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Annals of Community Medicine and Practice

Short Communication

Larvicidal Activity of the Lignan desoxypodophyllotoxin Against *Aedes albopictus*

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Submitted: 30 March 2017

Accepted: 19 April 2017

Published: 21 April 2017

ISSN: 2475-9465

Keywords

- *Aedes albopictus*
- Desoxypodophyllotoxin
- Lignan
- *Podophyllum hexandrum*
- Larvicidal activity

Abstract

Aedes (*Stegomyia*) *albopictus* (Skuse, 1894) (Diptera: Culicidae), the Asian tiger mosquito, is a less efficient vector of dengue compared to *Ae.* aegypti although, it is able to transmit other arboviruses and thus, should remain a concern for the public health. Dengue is regarded as the most rapidly spread mosquito-borne infectious disease world. Disease prevention is dependent on controlling the mosquito population. Plant-derived natural products have been investigated in the search for new insecticides and larvicides aiming to help in the control of insects and their larvae. Lignans are phenolic compounds mainly distributed in plants even though they are found in other organisms and the insecticidal properties have been reported. This communication describes the evaluation of the activity of desoxypodophyllotoxin (1), isolated from the rhizomes and roots of *Podophyllum hexandrum* against the mosquito *Ae. albopictus*. Bioassays were performed on 25 larvae (L3) of *Ae. albopictus*. The desoxypodophyllotoxin was dissolved in a mixture of acetone: dimethylsulfoxide (DMSO) (1:1) and applied at final concentrations of 1-30 µg/mL. The mortality (100%) of the *Ae. albopictus* larval was observed in all the concentrations occurred. This study showed the larvicidal activity of the lignan desoxypodophyllotoxin (**1**) against the larvae of *Ae. albopictus*, and to some extent, confirms its potential as an application in the control of mosquitoes, the main vectors of arboviruses.

INTRODUCTION

Aedes (*Stegomyia*) *albopictus* (Skuse, 1894) (Diptera: Culicidae), is a mosquito originally from Asia which is also an invasive species that can also be found in areas of tropical, subtropical and temperate climates [1]. It has been reported that this Asian tiger mosquito is a less efficient vector of dengue compared to *Ae. aegypti* although, it offers a special concern to the public health implications because it is able to transmit other arboviruses such as chikungunya [2]. It can also transmit heartworm parasites [1] in dogs. Dengue is regarded as the most rapidly spread mosquito-borne infectious disease world. Despite its consequences, there is no effective treatment for patients who rely on supportive care. Moreover, disease prevention is dependent on controlling the mosquito population [3]. The use of conventional insecticides is not safe since some of them are toxic to humans and to non-target organisms and limited success has been achieved with them. In addition, due to repeated applications, the emergence of insecticide resistance in mosquitoes has increased and this causes environmental damage. Furthermore, vector control is costly and has only been partially successful in reducing transmission of the disease. Controlling the mosquito at the larval stage may be an alternative.

Plant-derived natural products have been investigated in the search for new insecticides and larvicides aiming to help in the control of insects and their larvae. It is desirable to find an environmentally safe, biodegradable and target insecticide. Many natural products are highly active against arthropods particularly alkaloids, phenolic compounds and terpenoids [4].

Lignans are phenolic compounds mainly distributed in plants even though they are found in other organisms. Their biological activities were fully reviewed by MacRae and Towers (1984) [5], and include antibacterial, antifungal, antiviral and antioxidant activity. Besides the insecticidal properties of lignans have also been reported [6].

Podophyllum species have well known lignan profiles [7], and

podophyllotoxin and its derivatives have received more attention due to their medicinal applications [8]. On the other hand, the spectrum of activities for these compounds is being expanded due to their effect on insects by known lignans [9]. In a recent study, we have reported that the larvicidal activity of podophyllotoxone against the larvae of *Ae. aegypti* made a possible contribution to the delayed development of its larvae [10].

Our research group is currently investigating the insecticidal and larvicidal potential of plants containing lignans and their isolated compounds and their use against vectors of great concern to the public health. This communication describes the evaluation of the larvicidal activity of desoxypodophyllotoxin (1) (Figure 1), against the mosquito *Ae. albopictus*. In a previous study [10], we reported on the isolation and identification of this compound from an ethanolic (EtOH) extract from the rhizomes and roots of *Podophyllum hexandrum*.

MATERIALS AND METHODS

Ae. albopictus eggs were obtained from the Núcleo Operacional Sentinela de Mosquitos Vetores, Instituto Oswaldo Cruz/FIOCRUZ, Rio de Janeiro. The bioassays were held in the Laboratório de Insetos Vetores, Universidade Severino Sombra, Rio de Janeiro. The larvae at 3rd stage (L3) were pooled and separated for the bioassays.

Desoxypodophyllotoxin (1) was dissolved in a mixture of acetone: dimethylsulfoxide (DMSO) (1:1) and applied at final concentrations of 1, 10 and 30 µg/mL in a receptacle containing mineral water (25 mL) and a diet of fishmeal (Alcon Guppy) at a dose of 0.3 mg/larva. Twenty-five third-stage (L3) larvae were used per group: test, control (without desoxypodophyllotoxin (1) and without a mixture of acetone: DMSO) and testimony control (with a mixture of acetone: DMSO). The experiments were performed in triplicate, totaling 75 larvae (L3) per group, with three repetitions. The larvae were maintained in a climate-controlled chamber at 28 ± 1 °C and 70 ± 10% relative humidity. The bioassays followed the methodology described by Maleck and collaborators (2017) [10], which were adapted from WHO (2005) [11]. The data were analyzed by means of one-way ANOVA with means separated using the Tukey test with a significance level of 5% [12], and standard deviations were calculated using the averages from the experiments through GraphPad Instat 3.05 [13]. The LC_{50} value was calculated by means of Trimmed Spearman-Karber analysis [14].

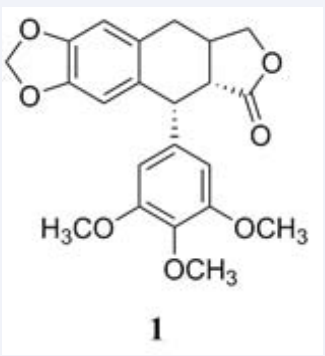

Figure 1 Desoxypodophyllotoxin (**1**) isolated from *Podophyllum hexandrum.*

RESULTS AND DISCUSSION

In this study, the treatment with desoxypodophyllotoxin (1) showed that the L3-L4 larval development was reduced (1-5 d) at the concentrations of 1 µg/mL, 10 µg/mL, and 30 µg/mL when compared with both controls which showed delayed development of larvae (1-10 d) (Table 1A).

The larvae (L3) of *Ae. albopictus* treated with desoxypodophyllotoxin showed L3-L4 viability of 52% (1 µg/mL), 25% (10 µg/mL) and 28% 30 µg/mL (Table 1B). Both controls presented 100% viability of the L3-adult, however at the same concentrations evaluated (1, 10 and (30 µg/mL)) the larvae did not reach the adult phase as shown (Table 1B).

Regarding mortality, the concentration of 1 µg/mL of compound (1) affected 48% (12 ± 8.7) of the L3 larvae between the 1st to 3rd day. The high mortality rate above 70% for L3 was observed at 10 µg/mL (15.3 ± 11.1) (2-7 d) and 30 µg/mL (18 ± 7) (1-6 d) concentration. The data showed a toxic activity with an LC_{50} of 1.1 µg/mL. The mortality (100%) of the *Ae. albopictus* larval (L3+L4) was observed in all the concentrations occurred of the 1st to 7th (Table 1C).

Studies with lignans have shown their effects on the insects. In studies with podophylltotoxin and deoxypodophyllotoxin, both compounds have shown insecticidal activity against larvae of *Epilachna sparsa orientalis* [15]. According to the authors, deoxypodophyllotoxin was also active against adult females of *Culex pipiens molestus*, *Musca domestica*, *Blatella germanica* and *Periplaneta fuliginosa* [15].

Cabral and collaborators (2000) [9] evaluated some lignans and neolignans as inhibitors of ecdysis on four instar larvae of *Rhodnius prolixus*, a major vector of Chagas disease. Podophyllotoxin caused a high moulting inhibition and significant toxicity when applied either orally and topically. Whereas the high percentage of ecdysis inhibition (58%) was observed for pinoresinol applied orally at a concentration of 100 mg/mL. Moreover, podophyllotoxin and burchellin reduced the excretion of the insect in 24 h while the other evaluated lignans did not have any effect on the excretion.

Burchellin, a lignan isolated from the stems of *Ocotea cymbarum* (Lauraceae) was very effective against *Ae. aegypti* and showed larval mortality of 100% at a concentration of 30 µg/mL, between 24 and 78 h after the treatment [16].

Overall, desoxypodophyllotoxin demonstrated 100% larval mortality on *Ae. albopictus* at all the concentrations evaluated until 7 days after treated. These results corroborate with our previous work, where this compound has shown a 100% larval mortality for *Ae. aegypti* at the same concentrations [10]. In addition, desoxypodophyllotoxin did not demonstrated toxicity toward the peritoneal macrophage cells of BALB/c mice, and thus, is worthy of further studies [10].

CONCLUSION

This study showed the larvicidal activity of the lignan desoxypodophyllotoxin (1) against the larvae of *Ae. albopictus*,

Table 1: Duration of development (A), viability (B) and mortality (C) among *Aedes albopictus* larvae (L3) treated with desoxypodophyllotoxin **(1).**

Treatment	L3-L4 (days)		Pupae (days)		L3-adult (days)	
A	X ± SD	VI	X ± SD	VI	X ± SD	VI
Control	2.1 ± 1.5a	1-10	11.2 ± 3a	5-18	18.8 ± 3a	7-20
Testimony	3.3 ± 2.1bc	1-10	11 ± 2.3a	5-17	13.6 ± 2.2a	8-18
1 µg/mL	2.8 ± 0.4ac	2-3	0	0	0	0
10 µg/mL	2.1 ± 0.6ad*	1-4	0	0	0	0
30 µg/mL	3.8 ± 1bc	2-5	0	0	0	0

	L3	L3-L4		L4-Pupae		L3-adult	
B	X ± SD	X ± SD	%	X ± SD	%	X ± SD	%
Control	25 ± 0a	25 ± 0a	100	25 ± 0a	100	25 ± 0	100
Testimony	25 ± 0a	25 ± 0a	100	25 ± 0a	100	25 ± 0	100
1 µg/mL	25 ± 0a	13 ± 8.7a	52	0	0	0	0
10 µg/mL	25 ± 0a	6 ± 6b*	25	0	0	0	0
30 µg/mL	25 ± 0a	7 ± 7b*	28	0	0	0	0

	Larvae (L3+L4)			Pupae		
C	X ± SD	VI	%	X ± SD	VI	%
Control	0a	0	0	0	0	0
Testimony	0a	0	0	0	0	0
1 µg/mL	25± 0b***	1-7	100	0	0	0
10 µg/mL	25± 0b***	2-7	100	0	0	0
30 µg/mL	25± 0b***	1-6	100	0	0	0

Experiments with 25 larvae (L3) of *Ae. albopictus*, for each test group and control, in triplicate, with three repetitions (n = 75). Mean and standard deviation (X ± SD). Range of variation (VI). Values followed by the same letter (a = a, b = b, c = c) have no significant differences. Significance levels according to the Tukey test, represented as ***P < 0.001, *P <0.1 vs. acetone: dimethyl sulfoxide (DMSO) (1:1) (testimony).

and to some extent, confirms its potential as an application in the control of mosquitoes, the main vectors of arboviruses.

ACKNOWLEDGEMENTS

The authors thanks FUSVE/USS; Fundação de Amparo a Novas estratégias para o controle do mosquito *Aedes aegypti*, vetor da Dengue, Chikungunya e do virus Zika: uma abordagem integrada/RedeZIKA#1" and FIOCRUZ/CAPES-Brasil Sem Miséria. In addition, CGS is grateful to Dr Patrick H Huddleston (Nottingham Trent University, UK) for discussing the results of the isolation and idenfication of lignans from *Podophyllum* species.

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Cite this article

Maleck M, da Silva Pedra Parreira W, Serdeiro MT, Tavares Vieira RR, Honório NA, et al. (2017) Larvicidal Activity of the Lignan desoxypodophyllotoxin Against Aedes albopictus. Ann Community Med Pract 3(2): 1022.