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Evaluation of chemical composition of the leaves of *Ocimum gratissimum* and *Vernonia amygdalina*

S.A. ADENIYI [1*], C.L. ORJIEKWE [1], J.E. EHIAGBONARE [2] and B.D. ARIMAH [3]

[1]*Department of Chemical Sciences, College of Natural and Applied Sciences, Igbinedion University, Okada, Nigeria.*
[2]*Depatment of Biological Sciences, College of Natural and Applied Sciences, Igbinedion University, Okada, Nigeria.*
[3]*Department of Pharmaceutical Microbiology, College of Pharmacy, Igbinedion University, Okada, Nigeria.*
[*]*Corresponding author, E-mail: gokeomen@yahoo.com*

ABSTRACT

The leaves of *Ocimum gratissimum* and *Vernonia amygdalina* were analyzed for their chemical composition, minerals and vitamins. The results obtained from the analysis of the two plants were compared. The results revealed the presence of bioactive constituents comprising alkaloids (1.90 to 2.68 mg/100 g), tannins (0.04 to 0.07 mg/100g), saponins (0.06 to 2.94 mg/100 g), flavonoids (1.88 to 2.98 mg/100 g) and phenols (0.03 to 0.07 mg/100g). These plants contained ascorbic acid (241.06 to 310.62 mg/100 g), niacin (0.29 to 0.80 mg/100 g), riboflavin (0.08 to 0.09 mg/100 g) and thiamine (0.03 to 0.08 mg/100 g). These plants are good sources of minerals such as Ca, P, K, Mg, Na, Fe and Zn. The importance of these chemical constituents with respect to their roles in ethnomedicine in Nigeria was also discussed.

Keywords: Bioactive compounds, *Ocimum gratissimum*, *Vernonia amygdalina*.

INTRODUCTION

There is growing interest in exploiting plants for medicinal purposes especially in Africa. Medicinal plants such as *Ocimum gratissimum* and *Vernonia amygdalina* have been asserted to provide various medicinal properties (Okigbo and Igwe, 2007; Nwinyi et al., 2009). *Ocimum gratissimum* L; family Leguminoceae is grown in gardens and used as a tea leaf for fevers. It is widely distributed in tropical and warm temperature regions (Okigbo and Ogbonnaya, 2006). *Ocimum gratissimum* is commonly used in folk medicine to treat different diseases such as upper respiratory tract infection, diarrhoea, skin diseases, pneumonia, cough and conjunctivitis (Matasyoh et al., 2007). *Ocimum gratissimum* is grown for the essential oils in its leaves and stems. Eugenol, thymol, citral, geraniol and linlool have been extracted from the oil (Martins and Alvarenga, 2008). Essential oils from the plant have been reported to possess an interesting spectrum of antifungal properties (Matasyoh et al., 2007). The antinociceptive property of the essential oil of the plant has been reported (Rabelo et al, 2003). The whole plant and the essential oil are used in traditional medicine especially

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in Africa and India. The essential oil is also an important insect repellent. *O. gratissimum* is germicidal (Pessoa et al, 2003; Holets et al, 2003) and has found wide use in toothpastes and mouth washes as well as topical ointments. It is used as an excellent gargle for some throats and tonsillitis. It is also used as an expectorant and a cough suppressant. The plant extract is used against gastrointestinal helminths of animals and man (Chitwood, 2003). In addition, *O. gratissimum* carminative properties make it a good choice for upset stomach. It is used as an emetic and for hemorrhoids. The plant is also used for the treatment of rheumatism, paralysis, epilepsy, high fever, diarrhoea, sunstroke, influenza, gonorrhea and mental illness (Okigbo and Ogbonnanya, 2006; Martins and Alvarenga, 2008). In addition, the plant is used as a spice and condiment in the southern part of Nigeria. From recent findings, *O. gratissimum* has proved to be useful in the medication for people living with Human Immuno deficiency Virus (HIV), and Acquired Immuno Deficiency Syndrome virus AIDs (Nwinyi et al, 2009).

Vernonia amygdalina is a shrub or small tree of 2-5 m with petiolate leaf of about 6 mm diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste. It grows under a range of ecological zones in Africa and produces large mass of forage and is drought tolerant. There are about 200 species of *Vernonia*. The leaves are used for human consumption and washed before eating to get rid of the bitter taste. They are used as vegetable and they stimulate the digestive system, as well as they reduce fever (Udeme and Owunari, 2009). Iwalokun et al. (2004) have also reported that *Vernonia amygdalina* possesses antibacterial, antifungal, antiplasmodial and nematicidal properties.

The present study was designed to evaluate the secondary metabolite constituents, minerals and vitamins of *O. gratissimum* and *V. amygdalina* commonly used in alternative herbal medicine in Nigeria, in a view to highlight their roles in ethnomedicine.

MATERIALS AND METHODS

Plant materials

The experimental leaves were collected from the Crown Estate, Okada in Ovia North East Local Government Area of Edo State, Nigeria on the 12th March 2011. The plant leaves were identified and authenticated by a taxonomist at the Biological Sciences Department, Igbinedion University, Okada, Nigeria. A voucher specimen (Voucher Specimen Number 321) has been deposited at the herbarium of the Department. The leaves were air-dried at room temperature (26 ^{o}C) for 8 weeks, and milled into uniform powder with the aid of an electrical grinder and finally stored in airtight bottles before analysis.

Preparation of fat free sample

Two grams of the sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h.

Phytochemical analysis

Chemical tests were carried out on the samples for the quantitative determination of phytochemical constituents.

Alkaloid determination

The alkaloid content was determined gravimetrically. Five grams of the sample were weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration using Whatman filter paper No. 42 (125 mm) and weighed (Obadoni and Ochuko, 2001).

Saponin determination

Saponin content was determined using the method described by Obadoni and Ochuko (2001). Twenty grams of each ground samples were dispersed in 200 ml of 20% ethanol. The

suspension was heated over a hot water bath for 4 h with continuous stirring at about 55 ^{o}C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ^{o}C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The mixture of n-butanol and extracts was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath at about 90 ^{o}C. The samples were dried in an oven at 100 ^{o}C until a constant weight is obtained. The saponin content was calculated in percentage (Obadoni and Ochuko, 2001).

Tannin determination

Tannin content was determined going by the method described by Van-Burden and Robinson (1981). Five hundred miligrams of the sample was weighed into 100 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a tube and mixed with 3 ml of 0.1M $FeCl_3$ in 0.1 N HCl and 0.008M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelength, within 10 min. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured (Van-Burden and Robinson, 1981).

Flavonoid determination

Flavonoid content was determined using the method described by Boham and Kocipai (1994). Ten grams of the ground samples were extracted repeatedly with 300 ml of methanol:water (80:20) at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Boham and Kocipai, 1994).

Determination of total phenols

Total phenols were determined using the method described by Obadoni and Ochuko (2001). For the extraction of phenolic component, the fat free sample was boiled with 50 ml of ether for 15 min. 5 ml of the extract was pipette into a 50 ml volumetric flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths (Obadoni and Ochuko, 2001).

Mineral analysis

The major elements, comprising calcium, magnesium, sodium, potassium and trace elements (iron and zinc) were determined according to the method of Shahidi et al. (1999). The ground samples were sieved with a 2 mm rubber sieve and 2 g of each of the samples were weighed and subjected to dry ashing in a well-cleaned porcelain crucible at 550 ^{o}C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO_3 / HCl / H_2O (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated. The solution in each crucible was transferred into a 100 ml volumetric flask by filtration through a Whatman No 42 filter paper and the volume was made to the mark with deionized water. The solution was used for elemental analysis in an Alpha 4 Atomic Absorption Spectrophotometer (Chem Tech Analytical) attached to Alpha graphite atomizer A270 with a Gateway 2000 computer printer (Shahidi et al., 1999).

Vitamin analysis

Determination of ascorbic acid (Vitamin C)

Vitamin C content was determined according to the method of Baraket et al.

(1973). Five grams of the sample was weighed into an extraction tube and 100 ml of EDTA/TCA (2:1) extracting solution were mixed and the mixture shaken for 30 min. This was transferred into a centrifuge tube and centrifuged at 3000 rpm for 20 min. It was transferred into a 100 ml volumetric flask and made up to 100 ml mark with the extracting solution. 20 ml of the extract was pipetted into the volumetric flask and 1% starch indicator was added. These were titrated with 20% $CuSO_4$ solution to get a dark end point (Baraket et al., 1973).

Determination of niacin

Niacin content was determined according to the method of Okwu and Josiah (2006). Five grams of the sample was treated with 50 ml of 1 N sulphuric acid and shaken for 30 min. 3 drops of ammonia solution (0.1N) were added to the sample and filtered. 10 ml of the filtrate was pipetted into a 50 ml volumetric flask and 5 ml potassium cyanide was added. This was acidified with 5 ml of 0.02 N H_2SO_4 and absorbance measured in the spectrophotometer at 470 nm wavelength (Okwu and Josiah, 2006).

Determination of riboflavin

Riboflavin content was determined according to the method of Okwu and Josiah (2006). Five grams of the sample was extracted with 100 ml of 50% ethanol solution and shaken for 1 h. This was filtered into 100 ml flask; 10 ml of the extract was pipetted into 50 ml volumetric flask. 10 ml of 5% potassium permanganate and 10 ml of 30% H_2O_2 were added and allowed to stand over a hot water bath for about 30 min. 2 ml of 40% sodium sulphate was added. This was made up to 50 ml mark with deionized water and the absorbance measured at 510 nm in a spectrophotometer (Okwu and Josiah, 2006).

Determination of thiamine

Thiamine content was determined according to the method of Okwu and Josiah (2006). Five grams of the sample were homogenized with ethanolic sodium hydroxide (50 ml). It was filtered into a 100 ml flask. 10 ml of the filtrate was pipetted and the colour developed by addition of 10 ml potassium dichromate and read at 360 nm. A blank was prepared and the colour also developed and read at the same wavelength (Okwu and Josiah, 2006).

Statistical analysis

Three analytical determinations were carried out on each independent replication for every parameter. Three independent replicates (n = 3) were obtained from each treatment and the results presented in tables and are reported as means ± standard deviation (SD). Data were analysed by t-test ($P < 0.05$).

RESULTS

Table 1 summarizes the quantitative determination of phytochemical constituents of *O. gratissimum* and *V. amygdalina*. The result of the phytochemical analysis reveals that both plants contain substantial amount of alkaloids, flavonoids and saponins. These are known to exhibit medicinal activity as well as physiological activity (Sofowora, 1993). The trace amount of saponin in *O. gratissimum* in this study is in agreement with the results of Adeniyi et al. (2010).

The mineral contents of both plants are shown in Table 2. Potassium was the most abundant macroelement present ranging from 3.13 mg/100g in *O. gratissimum* to 5.86 mg/100g in *V. amygdalina* while calcium was the least abundant macroelement present ranging from 0.88 mg/100g in *O. gratissimum* to 0.97 mg/100g in *V. amygdalina*. Among the microelements, Iron was the most abundant ranging from 0.84 mg/100g in *O. gratissimum* to 2.73 mg/100g in *V. amygdalina*. Zinc was present at 0.52 mg/100g in *O. gratissimum*, while *V. amygdalina* contained 0.51 mg/100g of zinc.

The results of analysis of *O. gratissimum* and *V. amygdalina* showed that the plants are rich in vitamins (Table 3). Ascorbic acid (vitamin C) was found to be 310.62 mg/100g in *V. amygdalina* and 241.06 mg/100g in *O. gratissimum*. Niacin, Riboflavin and thiamine were also detected in both plants.

Table 1: Phytochemical composition of the leaves of *O. gratissimum* and *V. amygdalina* expressed as mg/100 g dry weight.

Phytochemicals	*Ocimum gratissimum*	*Vernonia amygdalina*
Alkaloid	2.68 ± 0.20	1.90 ± 0.11
Saponin	1.02 ± 0.02	2.94 ± 0.20
Tannin	0.04 ± 0.02	0.07 ± 0.02
Flavonoid	1.88 ± 0.11	2.98 ± 0.22
Phenol	0.03 ± 0.02	0.07 ± 0.02

Data are mean of triplicate determinations on a dry weight basis ± standard deviation.

Table 2: Mineral composition of the leaves of *O. gratissimum* and *V. amygdalina* on mg/ 100 g dry weight.

Minerals	*Ocimum gratissimum*	*Vernonia amygdalina*
Macroelements		
Calcium	0.88 ± 0.10	0.97 ± 0.11
Magnesium	0.93 ± 0.10	1.15 ± 0.10
Sodium	0.89 ± 0.11	1.39 ± 0.10
Potassium	3.13 ± 0.20	5.86 ± 0.30
Microelements		
Iron	0.84 ± 0.11	2.73 ± 0.10
Zinc	0.52 ± 0.11	0.51 ± 0.11

Data are mean of triplicate determinations on a dry weight basis ± standard deviation.

Table 3: Vitamin composition of *O. gratissimum* and *V. amygdalina* in mg/100 g dry weight.

Vitamin	*Ocimum gratissimum*	*Vernonia amygdalina*
Ascobic acid	241.06 ± 0.20	310.62 ± 0.30
Niacin	0.29 ± 0.10	0.80 ± 0.11
Rboflavin	0.08 ± 0.02	0.09 ± 0.10
Thiamin	0.03 ± 0.02	0.08 ± 0.02

Data are mean of triplicate determinations on a dry weight basis ± standard deviation.

DISCUSSION

The presence of alkaloid showed that both plants can be used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects (Stray, 1998; Okwu and Okwu, 2004). Flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity (Salah et al., 1995; Del-Rio et al., 1997). Flavonoids are also known to have anti-inflammatory, anti-allergic and anti-viral properties. They can lower the risk of arthritis, osteoporosis, allergies and viral disease caused by herpes simplex virus, parainfluenza virus and adenovirus (Okwu, 2004). Flavonoids can help prevent atherosclerosis, which is a disease characterized by the deposition of fats inside the arterial wall. Such deposition narrows the arteries and thereby, hinders

blood flow to the vital organs of our body, like heart and brain. So, this disease increases the risk of heart attack and stroke. Flavonoids, by preventing atherosclerosis, lower the risk of coronary heart diseases. The presence of saponin is an indication that the plants possess the property of precipitating and coagulating red blood cells. Some of the characteristics include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo et al., 2000; Okwu, 2004). The trace amount of tannins and phenolic compounds in both plants indicates that the plants may not have stringent properties and cannot be used as antimicrobial agent (Ofokansi et al., 2005).

Minerals are important for vital body functions such as acid, base and water balance. Calcium is good for growth and maintenance of bones, teeth and muscles (Dosunmu, 1997; Turan et al., 2003). Normal extracellular calcium concentrations are necessary for blood coagulation and for the integrity, intracellular cement substances (Okaka and Okaka, 2001), therefore these vegetables could provide veritable sources of calcium. Sodium is an activator of transport ATP-ases in animals and possibly also in plants (Adeyeye, 2005). There is also direct relationship of sodium intake with hypertension in human (Dahl, 1972). Iron is an important constituent of hemoglobin (Onwordi et al., 2009). Therefore, this is probably why some people use these plants to build-up hemoglobin, especially when they are recovering from sickness. Zinc is needed in the body to help the pancreas produce insulin, to allow insulin to work more effectively, and to protect insulin receptors on cells. Therefore the presence of zinc in the vegetables could mean that the vegetables can play valuable roles in the management of diabetes, which results from insulin malfunction (Okaka and Okaka, 2001). Vegetables contribute to these minerals and enhance their availability in daily life. These vegetables can supplement the daily requirements of Ca, Fe and Zn which have been put by FAO/WHO at 260 mg/day, 0.425 mg/g and 0.099 mg/g respectively (Weigert, 1991).

These plants are good source of ascorbic acids, niacin, riboflavin and thiamine (Table 3). Natural ascorbic acid is vital for the body performance (Okwu, 2004]. Lack of ascorbic acid impairs the normal formation of intercellular substances throughout the body, including collagen, bone matrix and tooth dentine. The pathological change resulting from this defect is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intercellular substances (Hunt et al., 1980). Therefore, the clinical manifestations of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anemia, pains in the joints can be related to the association of ascorbic acid and normal connective tissue metabolism (Hunt et al., 1980; Okwu, 2004). This function of ascorbic acid also accounts for the requirement for normal wound healing. As a result of the availability of ascorbic acid in all the vegetables, they can be used in herbal medicine for the treatment of common cold and other diseases like prostrate cancer (Okwu, 2003).

Conclusion

This study has shown that *O. gratissimum* and *V. amygdalina* are good source of phytochemicals that are biologically important, thus they can be potential sources of useful drugs. Since these plants contain appreciable level of vitamins and minerals that are readily available, they could be consumed to supplement the scarce or non-available sources of nutrients.

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