ALKALOID, TANNIN PROFILES AND MINERAL ELEMENT COMPOSITION OF THE LEAVES AND STEM OF *VERNONIA AMYGDALINA* (BITTER LEAF) PLANT HARVESTED FROM WUKARI TOWN, TARABA STATE, NORTH-EAST NIGERIA

Odiba John Oko
Department of Chemical Sciences
Federal University, Wukari
Corresponding author:odibaoko@hotmail.com

Raphael Odoh
Department of Chemical Sciences
Federal University, Wukari
odoraf@gmail.com

Sylvester Mbaavaga Tongu
Department of Chemistry
Federal University of Agriculture, Makurdi
Tongusyl@gmail.com

Clara Obianuju Anidobu
Department of Chemical Sciences,
Federal University, Wukari
maryclaraandy@gmail.com

Ifeoma Sandra Udegbunam
Department of Chemical Sciences
Federal University, Wukari
Udegbunam@fuwukari.edu.ng
&
Hauwa Umar[1]
Department of Chemical Sciences
Federal University, Wukari

ABSTRACT

The composition of alkaloids and tannins in the leaves and stem of *Vernonia amygdalina* (bitter leaf) plant was determined using gas chromatographic technique coupled to flame ionization detector while the mineral elements; calcium, magnesium, potassium and zinc were determined by Atomic Absorption Spectroscopic technique. The study revealed the presence of twenty (20) alkaloids in both the leaves and stem extract. The % composition were in the range of 0.03 % - 34.00 % (leaves) and 0.04 % - 36.93 % (stem) respectively while four (4) tannins having % compositions ranging between 0.82 % - 44.40 % (leaves) and 5.14 % - 28.37 % (stem) were found in the plant. Lactucopicrin (34.00 %, 36.93 %) and lactucin (18.91 %, 20.27 %) were the most predominant alkaloids in the leaves and stem respectively while augustamine (0.40 %, 0.40 %) and crinamidine (0.03 %, 0.04 %) were the least available alkaloids. Tannic acid (44.40 %) and acertannin (28.37 %) were however the most predominant tannins in the leaves and stem respectively. Generally, the total concentration of the alkaloids in the leaves and stem were 0.3791 mg/100g and 0.2245 mg/100g while tannin concentrations were 0.0248 mg/100g and 0.0557mg/100g respectively. High concentrations of calcium (212.48 mg/100g, 208.42 mg/100g), magnesium (422.42 mg/100g, 411.13 mg/100g) and potassium (636.62 mg/100g, 601.62 mg/100g) respectively were found in the leaves and stem. Zinc concentration (3.72 mg/100g, 3.16 mg/100g) respectively were however very low compared to the other mineral elements analyzed for. *Vernonia amygdalina* leaves and stem could therefore be very useful in treatment of diseases both in humans and animals if adequate amounts are consumed.

Keywords: Alkaloids, Bitter leaf, Composition, Gas chromatography, Phytochemicals, Tannins.

INTRODUCTION

Phytochemicals are chemicals which are obtained from plants (Eleazu *et al*., 2012). They have been shown to have medicinal values since they produce physiological actions on the human body (Surjowardojo *et al.,* 2014). Some phytochemicals that have medicinal values include alkaloids, tannins, saponins and flavonoids. Phytochemicals such as saponins and alkaloids, terpenes, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, edotides and sesquiterpenes have been reportedly extracted and isolated from *Vernonia amygdalina* (Farombi and Owoeye, 2011).

LITERATURE REVIEW

The bitter leaf plant (*Vernonia amygdalina*) is a plant that is grown around the world especially in Nigeria. It is a shrub which can grow as tall as 23 feet when fully grown (Echem and Kabari, 2013). It is known to different ethnic groups in Nigeria by various names. These include ewuro (Yoruba), olugbu (Igbo) and shuwakaa (Hausa) in parts of Nigeria (Okeke *et al*., 2015) and ityuna (Tiv), olubu (idoma). The leaves are green and have characteristic odor and bitter taste (Udochukwu *et al*., 2015). *Vernonia amygdalina* has been proven to exhibit medicinal properties. The range of medicinal applications of the plant include antihelminthic, antioxidant properties, antidiabetic, hypolipidemic, anticancer, antifertility, antimicrobial, antimalaria, analgesic, spematogenic effects among others (Egharevba *et al*., 2014). Aqueous extract of the leaves has also been reported to contain saponins, flavonoids, alkaloids, tannins among others (Oseni *et al*., 2014). The bitter taste reported for *Vernonia amygdalina* plant has been attributed to anti-nutritional factors (Anacletus *et al*., 2015). Antinutritional factors are substances that when present tends to reduce the availability of one or more nutrients. They include alkaloids, tannins, saponins, phytic acids, oxalic acids, flavonoids, glucosinolates among others.

Tannin was reported to exhibit antiseptic action (Obeta, 2015). Furthermore, tannins have recently been discovered to draw interest because of the incidence of diseases such as cancer and HIV/AIDS (Karamall and Tennis, 2001). High tannin concentrations have been reported in almost all parts of plants such as the barks, leaves, fruits, roots and seeds (Karamall and Tennis, 2001). Vernolide and vernodalol which was isolated from *Vernonia amygdalina* had been shown to have antioxidant activity and this is manifested as reducing capacity and free radical scavenging activity (Erasto *et al*., 2007). In addition, *Vernonia amygdalina* had also been reportedly used as a corrosion inhibitor on aluminium in acidic media because it was found to be eco-friendly (Oluseyi *et al*., 2012).

Furthermore, mineral elements play vital roles in the human body and it is necessary that adequate amounts are consumed daily. The absence or shortage of these mineral elements in the body can open doors to a variety of diseases in the body. Usually, a characteristic syndrome is exposed indicating which function a particular mineral plays in the metabolism of the animal (Soetan *et al.,* 2010). Potassium plays a role in protein synthesis, enzyme activation and maintenance of water balance. Calcium on the other hand helps in the stabilization of the cell walls and in maintaining membrane structure as well as cell permeability while magnesium and zinc help in the activation of some enzymes.

Despite the fact that *Vernonia amygdalina* leaves have been assessed for their phytochemical constituents to include alkaloids and tannins along with the determinations of their total contents (Okeke *et al*., 2015) , the individual constituent alkaloids and tannins have not been

reported. In addition, the quantity of mineral elements in the plant harvested in Wukari has also not been reported. It is against this background that the present study was conducted to ascertain and quantify the alkaloid and tannin profiles alongside the mineral element composition in the leaves and stem of *Vernonia amygdalina* plant grown in Wukari town ; Taraba State, North Eastern Nigeria.

Materials and methods

Sample collection and preparation

The plants of *Vernonia amygdalina* were harvested randomly from three locations of Wukari town (Roger road, Kantanawa and Marmara) in April, 2016. Each location was visited thrice. The leaves and stem parts of the plants were isolated, air dried, pulverized in a mortar and then packed into an air tight polythene material after identification by a botanist in the biological Sciences department of the Federal University Wukari, Taraba State.

Procedure for extraction of tannins

A method of Swain (1979) as described by Mboso (2013) was adopted with modifications for extracting tannins. 0.5g of the pulverized sample was measured into a 50 mL borosilicate beaker; 20 mL of 50 % methanol was transferred into the beaker containing the sample then covered with a cotton wool. This was placed in a water bath at 80^{o}C for 1 hour. The extract was then filtered with a double layered Whatman No 1 filter paper into a 100 mL volumetric flask then rinsed with 50 % methanol. After which the extract was concentrated to 2 mL. 1µL was injected into the injection port of the gas chromatograph.

Procedure for extraction of alkaloids

The extraction of alkaloids was carried by adopting the method of Ngounou *et al* (2005) with modifications. 5.0g of the pulverized sample was macerated in hexane of 25 mL for about 72 hours. The extract was then filtered and the residue air dried, treated with 10 % aqueous NH_3 and macerated in $CHCl_3$ for 24 hours. After filtration and evaporation at reduced pressure, the resultant crude extract was treated with 7.5 mL of aqueous HCl. The aqueous phase was made alkaline with aqueous NH_3 and then extracted thrice with chloroform. The chloroform fraction was washed with water. The extract was poured into a round bottomed flask of the rotary evaporator to drive off the solvent from the extract. The water was then removed from the concentrated extract using anhydrous sodium sulphate before chromatographic analysis.

Conditions for the operation of the gas chromatograph

The Gas chromatograph used for this analysis is HP 6890 which is powered with HP chemstation 1206 software. The split ratio is 20:1; carrier gas is Nitrogen and the inlet temperature is 250^{o}C. The column dimension is 30m×0.25mm× 0.25µm while the oven was programmed to an initial temperature of 60^{o} C for 5 minutes and the 1st and 2nd ramping was 10^{o}C/min for 20 mins and 15^{o} C/min for 4 mins respectively. The detector employed is flame ionization detector (FID) which operated at a temperature of 320^{o}C.

Digestion of samples

5g of pulverized sample was weighed and ashed in a muffle furnace at 550ºC for 4 hours until a constant weight was attained; 0.5g of the ashed sample was weighed and transferred into a pre-cleaned 250ml capacity beaker for digestion. 30 ml of concentrated nitric acid was added into the weighed sample in the beaker. The sample with the nitric acid in it was placed on a hot plate for digestion in a fume cupboard. The beaker and its content after the digestion were allowed to cool. Another 20 ml of the concentrated nitric acid was added and the mixture digested further in the fume cupboard. The mixture was allowed to cool to room temperature

and then filtered with Whatman No 42 into a 250ml volumetric flask. The filtrate was then made up to the mark with deionised water.

Mineral element Analysis

The samples were analyzed using atomic absorption spectrophotometer (UNICAM 969) powered by SOLAAR software after calibrations with standards of calcium, magnesium, potassium and zinc.

RESULTS AND DISCUSSION

The % composition of the alkaloids present in the leaves of *Vernonia amygdalina* plants are presented in Table 1. In addition, the concentrations of the alkaloids in the leaves are shown in Figure 1. The total concentration of alkaloids in the leaves was found to be 0.3791 mg/100g.

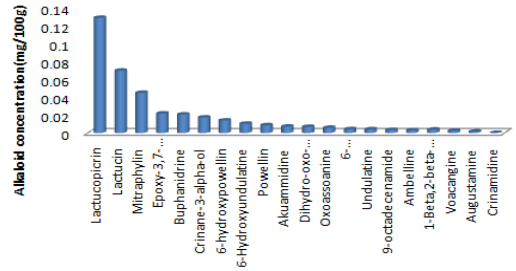

Figure 1: Alkaloid concentrations (mg/100g) in the leaves of *Vernonia amygdalina* plant

Table 1:% composition of alkaloids in the leaves of *Vernonia amygdalina* plant

S/No	Alkaloid	% Composition
1.	Lactucopicrin	34.00
2.	Lactucin	18.41
3.	Mitraphylin	11.84
4.	Epoxy-3,7-dimethoxycrinane-11-one	5.75
5.	Buphanidrine	5.46
6.	Crinane-3-alpha-ol	4.56
7.	6-hydroxypowellin	3.69
8.	6-Hydroxyundulatine	2.74
9.	Powellin	2.29
10.	Akuammidine	1.90
11.	Dihydro-oxo-demethoxyhemanthamine	1.79
12.	Oxoassoanine	1.53
13.	6-Hydrooxybuphanidrine	1.13
14.	Undulatine	1.08
15.	9-octadecenamide	0.90
16.	Ambelline	0.82
17.	1-Beta,2-beta-Epoxyambelline	1.00
18.	Voacangine	0.66
19.	Augustamine	0.40
20.	Crinamidine	0.03

Similarly, the alkaloid profiles in the stem are presented as % compositions in Table 2 while their concentrations (mg/100g) are presented in Figure 2. The total alkaloid concentration in the stem is 0.2245 mg /100g.

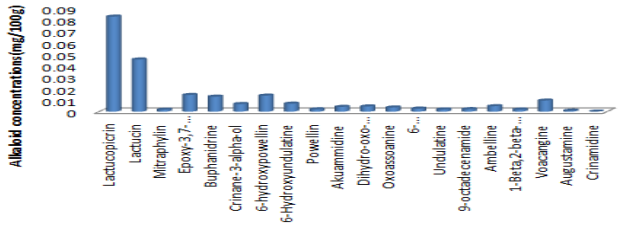

Figure 2: Alkaloid concentrations (mg/100g) in the stem of *Vernonia amygdalina* plant

Table 2:% composition of alkaloids in the stem of *Vernonia amygdalina* plant

S/No	Alkaloid	% Composition
1.	Lactucopicrin	36.93
2.	Lactucin	20.27
3.	Mitraphylin	0.80
4.	Epoxy-3,7-dimethoxycrinane-11-one	6.50
5.	Buphanidrine	5.84
6.	Crinane-3-alpha-ol	2.98
7.	6-hydroxypowellin	6.24
8.	6-Hydroxyundulatine	3.12
9.	Powellin	0.94
10.	Akuammidine	1.87
11.	Dihydro-oxo-demethoxyhemanthamine	2.05
12.	Oxoassoanine	1.65
13.	6-Hydrooxybuphanidrine	1.20
14.	Undulatine	0.89
15.	9-octadecenamide	0.98
16.	Ambelline	2.14
17.	1-Beta,2-beta-Epoxyambelline	0.85
18.	Voacangine	43.20
19.	Augustamine	0.40
20.	Crinamidine	0.04

The % composition of Lactucopicrin was highest both in the leaves (34%) and stem (37%) while augustamine and crinamidine had the lowest % compositions in both the leave and stem. This suggests that lactucopicrin is highly stable in the plant and hence not prone to being converted to other compounds while augustamine and crinamidine may have being prone to conversions that deplete their concentrations in the plant parts studied. In a study conducted on seeds and leaves of *Lupinus aschenborni Schaner* analysed with GLC and GLC-MS, 24 quinolizidine alkaloids like 13α-hydroxylupanine, augusifoline, tetrahydrorhomifoline, lupanine, multiflorine, sparteine, albine among others were detected (Torres *et al*., 1999). However, none of these alkaloids were found in the present study. Furthermore, the alkaloid content determined in the leaves and flowers of *Tabernaemonata heyneana* were 14.6 mg/g and 7.9 mg/g respectively which are higher than the concentration determined in the leaves and stem of *Vernonia amygdalina*. Alkaloids have been reported to have higher concentrations in storage tissues such as roots, fruits and seeds than leaves (Khan *et al*., 2011). The present study however revealed that alkaloid concentrations in the leaves were higher than its concentration in the stem. A study on the root extracts of *Cichorium*

intybus revealed that it contains lactucin and lactucopicrin known to be good antimalarial compounds (Bischoff *et al*., 2004). This could be the reason for the antimalarial activity exhibited by *Vernonia amygdalina* plant. Voacangine, one of the alkaloids determined in the leaves and stem of the *Vernonia amygdalina* plant is reported to be an anticancer and has served as a drug for onchocerciasis (Borakaeyabe *et al*., 2015) and was also determined in *Tabernaemontana fuchsiaefolia* (Zocoler et al., 2005).

The % composition of the various tannins in the *vernonia amygdalina* plant is presented in Table 3 while the tannin concentrations in (mg/100g) are shown in Figure 3.

Table 3: % composition of Tannins in the leaves and stem of *Vernonia amygdalina* plant

S/No	Tannin	Leaves	Stem
1.	Tannic Acid	44.40	53.14
2.	Acertannin	34.68	28.37
3.	Hamamelitannin	0.82	13.10
4.	Leucopetunidin-3-glucoside	8.47	5.39

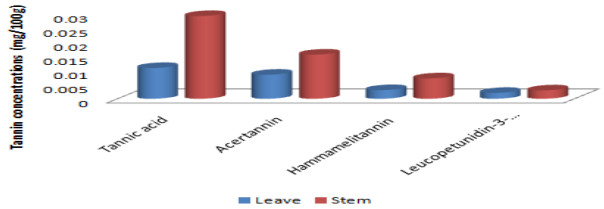

Figure 3: Tannin concentrations in the leaves and stem of *Vernonia amygdalina* plant

There are four tannins detected in *Vernonia amygdalina*, of these, the % composition of tannic acid and acertannin is more in both the leaves and stem compared to Hamamelitannin and leucopetunidin-3-glucoside. Nevertheless, the concentration of acertannin and leucopetunidin-3-glucoside is more in the leaves than in the stem but tannic acid and Hamameltannin concentration was more in the stem than in the leaves. The reason for this is not clear but it may suggest that the tannins tend to deposit better in the stem than the leaves. Generally, the total concentration of tannins in the stem (0.0557 mg/100g) is more than in the leaves (0.0248 mg/100g). In relative terms, the alkaloid content of the leaves and stem is more than that of tannins in the plants which could suggest that alkaloids may be less prone to reactions that convert them to other forms than their present form. This may account for the difference in their concentrations in the plants. The chromatograms for the analysis are shown below;

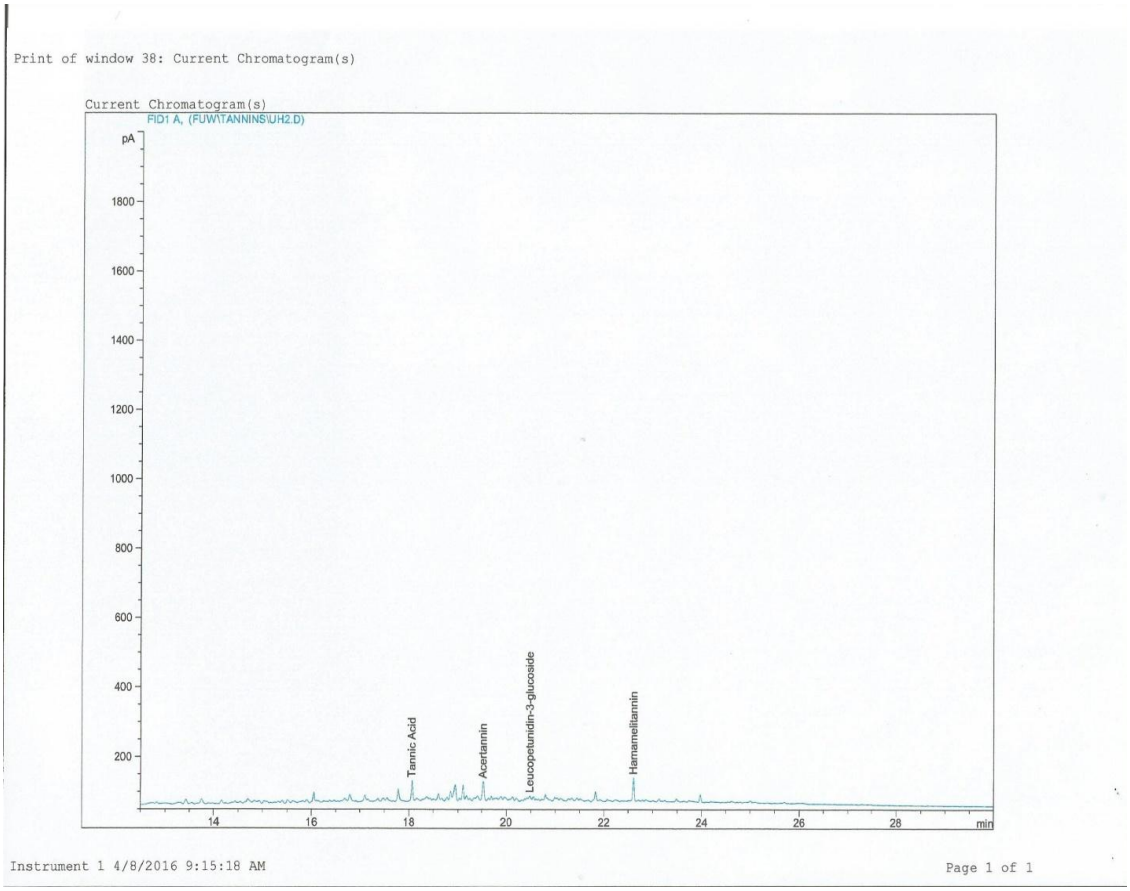

Fig 4: Chromatogram for tannins in the leaves of *Vernonia amygdalina* Plant.

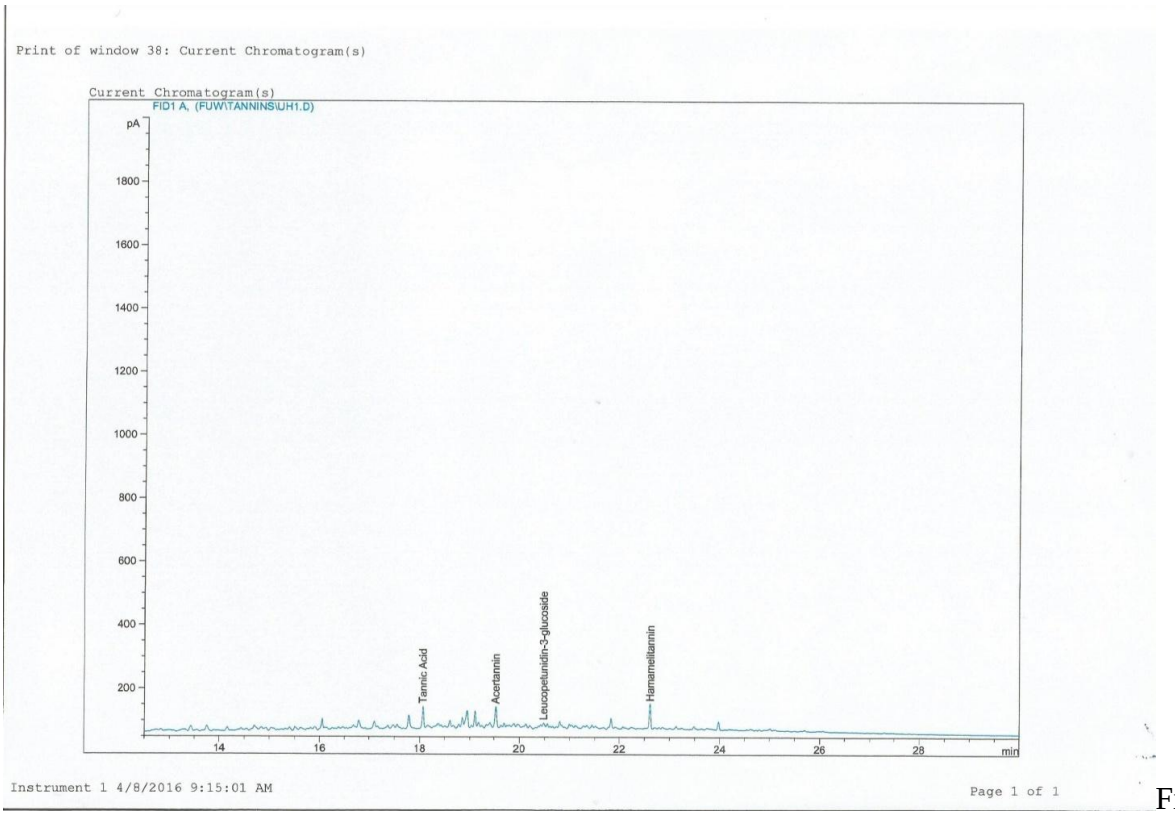

Fig 5: Chromatogram for tannins in the stems of *Vernonia amygdalina* Plant.

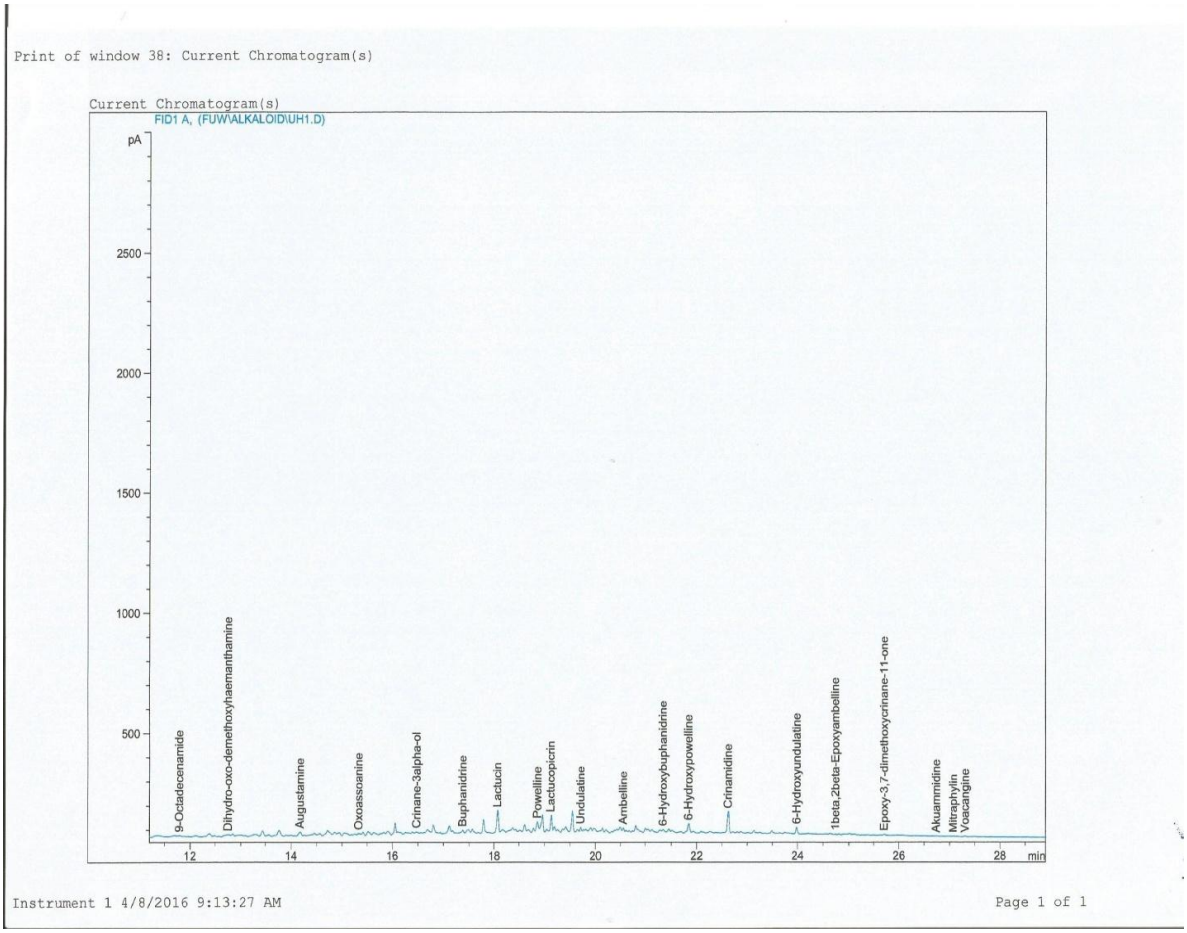

Fig 6: Chromatogram in alkaloids in the leaves of *Vernonia amygdalina*

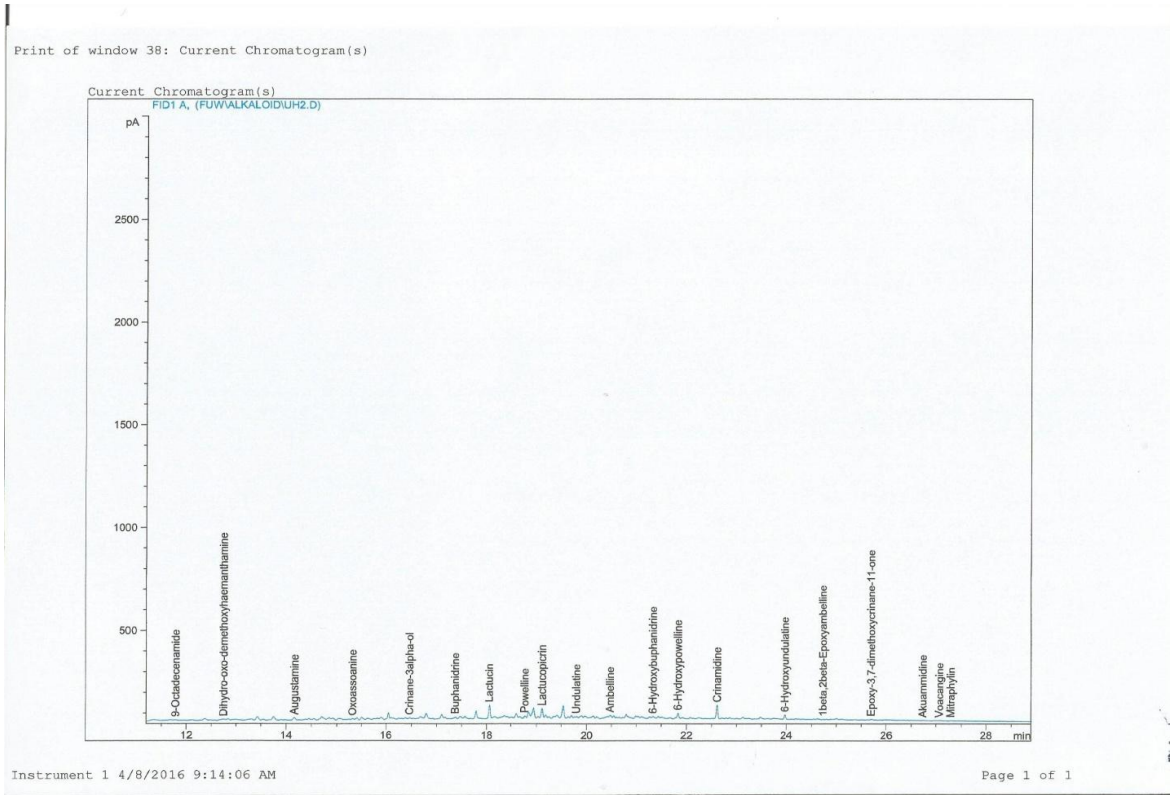

Fig 7: Chromatogram of alkaloids in the stem of *Vernonia amygdalina*

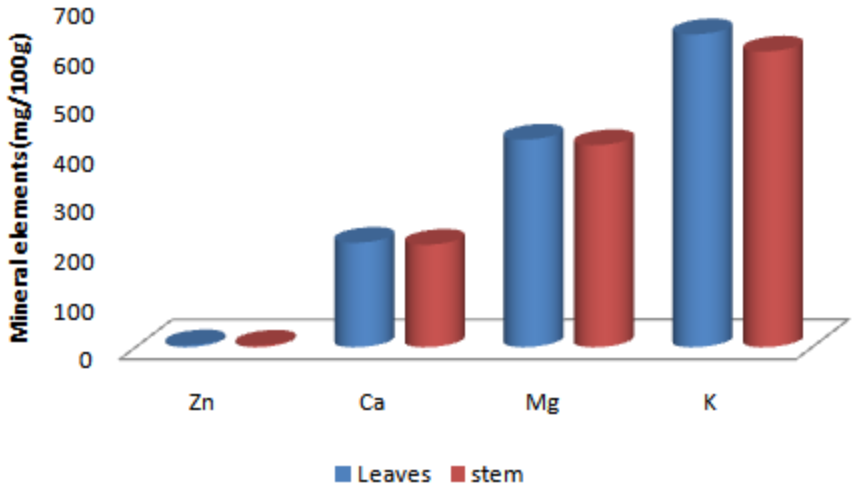

Figure 8: Concentration (mg/100g) of mineral elements in the leaves and stem of *Vernonia amygdalina* plant

The concentrations of the mineral elements in the bitter leaf plant are presented in Figure 8. The results indicate that potassium had the highest concentrations (636.62 mg/100g, 601.62 mg/100g) in the leave and stem respectively while Zn had the lowest concentrations (3.72 mg/100g, 3.16 mg/100g). Generally, the concentration in the leaves and stem do not differ much, nevertheless the concentrations were slightly higher in the leaves than in the stem.
Mineral elements have also been determined in bitter leaf from other locations in Nigeria. A study conducted on bitter leaf from Erelu, Oyo State reported; Ca (151± 1.40), K (61.5 ± 0.38), Mg (96.5± 0.96) and Zn (1.1± 0.11) (Sodamade, 2013). The concentrations determined for the *Vernonia amygdalina* leaf from this settlement is far lower than the concentrations determined for the same elements in the present study. Similarly, in a study conducted on bitter leaf among other leafy vegetables in Igala land, Kogi State these elements have the following concentrations (mg/20g); K (0.006 ± 0.03), Ca (0.02±0.006) and Mg (0.03±0.006) (Opega *et al*., 2016). These concentrations are far lower than determined in Erelu and in the present study. Furthermore, the mineral constituents in an unprocessed bitter leaf sample harvested from Umuahia had the following concentrations in mg/100g; Ca (13.11± 0.99), Mg (19.01±0.03), K (9.00±0.01) and Mn (1.01± 0.01). The concentrations of the mineral elements in this sample were also lower than those found in the present study. The very high amounts of potassium, magnesium and calcium as against zinc in the leaves and stem may suggest that soil on which the plant was harvested from for analysis contained a good quantity of these mineral elements. A study on Wukari waters revealed that the water from hand dug wells and boreholes reflected hardness even after a week of storage (Oko *et al*., 2014). Hardness of water depends on the calcium and magnesium composition of soil in an environment. This could be a further confirmation that the high calcium and magnesium concentrations recorded in the *Vernonia amygdalina* leaves and stem is attributed to the level of calcium and magnesium in the soils of Wukari town.

CONCLUSION

The study which quantified the composition of alkaloids and tannins in the stem and leaves of the bitter leaf (*Vernonia amygdalina*) plant using gas chromatographic analysis revealed 20 alkaloids and 4 tannins. The total alkaloid concentrations in the leaves and stem were 0.3791 mg/100g and 0.2245 mg/100g respectively while total tannin concentrations were 0.0248

mg/100g and 0.0557 mg/100g. This indicates that tannin concentration is more in the stem than leaves while alkaloid concentration was more in the leaves than stem. The leaves and stem also contain high concentrations of calcium, magnesium and potassium which are required for enzyme activity and maintenance of body cells. The implication of this is that both the leaves and stem of *Vernonia amygdalina* would be useful in treating diseases in humans if adequate amounts are used.

REFERENCES

Anacletus, F., Adisa, O.I and Uwakwe, A.(2015) Investigations on implications of gas flaring on some phytochemicals and Trace metal content of Bitter leaf (Vernonia amygdalina). *Journal of Environment and Earth Science*, 5(10):1-5.

Agomuo,J.K.,Akajiaku,L.O.,Alaka,I.C and Taiwo,M.(2016) mineral and Antinutrients of fresh and squeezed-washed bitter leaf (Vernonia amygdalina) as affected by traditional debittering methods. *European Journal Of Food Science and Technology*,4(2):21-30.

Bischoff,A.T., Kelley,C.J., Karchesy,Y., laurantos,M., Nguyen-Dinh, P and Ghafoor, A. (2004) Antimalarial activity of Lactucin and Lactucopicrin:Sesquiterpene lactones isolated from Cichorium intybus. *Journal of Ethnopharmacology*,95(2,3):455-457.

Borakaeyabe, S.B., Mbah, J.A., Cho-Ngwa, F., Metuge, J.A and Efange, S.M.A. (2015) isolation and characterization of filaricidal compounds from the stem bark of Voacanga Africana, a plant used in the traditional treatment of onchocerchiasis in Cameroon. *Journal of Medicinal Plants Research*, 9(4):471-478

Echem,O.G and Kabari,L.G (2013)Heavy metal content in Bitter leaf (*Vernonia amygdalina*) grown along Heavy traffic routes in Port Harcourt. *Agricultural Chemistry*, 201-210.

Egharevba,C.,Osayemwenre, E., Imieje,V.,Ahomafor,J., Akunyili,C.,Udu-Cosi,A.A., Onyekaba,T.,Osakue,J.,Ifitikhar,A and Abiodun,F. (2014) Significance of Bitter Leaf (vernonia amygdalina)in tropical diseases and Beyond: A review. *Malaria Chemotheraphy Control & Elimination*, 3:1

Eleazu, C.O., Eleazu, K.C., Awa, E and Chukwuma, S.C. (2012) Comparative study of the phytochemical composition of the leaves of five Nigerian medicinal plants. *Journal of Biotechnology and Pharmaceutical Research*, 3(2):42-46.

Erasto,P.,Grierson,D.S and Afoloyan,A.J. (2006) Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina.* Journal of Ethnopharmacology., 106:117-120.

Farombi,E.O and Owoeye,O.(2011) Antioxidative and Chemopreventive properties of vernonia Amygdalina and Garcinia biflavonoid. *International Journal of Environmental Research and Public Health*, 8(6):2533-2555.

Karamall, K. and Tennis, V.R. (2001) Tannins: Classification and Definition. *Nat.Prod.Rep*., 18:641-649.

Khan, A.M., Quereshi, R.A, Ullah, F., Gilani, S.A., Nosheen, A., Sahreen,S., Laghari, M.K., Laghari, M.Y., Rehman, S.U., Hussain, I. and Murad,W.(2011) Phytochemical analysis of selected medicinal plants of Margalla hills and Surrounding. *Journal of Medicinal Plants Research*, 5(25):6017-6023.

Mboso, O.E., Eyong, E.U., Odey, M.O. and Osakwe, E. (2013) Comparative Phytochemical screening of Ereromastax Speciosa and Ereromastax polysmema. *Journal of Natural Products and Plant Resources*, 3(2):37-41.

Ngounou, F.N., Manfouo, R.N., Tapondjou, L.A., Lontsi, D., Kuete,V., Penlap,V., Etoa, F.X., Dubois, M.A.L. and Sondengam, B.L (2005) Antimicrobial Diterpenoid Alkaloids from erthrophleum Suaveolens (Guill & Perr) Brenan, *Bull.Chem.Soc.Ethiop*.,19(2):221-226.

Obeta, N.A. (2015)Effect of moist heating and drying processing on the proximate and phytochemical composition of Vernonia amygdalina and gongronema Latifolium leaves. *Agricultural Science Research Journal*, 5(11):153-165.

Okeke, C.U., Ezeabara, C.A., Okoronkwo, O.F., Udechukwu, C.D., Uka, C.J and Aziagba, B.O (2015).Determination of Nutritional and phytochemical compositions of two variants of bitter leaf (*Vernonia amygdalina Del*). *Journal of Human Nutrition & Food Science*, 3(3):1065.

Oko,O.J.,Aremu,M.O.,Odoh,R.,Yebpella,G and Shenge,G.A.(2014) Assessment of water quality index of Borehole and well water in Wukari Town,Taraba State, Nigeria. *Journal of Environment and Earth Sciences*,4(5):1-9.

Opega,J.L.,Ojo,O.C.,Awodi,Y.P and Alfa,I.N.(2016)Proximate composition,mineral and Phytochemical contents of some leafy vegetables native to Igala Kingdom, Kogi State, Nigeria. *International Journal Of Biochemistry Research and Review*,15(4):1-11

Oseni, L.A., Abugri, J., Sumabe, B.K. and Onilimor,P.J. (2014)Leaf extracts of Vernonia amygdalina Del from Northern Ghana contain Bioactive agents that inhibit the growth of some beta-Lactamase producing bacteria in Vitro. *British Journal of Pharmaceutical Research*, 4(2):192-202.

Surjowardojo, P., Sarwiyono, Thohari, I. and Ridhowi, A. (2014) Quantitative and qualitative phytochemicals analysis of *Muntingia calabura. Journal of Biology, Agriculture and Healthcare*,4(16):84-88.

Sodamade,A(2013)Proximate analysis,Mineral content,Amino Acid composition and Functional properties of Vernonia amygdalina vegetable leaf protein concentrate. *Greener Journal of Agricultural Sciences*,3(3):204-210.

Soetan,K.O.,Olaiya,C.O and Oyewole,O.E.(2010)The importance of mineral elements for humans, domestic animals and Plants: A review, *African Journal of Food Science*,4(5):200-222

Torres, K.B., Quintos, N.R., Herrera, J.M., Tei, A and Wink, M. (1999)Alkaloid profiles of leaves and seeds of Lupinus Aschenbornii Schauer from Mexico in Santen,V.E.,Wink, M., Weissmann, S and Roemer, P.(Eds) Lupin, ancient Crop for the new Millenium. Proceedings of the 9th International Lupin Conference. New Zealand: International Lupin Association.

Udochukwu, U., Omeje, F.I., Uloma, I.S and Oseiwe,F.D.(2015) Phytochemical analysis of Vernonia amygdalina and Ocimum gratissimum extracts and their antibacterial activity on some drug resistant bacteria. *American Journal of Research Communication*, 3(5):225-235.

Zocoler,M.A.,de oliveira,A.J.B.,Sarragiotto,M.H.,Grzesius,V.L and Vidotti,G.L (2005) Qualitative determination of indole alkaloids of tabernaemontana fuscsiaefoli(Apocycynaceae).Journal of the Brazilian Chemical Society,16(6b): ISSN 1678-4790.