International Journal of Scientific World, 4 (1) (2016) 11-14

International Journal of Scientific World

Website: www.sciencepubco.com/index.php/IJSW
doi: 10.14419/ijsw.v4i1.5845
Research paper

Phytochemical analysis and proximate composition of *Vernonia amygdalina*

Usunobun Usunomena[1]*, Okolie P. Ngozi[2]

[1]*Department of Basic sciences (Biochemistry unit), Faculty of Basic and Applied sciences, Benson Idahosa University, P.M.B 1100, Benin City, Edo state, Nigeria*
[2]*Department of Biochemistry, Faculty of Life sciences, University of Benin, Benin city*
**Corresponding author E-mail:uusunobun@biu.edu.ng*

Abstract

Background: *Vernonia amygdalina* is used to prepare dishes as well as a multi-purpose fodder tree with high biomass yield. This study was conducted to determine phytochemicals and proximate composition of *Vernonia amygdalina* leaves.
Methods: Evaluation of *Vernonia amygdalina* for phytochemicals and proximate composition were conducted using standard methods.
Results: Result showed that *Vernonia amygdalina* contain phytochemicals such as alkaloids, tannins, flavonoids, saponins, triterpenoids, steroids, cardiac glycosides, and reducing sugar. Results obtained on the proximate composition shows that *Vernonia amygdalina* in percentage (%) contain dry matter (90.68 ± 0.77), crude protein (22.81 ± 0.17), crude fiber (18.17±0.06), moisture (9.32 ± 0.67), ash (16.65 ± 0.09), crude fat (4.34 ± 0.04) and carbohydrate (38.03 ± 0.06).
Conclusion: The presence of phytochemicals like saponins, tannins, alkaloids and flavonoids explains the medicinal potentials of *Vernonia amygdalina* leaves in therapeutic uses. Also the leafy vegetables if consume in sufficient amount would contribute greatly to the nutritional requirement for human health and to the food security of Nigerian population.

Keywords:*Vernonia amygdalina; Phytochemicals; Proximate; Ethanol.*

1. Introduction

Vernonia amygdalina, variously known as bitter leaf (English), *oriwo* (Edo), *ewuro* (Yoruba), *shikawa* (Hausa), and *olubu* (Igbo), is a tropical shrub, 1-3m in height with petiole leaf of about 6mm in diameter, and elliptic in shape [1]. The leaves are dark green coloured with a characteristic odour and a bitter taste. The species is indigenous to tropical Africa and is found wild or cultivated all over sub- Saharan Africa [2]. The leaves are eaten, after crushing and washing thoroughly to remove the bitterness [3]. All parts of the plant are pharmacologically useful. Both the roots and leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort, among others [4]. The stem and root divested of the bark are used as chew-sticks in Nigeria. More importantly, the leaves are used to prepare the very popular bitter leaf soup in Nigeria, and are also reportedly consumed by goats in some parts of Nigeria [5]. Antihelmitic and antimalarial properties [6] as well as antitumourigenic properties [7], have also been reported for extracts from the plant. Other studies have demonstrated hypoglycaemic and hypolipidaemic effects of the leaf extract in experimental animals [8-10]. This study was conducted to determine nutritional and physicochemical properties *Vernonia amygdalina* leaves.

2. Materials and methods

2.1. Collection, identification and preparation of plant materials

Vernonia amygdalina leaves were purchased from a local market in Benin City, Edo state, Nigeria. The fresh leaves were identified, separated from the stalk, washed and air-dried at room temperature (24°C) and then pulverized, crushed into fine powder and weighed.

2.2. Extraction of the plant leaves

Ethanolic extracts of the *Vernonia amygdalina* leaves was prepared by soaking 400g of the dry powdered plant leaves in 1000ml of absolute ethanol at room temperature for 48hrs. The extract was thereafter filtered first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extract was then concentrated using a rotary evaporator with the water bath set at 40◦C to one-tenth its original volume and finally with a freeze drier. The dried residue was then stored at 4ºC. Portions of the crude plant extract residue were weighed and dissolved in distilled water for experimental analysis.

2.3. Methods for proximate analysis

The dry matter, moisture, ash, crude fat, crude protein (nitrogen x 6.25) and crude fibre contents were determined in powdered *Vernonia amygdalina* leaves using the standard methods of the Association of Official Analytical Chemists [11] while carbohydrate content was calculated based on the net difference between the other nutrients and the total percentage composition.

2.4. Methods for phytochemical screening

Phytochemical screening was performed using standard procedures [12-14].

2.4.1. Test for saponins

To 0.5g of extract, 5ml of distilled water was added in a test tube and the solution shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

2.4.2. Test for triterpenoids

0.5g of the extract was dissolved in 1ml of chloroform and 1ml acetic anhydride added, followed by the addition of 2ml of concentrated H_2SO_4. Triterpenoids was indicated by formation of reddish violet colour.

2.4.3. Test for tannins

About 0.5g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and the solution observed for brownish green or a blue-black colouration

2.4.4. Test for reducing sugar (fehling`s test)

0.5g of the extract was dissolved in 5ml distilled water and filtered. The filtrate was hydrolysed with dilute HCl, neutralized with alkali (NaOH) and heated with Ferling`s A and B solutions. Formation of red precipitate indicated the presence of reducing sugars.

2.4.5. Test for anthraquinones

0.5g of the extract was boiled with 10ml of H_2SO_4 and filtered while hot. The filtrate was shaken with 5ml of chloroform, the chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

2.4.6. Test for steroids

0.5g of the extract was dissolved in 10ml of chloroform and equal volume of concentrated H_2SO_4 was added by the sides of the test tubes. Reddish upper layer and yellowish sulphuric acid layer with green fluorescence indicate the presence of steroids.

2.4.7. Test for cardiac glycosides (keller-killiani test)

To 0.5g of extract dissolved in 5ml water was added 2ml of glacial acetic acid solution containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of a deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

2.4.8 Test for flavonoids

Dilute ammonia (5ml) was added to a portion of an aqueous filtrate of the extract. Then, concentrated sulphuric acid (1ml) was added. A yellow colouration indicated the presence of flavonoids.

2.4.9. Test for alkaloids

Extract was dissolved in dilute HCl and filtered. Filtrates were treated with Mayer`s reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

3. Results and discussion

3.1. Phytochemical analysis

Phytochemicals also known as phytonutrients are naturally occurring substances found in plants [15]. The result of the phytochemical screening of the ethanolic leaf extract of Vernonia amygdalina showed presence of flavonoids, alkaloids, saponins, tannins, triterpenoids, steroids, reducing sugars and cardiac glycosides and absence of anthraquinones (Table 1). These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals. Flavonoids have been reported to interfere with the activities of the enzymes involved in ROS generation, quenching of free radicals, chelating transition metals and rendering them redox inactive in the fenton reaction [16-17]. Anti-microbial, as well as many physiological activities such as stimulation of phagocytic cells, host-mediated tumor activity and a wide range of anti-infective actions have been attributed to tannins [18]. Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues [19-20]. Most alkaloids have a strong bitter taste and are very toxic and for these reasons they are used by plant to defend themselves against herbivory, and attacks by microbial pathogens and invertebrate pests [21]. Saponins as a class of natural products are involved in complexation with cholesterol to form pores in cell membrane bilayers [22], and as such may be used as anti-cholesterol agents or cholesterol lowering agent. Steroids are very important compounds especially due to their relationship with compounds such as sex hormone, thus its importance and interest in pharmaceutical companies [23-24]. Cardiac glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure [25].

Table 1: Phytochemical Screening of *Vernonia amygdalina* Ethanolic Leaf Extract

Phytochemicals	*Vernonia amygdalina*
Flavonoids	Present
Saponins	Present
Tannins	Present
Steroids	Present
Alkaloids	Present
Triterpenoids	Present
Anthraquinone	Absent
Reducing sugar	Present
Cardiac glycosides	Present

3.2. Proximate composition

The leaves of *Vernonia amygdalina* contained crude protein value of 22.81% which was in agreement with the report that *Vernonia amygdalina* contained 17 to 33% of crude protein out of dry matter [26]. Also the crude protein value of *Vernonia amygdalina* leaves obtained is higher than protein content of *Momordica foecide* (4.6%) leaves consumed in Nigeria and Swaziland, Lesiantheraafricanas (13.1%) [27-29], *Amaranthus candatus* (20.5% DW), but lower than *Piper guineeses* (29.78% DW) and *T. triangulare* (31.00% DW) [30-32]. Proteins are building block units and the food protein is needed to make vital hormones, important brain chemicals, antibodies, digestive enzymes, and necessary elements for the manufacture of DNA. Some proteins are involved in structural support, while others are involved in bodily movement, or in defense against germs [33].

The leaves of *Vernonia amygdalina* contained crude fibre value of 18.17% which was in agreement with the report that *Vernonia amygdalina* contained 6.5 to 29.2% of crude fibre out of dry matter [26]. Also the crude fibre value of the *Vernonia amygdalina* leaf is high when compared to *Ipomea batatas* (7.20%), *T. triangulare* (6.20%) *P. guineensis* (6.40%), and *Corchorus olitorius* (7.0%), [30-31]. Fibre cleanses the digestive tract by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Fibre also adds bulk to the diet and prevents the intake of excess starchy food [34] and may therefore guard against metabolic conditions such as hypercholesterolemia and diabetes mellitus [35]. Other findings [36] also showed evidence

that a high intake of dietary fiber is associated with enhanced insulin sensitivity and therefore may have a role in the prevention and control of Type 2 diabetes. The substantial amount of fibre in *Vernonia amygdalina* leaves shows that they can help in keeping the digestive system healthy and functioning properly.

Vernonia amygdalina had ash value of 16.65% which is a reflection of the mineral contents preserved in the plants leaf. The ash content obtained compare favorably with the values reported for *Vernonia colorate* (15.86%) and *Moringa oleifera* (15.09%) [31], [37] and lower than that of some leafy vegetables commonly consumed in Nigeria such as *Talinum triangulare* (20.05%) but higher than some other vegetables such as *Occimum graticimum* (8.00%) and *Hibiscus esculentus* (8.00%) [30]. the result therefore suggests a high deposit of mineral elements in the leaves [31].

Vernonia amygdalina had carbohydrate value of 38.03%, lower than reported values for *Corchorus tridens* (75.0% DW) and sweet potatoes leaves (82.8%) [38] but higher than 20% and 23.7% reported for Senna obtusfolia and *Amaranthus incurvatus* respectively [27, 39]. Thus the high carbohydrate content contributes the highest Kilojoules to the energy value in *Vernonia amygdalina*. Carbohydrates produced by plants are one of the three main energy sources in food, along with protein and fat. When animals eat plants, energy stored in carbohydrates is released by the process of respiration, a chemical reaction between glucose and oxygen to produce energy, carbon dioxide, and water.

Vernonia amygdalina had a crude fat value of 4.34% which was in agreement with Yeap et al (26) reported crude fat value range of 2 to 15%. Also the 4.34% *Vernonia amygdalina* crude fat value obtained compares favourably when compared to those of *Talinum triangulare* (5.90%), BaseilaAlba (8.71%), *Amaranthus hybridus* (4.80%), *Calchorus africanum* (4.20%) [30]. A diet providing 1 - 2% of its caloric of energy as fat is said to be sufficient for human being as excess fat consumption is implicated in certain cardiovascular disorders [31].

The moisture content value of *Vernonia amygdalina* leaves was relatively low. The low moisture content would therefore hinder the growth of spoilage microorganisms and enhance shelf life.

Table 2: Proximate Analysis of *Vernonia amygdalina* Leaves

Proximate composition	*Vernonia amygdalina* (%)
Dry matter	90.68 ± 0.77
Moisture Content	9.32 ± 0.67
Crude protein	22.81 ± 0.17
Crude fiber	18.17 ± 0.06
Ash content	16.65 ± 0.09
Crude fat/oil	4.34 ± 0.04
Carbohydrate	38.03 ± 0.06

Values are means ± SD for 3 determinations.

In conclusion, the broad distribution of nutrients and phytochemicals in the *Vernonia amygdalina* leaves studied support, as well as provide a basic rationale for their use as in folk medicine. This study also indicates that *Vernonia amygdalina* leaves, besides serving as good source of pharmacologically active phytochemicals may also be useful as supplements in human and animal nutrition.

References

[1] Igile GO, Oleszek W, Jurzysta M, Burda S, Fafunso M, & Fasanmade AA. (1995). Nutritional assesment of *Vernonia amygdalina* leaves in growing mice. Journal of Agriculture and Food Chemistry43, 2162-2166.http://dx.doi.org/10.1021/jf00056a038.

[2] Bosch CH, Borus DJ & Siemonsma JS. (2005). *Vegetables of Tropical Africa. Conclusions and Recommendations Based on PROTA 2: 'Vegetables'*. PROTA Foundation, Wageningen, Netherlands. 10 modules, pp68.

[3] Mayhew S & Penny A. (1988). *Macmillan Tropical and Subtropical Foods*. Macmillan Publishers, London, p107.

[4] Gill LS (1992). Ethnobotanical uses of plants in Nigerial. University of Binin press, Benin City, 350.

[5] Argheore EM, Makkar HPS & Becker K. (1998). Feed value of some browse plants from the central zone of Delta State Nigeria. Tropical Science38(2), 97–104.

[6] Abosi AO &Raseroka BH. (2003). In *vivo* antimalarial activity of *Vernonia amygdalina*. British Journal of Biomedical Science 60(2), 89- 91.

[7] Izevbige EB, Bryant TL, & Walker AA (2004). novel natural hibitor of extracellular signal regulated kinases and human breast cancer cell growth. Experimental Biology andMedicine 229(2), 163-169.

[8] Akah PA & Okafor CI (1992).Hypoglycemic effect of *Vernonia amygdalina* in experimental rabbits Plant Medicine andResearch1, 6–10.

[9] Nwanjo HU (2005). Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic ratmodels. Nigerian Journal of Physiological Science 20, 39-42.

[10] Ebong PE, Atangwho IJ, Eyong EU, &Egbung GE. (2008). the antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica*(A. Juss) (Neem) and *Vernonia amygdalina* (Del.) (African bitter leaf). American Journal of Biochemistry and Biotechnology 4, 239-244.http://dx.doi.org/10.3844/ajbbsp.2008.239.244.

[11] AOAC (Association of Official Analytical Chemists). (2000). *Official method of analysis*. 15th Edition, Washington D.C. p212.

[12] Sofowora LA. (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, pp55-71.

[13] Trease GE & Evans WC. (1985). Pharmacognosy 11th Ed., Tindall Ltd, London, pp60-75.

[14] Ayoola, GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC &Atangbayila TO. (2008).Phytochemical screening and antioxidant activities of some selected medicinal plantsused for malarial therapy in South-Western Nigeria. Tropical Journal ofPharmaceutical Research 7, 1019-1024.

[15] Ugwu OPC, Nwodo OFC, Joshua PE, Bawa A, Ossai EC &Odo CE.(2013). Phytochemical and Acute Toxicity Studies of *Moringaoleifera*Ethanol Leaf Extract. *International Journal of Life SciencesBiotechnology and Pharma Research* 2(2), 66-71.

[16] Aiyegoro OA &Okoh AI. (2009). Phytochemical screening and polyphenolic antioxidant activity of aqueous crude leaf extract of *Helichrysumpedunculatum*. International Journal of Molecular Science 10, 4990–5001.http://dx.doi.org/10.3390/ijms10114990.

[17] Omoregie ES,Osagie AU & Iruolaje EO (2011). *In Vitro* Antioxidant Activity And The Effect Of Methanolic Extracts Of Some Local Plants On Nutritionally Stressed Rats. Pharmacologyonline1, 23-56.

[18] Haslam E. (1996). Natural Polyphenols (vegetable tannins) as drugs: possible modes of action. *Journal of National Productivity* 59, 205- 215.http://dx.doi.org/10.1021/np960040+.

[19] Okwu DE. & Emenike IN. (2006). Evaluation of the phytonutrients and vitamin contents of Citrusfruits. *International Journal ofMolecular Medicine and Advance Sci*ience 2, 1–6.

[20] Adegboye MF, Akinpelu DA. &Okoh, A. (2008). The bioactive and phytochemical properties of *Garcinia kola* (Heckel) seed extract on some pathogens. African Journal of Biotechnology 7(21), 3934-3938.

[21] Harbone, JB. (1998). Methods of extraction and isolation. In: Phytochemical Methods. Chapman and Hall, London;Pp60-66.

[22] Francis C, George G, Zohar K, Harinder PS, Makhar LM, & Klaus B. (2002). The biological action of saponins in animal system: a review. British Journal of Nutrition88(6),587-605l.http://dx.doi.org/10.1079/BJN2002725.

[23] Okwu DE.(2001).Evaluation of the chemical composition of medicinal plants belonging to Euphorbiaceae. Pakistan Veterinary Journal 14, 160-162.

[24] Aiyegoro OA. &Okoh AI. (2010). Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium*DC. BMC Complementary and Alternative Medicine 10, 21-32.http://dx.doi.org/10.1186/1472-6882-10-21.

[25] Yukari I, Youichi F, Ikuko N, &Itsuru Y. (1995). Quantitative HPLC analysis of cardiac glycosides in *Digitalis purpurea*leaves. JournalNational Productivity58(60), 897-901.

[26] Yeap SK, Ho WY, Beh BK, Liang WS, Ky H, Yousr AHN &Alitheen, NB. (2010). *Vernonia amygdalina*, an ethnoveterinary and ethnomedical used green vegetable with multiple bio- activities. Journal of Medicinal Plants Research 4(25), 2787-2812.

[27] Hassan LG & Umar KJ (2006).Nutritional value of Balsam Apple (*Momordica balsamina*L.) leaves. *Pakistan Journal Nutrition* 5(6), 522-529.http://dx.doi.org/10.3923/pjn.2006.522.529.

[28] Isong EU. AndIdiong. (1997). Comparative studies on the nutritional and toxic composition of three varieties of *Leianthera Africana*, Plants.Food and Human Nutrition51, 79-84.http://dx.doi.org/10.1023/A:1007922308985.
[29] Ogle BM & Grivetti LE (1985).Legacy of the chameleon: Edible wild plants in the Kingdom of Swaziland, Southern Africa. A cultural, ecological nutritional study. Part IV: Nutritional analysis and conclusion, Ecology and Food. Nutrition17, 41-64.http://dx.doi.org/10.1080/03670244.1985.9990881.
[30] Akindahunsi AA & Salawu.SO. (2005). Photochemical screening and nutrient-anti-nutrient composition of selected tropical green-vegetables, African Journal of Biotechnology4, 497-501
[31] Antia BS, Akpan EJ, Okon PA &Umoren IU.(2006). Nutritive and Anti-Nutritive Evaluation of Sweet Potatoes (*Ipomoea batatas*) Leaves. Pakistan Journal of Nutrition5,166-168http://dx.doi.org/10.3923/pjn.2006.166.168.
[32] Etuk EU, Bassey MN, Umoh UO & Inyang EG. (1998). Comparative nutritional studies on three local varieties of *Heinsiacrinita*, Plant Varieties Seeds11, 151-158.
[33] Bailey R (2008), the Role of Proteins in the Body. About.com Guide to Biology.
[34] Mensah JK, Okoli RI, Ohaju-Obodo JO and Eifediyi K. (2008).Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. African Journal of Biotechnology7, 2304-2309.
[35] Henry S. (2004). Fiber: An All Natural "Medicine" for Type 2 Diabetes? Consumer Health Interactive.http://www.ahealthyme.com
[36] Ylonen K, Saloranta C, Kronberg C, Leif G, Antti A, Suvi M, &Virtanean M. (2003). Associations of Dietary Fiber with Glucose Metabolism in Non-diabetic relatives of subjects with Type 2 Diabetes. Diabetes care 26, 1979-1985.http://dx.doi.org/10.2337/diacare.26.7.1979.
[37] Lockeett CT, CalvertCC and Grivetti LE (2005). Energy and micronutrient Composition of dietary and Medicinal wild plants Consumed during drought: Study of Rural Fulani, Northeastern Nigeria. International Journal ofFood. Science and Nutrition51, 195-208.
[38] Asibey-BerkoE,&Tayie FAK. (1999). Proximate Analysis of some under -utilized Ghanian vegetables. Ghana Journal ofScience 39, 8-16.
[39] Faruq UZ, Sani A and Hassan LG (2002). Proximate composition of sickle pod. (*Senna obtusfolia*) leaves,Nigerian Journal of Basic andApplied Sciences11, 157-164.