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Assessment of Free Radical Scavenging Potency and Nutritional Profile of the Formulation of *Ocimum Gratticimum* (Scent Leaf) and *Vernonia amygdalina* (Bitter Leaf)

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ABSTRACT

Bitter leaf (*Vernonia amygdalina)* and scent leaf (*Ocimum gratticimum)* are well known vegetables with lots of medicinal potency, commonly grown in most part of West Africa, but consumed in little amount due to the bitter taste of bitter leaf and availability of alternatives for scent leaf. These vegetables are underutilized, owing to their countless medicinal potency.The study was undertaken to estimate proximate, mineral composition and antioxidant potentials of the formulation of Bitter leaf and Scent leaf vegetables using standard methods. The formulation (1:1) of *V. amygdalina* and *O. gratissimum* contained in percent, moisture (9.15), ash (11.78), crude protein (40.22), crude lipid (6.08), crude fibre (7.69), carbohydrate (45.44) and calorific value (1723.18 kcal). Elemental analyses of the formulation (in mg/100g) revealed that the formulation contained: Sodium (536.67), Potassium (3866.67), Calcium (3255.33), Magnesium (4014.67), Iron (612.13), Nitrogen (6.44) and Phosphorus (2135.07). The qualitative screening for free radical scavenging compounds using 1,1-Diphenyl-2-PicrylHydrazyl (DPPH) was carried out on TLC. The result showed greater activity in the ethanol extract. *In vitro* quantitative determination of free radical scavenging activity using DPPH at 25, 50, 100, 200 and 400 µg/mL also showed ethanol extract with the highest activity (IC_{50} of 48.73 µg/mL). The results of nutritional analyses showed that the formulation is a good source of plant protein, minerals, crude fibre, carbohydrate and calories. From the available data, the nutrient content of the formulation will contribute to nutritional requirements for good health in humans. Also, the results obtained for antioxidant potentials are indications that the formulation may be used in combating free radical related diseases often orchestrated by oxidative stress.

Keywords: *Ocimum gratticimum, Vernonia amygdalina,* Proximate, Antioxidant, Formulation,

1.0 INTRODUCTION

In Africa, many studies have indicated that a vast number of indigenous plants play a significant role in the diet of the populace (Muhammad *et al*., 2011). Plants are common sources of medicine and nutrient, and the dependence of human beings on plants date back to centuries. Common plants can serve as rich resources of natural drug research and development (Kong *et al*., 2008). The medicinal use of these plants ranges from the administration of the plant roots, stem, stem- bark, leaves and seed as extracts in forms of infusion or decoction (Ogbulie *et al*., 2004).

Vegetables are the cheapest and most available sources of important medicine and nutrients, supplying the body with minerals salts, vitamins and certain hormone precursors, protein, energy and essential amino acids (Okafor, 1983; Amaechi, 2009). Most vegetables from leaves, roots or stems of plants are good sources of medicine, vitamins, proteins, fibers and mineral elements for human nutrition (Abdulraham *et al.*, 2003).

The knowledge of various medicinal plants brings about the concept of Polyherbalism or polyherbal formulations. Formulations containing two or more than two herbs are called polyherbal formulation.

Even though single herbal formulations have been well established due to their active phytoconstituents, they are usually present in minute amount and sometimes, they are insufficient to achieve the desirable therapeutic effect (Subramani *et al.,* 2014).

Scientific studies have revealed that plants with varying potency when combined may theoretically produce a greater result, as compared to individual use of the plant and also the sum of their individual effect. Thus, positive herb to herb interaction produces synergism, which could be pharmacokinetics synergism or pharmacodynamics synergism. The popularity of polyherbal formulation is due to their high effectiveness in a vast number of diseases. They have a wide therapeutic range (effective at low dose and safe at high dose), fewer side effect, eco-friendly, cheaper and readily available (Tayade and Patil, 2015).

The plants *Vernonia amygdalina* and *Ocimum gratissimum* belongs to the groups of plants known as daisy and spices respectively. Due to their peculiar flavour, preservative nature, taste and aroma, both plants are classified as condiment vegetables and often used to complement staple foods (Ishola *et al.,* 2017). Leaves of *V. amygdalina* contain antioxidant and anti-microbial properties (Faronbi and Owoeye, 2011). It is rich in vitamins A, C, E, B_1 and B_2 and hence, it is effective in the treatment of a variety of common ailments (Adenuga *et al.,* 2010). Similarly, *O. gratissimum* has medicinal properties (Ajao *et al.,* 2017). Even though both plants are of economic value, only a few people consume *V. amygdalina* because of the bitter taste of its leaf juice compared to other vegetables. Both plants have applications in traditional medicines in Africa and India (Anonymous, 2015). The paper evaluates the nutritional profile and antioxidant activity of the formulation of *Vernonia amygdalina* and *Ocimum gratissimum*.

2.0 MATERIALS AND METHODS

2.1 Sample collection and Treatment

Vernonia amygdalina and *Ocimum gratissimum* plants were collected in November, 2018 from Anyigba in Dekina Local Government Area and Okabo in Ofu Local Government Area of Kogi State, Nigeria. Prior to analyses, the samples were identified at the Botany department, Kogi State University, Anyigba, Nigeria. The specimen voucher numbers are SN-412-90-CD-382 for *O. gratissimum* and SN-38-307-CD-400 for *V. amygdalina.* The samples were air dried and pulverized separately, it was then stored in a polythene bag to prevent moisture.

2.2 Proximate Analysis

The methods of the Association of Official Analytical Chemists (AOAC, 2010) were employed in the determination of moisture, ash, crude lipid, crude protein, crude fibre, available carbohydrate contents and energy value of the formulation.

2.3 Mineral Analysis

The mineral content was determined according to the method described by (AOAC, 2010). Mineral elements (Ca, Mg and Fe) were determined using Atomic Absorption Spectrometry. After acid digestion of the formulation, sodium and potassium were determined by Flame Emission Spectrometry. The phosphorus content was determined using Molybdate method with the aid of colorimeter.

2.4 Preparation and Extraction of Plant Sample

A mass of 100 g pulverized sample of *O. gratissimum* and 100 g of pulverized sample of *V. amygdalina* were measured into two 1000 mL beakers labelled A and B. To beaker A, 800 mL of Chloroform was added and to beaker B, 800 mL of Ethanol was added. The contents of both beakers were thoroughly stirred, covered with a foil paper and left for 72 hours to attain maximum extraction of plant constituents. Both mixtures were filtered separately, and the filtrates were allowed to evaporate to obtain crude extracts.

2.5 Qualitative Determination of Free Radical scavenging compounds using DPPH

Freshly prepared DPPH solution (0.00591 g DPPH in 50 mL methanol) was sprayed on freshly developed TLC plate of each extract. Formation of yellow or white spot(s) against purple background indicates the presence of free radical scavenging compound(s).

2.6 Determination of *in vitro* Free Radical scavenging capacity using DPPH

The free radical scavenging capacity of the extracts were tested against a solution of DPPH (DPPH in methanol) using the method reported by Adesegun *et al.* (2008). Various concentration of the extracts were prepared in methanol (25, 50, 100, 200, 400 µg/mL). The samples of the different concentration were mixed with 2 mL of the prepared DPPH. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 20 minutes. The absorbance was measured at 517 nm using UV-Visible Spectrophotometer for both samples and blank. Ascorbic acid (Vitamin C) at various concentrations was used as standard. Inhibition of free radical scavenging activity by DPPH in percent (I %) was calculated using the equation below:

$$\%I = \{(A_{blank} - A_{sample})/A_{blank}\} \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

3.0 RESULTS

Table 1: Proximate composition of the formulation

Parameters	(O : V = 1 : 1)	*O. gratissimum*	*V. amygdalina*
Moisture (% wet weight)	9.15 ± 0.05	6.48 ± 0.55	2.45 ± 0.05
Ash (% DW)	11.78 ± 0.03	4.25 ± 0.02	4.36 ± 0.03
Crude protein (% DW)	40.22 ± 0.06	14.73 ± 0.38	42.52 ± 0.25
Crude lipid (% DW)	6.08 ± 0.03	2.25 ± 0.03	6.50 ± 0.29
Crude fibre (% DW)	7.69 ± 0.08	5.75 ± 0.30	3.85 ± 0.02
Carbohydrate (% DW)	45.44 ± 0.14	66.34 ± 0.27	40.47 ± 0.07
Calorific value (kcal)	1723.18	1441.51	1633.68

Mean ± SE of three replicates.

Table 2: Elemental composition of the formulation

	Concentration (mg/100g)		
Element	**(O : V = 1 : 1)**	***O. gratissimum***	***V. amygdalina***
Sodium	536.67 ± 4.04	138.34 ± 0.20	128.48 ± 0.08
Potassium	3866.67 ± 5.77	972.30 ± 1.26	3260.10 ± 2.45
Calcium	3255.33 ± 2.89	3217.63 ± 2.68	2766.90 ± 4.12
Magnesium	4014.67 ± 2.31	260.63 ± 0.28	253.15 ± 0.09
Iron	612.13 ± 0.98	590.40 ± 1.30	500.00 ± 0.53
Nitrogen	6.44± 0.006	2.36 ± 0.03	6.80 ± 0.25
Phosphorus	2135.07 ± 0.92	428.20 ± 0.16	625.30 ± 0.03

Mean ± SE of three replicates. O = *Ocimum gratissimum* and V = *Vernonia amygdalina*

Extracts	Inference
Chloroform	++
Ethanol	+++

Table 3: Qualitative Free Radical Scavenging Activity of the formulation on DPPH

Key: ++ = moderately active +++ = highly activ

Table 4: Quantitative Free Radical Scavenging Activity of the formulation using DPPH.

S/N	Concentration ($\mu g/cm^3$)	Zone of inhibition (%)		
		Chloroform Extract	Ethanol Extract	Ascorbic Acid
1	25	10.5±0.5	12.5±0.5	86.5±0.5
2	50	22.5±0.5	12.5±0.5	87.5±0.5
3	100	29.8±0.3	14.8±0.3	87.5±0.5
4	200	32.8±0.3	37.5±0.5	88.5±0.5
5	400	38.8±0.3	65.8±0.3	99.5±0.5

Table 5: IC_{50} Values of the formulation

Extracts	IC_{50}(µg/mL)
Chloroform	84.59
Ethanol	48.73
Ascorbic acid	14.52

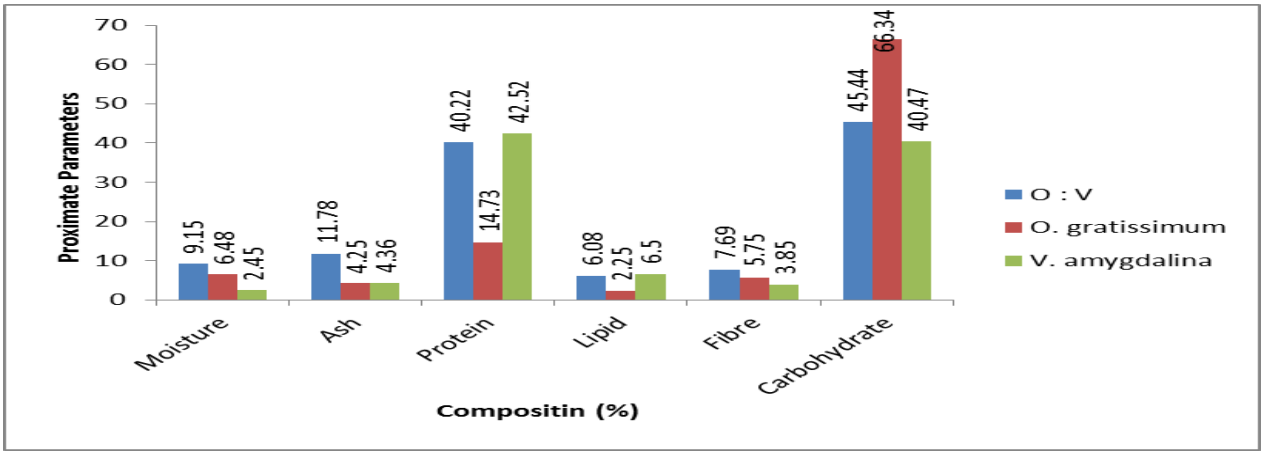

Figure 1: The proximate analyses values of the formulation

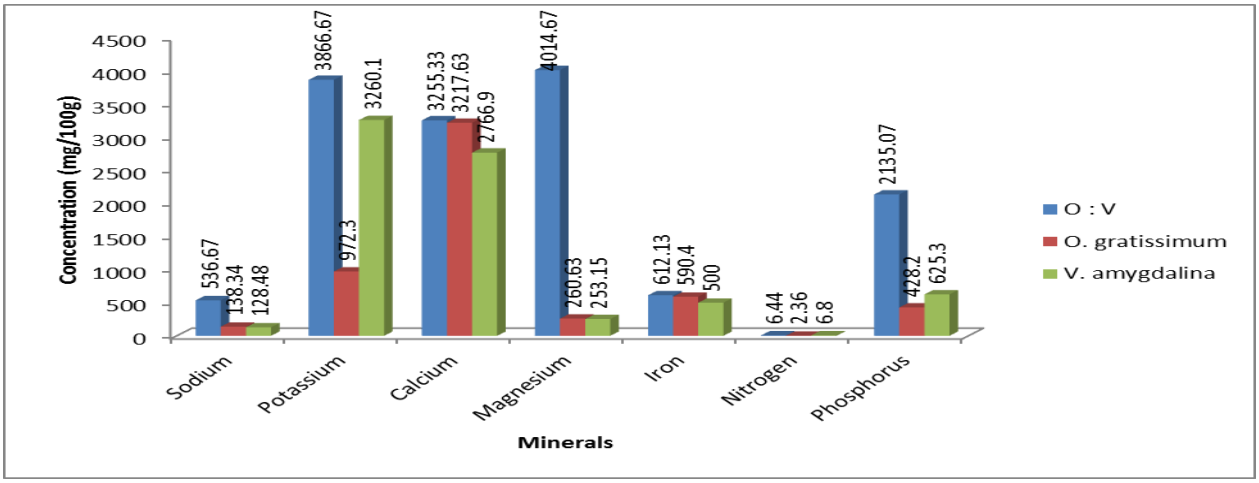

Figure 2: Mineral composition of the formulation

Figure 3: Qualitative Analysis of Free Radical Scavenging Activity of the formulation

Plate 1: Ethanol Extract

Plate 2: Chloroform Extract

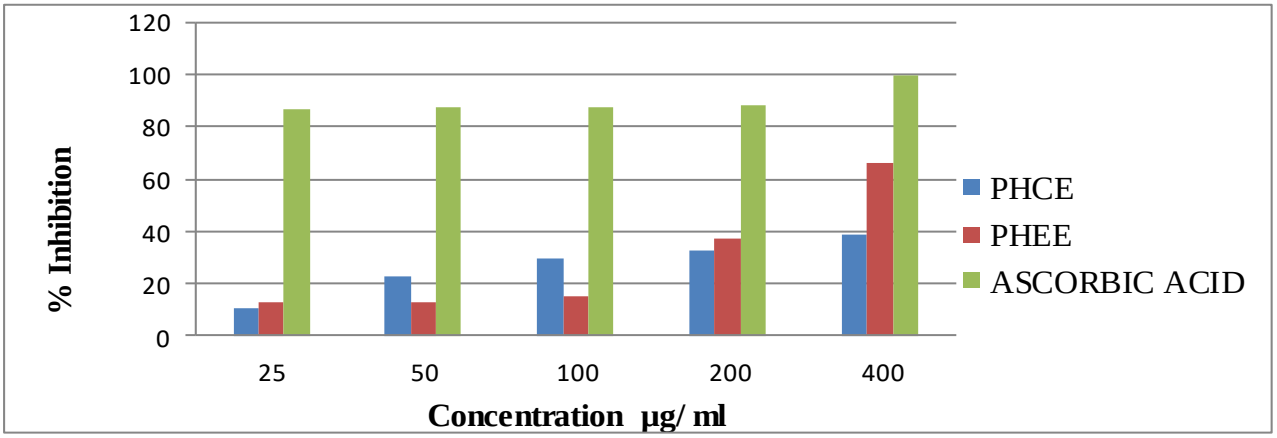

Figure 4: Showing the Free Radical Scavenging activity of the formulation

4.0 DISCUSSION

The proximate composition of the formulation (Table 1 and Figure 1) shows ash content of 11.78±0.03, which represents the index of mineral elements present in the formulation. The value obtained for the ash showed that the formulation contained considerable amounts of inorganic elements. The crude protein content of the formulation is 40.22±0.06; this shows that there is a synergy between the two plants in the formulation. This implies that, the formulation can serve as protein supplement. The crude fibre content of the formulation is 7.69±0.08. The value is higher when compared with the values obtained for the individual plant. Fibre diet promotes the movement of food through the intestine; high fibre in food expands the wall of colon, easing the passage of waste. Thus, making it an effective laxative (Anhawange *et al*., 2004). The available carbohydrate content of the formulation is 45.44±0.14. This value reveals a synergy between the two plants used in the formulation. Carbohydrate is an important part of food because; it is the main source of energy for human body to carry out some important metabolic functions (Carpi, 2003). The formulation may be considered as a good source of calories.

The results of elemental composition (Table 2 and Figure 2) revealed that magnesium is the most abundant in the formulation (4014.67 mg/100g), followed by potassium, calcium, phosphorus, iron and sodium with the values in mg/100g of 3866.67, 3255.33, 2135.07, 612.13 and 536.67 respectively. The values are higher when compared with the individual plant. Concentrations of the mineral elements in the formulation are higher when considering the recommended daily allowance (FND, 2002). This implies that the formulation is a good source of mineral elements.

The presence of free radical scavenging compounds of the formulation was detected in the chloroform and ethanol extracts. However, a different degree of free radical scavenging activity was observed from the TLC plates (Table 3, Plates 1 and 2). It has been reported that free radical scavenging compounds show yellow or white spots on the TLC plate (Halilu *et al*., 2008). The ethanol extract shows high potency as shown on the TLC plate.

The *in vitro* free radical scavenging activity of the formulation (Table 4 and Figure 4), revealed that, the free radical scavenging potential of the extracts are concentration dependent. The ethanol extract demonstrated the highest free radical scavenging potential (at 200 and 400 µg/mL) when compared with the standard (Ascorbic acid). On the other hand, the chloroform extract shows high potency (at 50 and 100 µg/mL) when compared with the standard. The IC_{50} values revealed ethanol extract to be more potent than the chloroform extract. Ethanol extract had IC_{50} value of 48.73 µg/mL (Table 5). The lower the IC_{50} value the more potent is the extract.

5.0 CONCLUSION

The results of the present study revealed that the formulation is a good source of plant protein, minerals, crude fibres, carbohydrate and calories. Thus, the formulation will contribute to nutritional requirements for good health in humans. The extracts also demonstrated antioxidant activities with ethanol extract showing the highest potency.

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