

Short Communication

Larvicidal Activity of the Lignan desoxypodophyllotoxin Against *Aedes albopictus*

Marise Maleck^{1-4*}, Walquiria da Silva Pedra Parreira¹, Michele Teixeira Serdeiro^{1,5}, Richard Raphael Borges Tavares Vieira¹, Nildimar Alves Honório⁶, and Cláudia Gontijo Silva⁷

¹Laboratório de Insetos Vetores, Universidade Severino Sombra, Brazil

²Mestrado Profissional em Ciências Aplicadas em Saúde, Universidade Severino Sombra, Brazil

³Mestrado Profissional em Ciências Ambientais, Universidade Severino Sombra, Brazil

⁴Laboratório de Entomologia Médica e Forense, Instituto Oswaldo Cruz/FIOCRUZ, Brazil

⁵Laboratório Interdisciplinar de Vigilância Entomológica em Díptera e Hemiptera, Instituto Oswaldo Cruz/FIOCRUZ, Brazil

⁶Laboratório de Transmissores de Hematozoários e Núcleo Operacional Sentinela de Mosquitos Vetores, Instituto Oswaldo Cruz/FIOCRUZ, Rio de Janeiro, RJ, Brazil.

⁷Serviço de Biotecnologia Vegetal, Divisão de Ciência e Inovação, Fundação Ezequiel Dias, Brazil

*Corresponding author

Dra Marise Maleck, Laboratório de Insetos Vetores, Universidade Severino Sombra, Avenida Expedicionário Oswaldo Almeida Ramos, 280, 27700-000, Vassouras, RJ, Brazil, Tel: 55 24 2471 8351; Email: marise.maleck@gmail.com

Submitted: 30 March 2017

Accepted: 19 April 2017

Published: 21 April 2017

ISSN: 2475-9465

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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 desoxy-podophyllotoxin (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.

Desoxy-podophyllotoxin (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 desoxy-podophyllotoxin (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

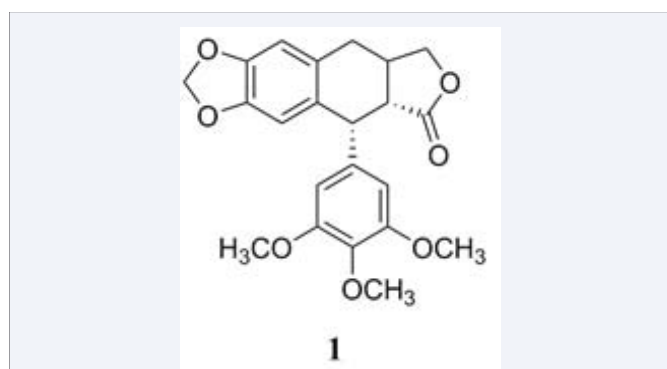


Figure 1 Desoxy-podophyllotoxin (1) isolated from *Podophyllum hexandrum*.

3.05 [13]. The LC₅₀ value was calculated by means of Trimmed Spearman-Kärber analysis [14].

RESULTS AND DISCUSSION

In this study, the treatment with desoxy-podophyllotoxin (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 desoxy-podophyllotoxin 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 LC₅₀ 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 podophyllotoxin and desoxy-podophyllotoxin, both compounds have shown insecticidal activity against larvae of *Epilachna sparsa orientalis* [15]. According to the authors, desoxy-podophyllotoxin 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 pinorelinol 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, desoxy-podophyllotoxin 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, desoxy-podophyllotoxin 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 desoxy-podophyllotoxin (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 desoxy-podophyllotoxin (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 vírus 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 identification of lignans from *Podophyllum* species.

REFERENCES

- Cdc.gov [homepage on the Internet]. Atlanta: Centers for Disease Control and Prevention. [updated 2016 April 5; cited 2016 Dec 6].
- Bonilauri P, Bellini R, Calzolari M, Angelini R, Venturi L, Fallacara F, et al. Chikungunya virus in *Aedes albopictus*, Italy. *Emerg Infect Dis*. 2008; 14: 852-854.
- Schwartz LM, Halloran ME, Durbin AP, Longini Jr IM. The dengue vaccine pipeline: implications for the future of dengue control. *Vaccine*. 2015; 33: 3293-3298.
- Mann RS, Kaufman PE. Natural product pesticides: their development, delivery and use against insect vectors. *Mini Rev Org Chem*. 2012; 9: 185-202.
- MacRae WD, Towers GHN. Biological activities of lignans. *Phytochemistry*. 1984; 23: 1207-1220.
- Saguez J, Attoumbré J, Giordanengo P, Baltora-Rosset S. Biological activities of lignans and neolignans on the aphid *Myzus persicae* (Sulzer). *Arthropod Plant Interact*. 2013; 7: 225-233.
- Jackson DE, Dewick PM. Tumour-inhibitory aryltetralinlignans from *Podophyllum pleianthum*. *Phytochemistry*. 1985; 24: 2407-2409.
- Silva CG, Almeida VL, Campana PRV, Rocha MP. Plant Cell Cultures as Producers of Secondary Metabolites: *Podophyllum* Lignans as a Model. In: Jha S (ed.), *Transgenesis and Secondary Metabolism*, Reference Series in Phytochemistry (Mérillon JM, Ramawat KG, series eds.). Springer. 2016; 1-36.
- Cabral MMO, Azambuja P, Gottlieb OR, Garcia ES. Effects of some lignans and neolignans on the development and excretion of *Rhodnius prolixus*. *Fitoterapia*. 2000; 71: 1-9.
- Maleck M, de Oliveira Hollanda P, Serdeiro MT, de Araújo Soares RO, Honório NA, Silva CG. Toxicity and larvicidal activity of *Podophyllum*-based lignans against *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol*. 2017; 54: 159-166.
- World Health Organization. Dept. of Communicable Disease Prevention, Control and Eradication. WHO Pesticide Evaluation Scheme Guidelines for laboratory and field testing of mosquito larvicides. 2002. 39.
- Sokal RR, Rohlf FJ. *Principios y Metodos Estadísticos em la Investigación Biológica*. Madrid, España: H. Blume Ed. 1979; 223.
- Motulsky HJ. *Analyzing data with GraphPad Prism*, GraphPad Software Inc, San Diego, CA. 2002.
- Hamilton MA, Russo RV. Trimmed Spearman-Kärber method for estimating median lethal concentrations in bioassays. *Thurston*

- Environ Sci Technol. 1978; 11: 714-719.
15. Inamori Y, Tsujibo H, Oki S, Kodama Y, Ogawa K. Mechanism of action of deoxypodophyllotoxin (Anthricin). III. The mode of delayed insecticidal action of deoxypodophyllotoxin. Chem Pharm Bull. 1986; 34: 2247-2250.
16. Narciso JOA, Soares ROA, Mallet JRS, Guimarães AE, Chaves COM, Barbosa-Filho JM, et al. Burchellin: study of bioactivity against *Aedes aegypti*. Parasit Vectors. 2014; 7: 172.

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.