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Comparative study of proximate, mineral and phytochemical analysis of the leaves of *Ocimum gratissimum, Vernonia amygdalina* and *Moringa oleifera*

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The aim of this study is to make a comparative analysis of the proximate, mineral and phytochemical compositions of the leaves of *Ocimum gratissimum, Vernonia amygdalina* and *Moringa Oleifera* plants found within our vicinity in Nigeria. The analyses were investigated in accordance with standard procedures and compared. Of the 3 plants, *M. oleifera* had highest (P<0.05) crude fat, crude protein and total ash but lowest in carbohydrate content, whereas, *V. amygdalina* with highest fiber had the lowest crude fat and total ash. Crude fiber and crude protein were lowest in *O. gratissimum*. Also, *O. gratissimum* showed the highest composition of calcuim, potassium, copper and zinc content compared to *V. amygdalina* and *M. oleifera*. Phytate and saponin were significantly predominant (P<0.05) in *O. gratissimum* relative to *V. amygdalina* and *M. oleifera*, whereas flavonoid and tannin were highest (P<0.05) in *V. amygdalina*. However, *M. oleifera* had the highest content of alkaloids among the three plants. The results showed that three leaves evaluated contained varying amount of the proximate, minerals and phytochemicals, hence, the regular use of these leaves are recommended.

Key words: Proximate, minerals, phytochemical, phytate, saponin, tannin.

INTRODUCTION

In Africa, many studies have indicated that a vast number of indigenous plants play a significant role in the diet of the populace (Muhammed et al., 2011). Plants are the cheapest and most available sources of important nutrients, supplying the body with minerals, vitamins and some hormone precursors, protein, energy and essential amino acids (Amaechi, 2009). Medicinal plants are plants that contain substances that can be used for therapeutic purposes or for synthesis of drugs (Sofowora, 2008). Most tropical countries are blessed with a diversity of foodstuffs which play a basic role in nutrition and healthy body development. Unfortunately, an estimate of 789 million people in developing countries still suffers from malnutrition, especially infants and children of rural areas (WHF, 2005). Malnutrition can be tremendously reduced with an increased use of foods rich in energy, proteins, iron and vitamin A most especially those from the rural environment. The lack of nutritional information and

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inadequate development of nutritionally improved products from local raw materials have direct bearing on nutrition. Much effort has been concentrated on seeds while leafy vegetables have largely been ignored. Recently in Africa, increased interest has been observed in the use of herbs to improve health; herbs could be regarded as one of the first real functional food, but has largely become forgotten food in the modern westernized diet. Culinary herbs are as important today as they were in ancient times for enhancing the flavour and taste of our foods as well as serving as a source of dietary medicine (Uhegbu et al., 2011). Ignorance concerning the nutritional properties and presence of some phytochemicals are the major reasons for under-utilization of these herbs. So many people consume vegetables because of their flavors and taste, and do not concern themselves with their nutritional composition (Oshodi, 1992; Ejoh et al., 1996).

Ocimum gratissimum (scent leaves) belongs to the family Lamiaceae and is found mostly in the tropical countries including; Nigeria, India, North and South America, Mexico and Brazil. It is a full developed flowering plant with roots, stem and leaves systems (Iwu, 1993). It prefers moist and fertile soils during growth, but can tolerate drought after flowering. It is naturally and traditionally used to relief pains and in the treatment of rheumatism, diarrhea, high fever, convulsions, diabetes, eczema, piles, skin infections, gastroenteritis, stomachache, cuts, wounds, inflammation, diuretic and as a repellant (Chitwood, 2003; Ilori, 1996).

Vernonia amygdalina (VA) is a shrub or small tree that grows throughout tropical Africa, and is popularly called bitter leaf because of its abundant bitter taste (Ekpo et al., 2007). The leaves contain a considerable amount of anti-nutritional factors like high level of tannic acid and saponin. Research has shown that *V. amygdalina* have some beneficial effect in disease management of poultry (Dakpogan, 2006) such as anti-bacterial and anti-parasitic and anti-oxidant (Erasto et al., 2009) and as growth promoter by enhancing the gastro intestinal enzymes thus increasing feed conversion efficiency (Olabatoke and Oloniruba, 2009). Beside that it is used as an indigenous vegetable in human nutrition, the plant has also acquired significant relevance in human medicine having been proven to possess potent anti-malarial as well as anti-tumorigenic properties (Izevbigie, 2003).

Moringa oleifera belongs to the Moringaceae family and is considered to have its origin in the north-west region of India, south of the Himalayan Mountains. It is now widely cultivated and has become naturalized in many locations in the tropics (Fahey et al., 2001). It is a rapidly-growing tree also known as horseradish tree or drumstick tree. All parts of the Moringa tree are edible and have long been consumed by humans. Recently, there has been interest in the utilization of Moringa as a protein source for livestock (Sarwatt et al., 2002). Furthermore, there is the possibility of obtaining large amount of high quality forage from Moringa without expensive inputs due to favorable soil and climatic conditions for its growth. Sarwatt et al. (2004) reported that Moringa foliage's are a potential inexpensive protein source for livestock feeding. The advantages of using Moringa for a protein resource are numerous, and include the fact that it is a perennial plant that can be harvested several times in one growing season and also has the potential to reduce feed cost.

Despite various reports available on medicinal plants, detailed comparative nutritional information on these three leaves is not available. However, to understand the roles played in human and animal nutrition, knowledge of proximate, mineral and phytochemical composition is fundamental to the understanding of the mode of action of these medicinal plants in general. This will be useful for the nutritional education of the public as a means to improve the nutritional status of the population. Therefore, this study quantitatively analyzed the proximate, mineral and phytochemical compositions of the leaves of *O. gratissimum, V. amygdalina* and *M. oleifera* that are commonly found within our vicinity.

MATERIALS AND METHODS

Leaf meal preparation

Mature fresh leaves of *O. gratissimum, V. amygdalina* and *M. oleifera* were identified by the Agronomy Department and harvested around the Teaching and Research Farm of Department of Agriculture and Industrial Technology, Babcock University, Ilishan-Remo, Ogun State. Harvesting was done between the hours of 16:00 and 17:00 when the plants must have completed their light stage of photosynthetic process for the day. The quantity of leaves needed was air dried at an average room temperature of 27°C for seven days and then milled with a hammer mill sieve of 0.02 mm pore size to obtain a fine powdery dust. The powdered test samples were stored in a dry, clean container with lid for further analysis.

Chemical analysis

Proximate analysis

The moisture content was determined by drying at 105°C in an oven until a constant weight was reached. For total ash determination, the leaves samples were weighed and converted to dry ash in a muffle furnace at 450 and at 550°C for incineration. The crude fat content was determined by extraction with hexane, using a Soxhlet apparatus. All these determinations were carried out according to AOAC (1990). Kjeldahl method was used for crude protein determination. Carbohydrate content was determined by calculating the difference between the sums of all the proximate compositions from 100%. Energy values were obtained by multiplying the carbohydrate, protein and fat by the Atwater conversion factors of 17, 17 and 37, respectively (Kilgour, 1987).

Minerals analysis

Mineral analyses were carried out according to Martin-Prevel et al. (1984). Elemental analyses were carried out using an atomic absorption spectrophotometer and flame photometer to determine calcium, sodium, potassium and magnesium content. Iron, copper

Table 1. Proximate composition of the leaves of *O. gratissimum, V. amygdalina* and *M. Oleifera*.

Parameter	Percentage composition (%) of leaves			
	Ocinum gratissimum	***Vernonia amygdalina***	***Moringa oleifera***	***SEM***
Moisture	13.60	14.50	14.20	1.15
Crude fat	4.20[b]	3.60[c]	4.60[a]	0.61
Crude protein	14.35[c]	21.00[b]	25.90[a]	2.29
Total ash	10.50[b]	7.10[c]	15.60[a]	1.83
Crude fiber	7.60[c]	8.90[a]	8.00[b]	0.01
Carbohydrate	49.75[a]	44.90[b]	31.70[c]	7.80

Means with similar alphabets along the rows were not statistically different ($p \leq 0.05$) level of probability.

Table 2. Mineral composition of leaves of *O. gratissimum, V. amygdalina* and *M. oleifera.*

Parameter	Percentage composition (%) of leaves			
	Ocinum gratissimum	***Vernonia amygdalina***	***Moringa oleifera***	***SEM***
Calcium	1.92[a]	1.27[b]	1.25[b]	0.11
Magnesium	0.07	0.06	0.06	0.01
Potassium	4.30[a]	3.70[b]	2.25[c]	0.30
Sodium	0.16	0.12	0.14	0.01
Iron	1.52[b]	2.48[a]	1.55[b]	0.41
Copper (mg/g)	17.60[a]	16.55[b]	3.90[c]	0.01
Zinc (mg/g)	57.50[a]	45.80[b]	23.40[c]	4.98

and zinc were determined calorimetrically. The concentration of each element in the leave sample was calculated on a dry matter basis.

Phytochemical analysis

Phytochemical analysis was conducted to determine the presence of phytate, saponin, flavanoid, tannin and alkaloid while the quantification of saponin was done by afrosimetric method (Koziol, 1991). The gravimetric method (Haborne, 1993) was used in determination of alkaloid and flavoniod contents. All the analyses were done using triplicate samples.

Statistical analysis

All the data were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences version 17.0 for windows, SPSS Inc. Means were separated using Duncan Multiple Range Test where significant.

RESULTS

Proximate composition

The proximate composition of the leaves of *Ocinum gratissimum, V. amygdalina* and *M. oleifera* presented in Table 1 showed that the moisture content value of the leaves were not significantly different but ranged from 13.60 – 14.50%. The highest moisture content value was obtained from *amygdalina* (14.50%) while the least value was obtained from *O.gratissimum* leaves.

Of the three leaves, *M. oleifera* had the highest crude fat (4.60%), crude protein (25.90%) and total ash (15.60%) content but lowest carbohydrate composition (31.70%); whereas, *V. amygdalina* with the highest crude fiber (8.90%) had the lowest crude fat (3.60%) and total ash (10.71%). *O. gratissimum* demonstrated the lowest content of crude protein and crude fiber (14.35 and 7.60% respectively).

Mineral composition

The result of mineral composition of the leaves of *Ocinum gratissimum, V. amygdalina* and *M. oleifera* were shown in Table 2. The results showed that *O. gratissimum* was significantly highest ($P<0.05$) in calcuim, potassium, copper and zinc (1.92%, 4.30%, 17.60, 57.50 mg/g respectively) content compared to the other two plant leaves. However, *V. amygdalina* was significantly highest in iron (2.84%) while the lowest values of potassium (2.25%), copper (3.90 mg/g) and zinc (23.40 mg/g) were obtained from *M. oleifera* leaves. The magnesium and sodium contents were similar in the three leaves and the value ranged from 0.06 – 0.07% and 0.12 – 0.16% respectively. Iron values obtained ranged from 1.52 - 2.48%. The lowest value was obtained from *O. gratissimum* while the highest value was obtained from *V. amygdalina* leaves. The three leaves were significantly

Table 3. Phytochemicals of the leaves of *O. gratissimum, V. amygdalina* and *M. oleifera.*

Parameter	Percentage composition (%) of leaves			
	Ocinum gratissimum	***Vernonia amygdalina***	***Moringa oleifera***	***SEM***
Phytate	10.44[a]	7.84[c]	9.05[b]	0.01
Saponin	1.10[a]	0.60[b]	0.40[b]	0.15
Flavanoid	25.30[b]	30.45[a]	26.15[b]	1.01
Tannin	0.03[b]	0.17[a]	0.02[b]	0.00
Alkaloid	2.18[b]	2.04[c]	2.80[a]	0.01

high in zinc. The value ranged from 23.40 – 57.50 mg/g. The lowest value was obtained from *M. oleifera* and the highest value from *O. gratissimum* leaves.

Phytochemical

The phytochemical analysis as shown in Table 3 revealed that phytate, saponin, flavanoid, tannin and alkaloid were present in the leaves. The phytate and saponin were significantly predominant (P>0.05) in *O. gratissimum* (10.44 and 1.10%), relative to *V. amygdalina and M. oleifera,* whereas flavonoid (25.30%) and tannin (0.03%) were highest (P<0.05) in *V. amygdalina* (30.24 and 0.17%). However, *M. oleifera* had the highest content of alkaloid among the three plants.

DISCUSSION

Proximate composition

The moisture content recorded in this study is significantly higher than the value obtained for *O. gratissimum* (6.67%) and (7.90%) for *V. amygdalina* leaves by Belewu et al. (2009) but lower than the value (20.08%) obtained by Ejoh et al. (2007). The moisture content value obtained for Moringa in this study (14.20%) was however higher than (5.90%) obtained by Yameogo et al. (2011) for *M. oleifera* leaves. The low level of moisture in all the samples investigated suggests that the leafy vegetables would store for long without spoilage since a higher water activity could enhance microbial action bringing about spoilage. In this study, the air dried leaves low moisture contents will favour their preventive properties against microbial attacked and thus the storage life of the air-dried leaves will be high. Although, these values were found to be low when compared to the 24.0% recorded for *A. senegalensis* (Yameogo et al., 2011). The fat content was also comparably low to the values obtained by Belewu et al. (2009) for *O. gratissimum* (11.75%) and *V. amygdalina* (13.40%), and the value reported by Ejoh et al. (2007) for *V. amygdalina* (4.70%). The low fat content of the samples studied suggests that the plant cannot serve as oil vegetables but may be useful for individuals on weight-reducing diets (Emebu and Anyika, 2011).

The high protein content values recorded for both *V. amygdalina* and *M. oleifera* leaves were found to be similar to that reported by Belewu et al. (2009) for *O. gratissimum* (20.78%) and *V. amygdalina* (28.88%) but higher than the value 19.23% obtained by Ejoh et al. (2007). The crude protein obtained in this study for Moringa (25.90%) was however lower than that (27.50%) obtained by Sun et al. (2018). The high protein values recorded for *V. amygdalina* and *M. oleifera* in this study suggest that the leaves can be ranked as a potential source of plant protein and therefore be used as a protein supplement in the diet.

The total ash values obtained for *O. gratissimum* and *V. amygdalina* were favorably compared to the range of 5.43 - 5.75% reported for some edible woody plants (Emmanuel et al., 2011) and higher than the range of 0.38 - 1.9% for selected vegetables grown in Peshawar (Bangash et al., 2011). The values obtained in this study were also higher than that obtained by Belewu et al. (2009) for *O. gratissimum* (3.58%) and *V. amygdalina* (4.85%). The values of ash observed in all the leaves is an indicator that these samples are good sources of minerals when compared to the values obtained for cereals (FAO, 1980).

The present study reports crude fiber values that are slightly lower than values reported by Ejoh et al. (2007) and Belewu et al. (2009) for *V. amygdalina* and *O. gratissimum* leaves. Also, the value obtained in this study for *M. oleifera* (8.00%) was lower than the value (9.40%) obtained by Sun et al. (2018). The differences in values obtained could be due to geographical location or soil type. This indicates that these leaves can be included in the diets without any deleterious effects; however, the carbohydrate content of *M. oleifera* leaves obtained in this study was lower (31.70) compared to that obtained (54.61) by Mónica et al. (2015). The value obtained for carbohydrate in the leaves of *V. amygdalina* was similar to that obtained by Okeke et al. (2015).

Total carbohydrate levels in these leaves were relatively high except that of Moringa. These carbohydrate sources are not generally used because most of them remain undigested. The difference observed may be due to the physiological state of the plant before harvesting.

(Singhal and Kulkarni, 2007).

Mineral

The calcium values obtained in this study are relatively high, *O. gratissimum* had the highest level of calcuim, while that of *V. amygdalina* and *M. oleifera* were not significantly different. Calcium is necessary for the strong bones and teeth. This indicates that the three leaves can provide part of the daily required calcuim in the body when consumed. Magnesium and sodium percentages obtained in this study were not comparatively different among the leaves. The potassium content of the leaves of *O. gratissimum, V. amygdalina* and *M. oleifera* differ significantly in values, with the highest obtained from *O.gratissimum* (4.30) and the lowest was obtained from the leaves of *M. oleifera* (2.25). Potassium is responsible for nerve action and some osmo-regulation in the body fluid. (Odoemena and Ekanem, 2006). The values of calcuim, magnesium, sodium and iron content fell within the range of 0.05 - 0.53% and 0.04% reported for maize and millet grains (Brou et al., 2009) and Hymenocardia ulmoides and V. ferruginea leaves (Andzouana and Mombouli, 2011), respectively. The iron content of the tested leaves were significantly different with the highest value obtained from *V. amygdalina* (2.48) , followed by that of *Moringa* (1.55) which was not significantly different from the value obtained from *O. gratissimum* (1.52). Iron is an essential trace element for hemoglobin formation, normal functioning of central nervous system and in oxidation of carbohydrates, protein and fats. (Adeleye and Otokiti, 1999). This indicates that *V. amygdalina* can contribute this mineral and enhance their availability in daily life. The values of copper in the leaves of Ocimum, Vernonia and Moringa varied from 3.90 -17.60 mg/g. Copper deficiency has been reported to cause cardiovascular disorders as well as anaemia. Significant difference was also observed in the values of zinc obtained for the three leaves with the least value obtained from Moringa (23.40 mg/g), while the highest value was obtained from the leaves of *O. gratissimum* (57.50 mg/g).

Phytochemicals

The analysis of the three leaves revealed that they contained appreciable amount of phytochemical. The presence of saponin in the samples agreed with the results reported by Edeoga et al. (2006). Saponin is used in medicine and pharmaceutical industries because of its foaming ability with frothy effect. Flavanoid were strongly present in all the leaves, the values obtained were significantly different. The highest value were obtained from the leaves of *V. amygdalina* (30.45%) followed by the *M. oleifera* (26.15) and the least value from *O. gratissimum* (25.30) leaves. The presence of flavonoid in the leaves supported the report of Edeoga et al. (2006); flavonoids are well known antioxidants (Tiwari and Rao, 2002). Although tannins are present in the tested leaves in minute levels, *V. amygdalina* had the highest percentage (0.17) while the lowest value was obtained from the leaves of *M. oleifera* (0.01). This is in agreement with the findings of Belewu et al. (2009), and also in line with the previous reports of Tiwari and Rao (2002) who reported high level of tannin and saponin for *V. amygdalina* which was responsible for the bitter taste of the plant. Tannins are known to have antiviral, antibacterial, antitumor and antidiabetic properties via carbohydrate modulation on the GIT due to the presence of these phytochemicals (Bnouham et al., 2006). The presences of tannins are responsible for the astringent flavour in these leaves. Alkaloids values obtained in this study were significantly different. The lowest values were obtained from the leaves of *V. amygdalina* (2.04%) followed by *O. gratissimum* (2.18%), while the highest values were obtained from the leaves of *M. oleifera* (2.80%).The presence of alkaloids in these leaves samples supported the reports of Edeoga et al. (2006). Alkaloids are known to play some metabolic roles. The compounds have protective role in animal and its constituent of most valuable drugs.

Conclusion

The study revealed that all the leaves are good sources of crude proteins, crude fat and minerals with levels particularly higher in most cases in M. oleifera, followed by *O. gratissimum* and the least by *V. amygdalina*. *O. gratissimum* had the highest level of calcuim and potassium followed by Moringa but *V. amygdalina* has a higher level of iron. These leaves also contain high levels of some antinutritional factors. *V. amygdalina* had highest level of flavanoid and tannin, while phytate and saponin were higher in *O. gratissimum*. In conclusion, these commonly available plants leaves are good sources and potent bioactive compounds which could be used for therapeutic purpose or as precursors of synthetic drugs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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