



Short Communication

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Larvicidal activity of crude extract of *Morinda morindiodes* (Jologbo) leaves on *Anopheles* mosquitoes larvae

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ABSTRACT

Mosquitoes are known vectors for transmitting malarial parasites. To prevent proliferation of mosquito borne diseases and to improve public health, mosquito control has been employed using unfriendly synthetic insecticides. Alternative to these synthetic agents are natural products. The present study was designed to investigate the larvicidal activity of the Pet-ether crude extract of the leaves of *Morinda morindiodes* (Jologbo), Rubiaceae family, against the fourth instar larvae of *Anopheles* mosquitoes. A quantity of the dried and powdered leaves was exhaustively extracted by the Soxhlet extraction method using petroleum ether (60-80%). Samples of larvae were transferred into the extract at the various concentrations (50, 100, 150, 200, and 250 ppm). The mortality was observed and the dead larvae counted after 15 min., 30 min, 40 min, 1 hr, 3 hrs and 5 hrs. There were progressive increases in the lethal effect on the *Anopheles* larvae with respect to time and concentration. Lethality was observed from 1 hr, at a concentration of 200 ppm and by the 5th hour, all the concentrations were lethal. After probit analysis, the calculated LC₅₀ (larvae) was 55.96 ppm/5 hrs.

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INTRODUCTION

Prevention of the proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been very successful due to human, technical, operational, ecological, and economic factors. This failure has also led to resistant strains of the mosquitoes. The above factors have resulted in the urge to look for environment friendly, cost-effective, biodegradable and

target specific insecticides against mosquito species (Gbolade, 2001).

Unlike organic insecticides which are based on single active ingredient, plant products comprise a number of chemicals which act concertedly on both behavioral and physiological processes. Various extracts of leaves, underground and aerial parts of plants are frequently being investigated for mosquitocidal activities (Kalyanasundaram and Dass, 1985; Gbolade, 2001). These plants belonged to the families of Asteraceae, Rubiaceae, Ranunculaceae, Euphorbiaceae, Verbenaceae and Liliaceae (in decreasing order) rank high among the families that were screened (Anupam et al., 2012). The larvicidal

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activity of crude extract also varies with the methods of extraction and part of the plant (Prakash et al., 2002; Tonk et al., 2003; Sheeren 2006).

Jologbo (*Morinda morindiodes*, Rubiaceae family) is a shrub distributed over a considerable extent in Liberia and also in the sub-region of West Africa. *Jologbo* is a popular Liberian herbal medicinal plant used in treatment of falciparum malaria, fever and intestinal worms. *Jologbo* was reported to contain a lot of volatile oils and phenolic compounds (Kiazolu et al., 2013).

In view of the fact that larvicides play a vital role in controlling mosquitoes in their breeding sites, the development of insecticides of plant origin as an alternative to chemical insecticide, shall be a welcome phenomenon. This study was undertaken to assess the larvicidal potential of the extracts of *Jologbo* leaves on *Anopheles* sp larvae.

MATERIALS AND METHODS

Collection of plant materials

The leaves of the *Jologbo* plant (*Morinda morindiodes*, Rubiaceae family) was harvested at Congo Town, Monrovia. The plant was identified by Mr David Wah of the Faculty of Forestry, University of Liberia. A voucher specimen was kept in the School of Pharmacy Herbarium, University of Liberia. The leaves were dried at room temperature and crushed into powder form. A 40 g weight quantity of the *Jologbo* leaves was extracted in 600 ml petroleum ether by Soxhlet extraction technique for 48 hours. The extract was filtered and the filtrate was evaporated under reduced pressure on water bath to obtain the crude extract.

Collection of *Anopheles* larvae

The wetlands around the S. K. D. Boulevard and the Gaye town community on the Old Road were used to collect samples of *Anopheles* larvae from the natural habitat along with their immediate surrounding water. Sufficient quantity of pond or stagnant water

was drawn from the marsh environment and taken to the laboratory for investigation. Hand lens was used to observed stream of larvae moving in the water and for the counting. Only lively, highly motile larvae were used after allowing them to acclimatize to the laboratory conditions.

Preparation of working solution and Bioactivity testing

Stock solution was prepared by dissolving 1 g of crude extract in 10 ml petroleum ether (Pet-ether) and volume made up to 100 ml with distilled water. Five different dilutions of 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm were prepared in 200 ml de-ionized water in volumetric flask. These concentrations were placed in 250 ml beaker and specified amount of larvae were released into each. The number of death against time was taken at 15 min, 30 min, 40 min, 1 hr and 3 hrs 5 hrs, respectively. The beakers were kept in a temperature controlled room at $28 \text{ }^{\circ}\text{C} \pm 2 \text{ }^{\circ}\text{C}$. The mortality data were subjected to probit analysis to determine the lethal concentration to kill 50% of the treated larvae (Randhawa 2009). For the control experiment, the same numbers of larvae used above were placed in 200 ml water containing 0.1ml of petroleum ether in a 250 ml beaker and observed. Each treatment was replicated five times.

RESULTS AND DISCUSSION

The dried and powder *Jologbo* leaves were extracted in petroleum ether using the Soxhlet extraction method. A 10.80 g of the crude extract was obtained. A stock solution (10 mg/mL) was prepared and from this solution, serial dilutions were made (50, 100, 150, 200, and 250 ppm). Samples of larvae were transferred into each concentration and the mortality was observed after 15 min, 30 min, 40 min, 1 hr, 3 hrs and 5 hrs, respectively.

From the above results, it was observed that the crude extract of the leaves of the

Jologbo plant has activity against the larvae of the *Anopheles* mosquitoes. The toxic effects of the crude extract on the larvae depend on the concentration and duration of exposure. At the various concentrations, no lethal effect was observed until after about an hour, at the 200 ppm and 250 ppm respectively. There were progressive increases in the lethal effect on the *Anopheles* larvae after about five hours of exposure to the crude extract. This indicated that the toxicity levels of the extract are dependent on time of exposure of the larvae and the applied concentration. The percent mortality and lethal concentration were calculated (Table 2). At the concentration of 150 ppm, 200 ppm and 250 ppm, 88%, 96% and 100% mortality were observed respectively. The lethal concentration for 50% mortality (LC_{50}), after

probit analysis, was found to be 55.96 ppm at 5 hours. This is comparable to previous reports on larvicidal activities of plant extracts. Kamaraj et al. (2011) reported LC_{50} values of 93.80 and 104.94 for the methanol extract of *Annona squamosa* leaves and methanol extract of the leaves of *Chrysanthemum indicum* L, respectively, against *Anopheles subpicus*. Other similar reports include the larvicidal study on the components of the leaves of *Azadirachta indica* and *Artemisia annua* Linn, where the LC_{50} of crude extracts were reported to be 19.9 and 69.0 ppm, respectively (Shobhita et al, 2005), while Pushplatha and Muthukrishnan (1995) reported LC_{50} of 72 ppm and 136 ppm for crude extracts of *Vitex negundo* and *Nerium oleander* leaves.

Table 1: Bioassay testing of crude extract at different concentrations.

Concentration (ppm)	15 min	30 min	40 min	1 hr	3 hrs	5 hrs
50	-	-	-	-	-	+
100	-	-	-	-	-	+
150	-	-	-	-	+	+
200	-	-	-	+	+	+
250	-	-	-	+	+	+

Key: - = no noticeable lethal effect + = lethal effect observed

Table 2: Percent mortality of *Anopheles* mosquito larvae at various concentrations of crude extract after five hours.

Concentration (ppm)	Quantity of larvae	No of deaths	% Mortality
50	25	13	52
100	25	14	56
150	25	22	88
200	25	24	96
250	25	25	100



Figure 1: Photo showing the shrub *Morinda morindiodes* (Rubiaceae) (*Jologbo* plant).

Conclusion

The crude extract of the leaves of *Jologbo* could be used as one of the source of finding chemical substances to design insecticidal agents, especially anti-mosquito agents. It can be concluded that the crude extracts of the leaves of the *Jologbo* plant have insecticidal activity, especially mosquitocidal activity, against the larvae of the *Anopheles* mosquitoes. Therefore, it could be formulated and used as an ecological friendly natural product for anti-mosquito activity.

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