European Journal of Medicinal Plants

24(4): 1-9, 2018; Article no.EJMP.43030
ISSN: 2231-0894, NLM ID: 101583475

Larvicidal Activity of Crude Extracts of *Vernonia cinerea* Less *(Asteraceae)* against the Larvae of *Anopheles gambiae* in Bobo Dioulasso, Burkina Faso

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Authors' contributions

This work was carried out in collaboration between all authors. Author AS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FDH and EB managed the analyses of the study. Author AS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/43030
Editor(s):
(1) Dr. Paolo Zucca, Department of Biomedical Sciences, University of Cagliari, Italy.
(2) Dr. Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Esraa Ashraf Ahmed ElHawary, Ain Shams University, Egypt.
(2) Khalid Shawky Hamadah, Al-Azhar University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/25786

Original Research Article

Received 15th May 2018
Accepted 23rd July 2018
Published 4th August 2018

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ABSTRACT

Aims: This study aims to evaluate the larvicidal activity of lyophilized methanolic extracts, hydro-methanolic extracts and aqueous extracts of *Vernonia cinerea* Less against the 3rd and 4th instars larvae of *Anopheles gambiae*.

Place and Duration of Study: Pharmacognosy Laboratory, Department of Biomedical Sciences, Centre MURAZ /Research Institute for Health; Ministry of Health, Bobo-Dioulasso, between February 2017 and January 2018. Institut de Recherche en Sciences de la Santé/Direction Régionale de l'Ouest (IRSS) Bobo-Dioulasso, between March 2017 and January 2018.

Methodology: The whole plant material was collected in Banfora, located at West of Burkina Faso. The 80% methanolic, 50/50% hydro-methanolic and aqueous extracts were obtained by using the classical natural product extraction method of the laboratory. Extracts were lyophilized and a series of concentrations of the extracts ranging from 0.001 – 100 mg/L were prepared. The larvicidal activity of lyophilized extracts has been tested against the 3rd and 4th *Anopheles gambiae* larvae. The larval mortality was evaluated after 24 hours and 48 hours of exposure. The percent of means of mortality was calculated. Lethal Concentration LC50 and LC90 values were determined.

Results: In the laboratory, the results of methanolic extract at 100 mg/L achieved (100%) mortality against *Anopheles gambiae* after 24 hours of exposure.The same extract at 10 mg/L generated (95.85±1.26%) of mortality outside the laboratory. The 80% methanolic extract showed Lethal Concentration LC50 of 11.07 mg/L and Lethal Concentration LC90 of 81.38 mg/L (after 24 hours). The 50/50% hydro-methanolic extracts achieved Lethal Concentration LC50 of 22.27 mg/L against 3417.78 mg/L respectively inside and outside of the laboratory.

Conclusion: The methanolic extract is the most effective in killing on the larvae compared to the other extracts tested. *Vernonia cinerea* Less possesses larvicidal activity against *Anopheles gambiae* larvae. It may be a possible source of mosquito vector control. This study provided promising finding for using crude extracts of *Vernonia cinerea* Less in controlling larvae of malaria vectors.

Keywords: Vernonia cinerea Less; Anopheles gambiae; larvicidal activity; malaria; Banfora; Bobo-Dioulasso; Burkina Faso.

ABBREVIATIONS

Me : Methanolic extracts
MH : Hydro-methanolic extracts
AQ : Aqueous extracts
TM : Control
LC : lethal concentration
WHO : World Health Organization.

1. INTRODUCTION

Mosquitoes are vectors responsible for the transmission of diverse diseases such as filariasis, yellow fever, dengue, malaria and other infections [1]. Among these diseases, malaria causes many deaths in children under five years old and pregnant women. In 2016, the World Health Organization recorded 216 million cases and 445,000 associated deaths with 90% in sub-Saharan Africa [2]. Many hold back today are opposed to the efforts of the scientific community to fight malaria, namely: the recrudescence of the resistance of *Plasmodium* to antimalarial drugs, mosquito resistance against insecticides [3,4], precarious hygiene conditions in some of our large residential areas that promote the development of mosquitoes [5]. Faced with this scourge, which constitutes a real problem of public health, the World Health Organization has advocated the implementation of new strategies to fight this disease. In this way, many approaches have been developed to control the proliferation of the mosquito's vector. These approaches include the control of mosquitoes in the larval stage [6]. Current methods of mosquito control are based on the use of synthetic insecticides whose effectiveness and speed of action are without common measure. Unfortunately, in this arsenal of chemical products, some of them are ineffective against mosquitoes and show adverse effects for humans, animals and the environment [3,4,7–9]. In this context, it is very urgent to search for new insecticides as an alternative especially the development of biodegradable insecticides. This approach is all the more plausible as in some parts of the World, plants were traditionally used by communities against mosquito vectors and other insects [10,11]. These natural products used as larvicides have the advantage to be

harmless to organisms living in the environment unlike synthetic insecticides [12]. Among these plants included *Vernonia cinerea* Less (*Asteraceae*) an annual plant widely distributed in India and the Western region of Burkina Faso [13]. It is the subject of many therapeutic uses in different traditional medicines. Extracts of the plant were studied for various activities namely larvicidal activity [14], insect repellent [15], antibacterial activity [16], antipyretic, analgesic and anti-inflammatory activity [17], and its antiplasmodial activity [13,18]. This study aims to evaluate the larvicidal activity of lyophilized 80% methanolic extracts, 50/50% hydro-methanolic extracts and aqueous extracts of *Vernonia cinerea* Less against the 3rd and 4th instars larvae of *Anopheles gambiae*.

2. MATERIALS AND METHODS

2.1 Mosquitoes Larvae

Larvae from laboratory-reared of *Anopheles gambiae* were obtained from an outbred colony established in 2008 and repeatedly replenished with F1 from wild-caught mosquito females collected in Somousso (11°00'46"N, 4°02'45" W), located at 30 km northeast of Bobo-Dioulasso in southwestern Burkina Faso (West Africa), and identified by routine PCR-RFLP. Mosquitoes were held in 30 cm × 30 cm × 30 cm mesh-covered cages at the "Institut de Recherche en Science de la Santé/Direction Régionale de l'Ouest" (IRSS/DRO) insectary under standard conditions (27 ± 2°C, 70 ± 5% relative humidity, 12:12 LD). Females were maintained on rabbit blood by direct feeding, and adult males and females fed with 5% glucose. Larvae were reared at a density of about 300 first instar larvae in 700 ml of water in plastic trays and were fed with Tetramin Baby Fish Food (Tetra Werke, Melle, Germany). Mosquito larvae were collected from plastic trays with a plastic Pasteur pipette were placed in each test cup before to add bioassay solution.

2.2 Collection of Plant Material and Extraction

The collect of plant *V. cinerea* Less and extraction were already carried out according to Sourabie et al. [19]. Plant material was collected in March 2017 in Banfora (10.6400° N, 4.7588° W), located at 85 km West of Bobo-Dioulasso in western Burkina Faso (West Africa). All part of the plant was used for collection. After collect, the plant was washed with water and dried at room temperature. Then the plant was crushed and the raw material obtained was sent to IRSS/DRO. In the laboratory, crude organic extracts were prepared by maceration for 16 h successively with dichloromethane, 80% methanol and 50/50% hydro-methanol solvents. The plant powder (100 g) was used for these organic extraction methods with 1000 ml of each solvent. The dichloromethanolic extract was air dried at room temperature. The 80% methanolic and 50/50% hydro-methanolic extracts were freeze-dried with lyophilisator (Brand) after total evaporation of solvents. Aqueous extracts were prepared by boiling 200 g of plant powder in 2000 ml of purified water for 30 min. After cooling, solutions were filtered on cotton wool and freeze-dried. Plant powder was obtained by applying the classical natural product extraction method by Sanon et al. [20]. The extracts were lyophilized in CNRFP (Centre National de Recherche et de Formation sur le Paludisme). The lyophilizates were used to prepare the stock solution that was used for the larvicidal tests against mosquitoes larvae and stored at 4°C.

2.3 Experimental Doses Preparation

The preparation of experimental doses was made from an initial solution. The stock solution was prepared for each test extract by dissolving 100 mg of the extract in 10 ml of distilled water in a 15 ml plastic Falcon tube. The solution was thoroughly mixed to obtain a homogeneous mixture and serial dilutions of 100 mg/L, 10 mg/L, 1 mg/L, 0.1 mg/L, 0.01 mg/L and 0.001 mg/L were prepared.

2.4 Bioassay under Laboratory Conditions

The larvicidal bioassay was conducted by using the WHO standard protocol with little modification [21]. Each concentration was replicated five times with a control containing distilled water to validate the test. The concentrations used were noted on data recording forms. Thirty (30) larvae of 3rd and 4th instars larvae were collected from plastic trays using a plastic Pasteur pipette and placed in each test cup and control cup. All plastic cups were incubated under controlled conditions at a temperature of 27 ± 2°C, relative humidity of 70 ± 5% and 12-12 light-dark regime. The larvae were treated with 100 ml of bioassay solution for each dose of experiment and control were treated with distilled water added fish food. No nutritional supplements were added during the assays. The mortality of larvae was recorded after 24 hours and 48 hours of exposure. Dead larvae are those that cannot be induced to move

when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface or not showing the characteristic diving reaction when the water is disturbed. For calculating percentage mortality, moribund larvae are counted and added to dead larvae [20].

2.5 Bioassay Outside the Laboratory

The larvicidal bioassay outside the laboratory conditions was carried out using the same concentrations used in laboratory assays. Each concentration was carried out in five replicates with five control distilled water. For the larvicidal assay, 30 live 3rd and 4th instars larvae were placed in each assay replicate and in each control.

2.6 Statistical Analysis

The R software version 3.0.0 was used to calculate the percentage of larval mortality and standard error *(SE).* Mortality rates were calculated by the formula below:

$$\%M = \left(\frac{\text{Number of dead Larvae}}{\text{Number of total Larvae}}\right) x\ 100$$

Mortality was recorded after 24 and 48 hours and was corrected using Abbott's [22] formula when the control mortality ranged between 5-20 per cent.

$$Mc = \left(\frac{\%\ \text{Mortality in Treated} - \%\ \text{Mortality in control}}{100 - \%\ \text{Mortality in control}}\right) x\ 100$$

Lethal concentrations which caused 50 and 90% larval mortality (LC50, LC90) and R Squared (R^2) were determined by Excel software 2013 version and by the experimental curve of mean corrected mortalities according to the concentrations of the test extracts. The interpretation of the mortality rate of *Anopheles* larvae was based on WHOPES, [23] susceptibility tests is:

Mortality rate between 98 – 100% indicates susceptibility.

Mortality rate between 90 – 97% suggest possible resistance.

Mortality rate < 90% indicates resistance.

3. RESULTS

3.1 Bioassay under Laboratory Conditions

In the laboratory conditions, after 24 hours of exposure with 100 mg/L dose, 80% methanolic extracts achieved 100% mortality on *Anopheles gambiae.* The 50/50% hydro-methanolic extracts and aqueous extracts achieved 92.62±2.57% and 14.22±5.44% corrected mortality on *Anopheles gambiae* respectively. After 48 hours of exposure, 50/50% hydro-methanolic extract killed 96.25±1.05% and the aqueous extract achieved 27.19±5.51% corrected mortality respectively against *Anopheles gambiae* of the 3rd and 4th instars larvae (Table 1). Furthermore, LC50 and LC90 values of 80% methanolic extracts were 11.07 and 81.38 mg/L respectively (after 24 hours). The 50/50% hydro-methanolic extracts values were 22.27 and 95.62 mg/L (after 24 hours); 5.67 and 88.21 mg/L (after 48 hours) respectively. The aqueous extracts values were showed to be very considerable after 24 hours and 48 hours of exposure (Table 2).

3.2 Bioassay outside the Laboratory

The outside conditions, after 24 hours of exposure with 100 mg/L dose, the 80% methanolic extracts achieved 68.22±15.89% corrected mortality against *Anopheles gambiae*. The 50/50% hydro-methanolic extracts and aqueous extracts killed (8.86±5.72%) and (82.57±9.07%) of mosquitoes larvae respectively. After 48h of exposure, the 80% methanolic extracts showed (83.16±9.11%), the 50/50% hydro-methanolic extract was found (10.75±5.36%) and the aqueous extract showed (83.16±9.26%) corrected mortality of *Anopheles gambiae* of the 3rd and 4th instars larvae (Table 3). Lethal concentrations LC50 and LC90 percent values of 80% methanolic extracts were 3.25 and 160.29 mg/L (after 24 hours) and aqueous extracts 50.07 and 110.69 mg/L respectively (Table 4).

4. DISCUSSION

In the laboratory conditions, the mortality rates of *Anopheles gambiae* larvae recorded at the 24th hour at the various concentrations of 50/50% hydro-methanolic and aqueous extracts tested exhibited a resistance (mortality rate < 90%) to the extract. However, the mortality rate of 50/50% hydro-methanolic extract for concentration to 100 mg/L exhibited a possible resistance (mortality rate =92.62%) (Table 1). However, the mortality rate of 80% methanolic extract for concentration to 100 mg/L exhibited the higher susceptibility of larvae (mortality rate =100%). At 48 hours, the mortality rate of *Anopheles gambiae* larvae for concentrations of 0.001 to 100 mg/L showed that the larvae were

resistant (mortality rate < 90%) to extracts. In the outside conditions, mortality rates recorded at the 24 hours and 48 hours for all concentrations of extracts tested showed that *Anopheles gambiae* larvae are resistant (mortality rate < 90%). Excepted 80% methanolic extract recorded a possible resistance with mortality rate =95.42%. The results of the study suggest that the 80% methanolic extract of *Vernonia cinerea* Less exhibited larvicidal properties against *Anopheles gambiae* larvae. Malaria vector *Anopheles gambiae* larvae exhibited higher susceptibility to this extract as compared to other tested plant extracts.

Table 1. Survival of 3rd and 4th instars larvae *Anopheles gambiae* after 24 hours and 48 hours exposure to the extracts of *Vernonia cinerea* Less in laboratory conditions

Time of exposure (hours)	Treatment	Concentration (mg/L)	Mortality ± SE (%)	Corrected Mortality ± SE (%)	Replicates	Survival (%)
24	AQ	100	22.14±5.44	14.22 ± 5.44	5	77.85
		10	21.53±2.57	13.55± 2.57	5	78.46
		1	17.00±3.78	8.56±3.78	5	83.00
		0.1	15.00±4.10	6.35±4.10	5	84.99
		0.01	9.40±4.43	0.18±4.43	5	90.59
		0.001	13.18±1.90	4.35±1.90	5	86.81
	Me	100	100.00±0.00	100.00±0.00	5	0.00
		10	60.34 ± 11.62	56.30±11.62	5	39.65
		1	38.46 ± 9.91	32.20±9.91	5	61.53
		0.1	68.00 ± 9.69	64.74±9.69	5	32.00
		0.01	46.66 ± 7.30	41.23±7.30	5	53.33
		0.001	37.33 ± 4.13	30.95±4.13	5	62.66
	MH	100	93.31 ± 2.57	92.62±2.57	5	6.68
		10	45.75 ± 7.36	40.23±7.36	5	54.24
		1	50.62 ± 7.97	45.59±7.97	5	49.37
		0.1	44.18 ± 5.27	38.50±5.27	5	55.81
		0.01	34.85 ± 5.58	28.22±5.58	5	65.14
		0.001	47.86 ± 4.98	42.55±4.98	5	52.13
	TM	Control	9.23 ± 3.26	00.00±0.00	5	90.76
48	AQ	100	34.69 ± 5.51	27.19±5.51	5	65.30
		10	35.55 ± 1.79	28.15±1.79	5	64.44
		1	29.94 ± 4.50	21.89±4.50	5	70.05
		0.1	20.34 ± 5.63	11.20±5.63	5	79.65
		0.01	24.97 ± 4.99	16.36±4.99	5	75.02
		0.001	25.40 ± 2.55	16.84±2.55	5	74.59
	Me	100	100.00 ± 0.00	100.00±0.00	5	0.00
		10	74.45 ± 7.60	71.51±7.60	5	25.54
		1	69.41 ± 6.88	65.90±6.88	5	30.58
		0.1	88.00 ± 4.78	86.62±4.78	5	12.00
		0.01	66.66 ± 7.37	62.83±7.37	5	33.33
		0.001	79.33 ± 2.86	76.95±2.86	5	20.66
	MH	100	96.64 ± 1.05	96.25±1.05	5	3.35
		10	51.22 ± 7.52	45.62±7.52	5	48.77
		1	62.71 ± 4.90	58.43±4.90	5	37.28
		0.1	57.37 ± 5.19	52.48±5.19	5	42.62
		0.01	44.25 ± 3.70	37.85±3.70	5	55.74
		0.001	52.21 ± 6.26	46.72±6.26	5	47.78
	TM	Control	10.29 ± 3.11	00.00±0.00	5	89.70

Me: Methanolic extracts, MH: Hydro-methanolic extracts, AQ: Aqueous extracts, TM: Control

Table 2. The values of LC50 and LC90 of *Anopheles gambiae* larvae treatment after 24 hours and 48 hours exposure in the laboratory conditions

Time of exposure (h)	Treatment	Equation	R^2	LC50 (mg/L)	LC90 (mg/L)
24	Me	y = 0.5689x + 43.702	0.7643	11.07	81.38
	MH	y = 0.5453x + 37.853	0.9313	22.27	95.62
	AQ	y = 1.1375ln(x) + 9.1779	0.8172	3.85 E+15	7.2 E+30
48	Me	-	-	-	-
	MH	y = 0.4846x + 47.25	0.8741	5.67	88.21
	AQ	y = 1.2137ln(x) + 21.669	0.6158	1.37 E+10	2.82 E+24

Me: Methanolic extracts, MH: Hydro-methanolic extracts, AQ: Aqueous extracts, LC: Lethal concentration

Table 3. Survival of 3rd and 4th instars larvae *Anopheles gambiae* after 24 hours and 48 hours exposure to the extracts of *Vernonia cinerea* Less outside the laboratory conditions

Time of exposure (hours)	Treatment	Concentration (mg/L)	Mortality ± SE (%)	Corrected Mortality ± SE (%)	Replicates	Survival (%)
24	AQ	100	84.18±9.07	82.57±9.07	5	15.81
		10	33.47±13.10	26.70±13.10	5	66.52
		1	30.19±14.74	23.09±14.74	5	69.80
		0.1	-	-	-	-
		0.01	30.31±14.28	23.22±14.28	5	69.68
		0.001	11.47±2.73	2.47±2.73	5	88.52
	Me	100	71.16±15.89	68.22±15.89	5	28.83
		10	95.85±1.26	95.42±1.26	5	4.14
		1	28.28±7.80	20.98±7.80	5	71.71
		0.1	-	-	-	-
		0.01	-	-	-	-
		0.001	-	-	-	-
	MH	100	17.28±5.72	8.86±5.72	5	82.71
		10	20.22±7.95	12.10±7.95	5	79.77
		1	10.57±5.03	1.47±5.03	5	89.42
		0.1	14.96±5.42	6.31±5.42	5	85.03
		0.01	29.23±5.98	22.03±5.98	5	70.76
		0.001	-	-	-	-
	TM	Control	9.23±3.37	0.00±0.00	5	90.76
48	AQ	100	84.84±9.26	83.16±9.26	5	15.15
		10	33.47±13.10	26.10±13.10	5	66.52
		1	30.19±14.74	22.45±14.74	5	69.80
		0.1	-	-	-	-
		0.01	33.57±14.14	26.21±14.14	5	66.42
		0.001	15.19±2.14	5.79±2.14	5	84.80
	Me	100	84.84±9.11	83.16±9.11	5	15.15
		10	95.85±1.26	95.39±1.26	5	4.148
		1	29.66±8.18	21.87±8.18	5	70.33
		0.1	-	-	-	-
		0.01	-	-	-	-
		0.001	-	-	-	-
	MH	100	19.65±5.36	10.75±5.36	5	80.34
		10	20.22±7.95	11.38±7.95	5	79.77
		1	12.30±6.75	2.58±6.75	5	87.69
		0.1	25.8±3.95	17.63±3.95	5	74.14
		0.01	30.69±5.67	23.01±5.67	5	69.30
		0.001	-	-	-	-
	TM	Control	9.97±3.14	0.00±0.00	5	90.022

Me: Methanolic extracts, MH: Hydro-methanolic extracts, AQ: Aqueous extracts, TM: Control

Table 4. The values of LC50 and LC90 of *Anopheles gambiae* larvae treatment after 24 hours and 48 hours exposure in outside laboratory conditions

Time of exposure (h)	Treatment	Equation	R^2	LC50 (mg/L)	LC90 (mg/L)
24	Me	y = 10.258ln(x) + 37.92	0.3932	3.25	160.29
	MH	-	-	-	-
	AQ	y = 0.6598x + 16.961	0.92	50.07	110.69
48	Me	y = 13.309ln(x) + 36.162	0.6052	2.85	57.12
	MH	-	-	-	-
	AQ	y = 0.6502x + 18.307	0.9327	48.74	110.26

Me: Methanolic extracts, MH: Hydro-methanolic extracts, AQ: Aqueous extracts, LC: Lethal concentration

Several studies in the literature were described the larvicidal activity of *Vernonia cinerea* Less extracts against various species of mosquitoes [14,24–26]. Studies of Varum *et al*. (2013) have reported the potential larvicidal activity of the plant against *Anopheles stephensi* mosquito species. Even Arivoli *et al.* (2011) were shown the larvicidal proprieties of methanolic extracts of *Vernonia cinerea* Less against *Culex quinquefasciatus* larvae. In this study, results observed with a lethal concentration (LC_{50} =11.07 mg/L) are similar with studies of Tchoumbougnang et al. (2009) in Cameroun on *Cymbopogon citrus* extracts (LC_{50} =18±0.7 mg/L) against *Anopheles gambiae* larvae. In previous studies, Soma et al. (2017) reported that *Vernonia cinerea* Less plant extracts possess biocidal active compounds including triterpenes and sterols, alkaloids, tannins, anthracenosids and coumarins. The larvicidal activity exhibited by plant extract in this study may be caused by conjugated or independent action of these chemical compounds against *Anopheles gambiae* 3rd and 4th instars larvae. These results highlight the importance of the evaluation of natural substances as effective natural larvicides to control *Anopheles* larvae. Particularly in areas where vectors have developed resistance or have reduced susceptibility to conventional synthetic insecticides.

5. CONCLUSION

The results of this study have indicated that *Vernonia cinerea* Less possesses larvicidal activity and supports its traditional application as an insecticide. Furthermore, there is a difference in effectiveness depending on the extracts tested and the environment of the experiment. The 80% methanolic plant extract is the most effective on the larvae compared to the other extracts tested. It may be a possible source of mosquito vector control.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

FUNDING STATEMENT

The West African Centre for Cell Biology of Infectious Pathogens (WACCBIP) is the organism that financed the payment of reagents for laboratory tests.

ACKNOWLEDGEMENTS

We would like to thank West African Centre for Cell Biology of Infectious Pathogens (WACCBIP) in University of Ghana for their financial and reagents support. We also thank the Institut de Recherche en Science de la Santé (IRSS/DRO) for the lab work and Centre National de Recherche et de Formation sur le Paludisme (CNRFP) for the lyophilization of extracts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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