

PHYTOCHEMICAL AND TRACE ELEMENT ANALYSIS OF VERNONIA AMYGDALINA (BITTER LEAF) IN DIFFERENT LOCATIONS IN NIGERIA

Mokogwu ATH¹, Okorie UP², Digban KA², Abubakar A³, Mokogwu EE⁴.

1. Department of Chemical Pathology, School of Clinical Medicine, Igbinedion University, Okada, Nigeria
2. Department of Medical Laboratory Science, Faculty of Basic Sciences, Igbinedion University, Okada, Nigeria
3. Federal University of Technology, Minna, Nigeria
4. Department of Agricultural Extension and Management, FAHPT, NVRI, Vom, Nigeria

Corresponding Author: Mokogwu ATH

Email: tmokogwu@yahoo.com

Abstract

Aim: This study was to evaluate the phytochemical and mineral elements of Vernonia amygdalina leaf grown in Anambra, Imo, Delta and Edo States of Nigeria.

Methods: Standard methods were used for the phytochemical screening while the elemental analysis was done by the use of Atomic Absorption Spectrophotometer.

Results: The results revealed high concentrations in mg/100g of the bioactive constituents; Flavonoids (2165, 2080, 2250 and 2285), Alkaloids (1955, 2450, 2610 and 2375) and Saponins (1850, 2080, 2250 and 2285) in Anambra, Imo, Delta and Edo states respectively. The mineral analysis indicates higher concentrations of Cl⁻ (38.47mg/100g, 42.37mg/100g, 51.23mg/100g and 47.57mg/100g) in Anambra, Imo, Delta and Edo states followed by Fe⁺⁺ in mg/100g (6.57, 5.87, 7.23 and 7.77) in Anambra, Imo, Delta and Edo states respectively.

Conclusion: The leaf of Vernonia amygdalina contains mineral elements and phytochemicals that are nutritionally and medicinally important for human health; it also shows that those grown in Delta and Edo States are relatively richer in both their mineral and phytochemical contents than those grown in Anambra and Imo States.

Key words: Vernonia amygdalina leaf, Phytochemicals, Minerals.

INTRODUCTION

Vernonia amygdalina, a member of the Asteaceae family, is a small perennial shrub that grows in tropical Africa particularly in Nigeria. It grows to a height of 2-5m. The leaves are elliptical and up to 20cm long with a rough back. It is commonly called bitter leaf in English because of its bitter taste (Ijike, 2011). African common names include onugbu (Igbo), ewuro (Yoruba), chusar-doki (Hausa), grawa (Amharic), etidot (Ibibio), ityuna (Tiv), oriwo (Edo), mululuza (Luganda), labwori (Acholi), olusia (Luo) and Ndolé (cameroon) (Egedigwe, 2010; Kokware, 2009). Aliyu et al., (2008) documented that the leaves are being used as a valuable source of food and medicine for the prevention of illness and maintenance of human

health. Huffmna and Seifu (1989), documented that chimpanzees have been observed to ingest the leaves when suffering from parasitic infection. Challand and Willcoe (2009), also stated that the fresh leaves have been successfully used for the treatment of uncomplicated malaria. Vernonia amygdalina extracts have been known to possess potential pharmacological effects (Song et al., 2005; Sweeney et al., 2005). Its anti cancerous effects as well as influence on body estrogen have also been documented (Blanco et al, 2010; Opata and Izevbigie, 2006; Song et al., 2005; Izevbigie et al., 2004; Jisaca et al., 1993; Kupchan et al., 1969). Also its antioxidant activities as well as its effects on blood glucose and lipids have also

been documented (Erasto et al., 2007; Erasto et al., 2006; Nwanjo, 2005). Erasto et al., (2006) described its medicinal use in the treatment of leech and bilharzia as well as pneumonia, cough and as a laxative. The importance of Vernonia amygdalina in medicine remains even the greatest relevance with the current global shift to obtain drugs from plant source, as a result of which attention has been given to the medical values of herbal remedies for safety, efficacy and economy (Glombitza et al., 1993; Mahabir and Gulliford, 1997). The World Health Organization in a number of resolutions emphasizes the need to ensure quality control of plant products by using modern techniques and applying suitable standards (WHO, 1992). Bitter leaf is continually being utilized as therapeutic agent in formulations for treating diseases in the traditional ethno medicinal system in southern and Eastern parts of Nigeria. However, environmental, atmosphere, pollution, soil, harvesting and handling are some of the factors which may play important roles in contamination of Vernonia amygdalina leaves by metals. It is therefore of major interest to evaluate the phytochemicals and some trace elements in the leaves of Vernonia amygdalina because variations or elevated levels of these metals may influence their use in medical practice. This study was designed to evaluate the phytochemical and mineral contents of V. amygdalina leaves grown in four (4) states of Nigeria.

MATERIALS AND METHODS

Sample Collection

Fresh plants of bitter leaf (Vernonia amygdalina) were obtained (plucked) from four states of Nigeria namely; Anambra, Delta, Edo and Imo. They were properly labeled according to each of the states and air dried in the laboratory for 21 days. The leaves were pulverized to powder; firstly with dried long cup and secondly with the short cup home blender. They were stored in airtight bottles, properly labeled according to state from which samples were obtained before analysis.

Phytochemical Analysis

Quantitative Phytochemical Analysis

Tests for tannins, terpenoids, cardiac glycosides, flavonoids, alkaloids, phenolics, saponins were carried out using standard methods (Marcano and Hasenaira, 1991).

Test for Tannins

1g of sample was extracted with 25ml 80:20 acetone: 10% glacial acetic acid for 4 hours. It was then filtered and measured at 500nm absorbance. The absorbance of the reagent blank was also measured. A standard graph with 10, 20, 30, 40, 50mg/100g of tannic acid was made. The concentration of tannins was read taken into consideration of the dilution factor.

Test for Terpenoids

1g of sample was weighed into 250ml beaker and 10ml petroleum ether was added. It was allowed to extract for 15 min and was filtered. The absorbance was then read at 420nm.

Test for Cardiac Glycosides

1g of sample with 40ml of water was extracted and placed in an oven at 100°C for 15 min. Then, to 1ml of the extract dissolved in 5ml of water was added 2ml of glacial acetic acid followed by one drop of iron chloride (FeCl₃) and 1ml of H₂SO₄. The absorbance was then measured at 410nm.

Tests for Flavonoids

1g of sample was extracted with 10ml of 80% methanol and left to stand for 2 hours. It was filtered through Whatman filter paper into a petri-dish, evaporated to dryness in an oven at 40°C and weighed.

Test for Alkaloids

1g of each sample (W) was extracted with 20ml of 10% acetic acid in ethanol, mixed and allowed to stand for 4 hours. The extract was filtered through Whatman filter paper. The filtrate was evaporated to about a quarter of its original volume and one drop of concentrated ammonia was added. The extract was filtered through weighed (W₁) Whatman filter paper. The filter paper was dried in the oven at 60°C. The dried filter paper was weighed to a constant weight (W₂).

$$\% \text{ Alkaloids} = \frac{(W_2 - W_1)}{W} \times \frac{100}{1}$$

Test for Phenolics

2g of each sample was extracted with 20ml of acetone, 0.5% formic acid for 2 min and was filtered. 2ml of the extract was mixed with 0.5ml fochin-cioalteau reagent, mixed for 15 seconds and allowed to stand at 40°C for 30 min into develop a colour. The absorbance was measured at 765nm and expressed as mg/g Gallic Acid Equivalent (GAE).

Test for Saponins

1g of sample of each sample was dispersed in 15ml of 20% ethanol. The suspension was put inside the water bath at 55°C for 4hours. The mixture was filtered and the residue re-extracted with another 15ml of 20% ethanol twice. The extract was reduced to about 5ml in the oven. The concentrate was transferred into a 250ml separating funnel and 5ml of petroleum ether was added and mixed vigorously. The petroleum ether layer was discarded and 3ml of butanol was added to the aqueous layer. The extract was washed twice with 5ml of 5% sodium chloride. The remaining solution was poured into a weighed petri-dish, evaporated to dryness in the oven and the residue was weighed.

Elemental Analysis

The major trace elements comprising iron, manganese, copper, fluorine, chromium, iodine, selenium, molybdenum, cobalt and zinc were determined according to the method of Shahidi et al., (1999) with slight modification. The grounded samples were sieved with a 2mm rubber sieve and 2g of each of the samples were subjected to dry ash in a well cleaned porcelain crucible at 55°C in a muffle furnace. The resultant ash was dissolved in 5ml of HNO₃/H₂O₂ (1:1) and heated gently on hot plate until brown fumes disappeared. To the remaining material in the crucible, 5ml of de-ionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100ml volumetric flask by filtration through a Whatman filter paper and the volume the volume was made to mark with de-ionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer (AAS) and the concentration of each element was calculated on percentage of dry matter.

RESULTS

Table 1 below shows the location and mean values of elemental composition in mg/100g in the four states studied. From the analysis, in Anambra state: the mean value for the various elements are as follows: 6.57 (Fe⁺⁺), 0.02 (Mn⁺⁺), 0.63 (Cu⁺⁺), 0.00(F⁻), 38.47 (Cl⁻), 0.10(I⁻), 0.01 (Se⁺⁺), 0.00 (Mo⁺⁺), 0.01(Co⁺⁺) and 0.33 (Zn⁺⁺). For Delta State: the mean values are as follows:- 7.23 (Fe⁺⁺), 0.02 (Mn⁺⁺), 0.53 (Cu⁺⁺), 0.00 (F⁻), 51.23 (Cl⁻), 0.20 (I⁻), 0.01 (Se⁺⁺) 0.00 (Mo⁺⁺), 0.01 (Co⁺⁺) and 0.40 (Zn⁺⁺). In Edo State: the mean values are indicated as 7.77 (Fe⁺⁺), 0.02 (Mn⁺⁺), 0.60 (Cu⁺⁺), 0.00 (F⁻), 47.57 (Cl⁻), 0.10 (I⁻), 0.01 (Se⁺⁺), 0.00 (Mo⁺⁺), 0.01 (Co⁺⁺) and 0.20 (Zn⁺⁺) while Imo State: elemental analysis indicates 5.87 (Fe⁺⁺), 0.03 (Mn⁺⁺), 0.80 (Cu⁺⁺), 0.00 (F⁻), 42.37 (Cl⁻), 0.10 (I⁻), 0.02 (Se⁺⁺), 0.00 (Mo⁺⁺), 0.00 (Co⁺⁺) and 0.30 (Zn⁺⁺). Amongst the cations, the bitter leaf contains more of Fe⁺⁺, followed by Cu⁺⁺, Zn⁺⁺ and Mn⁺⁺ in that order. While it contains very high levels of Cl⁻ within the anions followed by I⁻ and zero level of F⁻ as well as zero level of Mo⁺⁺. Table 2 also below, shows the phytochemical concentration in Mg/100g of Vernonia amygdalina in the four states under study. In Anambra state: the mean value for tannins is 408.33, Terpenoids (255), glycosides (145.00), flavonoids (2165.00), Alkanoides (1955.00), phenols (42.63) and saponins (1850.0). Mean values of phytochemical in Delta state are as follows: Tannins (430.00), Terpenoids (1455.00), glycosides (150.00), flavonoids (2250.00), alkanoides (2610.00), phenols (40.50) and saponins (1895.00). Edo State has a mean for Tannins (416.67), Terpenoids (1430.00), Glycosides (135.00), flavonoids (2285.00), alkanoides (2375.00), Phenols (45.5) and Saponins (1795.00). The phytochemical contents in Imo State are: Tannins (418.33), Terpenoids (1460.00), glycosides (125.00), flavonoids (2080.00) alkanoides (2450.00), phenols (46.13) and saponins (1745.00). Table 3 and 4 below shows only the mean values obtained in both the phytochemicals and the elemental analysis in four states of Nigeria.

Table 1: The location and mean values of elemental components (mg/100g) in four states

	Fe ⁺⁺	Mn ⁺⁺	Cu ⁺⁺	F-	Cl-	I-	Se ⁺⁺	Mo ⁺⁺	Co ⁺⁺	Zn ⁺⁺
Anambra	6.57	0.02	0.63	0.00	38.47	0.10	0.01	0.00	0.01	0.33
Imo	5.87	0.03	0.80	0.00	42.37	0.10	0.02	0.00	0.00	0.30
Delta	7.23	0.02	0.53	0.00	51.23	0.20	0.01	0.00	0.01	0.40
Edo	7.77	0.02	0.60	0.00	47.57	0.10	0.01	0.00	0.01	0.20

Table 2: The Phytochemical concentration in mg/100g in the four states

Location	Cardiac						
	Tannins (mg/100g)	Terpenoids (mg/100g)	Glycosides (mg/100g)	Flavonoids (mg/100g)	Alkanoids (mg/100g)	phenolics (GAE/g)	saponins (mg/100g)
Anambra	408.33	255.00	145.00	2165.00	1955.00	42.63	1850.00
Imo	418.33	1460.00	125.00	2080.00	2450.00	46.13	1745.00
Delta	430.00	1455.00	150.00	2250.00	2610.00	40.50	1895.00
Edo	416.67	1430.00	135.00	2285.00	2375.00	45.23	1795.00

Table 3: The Mean values obtained in phytochemicals analysis in four states

	Anambra	Imo	Delta	Edo
Tannins	408.33	418.33	430	416.67
Terpenoids	255	1460	1455	1430
Cardiac Glycosides	145	125	150	135
Flavonoids	2165	2080	2250	2285
Alkanoids	1955	2450	2610	2375
Phenolics	42.63	46.13	40.5	45.23
Saponins	1850	1745	1895	1795

Table 4: The Mean values obtained in elemental analysis in four states

	Anambra	Imo	Delta	Edo
Fe ⁺⁺	6.57	5.87	7.23	7.77
Mn ⁺⁺	0.02	0.03	0.02	0.02
Cu ⁺⁺	0.63	0.8	0.53	0.6
F-	0	0	0	0
Cl-	38.47	42.37	51.23	47.57
I-	0.1	0.1	0.2	0.1
Se ⁺⁺	0.01	0.02	0.01	0.01
Mo ⁺⁺	0	0	0	0
Co ⁺⁺	0.01	0	0.01	0.01
Zn ⁺⁺	0.33	0.3	0.4	0.2

DISCUSSION

Vernonia amygdalina which belongs to the family Asteraceae has a unique characteristic, which is location. It is a perennial shrub that grows in the tropical Africa and is found in all parts of Nigeria. The results of the phytochemical quantitative estimation of the leaves indicated a very high concentration of flavonoids (2165mg/100g in Anambra, 2250 mg/100g in Delta, 2285mg/100g in Edo and 2080mg/100g in Imo States). The flavonoids content was higher in Edo State followed by Delta State, Anambra and Imo state in that order (Table 3). The abundance of flavonoids which are hydroxylated phenolic substances might be responsible for the therapeutic effectiveness against a wide array of microorganisms, probably due to their ability to complex with extracellular and soluble proteins and to complex with the bacterial cell wall (Cowan, 1999). Also flavonoids and other phenolic compounds are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, and have strong anticancer activity (Salah et al., 1995; Del-Rio et al., 1997; Okwu, 2004). The Alkaloids content of *Vernonia amygdalina* is equally very high (1955mg/100g in Anambra, 2610mg/100g in Delta, 2375mg/100g in Edo and 2450mg/100g in Imo States). From table 3, the alkaloids concentration is in the order; Delta > Imo > Edo > Anambra. Alkaloids are very important in medicine and constitute most of the valuable drugs. They are known to have marked physiological effect on animals (Edeoga and Eriata, (2001)). Therefore their high concentration indicates potential source of useful drugs. The concentration of saponins in the leaves of *Vernonia amygdalina* were equally high (1850mg/100g in Anambra, 1895 in Delta, 1795mg/100g in Edo and 1745 in Imo state in the order Delta > Anambra > Edo > Imo State). Saponin has the property of binding with cholesterol, bitterness (which is the characteristics of Bitter leaves) and hemolytic activity in aqueous solution (Sodipo et al, 2000). Alcotannins, terpenoids, cardiac glycosides and phenolics are found in tangible concentration (Tables 2 and 3). Many plants are used in traditional medicine for treatment of diseases, fever and cough (Mutandzi et al, 2012). The very and fairly high concentration of some analyzed phytochemicals in this work (Tables 1 and 3) suggest that *Vernonia amygdalina* leaves might be very effective in the treatment of some vital diseases in both man and animals.

Nutritional valuable trace element analysis has shown that the *Vernonia amygdalina* leaves are rich sources of chlorine (Cl), iron (Fe²⁺), copper (Cu⁺⁺), Zinc (Zn⁺⁺) and reasonable concentrations of iodine (I), manganese (Mn²⁺), selenium (Se²⁺), and cobalt (Co²⁺) while fluorine (F) and molybdenum (Mo²⁺) were not detected (Tables 1 & 4). These trace elements are very essential in enzymes metabolism particularly as they serve as co-factors for most enzymatic features in man and animals (WHO, 1996). Therefore, the addition of *Vernonia amygdalina* leaves in the diet might prevent the occurrence of certain disorders such as acrodermatitis enteropathica, Wilson's disease, Keshan disease, Kaschui-Beck disease, Vitamin-k deficiency, Parkinson's like disease, glucose intolerance and neuropathy (Crook, 2006). The concentration of these elements in *Vernonia amygdalina* leaves are quite high and comparable to the concentration reported in certain medicinal plants (Korc, 1988; Vanghan and Judd, 2003) though with variable differences from the individual states as seen in this work.

CONCLUSION

The findings provide quantitative estimation of the phytochemicals as well as elemental analysis of *Vernonia amygdalina* (bitter leaves) which are important in understanding the pharmacological and or toxicological actions of bitter leaves used as a medicinal plant.

REFERENCES

- Aliyu AB, Musa AM., Oshanimi JA, Ibrahim HA, Oyewale AO (2008). Phytochemical analysis and mineral elements composition of some medicinal plants of Northern Nigeria. *Nig. Journ. Pharm. Sci.* 7: 119-125
- Blanco JG, Gil RR, Bocco JL, Meragelman TL, Genti-Raimondi S, Flurry A. (2001). *J Pharmacol Exp. Ther.* 297 (3): 1099.
- Challand S, Willcox M (2009). "A clinical trial of the traditional medicine *Vernonia amygdalina* in the treatment of uncomplicated malaria". *J Altern Complement Med* 15 (11): 1231-7.
- Cowan MM (1999). Plant products and antimicrobial agents. *Clinical Microbiology Review*, 12(4):564-582.

- Del-Rio A, Obdulio BG, Casfillo J, Marin FG, Ortuno A (1997). Uses and Properties of Citrus flavonoids. *J. Agric Food Chem.* 45:4505-4515.
- Edeoga HO, Eriata DO (2001). Alkaloid, tannin and saponin contents of some Nigeria medicinal plants. *J. Med. Aromatic Plant Sci.* 23:344-349.
- Egedigwe CA (2010). Effect of dietary incorporation of *Vernonia amygdalina* and *Vernonia colorata* on blood lipid profile and relative organ weight in albino rats (Thesis). MOUAU, Nigeria.
- Erasto P, Grierson DS, Afolayan AJ. (2007). "Evaluation of Antioxidant activity and the fatty acid profile of the leaves of *Vernonia amygdalina* growing in South Africa". *Food Chemistry* 104: 636–642.
- Erasto P, Grierson DS, Afolayan AJ. (2006). Bioactive sesquiterpenes lactones from the leaves of *Vernonia amygdalina*. *J. Ethnopharmacol.* 106: 177-120
- Glombitza KW, Mahran GH, Mirhom YW, Michael KG, Mtawi TK (1993). Hypoglycemic and antihyperglycemic effects of *Ziziphyspina christi* in rats. *Plant Med.* 60:244-247.
- Huffman MA, Seifu M (1989). "Observations on the illness and consumption of a possibly medicinal plant *Vernonia amygdalina* (Del.), by a wild chimpanzee in the Mahale Mountains National Park, Tanzania". *Primates* 30: 51–63.
- Ijeh II, Ejike CECC (2011). Current perspectives on the medicinal potential of *Vernonia amygdalina* (Del). *J. Med. Plant Res.* 5(7): 1051-1061.
- Izevbigie EB, Bryant JL, Walker A (2004). "Natural inhibitor of extracellular signal-regulated kinases and human breast cancer cells". *Exp Biol & Medicine* 229: 163–169.
- Jisaka M, Ohigashi H, Takegawa K, Huffman MA, Koshimizu K. (1993). "Antitumoral and antimicrobial activities of bitter sesquiterpenelactones of *Vernonia amygdalina*, a possible medical agent used by wild chimpanzees". *BiosciBiochem* 57 (5): 833.
- Kokwaro J (2009). Medicinal plants of East Africa. 3rd (ed) Nairobi Kenya. University of Nairobi press ISBN 9966-846—84-0.
- Korc M. (1988). Manganese homeostasis in human and its role in disease states. In: Prasad, A.S.(ed) Essential and toxic trace elements in human health and disease.. Alan R. Liss Inc, New York. Pp.253-264.
- Kupchan SM, Hemingway RJ, Karim A, Werner D. (1969). "Tumor inhibitors. XLVII. Vernodalin and vernomygdin, two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina*". *J. Org. Chem* 34 (12): 3908.
- Mahabir D, Gulliford MC (1997). Use of medicinal plants for diabetes in Trinidad and Tobago. *Rev Panam Slud Publica*, 1:1-16
- Marcono L, Hasenaira D (1991). Analysis of phytochemicals in leaves and seeds. *Agronomy Journal* Vol. 83 Pgs. 445-452.
- Martin A. Crook (2006). *Clinical Chemistry and Metabolic Medicine* 7th edition. Chapter on Vitamins, Trace elements and Metals. Pg 222-232.
- Mulaudzi RB, Nahala AR, Kulkarni MG, Van Staden J (2012). Pharmacological properties and proteinbinding capacity of phenolic extracts of some Venda medicinal plants used against cough and fever. *J. Ethnopharmacol.*
- Nwanjo HU (2005). "Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models". *Nigerian J Physiological Sciences* 20 (1-2): 30–42.
- Okwu DE (2004). Phytochemicals and vitamin content of indigenous spices of South-eastern Nigeria. *J. Sustain Agric. Environ.* 6(1) L30-37.
- Opata MM, Izevbigie EB (2006). "Aqueous *V. amygdalina* extracts alter MCF-7 cell membrane permeability and efflux". *Int. J. Environ. Res. Public Health* 3 (2): 174–179.
- Salah N, Müller NJ, Pagange G, Tijburg L, Bolwell GP, Rice E, Evans C (1995). Polyphenolic flavonols as scavengers of aqueous phase radicals as chain breaking antioxidants. *Arch. Biochem. Biophys.* 2:3390346.
- Shahidi F, Chavan UD, Bal AK, McKenzie DB (1999). Chemical composition of beach pea (*Lathyrus nordest L*) plant parts. *Food Chem.* 64L39-44.
- Sodipo DA, Akiniyi JA, Ogunbamosu JA (2000). Studies on certain characteristics of extracts of bark of *Pansinystallama cruceras* (K

schemp) picre Exbeille. *Global J. Pure Appl. Sci.*, 83-87.

Song YJ, Lee DY, Kim SN, Lee KR, Lee HW, Han JW, Kang DW, Lee HY, Kim YK (2005). "Apoptotic potential of sesquiterpene lactone ergolide through the inhibition of NF- κ B signaling pathway. *J. Pharmacol*57 (12): 1591–1597.

Sweeney CJ, Mehrotra S, Sadaria MR, Kumar S, Shortle NH, Roman Y, Sheridan C, Campbell RA, Murray DJ, Badve S, Nakshatri H (2005). "The sesquiterpene lactone parthenolide in combination with docetaxel reduces metastasis and improves survival in a xenograft model of breast cancer". *Mole. Cancer Ther*4 (6): 1004

Vaughan JG, Judd PA (2003). *The Oxford book of health foods: a comprehensive guide to natural remedies* 1st Edition, Oxford University Press, New York.

WHO (1992). *World Health Organization: expert committee on specific action for pharmaceutical preparation report*, Geneva. WHO Technical Report Series 823. Pg. 44-96.

WHO (1996). *Trace elements in human nutrition and health*. WHO Technical Report Series. Geneva. Pg. 119-205