Larvicidal Property of the Secondary Metabolites of *Hannoa klaineana* against the Larvae of *Aedes aegypti*

Abstract

The vector mosquito is the main vector for malaria which is endemic in Nigeria and Africa at large. Use of synthetic materials for the control of this vector has always proven abortive. The introduction of plant metabolites which is biodegradable maybe successful and safer to the human health in the control programme of this vector since plants are very rich in bioactive secondary metabolites. Hence, we evaluated the larvicidal activity of the crude methanol extract, n-hexane, chloroform, ethyl acetate and methanol fractions of *Hannoa klaineana* against the IV instar larvae of *Aedes aegypti* mosquito using standard protocols. The most active fractions were n-hexane, chloroform and ethyl acetate fractions with LC50 values of 1.006, 1.479, and 3.198 mg/ml respectively; n-hexane fraction having the highest property with LC50 value of 1.006 mg/ml and followed by chloroform fraction with LC50 value of 1.479 mg/ml. The evaluation has shown that the secondary metabolites of *H. klaineana* can be used as an alternative for the synthetic larvicides which its use has proven abortive in the vector control programmes.

Keywords: *Hannoa klaineana*, *Aedes aegypti*, mosquito, larvicidal, fractions.

1. Introduction

Aedes aegypti mosquito is a causative vector for dengue fever and this is endemic in the Americas and Africa [1, 2]. The lack or poor drainage systems in this regions especially during rainy seasons give the *Ae. aegypti* mosquito an edge, hence a good breeding place for them [3]. It

is observed that the *Ae. aegypti* mosquito breeds and develops in containers which includes: overhead tanks, rain barrels, flasks, earthen pots, tyres, water closed, vases, bottles, cisterns, tin cans, jars, etc. and some natural sites like snail shells, leaf axils, coconut shells and treeholes [2, 4]. Plants have served as sources of medicines, biocides, foods, waxes, oils and for other human needs. Its isolation of the bioactive compounds in pure forms was only feasible in late 19th century; where the chemical structures were identified [5]. The well-known vegetable family which has close to 500,000 varieties of plant; yet only a few has been structurally identified. This current development came into existence because of the crucial need of knowing compounds or substances which has potential use for therapeutic and insecticidal programs [6].

The use of synthetic insecticides has not been successful due to some factors such as ecological, economic, human, technical, etc. And its use has also been limited recently in mosquito control programmes. This is also due to the lack of novel insecticides, its high cost, its effect on the environment, its harmful effects on humans and other non-target communities, its non-biodegradable nature, the high insecticidal resistance [7, 8].

It is imperative to seek for alternative means to replace the synthetic insecticides which are safe environmentally, biodegradable, low cost, and target specific insecticides [9]. The plant *Hannoa klaineana* belongs to the family of Simaroubaceae [10] and it leaflets grows up to 8-40 cm long. Its leaves can be wavy, glabrous, or leathery with pitted glands. It flowers in August – November and fruits in September – February. The flowers may be bisexual or unisexual [11]. The plant is believed to be active in the treatment of malaria, fever, cough, colic diseases [10] and it has anti-tumor [12], antiviral, antifeedant, anti-amoebic [13], anti-inflammatory [14], antibacterial, antifungal, insecticidal and arachnicidal activities or properties [15] but no report on the mosquito larvicidal activity. In this study, the stem barks of *H. klaineana* were extracted and

fractionated in various solvents and evaluated against 25 IV instar larvae of *Ae. aegypti* to establish the most active extract.

2. Materials and methods

2.1.Collection of plant material

The stem bark *Hannoa klaineana* were collected from its natural habitat in Orba, Nsukka, Enugu State of Nigeria in August, 2011 and identified by Mr A.O. Ozioko, a taxonomist. Impurities were removed, the stem bark was room dried for two weeks and powdered. This was stored until required for extraction.

2.2.Plant extraction and fractionation

The extraction procedure was performed based on Ajaegbu *et al* [16] method with slight modification. The plant material (3 kg) was extracted for 3 days by maceration in methanol shaking intermittently in the laboratory of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Agulu, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The maceration process was then repeated twice for maximal extraction. The suspension was filtered through Whatman No. 1 filter paper (size: 24 cm) using a Buchner funnel. The methanol crude extract (MCE) was concentrated to dryness in rotary vacuum evaporator RE300 (ROTAFLO, England). The MCE was adsorbed in silica gel (70–230 mesh size) and sequentially fractionated using hexane (HF), chloroform (CF), ethyl acetate (EF) and methanol (MF) following the solvent polarities. The same rotary evaporator was used to concentrate the fractions at 40 ± 5°C. The yields were 8.13, 5.70, 5.49 and 47.05% for HF, CF, EF and MF, respectively. The crude methanol extract and fractions were stored in the refrigerator at –4°C before use.

2.3.Test organisms

The *Ae. aegypti* larvae were reared according to the method of Ajaegbu *et al* [17] in the laboratory, Faculty of Pharmaceutical Sciences, Agulu, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The larvae of *Ae. aegypti* were collected from WHO/National Arbovirus and Vector Research Centre, Enugu, Enugu state, Nigeria and were colonised. The larvae of *Ae. aegypti* were fed with chicken feed (grower) mixed with fish feed in 3:1 ratio. On every alternate day, the water from the culture bowl was changed carefully until IV instar larvae used for bioassay. Adults were provided with 10% sucrose solution and a Guinea pig for blood meal. This study was given an ethical approval from Anambra State University Teaching Hospital, Amaku, Awka; Anambra State, Nigeria Ethics Review Committee with the reference No. ANSUTH/AA/ECC/36. Mosquitoes were maintained at 26 ± 3°C, 80 ± 4% RH and under 12 : 12 h (light : dark) photoperiod cycles.

2.4.Mosquito larvicidal bioassay

Larval bioassay was conducted according to the method of Ajaegbu *et al* [17] to determine the toxicity of the plant extract and fractions against *Ae. aegypti* IV instar larvae. Stock solutions were made using Tween 80 as emulsifier to facilitate the dissolving of materials in water. Exactly 1 g of each extract and fraction were dissolved in 2 ml Tween 80 as stock solutions which were further diluted up to 100 ml by adding tap water. Test concentrations ranging from 125–1000 ppm were prepared by serial dilution of each stock solution; and 1 ml of Tween 80 in 99 ml of tap water was set up as negative control for each replicate, extract and mosquito species. All the concentrations were chosen after a preliminary test for each product and mosquito species. Daksh insecticide (Diclorvos 100% EC w/v), bought from the local market at Awka market, Anambra State, Nigeria at 2500 ppm (recommended concentration) was used as positive control. In brief, 25 IV instar larvae were released into each 250 ml beaker containing

100 ml of the aliquot and mortality was recorded 24 h post-exposure. No food was provided to larvae either to the tests or controls during the experiments. The dead larvae were expressed as percentage mortality at each concentration. In cases where bioassay tests showed >20% negative control mortality, these were discarded and repeated. However, when negative control mortality ranged from 5–20%, the observed percentage mortality was corrected by Abbott's formula[18]. The larvae were considered as dead, if these were not responsive to a gentle prodding with a fine needle. All bioassays were carried out at room (temperature of 26 ± 2°C and relative humidity of 81 ± 2%). Experiments were set in four replicates along with controls.

2.5.Statistical analysis

The corrected mortality was determined using Abbott's formula [18] whenever required. The percentage of mortality data was subjected to ANOVA procedure using Statistical Package for Social Science (SPSS 17.0). The Student-Newman-Keuls (SNK) test at $p = 0.05$ was used for mean separation. Probit [19] analysis was applied to determine lethal dosages causing 50% (LC50) and 90% (LC90) mortality of larvae 24 h post-exposure, and other statistics included 95% upper confidence limit (UCL) and lower confidence limit (LCL).

3. Result and discussion

Severally, synthetic products has been used to control these mosquitoes and yet there is an increasing resistance to these synthetic materials due to its repeated [2, 20]. Presently, insecticides of natural product origin are been used against mosquito species, and it has proven to be effective and these are safer to use since it has no phototoxic effect and does not lead to a scummy environment [21]. Also the parts of plants has been established to contain secondary metabolites with certain biological property that acts as deterrents and attractants [22]. The effectiveness of some plant secondary metabolites against *Ae. aegypti* larvae may be attributed to

some varying factors such as: plant parts used, solvent used for extraction, availability of vector species, plant species, etc. [17, 23]. Larvicidal activity of the methanol crude extract and different fractions of the stem bark of *H. klaineana* used were shown in Tables 1 and 2. Five different solvent fractions were tested against *Ae. aegpyti* and 100 percent larval mortality was observed in n-hexane and chloroform factions at 5 mg/ml (Table 1). All plant fractions showed moderate toxic effect on *Ae. aegpyti* after 24 h of exposure. However at 5 and 2.5 mg/ml, the highest mortality was found in chloroform fraction, followed by n-hexane fraction, ethyl acetate fraction and the methanol crude extract.

In this study, we evaluated the toxic effect of different solvent fractions of *H. klaineana*. There were variations in the toxic effects according to their differences in solvents used for extraction. All the extract/fractions were effective to an extent, with hexane, chloroform and ethyl acetate fractions having high toxic effect against the *Ae. aegypti* mosquito larvae. This has also been observed in researches of some scholars. For instance, the larvicidal property of different extracts of *Eclipta alba* (L.) Hassk (Asteraceae) against *Ae. aegypti* showed their LC50 values as follows: 146.28, 165.10, , 127.64, 154.88 and 151.38 ppm for chloroform, ethyl acetate, hexane, methanol and benzene extracts, respectively [21]. Also, crude methanol extract, n-hexane, dichloromethane, ethyl acetate, acetone and methanol fractions of *Spondias mombin* tested against the larvae of *Ae. aegypti*, n-hexane fraction was the most active fraction with a LC50 value of 22.54 ppm, followed by dichloromethane with LC50 value of 42.13 ppm [17]. The chloroform, methanol, ethanol, hexane, aqueous and ethyl acetate extracts of *Hippophae rhamnoides* L. evaluated against the larvae of *Ae. aegypti* showed the ethanol extract to be the most effective extract with LC50 value of 1424.45 ppm after 24 h post-exposure[24].

Conclusion

H. klaineana extract/fractions can serve as an alternative to the synthetic larvicides which are sold in the market and also not biodegradable and safe to the ecosystem. I recommend further studies on the isolation, purification and structural elucidation of the secondary metabolites present in the extract and fractions and further bioassay on those pure secondary metabolites for maximal utilization.

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Table 1: Percentage mortality of *Ae. aegypti* larva exposed to different extract/fractions of *H. klaineana* stem bark after 24 hours

Concentration (mg/ml)	% Mortality (mean±SD)				
	N-hexane	Chloroform	Ethyl acetate	Methanol	Methanol crude extract
5	100±0.00c	100±0.00c	90±3.33d	23.33±3.33c	84.44±5.09c
2.5	86±3.33b	100±0.00c	43.33±3.33c	11.89±1.34b	42.22±10.71b
1.25	50±8.81a	43.33±3.33b	5±1.67b	0±0.00a	22.22±5.09a
0.625	48.89±8.39a	5±1.67a	0±0.00a	0±0.00a	13.43±1.50a
Control	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
F-value	**50.611*** **	**1.865*** **	**839.99*** **	**116.232*** **	**71.034*** **

Mean ± SD in the same column for the same category of fraction, followed by the same letter do not differ significantly at p=0.05. Each data represents the mean of four replicates of 25 larvae each. ***P<0.001. F value*** means statistically there is great difference

Table 2: Lethal concentration (mg/ml) values of different extract/fractions of *H. klaineana* stem bark

Extract/fractions	LC50 (Confidence limit 95%)	LC90 (Confidence limit 95%)
N-hexane	1.006 (0.016-1.776)	3.290 (2.442-4.842)
Chloroform	1.479 (0.590-2.303)	3.763 (2.842-5.543)
Ethyl acetate	3.198 (2.281-4.212)	5.483 (4.423-7.561)
Methanol	5.964 (3.963-7.712)	8.248 (6.562-10.605)
Methanol crude extract	2.841 (1.767-3.787)	5.125 (4.132-6.913)

LC50 – lethal concentration that kills 50 percent of the exposed larvae. LC90 – lethal concentration that kills 90 percent of the exposed larvae.