

Preliminary Phytochemical Screening of the N-Hexane, Ethanol and Distilled Water Extracts of *Vernonia amygdalina* (Bitter Leaf)

¹O. Iloh Emmanuel, ¹O.B. Onyema-Iloh, ³E. Omogo Sunday and ²K.N. Agbafor

¹Department of Pure and Industrial Chemistry,

Chukwuemeka Odumegwu Ojukwu University, P.M.B. 02 Uli Anambra State, Nigeria

²Department of Biochemistry, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria

³Department of Biochemistry, College of Medicine, University of Lagos, Nigeria

Abstract: This research work is based on the determination of the phytochemical constituents of *Vernonia amygdalina* and thus ascertain its medicinal values. The phytochemical screening of the leaf extracts of *Vernonia amygdalina* which was done both qualitatively and quantitatively revealed the presence of saponins (12.36%), flavonoids (5.34%), phytates (9.282%), alkaloids (3.94%), phenols (6.869%), heamagglutinin (28.38%), tannins (22.10%) and cardiac glycosides (1.40%). The presence of these phytochemicals in their percentage ratio confirms the medicinal values of the leaves of *Vernonia amygdalina*. It also went further to extract the sample using three different solvents; n- hexane, ethanol and distilled water which showed that ethanol and distilled water as solvents gave the maximum yield of the extract.

Key words: *Vernonia amygdalina* • Phytochemicals and medicinal plants

INTRODUCTION

Medicinal plants have been used by man for centuries [1]. They are still important especially in many developing countries where traditional remedies are commonly used in the treatment of numerous ailments [2]. A medicinal plant is any plant which in one or more of its parts contains bioactive compounds that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [3, 4]. Mankind has used plants in an attempt to cure disease and relieve physical suffering [5, 6]. A large number of plants grow on wasteland and forest, which are useful medicinally. Many plants products like terpenes, alkaloids, aromatic essential oils, fatty oils, resins and dyes are discovered from the plants growing widely. Also the complex organic compounds are suggestive of various other more useful compounds needed by synthetic industry [7-9]. As a result of new global interest to search for anti-effective agents from natural sources, many more Nigerian higher plants have been investigated than previously [10-12]. Medicinal plants have been found to contain bioactive compounds called phytochemicals and secondary metabolite that can protect humans against

diseases [13-15]. Some important groups of these phytochemicals (secondary metabolites) are involved in many in-vitro studies and assessment of hematological parameters, antioxidant activities, anti-microbial effect and analgesic effect [16]. *Vernonia amygdalina* is a small shrub that grows in the tropical Africa. *V. amygdalina* typically grows to a height of 2-5 m. The leaves are elliptical and up to 20 cm long. Its bark is rough. *V. amygdalina* is commonly called bitter leaf in English because of its bitter taste [17]. African common names include grawa (Amharic), ewuro (Yoruba), etidot (Ibibio), onugbu (Igbo), ityuna (Tiv), oriwo (Edo), chusar-doki (Hausa), mululuza (Luganda), labwori (Acholi), olusia (Luo) and ndoleh (Cameroon). The cooked leaves are a staple vegetable in soups and stews of various cultures throughout equatorial Africa [18-21]. It does not produce seeds and has to be distributed or propagated through cutting. It grows under a range of ecological zones in Africa and produces a lager mass of forage and it is drought tolerant, with about 200 species of Vernonia. It is majorly used for human consumption and has to be washed to remove the bitter taste. Its bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides [22,23]. It stimulates the digestive

system as well as reduces fever. Intestinal parasitic infestations are treated by African people using the bitter leaf [24]. The leaves of this herb are consumed by people but only after rinsing them thoroughly to do away with their bitter taste [25-29]. Bitter leaf greens are used in the form of a vegetable with a view to promote the digestive system, in addition to reducing fever [30-34]. The main objective of this research work is to determine the phytochemicals in the leaves of *Vernonia amygdalina* used for healing and curing of various diseases.

MATERIALS AND METHODS

Plant Material: The plant *Vernonia amygdalina* was collected from Umuoma Uli in Ihiala Local Government Area, Anambra State, Nigeria and was identified by Mr. Ilo E.O.

Equipments and Apparatus/ Reagents/ Chemicals: The equipments and reagents used were of analytical grade.

Preparation of Plant for Extraction: Fresh leaves of *Vernonia amygdalina* were collected, washed and air dried at room temperature to avoid changes that may result in the case of hot temperature and then powdered using a grinder which was then sealed in an airtight polyethylene bag and the sample for test was then taken from there.

Solvent Extraction: A sample (40 g) of the powdered plant material was soaked in ethanol (200 ml), as the solvent for 24 hours. After which the mixture was filtered using Whatman filter paper. The filtrate was then refluxed for about 2 hours and then distilled to recover part of the solvent and evaporated to get the extract in a slurry form at about 50°C. This procedure was repeated with n-hexane and distilled water as solvents using 40g of the powdered sample for each extraction. At the end of the extraction, each extract was cooled and stored in the refrigerator.

Phytochemical Screening: Phytochemical screening for major constituents was undertaken using standard qualitative methods as described by Trease and Evans (1989) and Harborne (1998).

RESULTS AND DISCUSSION

The phytochemical Screening, qualitative and quantitative estimation of the leaves of *vernonia amygdalina* revealed the presence of alkaloids,

Table 1: Qualitative phytochemical screening of *Vernonia amygdalina*

Phytochemicals	N-Hexane	Ethanol	Distilled water
Alkaloids	++	++	++
Flavonoids	-	++	++
Cardiac Glycoside	++	++	-
Tannins	-	++	++
Resins	--	++	++
Steroids	++	+	-
Proteins	--	+	++
Carbohydrates	++	++	++
Saponins	+	++	++

Note: "+ +" represents presence of the constituents in moderate concentration
 "+" represents presence in slight concentration
 "-" represents absence of the constituents

Table 2: Quantitative Estimates of phytochemical constituents of *Vernonia amygdalina*

Phytochemicals	Composition (%), mg/l
Saponins	12.36%
Flavonoids	5.34%
Tannins	22.10%
Phytates	9.28%
Alkaloids	3.94%
Cardiac Glycoside	1.40%
Phenols	6.8690mg/l
Oxalate	0.0216mg/l
Heamagglutinin	28.38mg/l

flavonoids, saponins, tannins, steroids, resins and carbohydrates as stated in (Table 1 and 2) indicating high level of its possible medicinal and dietary values. The result in Table 2 showed that the plant contains high level of saponin (12.36%). Saponin is beneficial to human health. It has the property of precipitating and coagulating red blood cells (i.e it function as natural antibiotic). It enhances the function of several organs in the body and also cures a variety of diseases. Other characteristics of saponin includes formation of foams in aqueous solutions, hemolytic activity and cholesterol binding properties. The above properties bestow high medicinal activities on the extract of *Vernonia amygdalina*. Tannins are attributed for their analgesic and anti-inflammatory activities. Apart from this, tannins exhibits astringency property (i.e. fastens the healing of wounds and inflamed mucous membrane gusting their usage in herbal medicine (Okwu *et al.*, 2006) [24]. Phytate content (9.282%) in *V. amygdalina* is responsible for its ability to improve respiration and also aid in the elimination of toxins and improve overall vitality. Flavonoids exhibits anti-inflammatory, anti-allergic, analgesic effect and anti-oxidant properties. Anti-oxidants

are compounds that protects cells against the damaging effect of reactive oxygen species like hydroxyl radicals and peroxy nitrile. Flavonoids also acts as free radical scavengers which prevents oxidative cell damage. This makes *V. amygdalina* a potential medicinal plant. Alkaloids present (3.94%) signifies the bioactive activities in the plant. Phenols have antiseptic properties. It should be noted that steroidal compounds are of great importance in pharmacy due to their relationship with sex hormones. In table 1, the presence of carbohydrate and protein confirms the nutritional value of the leaves of *vernonia amygdalina* as carbohydrate is one of the essential nutrient needed in human diet likewise protein (primary metabolite). It also contains cardiac glycoside which has a characteristic action on the contractile force of the heart muscle. In order words, cardiac glycoside is used medicinally to increase the force of contraction of heart muscle and to regulate heart beats. This also indicates the medicinal value of the sample.

CONCLUSION

From the results in tables 1, 2 and 3, *Vernonia amygdalina* appears to be rich in secondary metabolites widely used in traditional medicine to combat and cure various ailments. The therapeutic potentials (such as anti-inflammatory, anti-oxidant, anti-allergic, anti-analgesic etc.) of *V. amygdalina* can therefore be attributed to the presence of these secondary metabolites (flavonoids, alkaloids, steroids, tannins, saponins, cardiac glycoside, phytate, phenols etc.) These classes of compounds are also known to exert pharmacological and antagonistic effects while some are capable of protecting the active ingredients in herbs from decomposing either chemically or physiologically. The information obtained from the quantitative phytochemical constituents of *V. amygdalina* provides incentive for evaluation of the use of the plant in treatment of ailment and in medicine.

In Nigeria, the research for new drugs is on course hence more investigation should be done on the leaves of *Vernonia amygdalina* with the aim of verifying the antibacterial activities of the plant so as to find an alternative for the common antibiotics present in use and aslo to detect the dosage and toxicological effect of the plant.

REFERENCES

1. Abosi, A.O. and B.H. Raseroka, 2003. "In vivo antimalarial activity of *Vernonia amygdalina*". Br. J. Biomed. Sci., 60: 89-91.

2. Akubue, P., et al., Preliminary Pharmacology study of Some Nigerian Medicinal Plants. J Ethnopharmacol., 1: 53-63.
3. Atal, C.K., J.B. Srivastava, B.K. Wali, R.B. Chakravarty, B.N. Dhawan and K.P. Rastong, 1978. Screening of Indian Plants for Biological activity part III, Indian J. Exp. Biol., 16: 330-349.
4. Borris, R.P., 1996. Natural products research perspectives from a major pharmaceutical company. J. Ethnopharmacol., 55: 29-38.
5. Coombs, J., 1989. Macmillian Dictionary of Bio-technology Macmillian Press Limited, London, pp: 325.
6. Edeoga, H.O., D.E. Okwu and B.O. Mbaebie, 2005. Phytochemical Constituents of some Nigerian Medicinal Plants. African Journal of Biotechnology, 4(7): 685-688.
7. Fasuyi, A.O., 2006. Nutritional potentials of some tropical vegetable leaf meals. Chemical characterization and functional properties. Afr. J. Biotechnol., 5: 49-53.
8. Finar, I.L., 1986. Organic Chemistry: Terpenoids. 5th Edn., Longman group Limited, London, pp: 354-368.
9. Gill, L.S., 1992. Ethanomedical uses of plants in Nigeria. University of Benin Press, Benin City Nigeria, pp: 10-30.
10. Harborne, J.B., 1998. 'Phytochemical Methods'. A guide to modern techniques of plant analysis 3rd edn. Chapman and Hall, New York, pp: 1-150.
11. Huffman, M.A. and M. Seifu, 1989. "Observation on the illness and consumption of a possibly medicinal plant *Vernonia amygdalina* (Del) by a wild Chimpanzee in the Mahala Mountains National park, Tanzania". Primates, 30: 51-63. doi:10.1007/Bf02381210.
12. Ijeh, I.I. and C.E.C.C. Ejike, 2011. "Current perspective on medicinal potential of *Vernonia amygdalina* del". J med plant Res., 5(7): 1051-1061.
13. Iwu, M.M., 1993. Handbook of Africa Medicinal Plants C. R. C. Press Inc. Washington.
14. John, T., 1996. "Phytochemicals as Evolutionary Mediators of Human Nutritional physiology. International Journal of Pharmacognosy, 34(5): 237-344.
15. Kokwaw, John, 2009. Medicinal plants of east Africa 3rd ed. Nairobi, Kenya: University of Nairobi press. ISBN 996-846-84-0
16. Kucera, M., V.O. Marqis and Kuceroahk, 1972. Contribution to the knowledge of Nigeria medical plant J.L.C separation and quantitative evaluation of alstonia boonei alkaloid plants. Medica, 21: 343-346.

17. Kucera, M., V.O. Marqis, D.J. Newman, G.M. Cragg and K.M. Snader, 2003. Natural products as sources of new drugs over the period. J. Nat. Prod., 66(7): 1022-1037.
18. Kumar, A., R.I. Lavarasan, T. Jayachandran, M. Decaraman, P. Aravindhan and N. Padmanabhan, 2009. Investigation on a tropical plant, *Syzygium cumini* from Kattuppalayam, Erode District, Tamil Nadu, South India. Pak. J. Nutr., 8: 83-85.
19. Medicinal Plants. Wikipedia the free encyclopedia, Retrieved from <http://www.wikipedia.com>.
20. Miller, A.L., 1996. Antioxidant flavonoids: structure, functions and clinical usage. Altern. Med. Rev., 1: 103-111.
21. Ndukwe, E., 2003. Nasal Activity and Anti-inflammatory Effect of Medicinal plant consumption. Journal of plant Nutritional Revolution, 48: 111-113.
22. Nigerian Institute of Pharmaceutical Research and Development (NIPRD, 1993). A Phytochemical Test Procedure. Department of Medicinal Plants and Traditional Medicine. Abuja.
23. Okwu, D.E. and C. Josiah, 2006. Evaluation of the chemical composition of Nigerian medicinal plants. Afri. J. Biotech., 5: 357-361.
24. Pietta, P.G., 2000. Flavonoids as anti-oxidants, J. Nat prod, 63: 1035-1042.
25. R.F. Weiss, Herbal medicine.
26. Thankur, M., M.F. Melzig, H. Fuchs and A. Weng, 2011. Institute of laboratory Medicine, Clinical Chemistry and Pathobiochemistry Charite Universitymedizin Berlin, pp: 19-29. DOI:<http://dx.doi.org/10.2147/BTAT.517261>.
27. Kostyuk, V.A., A. Pstapovich, E.N. Vladykovskaya, L.G. Korkina and I.B. Afanasev, 2001. Arch. Biochem. Biophys., 385: 129-137.
28. WHO (World Health Organisation, 2002). Medicinal plant role in Health care [www.google-who/healthcare/Medicinal plant - html.com](http://www.google-who/healthcare/Medicinal%20plant-html.com).
29. World Health Organization, 1998. Quality Control Methods for Medicinal Plant Materials, WHO, Geneva.
30. Sofowora, A., 1982. Medical Plants and Traditional Medicine in Africa. John Wiley Co. 192.
31. Trease, G.E. and W.C. Evans, 1983. Pharmacognosy 12th edition. Bailliere Tindell, London.
32. Borris, R.P., 1996. Natural products research perspectives from a major pharmaceutical company. J. Ethnopharmacol., 51: 29-38.
33. Ugwu, Okechukwu P.C., Nwodo, F.C. Okwesili Joshua, Parker E. Bawa, aburbakar, C. Ossai Emmanuel and E. Odo Christian, 2013. Phytochemical and Acute Toxicity Study of *Moringa oleifera* Ethanol Leaf Extract. International Journal of Life Sciences Biotechnology and Pharma Research, 2(2): 66-7.
34. Afiukwa, C.A., Ugwu, P.C. Okechukwu, L.N. Ebenyi, H.A. Oketa, J.N. Idenyi and C. Ossai, Emmanuel, 2013. Phytochemical Analyses of the two wild Edible Mushrooms *Auricularia polytricha* and *Pleurotus ostreatus* that are Commonly Found in Ohaukwu Metropolis, Ebonyi State, Nigeria. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4(2): 1065-1070.
35. Adonu, Cyril, C., P.C. Ugwu, C.O. Okechukwu, Esimone, C. Ossai, Emmanuel, Bawa, Abubakar and U. Okorie, Chris, 2013. Phytochemical Analyses of the Menthanol, Hot water and n-hexane extracts of the Aerial Parts of *Cassytha filiformis* (linn) and Leaves of *Cleistopholis patens* (benth). Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4(2): 1143-1149.