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Research Article

Larvicidal Activity of Sweet Wormwood (*Artemisia annua* L.) Extracts on *Aedes aegypti* (Tiger Mosquito)

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ABSTRACT

The control of mosquito using synthetic insecticides has some negative impacts such as environmental pollution and problem of resistance. Thus this work was aimed at evaluating the larvicidal activity of sweet wormwood (*Artemisia annua* L.) against *Aedes aegypti* mosquito. Hydrothermal extraction method was used to obtain leave extracts using 200 ml of ethanol, methanol and distilled water, respectively. Larvicidal bioassay was carried out using twenty five fourth instar larvae at 0.1 ml, 0.2 ml and 0.3 ml concentrations for each solvent extract. Set of control experiments using only the solvents were also set up. Mortalities were recorded from the first 15 minutes to 24 hours of exposure. The ethanol and methanol extracts of *A. annua* show a significant difference ($P<0.05$) in the mortality rates of *Ae. aegypti* larvae. However, there was no significant difference ($P>0.05$) in the mortality rates of *Ae. aegypti* larvae in relation to water extract of *A. annua*. The larvicidal activity of ethanol, methanol and water extracts of *A. annua* gave LC50 values of 0.131 ml, 0.602 ml and 0.25 ml, respectively. The result of this study reveals that ethanol extract of *A. annua* is considered the best in terms of LC50 compared to methanol and water in causing mortality of the larvae. Thus, it has great potential as a bio pesticides against *Ae. aegypti*.

Keywords: Larvicidal, *Artemisia annua, Aedes aegypti*, extract, Larvae

1. Background

Artemisia annua (Family - Asteraceae) has been used in traditional medicine for treating fever and malaria. There are several species of *Artemisia* known as aromatic fragrance plants that have a characteristic scent and taste[1]. The herb of *Artemisia* has been used medicinally to treat fevers for more than 2,000 years and to treat malaria for more than 1,000 years in China. *Artemisia* used in Chinese traditional medicine for centuries, is today considered part of the solution where malaria has become resistant to other medicines. Artemisinin-based combination therapies (ACTs) have been recommended by World Health Organization (WHO) since 2001 in all countries where *falciparum* malaria - the most resistant form of the disease - is endemic[2]. Zhang *et al.*[3] also proved that *Artemisia* showed the strongest biological activity in July, and found that the acaricidal activity varied significantly with the development of the individual plant. Mosquitoes have long been known for

their importance as vectors of diseases[4]. Despite their small size, mosquitoes are of economic and medical importance. The genera *Aedes*, *Anopheles*, and *Culex* are important vectors of mosquito-borne diseases worldwide. Mosquito-borne diseases cause economic loss and are commonly found in tropical rather than temperate region[5]. *Aedes aegypti*, the primary carrier of the dengue virus, which is predominant in tropical regions, also transmits yellow fever in Africa and South America[6]. Cases of dengue fever and dengue hemorrhagic fever have increased every year and resulted in high number of deaths in Malaysia[7]. World Health Organization also reported that mosquitoes are one of the deadliest insects in the world. Their ability to carry and spread disease to humans causes millions of death every year. In 2015, malaria alone caused 438, 000 deaths. The worldwide incidence of dengue has risen 30-fold in the past 30 years, and more countries are reporting first outbreak of the disease. Zika, dengue, Chikungunya, yellow fever are all transmitted to humans by the *Aedes aegypti* mosquito, according to the World Health Organization[8]. Thus, this study was to evaluate the larvicidal activity of the leaves of sweet wormwood (*Artemisia annua* L.) on *Aedes aegypti* mosquito.

2. Materials and Methods

2.1. Study area

The larvicidal activities of sweet wormwood (*Artemisia annua* L.) on *Aedes aegypti* was carried out in the Insectary of the Department of Science Laboratory Technology, University of Jos, Nigeria (Latitude 09°57' 01''N and Longitude 08°53' 21'' E).

2.2. Collection of Plant Materials

The leaves of *A. annua* were collected from the Botanical Garden of the Department of Plant Science and Biotechnology, University of Jos, Nigeria.

2.3. Preparation of Plant Extracts

The collected leaves were dried at room temperature (27-37°C) for 10 -15 days. The dried leaves were crushed using electric blender. Twenty four grams (24 g) of the powdered leaves were orderly poured into three different dried and clean reagent bottles containing 200 ml of ethanol, distilled water and methanol, respectively. The samples were placed on a mechanical shaker (1000 rmm/min) for 3 hrs, after which the samples were left over night to settle and filtered the next day using funnel and filter paper to obtain the extracts. The extracts were then used to test for larvicidal activities on *Aedes aegypti* larvae.

2.4. Collection of Mosquito Larvae

Larvae of *Ae. aegypti* were collected from abandoned flower pots in Bauchi road campus of the University of Jos. The larvae were collected using standard dipping technique. The dipper was lowered at an angle of 45^0 to minimize disruption and the top of the water was skimmed so as to cause the nearby larvae to flow into the dipper. Care was taken not to spill water when raising the dipper from the water. However, if the flower pots have emerging vegetation, the water was disturbed so as to cause the larvae to swim downwards. Some of the vegetation were then removed using the dipper to create a clear spot for sampling. Larvae collected were transferred into a gallon for transportation to the insectary.

2.5. Larvicidal Bioassay

Concentration of 0.1 ml, 0.2 ml and 0.3 ml of extracts were tested. Two replications were done to ensure the validity of result. The larval mortality bioassay was carried out according to the test method for larval susceptibility proposed by the World Health Organization[9]. Twenty five fourth instar larvae of *Ae. aegypti* were placed in plastic bowls containing 40 ml of aqueous suspension of tested material at various concentrations. A set of control experiments using only the solvents were also set up. Mortality was recorded from first fifteen minutes to 24 hours of exposure and the larvae were not starved of food over this period.

Dose responses of larvicidal bioassay

The numbers of death larvae were counted from the first 15 minutes to 24 hours of exposure. The LC_{50} was calculated using probit analysis to find out the acute toxicity of the extracts.

Statistical Analyses

Data was analyzed using R Console software (Version 3.2.2). The observed mortality and proportions of observed mortality of *Aedes aegypti* larvae in relation to different concentrations of solvents used to extract the leaves of *Artemisia annua* were compared using Pearson's Chi-square test and the difference between the three solvents were compared using one way ANOVA. Result from the acute toxicity test using different concentrations of *Artemisia annua* extracts were subjected to probit analysis to determine the LC_{50} values for the different solvents used in the extraction of the plant. The p-values <0.05 were considered statistically significant.

2.6. Corrected mortality: mortality was corrected using Abbott's formula.

$$E' = [(E-C)/ (100-C)] \times 100$$

Where E is the (uncorrected) exposure mortality expressed in percentage and C is the control mortality expressed in percentage.

3. Results

Determination of the acute toxicity of extract of *A. annua* leaves on larvae of Aedes. *aegypti*

Ethanol: The acute toxicity of different concentrations of ethanol extracts of *A. annua* against larvae of *Ae. aegypti* show a significant difference (χ^2 = 6, df = 2, P = 0.04979) in the mortality rate of *Ae. aegypti* larvae at 0.1 ml, 0.2 ml and 0.3 ml respectively (Figure 1). The result revealed that there was 83.33% mortality at 0.1 ml, 94% mortality at 0.2 ml after 105 minutes and 96.66% mortality at 0.3 ml after 45 minutes (**Figure 1**).

Methanol: There was a significant difference (χ^2 = 4, df = 2, P = 0.03451) in the mortality rate of larvae of *Ae.aegypti* in relation to concentrations of methanolic extracts of *A.annua* (Figure 2). The breakdown of the results showed 70% mortality at 0.1 ml after 24 hours, 75% mortality at 0.2 ml after 24 hours and 95% mortality at 0.3 ml after 24 hours, respectively (**Figure 2**).

Water: There was no significant difference (χ^2 =4, df= 2, P = 0.1353) in the mortality rate of larvae of *Ae. aegypti* in relation to concentrations of water extracts of *A.annua* (Figure 3).The results depicted 76% mortality at 0.1 ml, 73.33% mortality at 0.2

ml and 86.66% mortality at 0.3 ml after 24 hours, respectively (**Figure 3**).

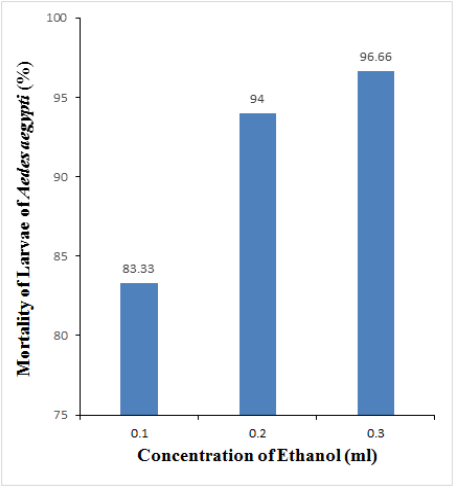

Figure 1: Mortality rate of Larvae of *Ae. aegypti* in relation to concentrations of ethanolic extract.

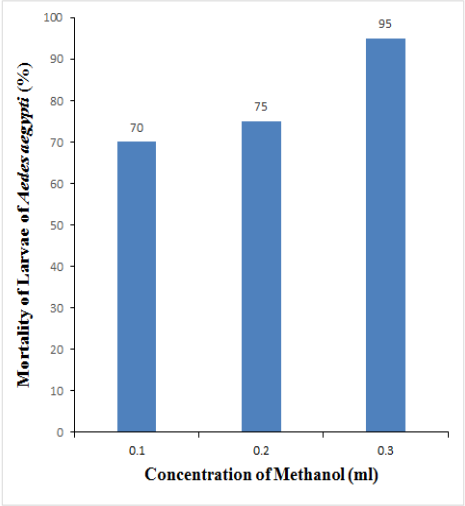

Figure 2: Mortality rate of Larvae of *Ae. aegypti* in relation to concentrations of methanolic extract.

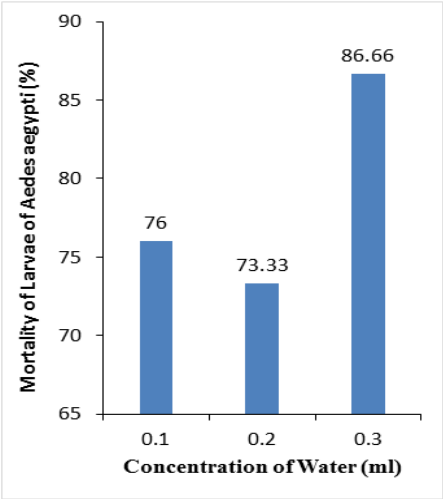

Figure 3: Mortality rate of Larvae of *Ae. aegypti* in relation to concentrations of Water extract.

Determination of the Lethal Concentration (Lc_{50}) of the plant extract on larvae of *Ae. aegypti*

The larvicidal activity of Ethanol extracts of *A. annua* gave an LC_{50} value of 0.131 ml (**Table 1**) indicating that ethanol extracts of *A. annua* exhibit larvicidal activity against *Ae. aegypti* larvae with a 24 hours LC_{50} value of 0.131 ml. However, the larvicidal activity of water extracts of *A. annua* recorded an LC_{50} value of 0.251 ml (Table1) demonstrating that water extracts of *A. annua* exhibit larvicidal activity against *Ae. aegypti* larvae with a 24 hours LC_{50} value of 0.25 ml. The larvicidal activity of methanol extracts of *A. annua* recorded an LC_{50} value of 0.602 ml (Table 1) exhibiting larvicidal activity against *Ae. aegypti* larvae with a 24 hours LC_{50} value of 0.602 ml.

Table 1: Larvicidal activity of extracts of *A. annua* against *Ae. Aegypti.*

Solvent	LC_{50} (ml)	Slope ± SD Chi-square	χ^2	Df	P value
Ethanol	0.131	0.65 ± 0.620	6	2	0.0497
Methanol	0.602	0.51 ± 0.351	4	2	0.0345
Water	0.251	0.42 ± 0.247	4	2	0.1353

Between solvents

$$\chi 2 = 6.592, df = 4, P = 0.159$$

4. Discussion

4.1. Determination of acute toxicity of ethanol, methanol and water extracts of *Artemisia annua* leaves against larvae of *Aedes aeqypti*

In this study, the significant difference in the acute toxicity of different concentrations of ethanol and methanol extracts of *A. annua* against larvae of *Ae. aegypti* (Figures 1 and 2) could be attributed to the analytical grade of the solvent used which is in agreement with the report of Ngwamah *et al.*[10] who worked on Comparative insecticidal activity of five Nigerian plant species against mosquito vectors in Yola, Adamawa state, Nigeria and reported a significant difference among methanol and petroleum ether extracts. The high mortality rate observed at 0.3 ml for ethanol and methanol after 24 hours of exposure is due to high concentration of the extract and this is consistent with the findings of Naimah[11] who worked on the larvicidal effect of *Artemisia annua* (Asterales: asteraceae) against the dengue fever mosquito vector *Aedes aegypti* (Diptera: Culicidae) and reported a high mortality rate of the larvae of *Ae. aegpti* at low concentrations of ethanol after 24 hours of exposure. Similar findings were also by Musa *et al.*[12] who studied the effect of aqueous and methanolic leaf extracts of *A. conyzoides* L and *Guiera senegalensis* L. against mosquito larvae in Zaria using different concentrations of 50, 100, 200 and 400 ppm, and reported 100% mortalities after 24 hours post treatment. The non-significant difference in the mortality rate of larvae of *Ae. aegypti* in relation to concentrations of water extracts of *A. annua* (Figure 3) in this study was consistent with the findings of Njila *et al.*[13] who worked on the potency of goat weed (*Ageratum conyzoides* L.) to *Culex quiquefasciatus* larvae and adults and revealed that water does not contain any chemical, it is mild, inactive and polar in nature. However, the high mortality rate at 0.3 ml concentration after 24 hours exposure (Figure 3) is similar to the findings of Paulo *et al.*[14] working on larvicidal activity of water extract of *Moringa oleifera* seeds against *Aedes aegypti* and its toxicity upon laboratory animals reported 99.2% larvae mortality within 24 hours at 5200 microg/mL. Hazrat *et al*[15]. who work on larvicidal activity of different plant extracts at different concentrations against 3rd instar larvae of *Aedes albopictus*, also reported high larvae mortality of 82% in *Clonorchis sinensis,* 58% in *Myristica fragrans,* 70% in *Matricaria chamomilla,* 62%

in *Mentha spicata and* 67% in *Zingiber officinale* at 800 ppm after 24 hours of exposure.

4.2. Determination of Lethal Concentration (Lc_{50}) of the leaves extract of *Artemisia annua* on larvae of *Aedes aegypti*

Ethanol, methanol and water extract of *Artemisia annua* were tested for larvicidal activity against larvae of *Aedes aegypti* mosquito. The larvicidal activites of ethanol extract of *A. annua* against larvae of *Ae. aegypti* shows larvicidal activity at LC_{50} value of 0.131 ml. Modise and Ashafa[16] reported similar larvicidal activity of *Foeniculum vulgare* against *Culex quinquefasciatus* mosquitoes at LC_{50} value of 0.10mg/mL after 24 hours of exposure. Naimah[11] also reported that ethanol extracts of *A. annua* exhibit toxicity against larvae of *Ae. aegypti* at LC_{50} value of 120.37 ppm after 24 hours of exposure. The larvicidal activities of methanol extract of *A.annua* shows larvicidal activity against larvae of *Ae. aegypti* at LC_{50} value of 0.60ml. Njila *et al.*[13] recorded similar larvicidal activity of methanolic extracts of *Ageratum conyzoides* at LC_{50} value of 1.09 ml after 24 hours of exposure. Extract of *Cymbopogon citrates* (lemon grass) exhibit the same level of or stronger larvicidal activity against *Cx. quinquefasciatus* at LC_{50} value of 3.495 g/l and 2.852 g/l[17]. The water extract gave the LC_{50} value of 0.25 ml indicating that the water extract is potent and exhibit larvicidal activity against larvae of *Ae. aegypti* at LC_{50} value of 0.25 ml. Japheth *et al*.[18] reported similar larvicidal activity of *Zanthoxylum gilletii* essential oil extracted by hydro-distillation against *Anopheles gambiae* at LC_{50} of 0.005773 mg/ml after 24 hours of exposure. The water extracts of *Moringa oleifera* seeds was tested against *Aedes aegypti* larvae with LC_{50} value of 1260 uglml[14]. It was observed that the water extract of *Moringa oleifera* seeds were relatively toxic to larvae of *Ae. aegypti.* Similar finding was made by Njila *et al*[13]. who recorded LC_{50} value of 1.38 ml of the water extract of *Ageratum conyzoides* on larvae of *Culex quinquefascatus.*

5. Conclusion

Plants are rich source of bioactive organic chemicals with an advantage over synthetic pesticides since they are less toxic, less prone to development of resistance and with easy biodegradability. The percentage mortalities observed in this study for ethanol and methanol leaf extracts of *Artemisia annua* shows significant difference and have the highest percentage mortality against *Ae. aegypti* larvae than water which shows no significant difference. Therefore different plants exhibit different level of potency against different mosquito species when extracted with different types of solvents. Lethal concentration (LC_{50}) values obtained in this study depicted that ethanol extract shows the strongest effect with an LC_{50} of 0.13 ml, water extract with the LC_{50} of 0.25 ml and methanol extract with the LC_{50} of 0.60 ml. This reveals that the leaves of *Artemisia annua* are very good, highly effective and a larvicide that is less harmful to the environment. The result of this study also reveals that the ethanol extracts of *A. annua* is considered the best in terms of LC_{50} as well as percentage mortality compared to methanol and water in controlling the larvae of *Ae. aegypti.*

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