vegetables. Proximate analysis and macro elements were determined using their various standard methods. The results of proximate analysis revealed the presence of moisture (12.28 ± 0.02% and 10.01± 0.01%) protein (35.37±0.11% and 22.20±0.02%) and total ash (6.00±0.20% and 5.75±0.10%) each in *V. amygdalina* and *O. gratisimum* respectively. The following minerals were present calcium, magnesium, potassium, sodium, iron, copper and zinc, with zinc being in the highest concentration (46.80± 0.03 mg/kg and 58.00±0.12 mg/kg) followed by copper (9.45±0.11 mg/kg and 16.60 ± 0.03 mg/kg) each in *V. amygdalina* and *O. gratissimum* respectively. There are higher values of phytochemicals and food components in *V. amygdalina* while *O. gratissimum* had higher mineral contents. These two vegetables are potential source of components for complementary medicine.

Keywords: Plant; food; bitter leaf; scent leaf; nutrients.

1. INTRODUCTION

Medicinal plants have been identified and known throughout human history as plants whose roots, stems, leaves and seeds possess therapeutic, tonic, and other pharmacological potentials. They are used as drug, food additives and for their nutritive values. Medicinal values of some plants lie in the chemical substances that produce definite physiological actions in their body .Among the chemical substances are phytochemicals which are bioactive natural compounds found in plants [1]. Many plants that are used in traditional medicine to alleviate symptoms of illnesses have been found to posses phytochemicals, examples of such plants include; *Az*adira indica (Dogonyaro), *Vernonia amygdalina* (Bitter leaf), *Allium sativum* (Garlic), [*Zingiber officinale* (Ginger)*, Moringa oliefera* (Moringa), *Ocimum gratissimum* (Scent leaf) etc. Chemical compounds present in these plants mediate their effects on human body through processes identical to those already well understood from chemical compounds in conventional drugs. Thus herbal medications do not differ greatly from conventional drugs in terms of their effects on the body .This enables herbal medicines to be effective as well as having the potentials to cause harmful side effects [2,3]. The use of herbs and search for drugs and dietary supplements derived from plants have accelerated in recent years because medicinal plants are known to contain some chemical substances which can be used for treatment purposes or to produce drugs [4]. Medicinal plants play vital roles in the health of individuals, in fact most modern drugs are derived from them [5,6]. Medicinal- plant -based drugs have the advantage of being simple, effective and have broad spectrum activity. An ethno-botanical and ubiquitous plant serves as rich resources of natural drugs for research development [7]. With the crises of covid-19 pandemic, researchers throughout the work are combing the earth searching for vaccines, remedies and possible breakthrough with the use of conventional drugs and medicinal plants.

Knowledge of the chemical constituents of plants is necessary for the discovery of the therapeutic agents in plants and for the purpose of disclosing new sources of economic materials like essential oils, gums, precursors etc for the synthesis of complex chemical substances [8]. Pharmacologists, natural products and environmental chemists, botanists, microbiologists, and other researchers are combing the earth looking for phytochemicals from plants that could be further developed for treatment of various diseases and for new leads to development. The revival of interest in the use and importance of medicinal plants by the WHO and other developing countries has led to intensified efforts on the documentation of ethno-medical data of medicinal plants as traditional herbalists keep no records, their knowledge is passed on verbally from one generation to the other leading to missing gaps in knowledge. All these intrigued the researchers to this topic as much information are needed on the phytochemicals, nutritional value and mineral constituents of medicinal plants hence the study focused on *Vernonia amygdalina* and *Ocimum gratissimum* two of the most commonly used vegetables in Southern Nigeria.

Ocimum gratissimum (Scent leaf) is a scented shrub with lime green fuzzy leaves which are used to flavor soup and spice food due to their strong aromatic odour [9]. *Ocimum gratissimum* is grown for the essential oil in its leaves and stems. The essential oil possesses antibacterial

properties [10], and is also used as an insect repellant so also the leaves when dry and burnt [10]. It is used in the traditional medicine for treatment of diarrhea [11], stomach ache, as a febrifuge, and components of anti-malaria remedies [12], general tonic, and antiseptic in wound dressing, skin infection, conjunctivitis and bronchitis. An infusion of the leaves called "Ocimum tea" is dispensed as a remedy for fever and diaphoresis. The roots are used as sedatives for children. The phytochemical screening of *Ocimum gratissimum* (scent leaf) shows that it is rich in alkaloids, tannins, phytates, flavonoids and oligosaccharides [13].

Vernonia amygdalina (Bitter leaf) is a small evergreen shrub that grows well in tropical Africa. It is called bitter leaf because of its bitter taste. The bitterness is as a result of the presence of the sesquiterpenes lactones and steroid glycosides [1]. *Vernonia amygdalina* is the most widely cultivated of the genus vernonia which has about 1000 species of the shrub [14], an ever green shrub with a characteristics odour and bitter taste .It is well known as a medicinal plant for diabetes. In Nigeria the leaves are used as vegetables for cooking the popular bitter leaf soup as it stimulates the digestive system.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Samples of *Ocimum gratissimum* and *Vernonia amygdalina* leaves were collected from a garden behind Federal Government College Okigwe in Imo State Nigeria, identified and authenticated in the Department of Crop Science, Micheal Okpara University of Agriculture Umudike Abia State Nigeria. The leaves of both samples were separately washed with distilled water, spread at room temperature, allowed to dry for two weeks and pulverized using Thomas Willey milling machine into fine powder and sieved through 2mm mesh sieve. The dried ground samples were separately stored in two different airtight containers at room temperature.

2.2 Extraction

100 grams of the dried ground bitter leaf samples were put in a 1000 ml beaker and 500 ml of deionised water added and stirred vigorously after which the plant residue was filtered through a muslin cloth and the obtained filtrate further filtered using Whatman No 1. Filter paper. The filtrate was evaporated to dryness using a rotatory evaporator [1,15,16]. The same procedure was carried out for *Ocimum gratissimum* (scent leaf).

2.3 Phytochemical Analysis

The qualitative and quantitative phytochemical screening of the extracts of *Ocimum gratissimum* and *Vernonia amygdalina* for secondary metabolites such as tannins, alkaloids, saponins, flavonoids, phenols, steroids, anthraquinones and terpenoids were carried out following the standard procedures according to the methods described by Abdullahi [17,18].

2.4 Proximate Analysis

The dried ground samples of *O. gratissimum* and *V. amygdalina* were analyzed for percentage moisture, ash, fibre, fat, carbohydrate and protein using the methods of AOAC and Mgbemena [19,20].

2.5 Mineral Analysis

The digested samples were analysed for minerals using their different methods of analysis. Flame photometer (Jenway Ltd, Dunmow Essex UK) was used to determine calcium, magnesium, potassium and sodium contents in the samples while Atomic Absorption Spectrometer (Bulk Scientific East Norwalk, USA) was used for the determination of iron, copper and zinc [20].

3. RESULTS AND DISCUSSION

The results of the qualitative and quantitative phytochemical analysis of the leaves extracts of *Vernonia amygdalina* and *Ocimum gratissimum* were shown in Table 1.

The results of the qualitative phytochemical analysis of *Ocimum gratissimum a*nd *Vernonia amygdalina* revealed the presence of saponin, tannin, flavonoids, steroids, terpenoids and alkaloids. *O.gratissimum* also contained phenols but lacked anthroquinones while *V. amygdalina* contained both anthroquinones and phenols. However, the quantitative phytochemical analysis revealed the presence of all the secondary metabolites in varied quantities in both leaves extracts. Saponin was the highest among the phytochemicals determined in both leaves extracts with the value 5.71 ± 0.12 mg/g in *V. amygdalina a*nd 3.52±0.01 mg/g in *O. gratissimum*, this is followed by terpenoids with

Table 1. The results of the Qualitative and Quantitative Phytochemical analysis of the water extract of the leaves of *Vernonia amygdalina* and *Ocimum gratissimum* (mg/100 g)

Phytochemicals	*Vernonia amygdalina*		*Ocimum gratissimum*	
	Qualitative	Quantitative	Qualitative	Quantitative
Saponin	+ + +	5.71 ± 0.12	+ +	3.52 ±0.01
Tannin	+ +	4.90±0.23	+	2.48 ± 0.12
Flavonoids	++	4.60±0.01	+	2.00± 0.03
Steroids	+	0.50 ±0.02	+	0.48 ± 0.02
Terpenoids	+ + +	5.64 ± 0.11	+ +	3.40 ± 0.11
Anthroquinones	-	0.06 ±0.14	-	0.07 ± 0.14
Alkaloids	+ +	3.16 ± 0.16	+	2.16 ± 0.02
Phenol	-	0.07 ± 0.06	+	0.73 ± 0.13

(+++) =highly present (++) = moderately present (+) =present (-) = absent

the value 5.64 ± 0.11 mg/g in *V. amygdalina* and 3.40±0.11 mg/g in *O. gratissimum*, tannin 4.90 ± 0.23 mg/g in *V. amygdalina* and 2.48 ± 0.03 mg/g in *O. gratissimum*, flavonoids 4.60± 0.01 mg/g in *V. amygdalina* and 2.00± 0.03 mg/g in *O. gratissimum*, alkaloids 3.16 ± 0.16 mg/g in *V. amygdalina* and 2.00 ± 0.02 mg/g in *O. gratissimum*, steroids 0.50 ± 0.02 mg/g in *V. amygdalina* and 0.48 ± 0.0 2 mg/g in *O. gratissimum*, phenol and anthroquinines were higher in *O. gratissimum* than in *V. amygdalina*. The results of phytochemical screening agreed with the works of Alexandra [21] and Udochukwu [22] that reported the abundance of bioactive components in these leaves. The presence of these metabolites showed the great potentials of these two vegetables as good sources of useful phytomedicines. The presence of saponins in plants is responsible for the various biological benefits like anti-inflammatory, anti- diabetic, anti- HIV and anti- atherosclerosis. It is very effective in maintaining liver function, lowering blood cholesterol, preventing peptic ulcer. It was also found to reduce nutrient utilization and conversion efficiency as in ruminants [23,24]. The non sugar part of saponin has direct anti-oxidant activities which may result in other benefits such as reduced risk of cancer and heart diseases in man [25]. Terpenoids which ranked second play a vital and diverse role in the field of foods, drugs (as it is exploited in the fight against cancer, malaria, inflammation and a variety of infectious diseases), cosmetics, hormones, vitamins etc [26,27]. Many alkaloids are used as drugs some of which include; nicotine, quinine, caffeine, cocaine and morphine. They have wide range of pharmacological activities such as anti-malaria (quinine), anti-asthma (ephedrine), anti-cancer, analgesics, (morphine), caffeine in tea, coffee stimulate and balance the nervous system [28]. Flavonoids are polyphenolic compounds which contribute to many other colours found in nature. They have been reported to have anti - viral and anti-allergic activities. Flavonoid, quercetin is known for its ability to relieve hay fever, eczema and asthma [29]. The anti-oxidants present in flavonoids may act in synergy with other phytochemicals in *V. amygdalina a*nd *O. gratissimum to* produce the medical benefits inherent in their leaves. The body immune systems, DNA are made from steroids. The type of steroids used to treat diseases is called corticosteroids. The results of qualitative and quantitative phytochemical analysis of this study revealed the presence of enough secondary metabolite in *V. amygdalina a*nd *O. gratissimum.*

Table 2 and Fig. 2 showed the result of proximate analysis of *V. amygdalina* and *O. gratissimum* leaves extracts, crude protein was 35.37 ± 0.11% in V.amygdalina and 22.20 ± 0.02% in O.gratissimum, crude protein was the highest of the food components in V.amygdalina . *V. amygdalina* had higher protein than *O. gratissimum*. Proteins are important in the body for the production of hormones, enzymes and blood plasma. They are immune boosters and can help in cell division as well as in growth [30]. Eating of *V. amygdalina* leaves are encouraged because of its high protein content. Carbohydrate was 32.28 ± 0.01 % in V.amygdalina and 51.85 ± 0.11% in O. gratissimum, carbohydrate was higher in O.gratissimum than V. amygdalina and also the highest of all the food components analysed. The result of carbohydrate agreed with the work of Mgbemena et al. [20] with the value of 51.7 ± 0.13% in the leaves of Irvingia gabonensis and 38.38 ± 0.14 % in Invingia wombulu.The high content of carbohydrates in the leaves of the two species makes them a good source of energy.

Moisture content was 12.28 ± 0.02% in *V. amygdalina* and 10.80 ± 0.01% in O. gratissimum. *O. gratissimum* had lower moisture content than *V. amygdalina* (Table 2). The moderate moisture contents showed that the leaves are less prone to deterioration since foods with high moisture are prone to deterioration [30] Moisture dissolves other substances and carries nutrients throughout the systems leading to effective performance of the organs [31]. Crude Fibre was 7.21±0.01% in *V. amygdalina* and 6.00 ± 0.03% in *O. gratissimum*. Generally dietary fibre helps in digestion and functions the body to slow down the rate of glucose absorption into the blood stream thereby reducing the risk of hyperglycemia, the levels of plasma choresterol and hence preventing colon cancer and cardiovascular diseases [32]. Crude fat was 6.00 ± 0.20% in *V. amygdalina* and 5.75 ± 0.10% in *O. gratissimum*. The value of crude fat was almost the same in the two species. Dietary fats are important not only because of their high energy value but also the fat-soluble vitamin and essential fatty acids contained in the fat of natural foods. Ash contents was 5.87 ± 0.03% in *V. amygdalina* and 3.4 ± 0.03 % in *O. gratissimum.* Ash content was higher in V. amygdalina than *O. gratissimum*. Higher ash content in the leaves of *V. amygdalina* is an indication of high minerals preserved in it [33].

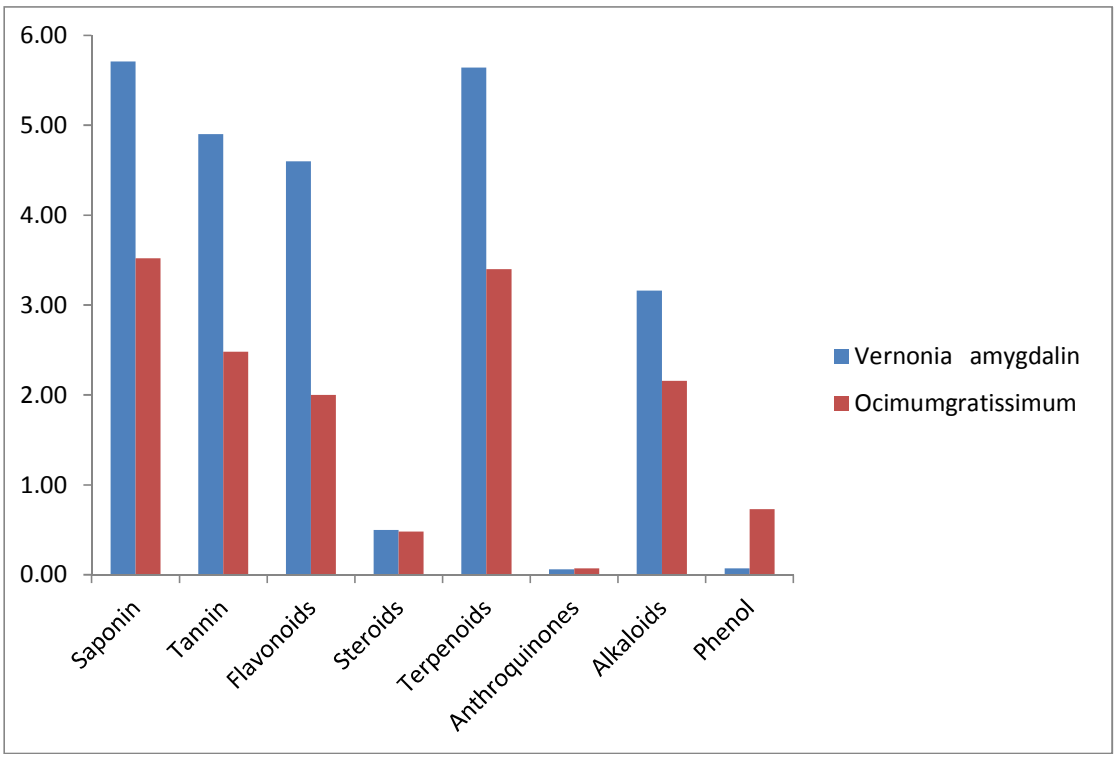

Fig. 1. Phytochemical compositions of *Vernonia amygdalina* and *Ocimum gratissimum*

Table 2. Proximate analysis of *Vernonia amygdalina* and *Ocimum gratissimum* (%)

Components	*Vernonia amygdalina*	*Ocimum gratissimu*
Moisture	12.28± 0.02	10.80 ±0.01
Crude Protein	35.37 ±0.11	22.20± 0.02
Crude Fat	6.00±0.20	5.75± 0.10
Crude Fibre	7.20± 0.01	6.00 ±0.03
Total Ash	5.87 ±0.03	3.40 ±0.11
Carbohydrate	32.28 ±0.01	51.85 ±0.11

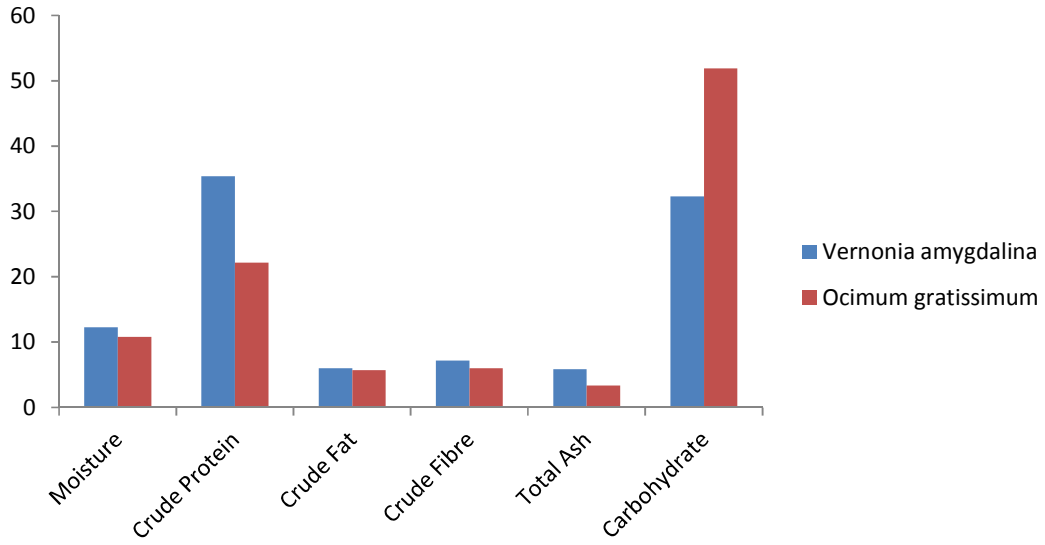

Fig. 2. Proximate compositions of *Vernonia amygdalina* and *Ocimum gratissimum*

Table 3 and Fig. 3 showed the mineral composition of *Vernonia amygdalina* and *Ocimum gratissimum* in mg/kg, the result revealed very high concentration of zinc compared to the other elements. The level of zinc in *O. gratissimum* was 58.00± 0.12 mg/kg and higher than that in V.amygdalina with the level 46.80 ± 0.03 mg/kg. The result was not in agreement with the work of Alexandra [29] that had low levels of zinc (0.20±0.06 mg/kg) in *O. gratissimum*. The high level of zinc is necessary as zinc is required for optimal functioning of physiological and biochemical processes, however, adequate care should be taken as excessive intake of zinc and iron is capable of causing vomiting, dehydration, electrolytic imbalance and lack of muscular co-ordination [34]. The concentration of copper in *O. gratissimum* (16.60. ± 0.03 mg/kg) was far higher than that of *V. amygdalina* (9.45 ± 0.11 mg/kg) and also the second highest among the minerals .The result was in agreement with the work of Olumide et al. [35] that had 17.60 for *O. gratissimum* and 10.55 for *V. amygdalina*. High concentration of copper is recommended as copper deficiency causes cardiovascular disorders as well as anemia. Cu is an essential element in the human body and exists as an integral part of Cu proteins cerulosmin, which is concerned with the release of iron from the cells into the plasma. The concentration of iron in *V. amygdalina* was 2.50 ± 0.01 mg/kg and 2.65± 0.02 mg/kg in O. gratissimum, the level of iron in *O. gratissimum* (2.65±0.02 mg/kg) was higher than in *O. gratissimum*. The level of Fe in this study did not agree with the work of Mgbemena et al. [20] that indicated low iron in the leaves of *I.gabonensis* and *I. wombulu*. The presence of Zn, Fe and Cu are necessary in leafy vegetables as they could serve as good sources of anti oxidants [36,37], thus the high presence of many phytochemicals in the two leaves are very advantageous. The study also revealed the presence of macro- elements calcium, magnesium, potassium and sodium in varying quantities. The levels of these macro-elements were higher in *O. gratissimum* than *V. amygdalina* (Table 3). Potassium had the highest concentrations among the macro-elements with the value 4.20 ± 0.01 mg/kg in *O. gratissimum* and 4.00 ± 0.02 mg/kg in *V. amygdalina*. The result of potassium agreed with the work of Olumide et al. [35] that had 4.30 for *O. gratissimum* and 3. 70 for *V. amygdalina* but did not agree with the work of Awe et al. [33] that had the value 2.28 in *O. gratissimum* and 0.08 in *V. amygdalina*. Potassium is actually needed in high amount as it is responsible for nerve action regulation and osmo- regulation in the body fluid. Potassium and sodium play important role in maintaining the water balance within cells [38]. Calcium was 1.87 ±0.01 mg/kg in *O.gratissimum* and 1.28 ± 0.03 mg/kg in *V. amygdalina*. Calcium is required for strong bones and teeth. Magnessium and sodium had low values. Magnessium helps in maintaining a normal heart rhythm [38].

Table 3. Mineral compositions of *Vernonia amygdalina* and *Ocimum gratissimum* leaves extracts (mg/kg)

Mineral	*Vernonia amygdalina*	*Ocimum gratissimum*
Calcium	1.28±0.03	1.84±0.01
Magnesium	0.07 ±0.01	0.08 ± 0.02
Potassium	4.00 ± 0.02	4.20 ± 0.01
Sodium	0.11± 0.12	0.14 ± 0.03
Iron.	2.50± 0.01	2.65 ±0.02
Copper	9.45± 0.11	16.60 ± 0.03
Zinc	46.80± 0.03	58.00 ± 0.12

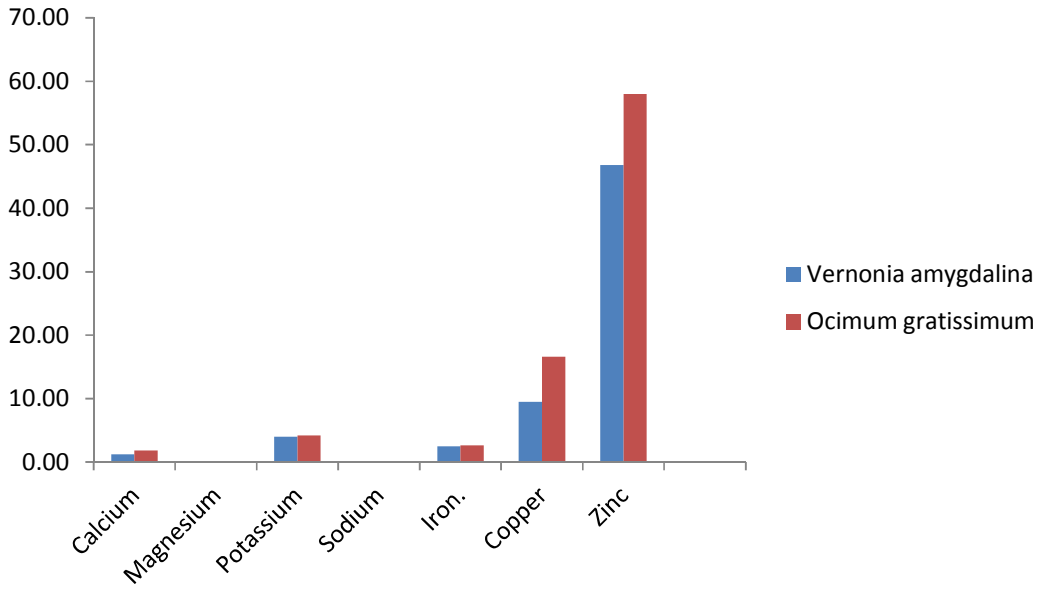

Fig. 3. Mineral compositions of *Vernonia amygdalina* and *Ocimum gratissimum*

4. CONCLUSION

Quantitative phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* reveals the presence of phytochemicals such as saponins, tannins, flavonoids, steroids, terpenoids, anthroquinones and phenols in varied quantities while qualitative phytochemicals reveals the absence of anthroquinones in both leaves and phenol in *V. amygdalina*. Saponin was found in highest quantity followed by terpenoids. *V. amygdalina* contained higher quantities of the phytochemicals than *O. gratissimum*. Proximate analysis revealed the presence of moisture, protein, crude fat, crude fibber ash and carbohydrates with protein and carbohydrate possessing higher percentage of all of them and protein higher in *V. amygdalina*. The use of *V. amygdalina* is highly recommended. Protein found higher in *V. amygdalina* is used to enhance the penetration of macromolecules through cell membrane and this confirms the need for the high use of *V. amygdalina*. The leaves of these two vegetables contained the following macro and micro minerals; calcium, magnesium, potassium, sodium, iron, copper and zinc. Potassium was the highest macro – elements while zinc was the highest micro elements. Zinc was the highest mineral found in the two leaves. *Ocimum gratissimum* contained higher mineral elements than *V. amygdalina* .The use of these two species of vegetables is therefore recommended because of their medicinal, mineral and food values.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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