International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

Check for updates

Phytochemical and larvicidal evaluation of stem bark of *Vernonia amygdalina*

Eberechi Okolie [1], Oghenekevwe Ikpan [1] and Ozadheoghene Eriarie Afieroho [1, 2, *]

[1] *Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt. Port Harcourt, Nigeria.*
[2] *Nuclei for Phytomedicines and Chemical Ecology (NuPaCE), Central Research Laboratory for Phytomedicines, Department of Pharmacognosy and Phytotherapy, University of Port Harcourt. Port Harcourt, Nigeria.*

International Journal of Science and Research Archive, 2023, 09(01), 250–256

Publication history: Received on 29 March 2023; revised on 24 May 2023; accepted on 26 May 2023

Article DOI: https://doi.org/10.30574/ijsra.2023.9.1.0360

Abstract

The *Aedes aegypti* mosquito is responsible for the transmission of yellow fever, and several arboviruses, which has led to an increasing disease burden. The development of resistance in mosquitoes has prompted researchers to investigate on biological larvicides that are biodegradable and ecofriendly. This study is aimed at evaluating the larvicidal activity of the stem bark of *Vernonia amygdalina* using the activity–guided fractionation approach. The powdered stem-bark (300g) was extracted by successive cold maceration first in dichloromethane and then in 70% aqueous ethanol for 72 hours with fresh replacement of solvent after every 24 hours. The extracts were separately concentrated using a rotary evaporator to obtain the dichloromethane extract (DCM) and 70% aqueous ethanol extract (AQE) respectively. The more active AQE was further partitioned with ethyl acetate to obtain the aqueous phase (AQP) and the organic phase (EAP). Larvicidal assay was done following the World Health Organisation protocol with modifications. Dichlorvos (2,3-dichlorovinyldimethylphosphate) was used as a reference larvicidal agent. Phytochemical screening was done using standard phytochemical screening reagents. The result from Larvicidal assay showed that the 70% ethanol extract gave a percentage mortality of 86.5%, at its highest concentration (10mg/ml) and an LC_{50} of 6.5mg/ml, while the highest percentage mortality recorded from the dichloromethane extract, was found to be 58%, at 10mg/ml. The EAP demonstrated a percentage mortality of 100%, at a concentration of 1mg/ml, while the AQP yielded a percentage mortality of 100% at concentration of 6.5mg/ml. With repect to phytochemical constituents, the crude plant sample contained deoxy sugars, triterpenoids, carbohydyrates, saponin and phenolics. The DCM lacked flavonoids and phenolics, but contained carbohydrates, triterpenoids and deoxy sugars. The AQE lacked free anthraquinones. The AQP contained carbohydrates, saponins, phenolics, whereas the EAP contained deoxy sugars, triterpenoids and flavonoids in addition. In conclusion, the 70% aqueous ethanol extract, as well as its organic and aqueous phases can be developed as bio-larvicides, for vector control of *Aedes aegypti* mosquitoes.

Keywords: Mortality; Insecticides; Ecofriendly; Arbovirus; Phenolic compounds

1. Introduction

The *Aedes aegypti* is also known as the yellow fever mosquito. This is because it is the primary vector of yellow fever, a disease which is prevalent in tropical South America and Africa. *Aedes aegypti* is also a known vector of several other viruses including dengue virus, chikungunya virus, and Zika virus. These arboviruses pose increasing global public health concerns because of their rapid geographical spread and increasing disease burden. Dengue is the most important arboviral disease and is widely distributed in the tropical and sub-tropical regions of the world. [1]. Thus, the effort towards mosquito control continues to be an important strategy in preventing the mosquito-borne diseases.

* Corresponding author: Ozadheoghene Eriarie Afieroho

Indeed, the present recrudescence of these diseases is due to the higher number of breeding places in today's throw-away society and to the increasing resistance of mosquitoes to current commercial insecticides. Although yellow fever has been reasonably brought under control with its vaccine, no vaccine is available for dengue. The only way of decreasing the incidence of this disease is thus the eradication of *A. aegypti*. Experience has shown that aerial toxicants for the eradication of this mosquito are not effective, since it is highly domesticated and many adults rest indoors in hidden places such as closets. The only successful way of reducing mosquito densities to a level where dengue or yellow fever epidemics do not occur is by attacking the larval breeding places[2]. Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control were in use since the 1920s [3]. Since then, the search for new bioactive compounds from the plant kingdom and an effort to determine their chemical structures and commercial production has been initiated. At present phytochemicals make up to 1 per cent of world's pesticide market [4]. Medicinal plants have been serving as source of rich natural components that are essential in the management of various ailments. The affordability and accessibility of these plants, along with their limited side effects, has prompted researchers to investigate more properties that can elicit pharmacological actions, such as antimicrobial activity, antipyretic effect, treatment of sexually transmitted diseases, antidiabetic properties, etc. This has led to a remarkable increase in the utilisation of herbal medicines, in recent times.

Some documented ethno medicinal uses of *Vernonia amygdalina* include: the leaves taken as an appetizer and the water extract as a digestive tonic and for treatment of fevers in Nigeria and some other African countries [5]. The young leaves are used in folk medicine as worm expeller and fertility inducer in sub-fertile women. Both the leaves and the roots are used traditionally in phytomedicine to treat fever, kidney and heart diseases, and stomach discomfort[6]. Sceintific reports on the biological activities such as: antihelminthic [7], antiplasmodial [8] and antimalaria [9], antimicrobial [10], antidiabetic[11], antioxidant[12], antianaemic [12], antidiarrhoeal [13], antiinflammatory[14] and anticancer [15] among others are reported in literature. These scientific reports are mostly on the leaves with scarce scientific report on the larvicidal properties of the stem bark. These emphasized the necessity for this study aimed to evaluate the larvicidal activity of the stem bark of *Vernonia amygdalina* (Asteraceae).

2. Material and methods

2.1. Collection of Plants

The stems of *Vernonia amygdalina* (Asteraceae) was collected from various farmlands within Port Harcourt. The fresh leaves were further identified and authenticated by a Taxonomist. A voucher specimen was deposited in the herbarium of the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt, with a voucher specimen number of UPHA0590.

2.2. Extraction of Plants Material

Successive extraction was done, by immersing 300g of the powdered plant sample in 1litre of dichloromethane. The mixture was shaken at intervals within a period of 24 hours, using a mechanical shaker, at 120rev/min. At the end of the 24hours, the solvent was drained out using a filter paper, the extract was obtained and kept in a glass bottle. This process was done for a duration of 72 hours, with the extract being filtered every 24 hours, and replaced with 1litre of fresh dichloromethane. The same procedure summarized above was carried out, using 70% ethanol. The various extracts obtained were concentrated, using a rotary evaporator, and they were furthered placed in crucibles in a water bath, to obtain the dried dichloromethane and aqueous ethanol extract respectively. Thereafter, their percentage yield was determined.

2.3. Larvicidal assay

Five ribbons of *Aedes aegypti* mosquito eggs were obtained from the National Arbovirus and Vectors Research Centre (NAVRC) Enugu. The eggs were dispersed into five plastic plates, containing warm water, and kept at room temperature. Thereafter, yeast was added to the water, to aid their incubation. The Eggs were fed biscuit after 24 hours and beyond, until they finally hatched into larvae, and ready for use. Larvicidal activity against *Aedes aegypti* Larvae was done, according to the World Health Organisation standard protocol [16] with modifications. Briefly, twenty (20) disposable plates were appropriately arranged and labelled according to their concentrations. Thereafter, 400 *Aedes aegypti* mosquito larvae, in the late third instar larva stage were collected with the aid of a dropper, into the plates, with each plate containing 20 larvae. Each concentration has 3 plates, with the fourth, serving as control. A stock solution of the 70% ethanol extract was prepared, by dissolving 5g of the extract in 500ml of distilled water, to yield a 10mg/ml solution. 300ml of the solution was poured out into a beaker, from which 100ml volume each was poured out into the first three plates. From the 200ml solution left, serial dilutions were made, to obtain a 5mg, 1mg, 0.5mg and 0.1mg/ml solution. 300ml of each were obtained, and 100ml was poured in triplicates, into the plates containing the larva. To the

fourth plate serving as control, 100ml of distilled water was poured into them. The same procedure as summarized above, was done, using the same quantity of the dichloromethane extract. At the end of 24, 48, and 72hours, the dead larvae were counted. Thereafter the median larvicidal concentration LC_{50} was determined using regression analysis. Test concentrations of: 10, 1, and 0.1mg/ml of 2,3 dichlorovinyl dimethyl phosphate were prepared and used as the positive control, against the late 3rd insta larva stages of *Aedes aegypti* mosquito. Mortality rate was recorded at the end of 24 hours.

2.4. Phytochemical Screening

The crude, 70% aqueous ethanol, and dichloromethane extracts were screened respectively using standard phytochemical screening methods[17-18] for the presence of: carbohydrates and reducing sugars, anthraquinones, triterpenoids, saponins, cardiac glycoside, phenolic compounds, flavonoids, and alkaloids.

2.5. Data analysis

Data expressed as mean ± standard deviation were analysed for statistical significance at p = 0.05 using one-way analysis of variance (ANOVA) and student t-test. The significance difference between the mean values of each extract at different concentrations and a control was established at P< or = 0.05 by the student t-test using SPSS software version 16 at 95% confidence interval. Comparison groups were achieved using ANOVA and Post HOC turkeys' multiple comparison test. LC_{50} was determined using sigmoid Emax model (Hill).

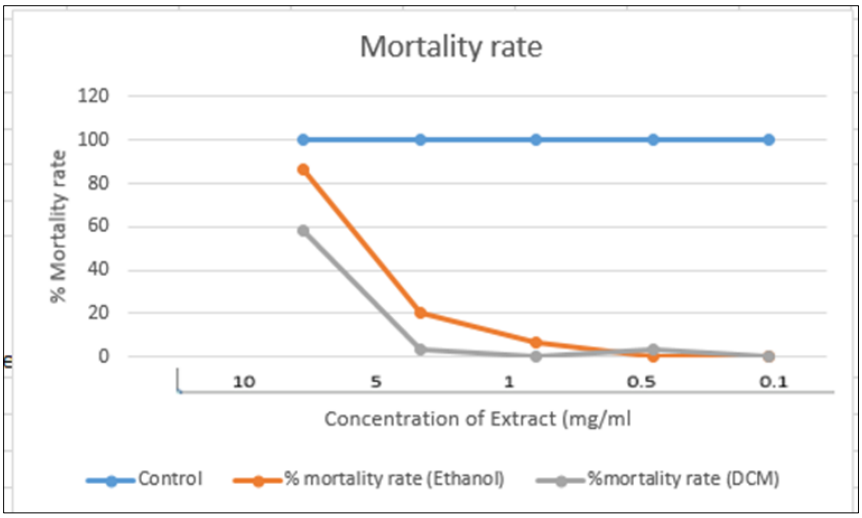
3. Results

Table 1 Average Mortality Rate and Percentage Mortality of 70% Aqueous Ethanol Extract, and Dichloromethane Extract

Test concentration (mg/ml)	Average Mortality Rate of Triplicates ± Standard Deviation						
	Aqueous Ethanol Extract			Dichloromethane extract			2,3-dichlorovinyl dimethyl phosphate
	24 Hours	48 Hours	72 Hours	24 Hours	48 Hours	72 Hours	24 Hours
10	14±0.8 (70%)	15.7±1.3 (78%)	17.3±2.1 (86.5%)	7±4.3(35%)	8.7±3.9 (43.5%)	11.7±2.9 (58%)	20±0(100%
5	1±0.8 (5%)	3.7±2.5 (18.5%)	4±2.2 (20%)	2.7±0.5 (13.5%)	4±0.8 (20%)	6.7±1.7 (3.5%)	NA
1	0.7±0.5 (3.5%)	0.7±0.5 (3.5%)	1.3±0.5 (6.5%)	0±0(0%)	0±0(0%)	0±0(0%)	20±0(100%)
0.5	0±0(0%)	0±0(0%)	0±0(0%)	0±0(0%)	0±0(0%)	0.7±0.5 (3.5%)	NA
1	0±0(0%)	0±0(0%)	0±0(0%)	0±0(0%	0±0(0%)	0±0(0%)	20±0(100%)

KEY: Values are represented as mean ± standard deviation of triplicate determination while values in parenthesis represents percentage mortality. NA signifies Non-Applicable

The LC_{50} extrapolated from the plot for the Active Aqueous Ethanol Extract is 6.5mg/ml



Key to Figure 1: Control: 2, 3 dichlorovinyl dimethyl Phosphate, DCM: Dichloromethane extract, Ethanol extracts: 70% acqueous ethanol extract

Figure 1 Concentration- dependent larvicidal activity profile of the DCM and 70% Aqueous Ethanol Extract

Table 2 Phytochemical Screening of the Crude, 70% Aqueous Ethanol Extract, and Dichloromethane Extract

Test	**Crude Sample**	**Extracts**	
		70% Aqueous Ethanol Extract	**Dichloromethane Extract**
Cardiac glycoside			
Kedde's test	Negative	Negative	Negative
Keller-killiani test	Positive	Positive	Positive
Alkaloids			
Mayer	Negative	Positive	Negative
Dragendoff	Negative	Negative	Negative
Hager	Negative	Negative	Negative
Triterpenoids			
Lieberman-burchard	Positive	Positive	Positive
Salkowski	Positive	Positive	Positive
Anthraquinones			
Free	Negative	Negative	Negative
Combined	Negative	Negative	Negative
Saponin			
Frothing test	Positive	Positive	Negative
Phenolics			
Ferric chloride test	Positive	Positive	Negative
Flavonoids			
Aluminium chloride	Positive	Positive	Negative
Shinoda test	NA	NA	NA

KEY: Positive indicates the presence of secondary metabolites Negative indicates the absence of secondary metabolites NA represents non applicable

4. Discussion

From the yield determination, the 70% aqueous ethanol extract was found to have a higher yield (8%) than the dichloromethane extract (3.73%). The results of the larvicidal activity in this study showed that *Vernonia amygdalina* is effective against *Aedes aegypti* mosquito larva. The 70% aqueous ethanol extract was found to be more efficacious against the larva, with the highest percentage mortality of 86.5% at 10mg/ml. On the other hand, the highest percentage mortality for the dichloromethane extract was found to be 58% which was exhibited at 10mg/ml. These were however relatively not as efficacious when compared with the result for the positive control (2,3 dichlorovinyl dimethyl phosphate) at test concentrations of 10, 1, and 0.1mg/ml which demonstrated a 100% mortality rate after 24 hours. The LC_{50} of the active aqueous ethanol extract, obtained from the plot was found to be 6.5mg/ml. These data showed that larvicidal activity can vary depending on the solvent used for extraction because each solvent can selectively extract a different group of constituents according to polarity. While the 70% aqueous ethanol extract showed a very significant larvicidal activity, that of the dichloromethane extract was quite low. This is because of the polarity of both solvents used. Previous researchers have attributed the larvicidal activity of various plants to phytochemicals such as saponins, phenolics, triterpenoids, and flavonoids. The ethyl acetate extract from the leaves of *A. aspera* from where a saponin was isolated showed larval mortality[19]. In a similar report on the saponins isolated from the root-derived callus of *B. aegyptiaca,* a 100% mortality at 500 ppm on the test larval population from emerging adults of the *A. aegypti* larvae was observed [20]. The finding of acute larvicidal effects of polyphenols against certain larval Culicidae, Chironomidae and Simuliidae has already suggested the prospect of using these polyphenols in dipteran pest control [21]. In this study as shown in Table 2, the phytochemical screening for the presence of cardiac glycoside using the Kedde's test for the aglycone of cardiac glycosides, exhibited a negative result for the crude extract and 70% aqueous ethanol extract, whereas they all tested positive to Keller Killiani's test. This infers that there is presence of deoxy sugars in the extracts, but absence of the cardenolide the aglycone moiety of cardiac glycoside. Also whereas triterpenoids were present in all extracts, the phytochemical screening result showed the presence of saponins, flavonoids, and phenolics, only in the crude *V. amygdalina* powder sample and its 70% aqueous ethanol extract but absent in the dichloromethane extract. Thus, it can be inferred that the larvicidal activity of the aqueous ethanol extract was due to the presence of phenolics and/or saponins with possible synergitic effect. It can also be inferred that the dichloromethane produced less larvicidal activity, because the solvent was not able to extract much of the phenol and saponin content from the plant sample. The moderate effect of the dichloromethane observed, could be attributed to the presence of the triterpenoids. Triterpenoids are the non-polar aglycone of saponins and this observed moderate larvicidal activity could plausibility underscore the structure activity relationship between the polar glycosidic form (the saponin) and the corresponding non-polar aglycone(triterpenoid). A study on *Kotschya africana* afforded the isolation of a triterpenoid, lupeol[22]. Lupeol is also known to be active against the larvae of *Aedes aegypti* with LC_{50} of 158.71 µg/mL after 24 hrs exposure time [23].The extracts and fractions obtained from plant species can exert their toxic effects on larvae through multiple methods, such as the inhibition of growth, reproduction, or fertility. Furthermore, studies have shown that the larvicidal potential of phenolic acids is influenced by the presence of a hydrocarbon chain on the phenolic ring and its degree of unsaturation [24].

Although the bio-larvicides are not as efficacious as the organic agents in control of mosquitoes, the bio-larvicides are easily bio-degradable, are ecofriendly and non-toxic to mammalian tissues. Therefore, they can be applied as larvicidal formulations in higher concentrations, in the elimination of mosquito larva, thereby reducing the harmful effects caused by the organic insecticides, reducing resistance in mosquitoes, and consequently curbing the disease spread.

5. Conclusion

In conclusion, both the 70% aqueous ethanol and dichloromethane extracts showed larvicidal activity with the polar 70% aqueous ethanol extract being more efficacious. Phenolics and saponins present in the 70% aqueous ethanol extract could offer a rationale for the observed trend of larvicidal activity with possible synergistic effect. Since *Vernonia amygdalina* is widely distributed in Africa, particularly in Nigeria, commercial exploitation could provide an important step in developing a novel plant-based larvicide.

Compliance with ethical standards

Acknowledgments

The authors are grateful to the technical staff in the Central Research Laboratory for Phytomedicines, Department of Pharmacognosy and Phytotherapy, University of Port Harcourt, Nigeria where the experimental stages were executed.

Disclosure of conflict of interest

With respect to this work, the authors declare a no conflict of interest

References

[1] Rigau-Pérez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV. Dengue and dengue haemorrhagic fever. Lancet. 1998; 352:971-977.

[2] Gluber DJ. Aedes aegypti and Aedes aegypti-borne disease control in the 1990's: top down or bottom up. American Journal of Tropical Medicine and Hygiene. 1989; 40(6):571-578

[3] Shahi M, Hanafi-Bojd AA, Iranshahi M, Vatandoost H, Hanafi-Bojd MY. Larvicidal efficacy of latex and extract of Calotropis procera (Gentianales: Asclepiadaceae) against Culex quinquefasciatus and Anopheles stephensi (Diptera: Culicidae). Journal of Vector Borne Diseases. 2010; 47: 185-8.

[4] Isman MB. Neem and other Botanical insecticides: Barriers to commercialization. Phytoparasitica. 1997; 25: 339-44.

[5] Abosi AO, Raseroka BH. In vivo antimalarial activity of Vernonia amygdalina. Br. Journal of Biomedical Science. 2003; 60:89–91

[6] Farombi, E.O. and Owoeye, O. (2011) Antioxidative and Chemopreventive Properties of Vernonia amygdalina and Garcinia bioflavonoid. International Journal of Environmental Research and Public Health. 2011; 8(6), 2533-2555.

[7] Ademola IO, Eloff JN. Anthelminthic activity of acetone extract and fractions of Vernonia amygdalina against Haemonchus contortus eggs and larvae. Tropical Animal Health and Production.2011; 43(2):521-7

[8] Abay SM, Lucantoni L, Dahiya N, Dori G, Dembo EG, Esposito F, Lupidi G, Ogboi S, Ouédraogo RK, Sinisi A, Taglialatela-Scafati O. Plasmodium transmission blocking activities of Vernonia amygdalina extracts and isolated compounds. Malaria Journal. 2015; 14(1):288.

[9] Yeshanew, S, Gete, W, Chilo, D. Evaluation of the Antimalarial Activity of Ethanol Extracts of the Leaves of Three Plant Species Collected from Yayu Cof-fee Forest Biosphere Reserve, Southwest Ethiopia. Journal of Experimental Pharmacology 2021; 13, 661–668. doi:10.2147/JEP.S304933

[10] Erasto P, Grierson DS, Afolayan AJ. Bioactive sesquiterpene lactones from the leaves of Vernonia amygdalina. Journal of Ethnopharmacology.2006; 106 (1):117-20

[11] Akah PA, Okafor CL, Blood sugar lowering effect of Vernonia amygdalina Del, in an experimental rabbit model. Phytotherapy Research. 6 (3);171–173.

[12] Omolola AA, Olorunfemi RM, Adetutu AD, Adeniyi SA.Ebenezer OF, Modulatory effect of methanolic extract of Vernonia amygdalina (MEVA) on tert-butyl hydroperoxide–induced erythrocyte haemolysis. Cell Biochemistry and Function, 2013; 31 (7);545–550

[13] Shittu, OB, Ajayi, OL, Bankole, SO, Popoola, TO. Intestinal ameliorative effects of traditional Ogi-tutu, Vernonia amygdalina and Psidium guajava in mice infected with Vibrio cholera. African Health Sciences 2016; 16 (2), 620–628

[14] Asante, DB, Henneh, IT, Acheampong, DO, Kyei, F, Adokoh, CK, Ofori, EG, Domey, NK, Adakudugu, E, Tangella, LP, Ameyaw, EO. Anti-inflammatory, anti-nociceptive and antipyretic activity of young and old leaves of Vernonia amygdalina. Biomedicine and Pharmacotherapy 2019; 111, 1187–1203.

[15] Hasibuan, P, Harahap, U, Sitorus, P, Satria, D. The anticancer activities of Vernonia amygdalina Delile. leaves on 4T1 breast cancer cells through phosphoinositide 3-kinase (PI3K) pathway. Heliyon 2020; 6 (7).

[16] WHO. (2005). The World Health Organisation Guidelines for laboratory and field testing of mosquito larvicied. Geneva: WHO, 2005. Pp 1-41

[17] Harborne, J.B. (1973). Phytochemical methods – a guide to modern techniques of plant analysis. 3rd ed. London Chapman and Hall.

[18] Houghton, P.J., Raman, A. (1998). Laboratory Hand book for the fractionation of Natural Extracts. London chapman and Hall.

[19] Bagavan A, A A Rahuman, C Kamaraj, Kannappan Geetha. Larvicidal activity of saponin from Achyranthes aspera against Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae). Parasitology research 2008; 103: 1. 223-229 Jun.

[20] Chapagain BP, Saharan V, Wiesman Z. Larvicidal activity of saponins from Balanites aegyptiaca callus against Aedes aegypti mosquito. Bioresource Technology 2008; 99(5):1165–1168

[21] Rey D., David J.P., Besnard G., Jullien J.L., Lagneau C.& Meyran J.C. Comparative sensitivity of larvalmosquitoes to vegetable polyphenols versus conventional insecticides. Journal of entomology. Exp.Appl. 2001; 98 (3): 361–367.

[22] Gitu, LM (2009). Biological and phytochemical studies of medicinal plants, Antidesma venosum (Euphorbiaceae) and Kotschya africana(Fabaceae) used in traditional medicine in Kenya. PhD thesis. Department of Chemistry. Jomo Kenyatta University of Agriculture and Technology. Nairobi, Kenya.

[23] Nobsathian, S; Bullangpoti, V; Kumrungsee, N; Wongsa, N; Ruttanakum, D. Larvicidal effect of compounds isolated from Maerua siamensis (Capparidaceae) against Aedes aegypti (Diptera: Culicidae) larvae. Chemical and biological techniques in agriculture. 2018; 5(1): 4-10.

[24] Lomonaco, D; Santiago, PMG; Ferreira, SY; Arriaga, CMA; Mazzetto, ES; Mele, G; Vasapollo, G. Study of technical CNSL and its main components as new green larvicides. Green Chemistry 2009; 11(1): 31-33