Short Communication

Preliminary evaluation of the larvicidal activity of extracts and fractions from Ocotea nutans (Nees) Mez against Aedes aegypti

Fernando Cesar Martins Betim[1], Camila Freitas de Oliveira[2], Deise Prehs Montrucchio[1], Obdulio Gomes Miguel[1], Marilis Dallarmi Miguel[1], Juliana Bello Baron Maurer[3] and Josiane de Fátima Gaspari Dias[1]

[1]. Universidade Federal do Paraná, Programa de Pós-Graduação em Ciências Farmacêuticas, Curitiba, PR, Brasil.
[2]. Universidade Estadual do Centro Oeste, Departamento de Farmácia, Guarapuava, PR, Brasil.
[3]. Universidade Federal do Paraná, Departamento de Bioquímica e Biologia Molecular, Curitiba, PR, Brasil.

Abstract

Introduction: Aedes aegypti is the main vector of dengue and yellow fever. Recently, the use of plant-sourced larvicides has gained momentum. Methods: The hydroethanolic extracts and fractions of Ocotea nutans leaves and stems were bioassayed to determine the larvicidal efficacy of these samples. Results: S-HEX (hexane fraction from the crude stem extract) demonstrated high potential for controlling third-stage larvae, with an LC50 of 14.14 µg.mL-1 (concentration required to inhibit 50% of the treated larvae). Conclusions: Extracts from O. nutans were effective against third-stage larvae of A. aegypti after 24 h of exposure.

Keywords: Ocotea nutans. Larvicidal potential. Aedes aegypti. Brazilian plant.
identification was performed by the forest engineer, Marcelo Leandro Brocco, from the Botanical Garden of Curitiba Herbarium, and the plant was compared with a voucher specimen deposited under number 56552 at the Herbarium of the Federal University of Paraná.

This study was authorized by SisGen, a legislative and deliberative body under the Ministry of Environment of Brazil, under the number A0EB51A, which grants permission to evaluate the bioactivities of extracts derived from Brazilian plants.

The leaves and stems were dried at 50 °C. A crude ethanolic extract was prepared with 96 °GL ethanol (1:10; w/v) in a Soxhlet apparatus under continuous reflux for 6 h at 80 °C. Fractions were obtained by employing the liquid-liquid partitioning method of using solvents in increasing order of polarity (n-hexane, chloroform, and ethyl acetate) in a modified Soxhlet apparatus. This technique was separately performed for leaves and stems to obtain crude ethanolic extracts from leaves (L-CEE) and stems (S-CEE).

After fractioning, four organic fractions were obtained from each crude extract: hexane fraction (L-HEX), chloroform fraction (L-CHL), ethyl acetate fraction (L-ETH), and a residual fraction (L-RES) from L-CEE; additionally, a hexane fraction (S-HEX), chloroform fraction (S-CHL), ethyl acetate fraction (S-ETH), and a residual fraction (S-RES) were obtained from S-CEE. Next, the extracts and fractions were vacuum filtered and concentrated in a rotary evaporator under reduced pressure at 40 °C.

The following methodology was adapted from Betim et al.\(^7\) and Garcez et al.\(^8\). The eggs of A. aegypti (Rockefeller strain provided by the Oswaldo Cruz Foundation) were reared under laboratory conditions (27±3 °C, relative humidity of 80%, and incubated in a Bio-Oxygen Demand incubator) by feeding with fish feed (Aldon basic, MEP 200 complex) from hatching until the third stage of larval development. The samples were diluted in 0.5% dimethyl sulfoxide (DMSO) and then dissolved in dechlorinated water to obtain the desired concentration (1000, 100, and 10 µg.mL\(^{-1}\)). After hatching, 10 larvae were treated with the controls (water and DMSO) as well as with extracts and fractions for 24h. Next, living and dead larvae were counted in triplicate for each treatment, reaching a total of 30 larvae for each sample dose.

The probit method was used to determine the lethal concentration (LC\(_{50}\) and LC\(_{90}\)) values as well as the corresponding 95% confidence intervals; the chi-square test was used for the A. aegypti assay. All statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA).

Based on our findings, it was confirmed that the extract and fractions obtained from O. nutans induced mortality in A. aegypti larvae (Table 1). Pronounced larvicidal effects were observed with the n-hexane fraction (S-HEX).

### Table 1: Induction of mortality in A. aegypti larvae by extracts and fractions from Ocotea nutans.

| Sample   | Concentration (µg.mL\(^{-1}\)) | Mortality (%) ± SD | LC\(_{50}\) (µg.mL\(^{-1}\)) (LCL - UCL) | LC\(_{90}\) (µg.mL\(^{-1}\)) (LCL - UCL) | x\(^2\) (df) |
|----------|-------------------------------|--------------------|----------------------------------------|----------------------------------------|-------------|
| L-CEE    | 10                            | 3.33 ± 0.57        | > 1000                                 | > 1000                                 | n.d.        |
|          | 100                           | 33.33 ± 0.57       | > 1000                                 | > 1000                                 | n.d.        |
|          | 1000                          | 40 ± 0.00          |                                       |                                       | n.d.        |
| L-HEX    | 10                            | 16.66 ± 0.57       | 111.32 (48.02 – 263.96)                | > 1000                                 | 2.38 (1) n.s.|
|          | 100                           | 53.33 ± 1.15       |                                       | > 1000                                 | 3.76 (1) n.s.|
|          | 1000                          | 76.66 ± 0.57       |                                       |                                       |             |
| L-CHL    | 10                            | 23.33 ± 0.57       | 171.41 (47.7 – 1129.1)                 | > 1000                                 | 1.91 (1) n.s.|
|          | 100                           | 50 ± 1.00          |                                       | > 1000                                 |             |
|          | 1000                          | 63.33 ± 0.57       |                                       |                                       |             |
| L-ETH    | 10                            | 0 ± 0.00           | > 1000                                 | > 1000                                 | n.d.        |
|          | 100                           | 0 ± 0.00           | > 1000                                 | > 1000                                 | n.d.        |
|          | 1000                          | 10 ± 0.00          |                                       |                                       |             |
| L-RES    | 10                            | 0 ± 0.00           | > 1000                                 | > 1000                                 | n.d.        |
|          | 100                           | 0 ± 0.00           | > 1000                                 | > 1000                                 | n.d.        |
|          | 1000                          | 1.0 ± 1.00         |                                       |                                       |             |
|          | 10                            | 16.66 ± 0.57       | > 1000                                 | > 1000                                 | n.d.        |
|          | 100                           | 0 ± 0.00           | > 1000                                 | > 1000                                 | n.d.        |
|          | 1000                          | 10 ± 0.00          |                                       |                                       |             |
| S-CEE    | 10                            | 23.33 ± 0.57       | > 1000                                 | > 1000                                 | n.d.        |
|          | 100                           | 23.33 ± 0.57       | > 1000                                 | > 1000                                 | n.d.        |
|          | 1000                          | 50 ± 1.00          |                                       |                                       |             |
| S-HEX    | 10                            | 46.66 ± 0.57       | 14.14 (4.3 – 23.18)                    | 207.55 (93.6 – 1120.9)                 | 2.20 (1) n.s.|
|          | 100                           | 76.66 ± 0.57       |                                       |                                       |             |
|          | 1000                          | 100.00 ± 0.00      |                                       |                                       |             |
| S-CHL    | 10                            | 6.66 ± 0.57        | 441.4 (209.3 – 2309.6)                 | > 1000                                 | 3.76 (1) n.s.|
|          | 100                           | 26.66 ± 1.52       |                                       | > 1000                                 |             |
|          | 1000                          | 63.33 ± 0.57       |                                       |                                       |             |
| S-ETH    | 10                            | 0 ± 0.00           | > 1000                                 | > 1000                                 | n.d.        |
|          | 100                           | 13.33 ± 1.15       | > 1000                                 | > 1000                                 | n.d.        |
|          | 1000                          | 23.33 ± 1.15       |                                       |                                       |             |
| S-RES    | 10                            | 0 ± 0.00           | > 1000                                 | > 1000                                 | n.d.        |
|          | 100                           | 0 ± 0.00           | > 1000                                 | > 1000                                 | n.d.        |
|          | 1000                          | 0 ± 0.00           |                                       |                                       |             |

Legend: Leaves crude ethanolic extract (L-CEE), leaves hexane fraction (L-HEX), leaves chloroform fraction (L-CHL), leaves ethyl acetate fraction (L-ETH), leaves residual fractions (L-RES). Stem crude ethanolic extract (S-CEE), stem hexane fraction (S-HEX), stem chloroform fraction (S-CHL), stem ethyl acetate fraction (S-ETH), stem residual fractions (S-RES). Notes: no mortality was observed in the negative controls; positive control killed 100% larvae in A. aegypti; (LC\(_{50}\)) lethal concentration that kills 50% of the exposed organisms; (LC\(_{90}\)) lethal concentration that kills 90% of the exposed organisms; (UCL) 95% upper confidence limit; (LCL) 95% lower confidence limit; \(x^2\) = chi-square statistic; df = degrees of freedom; (n.d.) not defined; (n.s.) not significant (p<0.05).
and chloroform fractions of leaves and stems. S-HEX demonstrated high potential for controlling third-stage larvae, with an LC₅₀ of 14.14 µg.mL⁻¹ (required to inhibit 50% of treated larvae). L-HEX, with an LC₅₀ of 111.32 µg.mL⁻¹, and L-CHL, with an LC₅₀ of 171.41 µg.mL⁻¹, revealed the greatest potential for controlling third-stage larvae. Additionally, S-CHL showed larvicidal potential with an LC₅₀ of 441.4 µg.mL⁻¹. Other extracts and fractions induced low or no mortality in third-stage larvae.

The larval mortality profile, with regard to the concentrations of extracts and fractions of *O. nutans*, is shown in Table 1. The mortality profile was accentuated for S-HEX, L-HEX, S-CHL, and L-CHL, mainly at concentrations of 1000 µg.mL⁻¹.

The Brazilian flora is rich in *Ocotea* plants, and extracts and fractions from several different species have already been evaluated for their larvicidal potential. Garcez et al.⁹ examined the potential larvicidal properties of ethanolic extracts obtained from the leaves, fruits, and trunk bark of *O. minarum* (Nees & C. Mart.) Mez against *A. aegypti*, reporting no mortality (LC₅₀ >1000 µg.mL⁻¹). The ethanolic extract was derived from the trunk of *O. suaveolens* (Meisn.) Benth. & Hook.f. ex Hieron. was evaluated against *A. aegypti* larvae, with no mortality recorded (LC₅₀ > 1000 µg.mL⁻¹).⁹ Notably, the ethanolic trunk bark extract from *O. velloziana* (Meisn.) Mez presented superior potential against *A. aegypti* larvae (LC₅₀ = 213.70 µg.mL⁻¹)⁹.

The low LC₅₀ of L-HEX and S-HEX shows that mortality was induced by this fraction because of the presence of a rich mixture of apolar compounds such as lignans, terpenes, and derivatives. In the literature, extracts produced with hexane indicate the possibility of apolar compound extraction. Narciso et al.¹⁰ examined the larvicidal properties of lignin extracted from the hexane fractions of stems from *O. cymbarum* Kunth and reported high mortality against *A. aegypti* larvae. Betim⁷ et al. examined essential oils (terpenoid-based constituents) from *O. nutans*, and this mixture could control third-stage larvae, with an LC₅₀ of 250 µg.mL⁻¹. Terpenes and derivatives have larvicidal properties, and it is suggested that their liposoluble characteristics have a strong influence on the mortality of larvae¹¹.

Mortality was induced by the L-CHL and S-CHL fractions due to the presence of a rich mixture of alkaloids and nitrogenated derivatives. Extracts produced with chloroform indicated the possibility of nitrogenated compound extraction (e.g., alkaloids), and these metabolites have great potential in larvicidal activities. Dicentrin⁹, an alkaloid isolated from *O. velloziana* and tested in the third stage of *A. aegypti* larvae, had an LC₅₀ of 30 µg.mL⁻¹.² The alkaloids showed larvicidal activity and had a mode of action similar to that of natural pyrethrin insecticides (basis for synthetic insecticides, e.g., pyrethroids). The phytolarvicidal action of alkaloids also has a similar action to the carbamate and organophosphate insecticides.² The genus *Ocotea* has a high possibility of containing the secondary metabolites derived from terpenoids and alkaloids, as described in the literature.

Notably, virus transmission by *A. aegypti* can be prevented or reduced through environmental management and by using synthetic insecticides belonging to pyrethroids or organophosphates, including temephos¹³, to minimize the spread of mosquitoes and human contact. However, the frequent use of synthetic pesticides to control the *A. aegypti* population can result in environmental and/or human contamination, along with the emergence of resistant insects¹⁴.

For vector control in public health, chemical control utilizing insecticides of organic or inorganic origin is one of the most frequently adopted strategies for sustainable, integrated management. However, its continued use has increased the appearance of resistant populations, leading to challenges in vector control. Moreover, resistance has been detected for all classes of insecticides, directly and profoundly affecting the re-emergence of vector-borne diseases. This is because despite important advances in the development of alternative methods, chemical insecticides remain an important tool in programs undertaking integrated control¹⁵.

Notably, the frequent use of these insecticides has resulted in phytotoxicity, human poisoning, and emergence of resistant insects⁵,⁸,⁹. Hence, researchers are attempting to develop alternative strategies to control *A. aegypti* proliferation, including the use of phytolarvicides composed of plant extracts or compounds³,⁷,⁸.

Plant-based compounds are already known to exhibit larvicidal properties, which could be useful for the development of eco-friendly larvicides⁵,¹⁴.

Under laboratory conditions, the hexane and chloroform fractions of the leaves and stems of *O. nutans* demonstrated larvicidal effects against *A. aegypti*. These results present an opportunity to replace synthetic pesticides with natural products in vector control programs for yellow fever, dengue, and, more recently, chikungunya. Moreover, the efficacy of the hexane stem fraction is promising. The potential of extracts derived from these plants as larvicides against *A. aegypti* represents an abundant and accessible alternative in southern Brazil, where increasing infestations and dengue cases have been reported in the last decade.

Furthermore, these extracts presented an alternative to the synthetic products recommended by the Ministry of Health for the control of dengue. Superior results can be obtained with additional studies to evaluate the larvicidal activity of pure compounds isolated from these plants.

**ACKNOWLEDGMENTS**

The authors thank forest engineer Marcelo Brotto for help with the identification of the plant material and Oswaldo Cruz Foundation (Fiocruz) for donation of material - *Aedes aegypti* eggs - for this research.

**FINANCIAL SUPPORT**

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

**AUTHORS’ CONTRIBUTION**

FCMB: performed the lab experiments, data collection and article writing. CFO: statistical analysis, and article writing. DPM: data analysis, and article writing. OGM: data analysis, and article writing.
writing. MDM: performed the lab experiments, data analysis, and article writing. JBBM: supplying the study conception and design, article review. JFGD: supplying the study conception and design, article review. All the authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Fonseca-Júnior DP, Serpa LLN, Barbosa GL, Pereira M, Holcman MM, Voltolini JC, et al. Vectors of arboviruses in the state of São Paulo: 30 years of Aedes aegypti and Aedes albopictus. Rev Saude Publica. 2019;53:84.
2. Lima EP, Goulart MOF, Rolim-Neto ML. Meta-analysis of studies on chemical, physical and biological agents in the control of Aedes aegypti. BMC Public Health. 2015;15:858.
3. Panneerselvam C, Murugan K, Kovendan K, Kumar PM, Subramaniam J. Mosquito larvicidal and pupicidal activity of Euphorbia hirta Linn. (Family: Euphorbiaceae) and Bacillus sphaericus against Anopheles stephensi Liston. (Diptera: Culicidae). Asian Pac J Trop Med. 2013;6(2):102-9.
4. Seetharaman PK, Chandrasekaran R, Gnanasekar S, Chandrakasan G, Gupta M, Manikandan DB, et al. Antimicrobial and larvicidal activity of eco-friendly silver nanoparticles synthesized from endophytic fungi Phomopsis liquidambaris. Biocatal Agric Biotechnol. 2018;16:22-30.
5. Silva EM, Roel AR, Porto KRA, Falco ME, Matias R. Insecticidal effect of the ethanol extract of Baccharis dracunculifolia (Asteraceae). Rev Biol Trop. 2017;65(2):517-23.
6. Flora do Brasil. Ocotea in Flora do Brasil 2020, em construção [Internet]. Jardim Botânico do Rio de Janeiro; 2020 [update 2020 Aug 05; cited 2020 Aug 05]. Available from: http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB8475.
7. Betim FCM, Oliveira CF, Souza AM, Szabo EM, Zanin SMW, Miguel OG, et al. Ocotea nutans (Nees) Mez (Lauraceae): chemical composition, antioxidant capacity and biological properties of essential oil. Braz J Pharm Sci. 2019;55:e18284.
8. Carvalho JLS, Cunico MM, Dias JFG, Miguel MM, Miguel OG. Termoestabilidade de processos extrativos de Nasturtium officinale R. Br., brassicaceae por sistema Soxhlet modificado. Quim Nova. 2009;32(4):1031-5.
9. Garcez WS, Garcez FR, da Silva LMGE, Hamerski L. Larvicidal activity against Aedes aegypti of some plants native to the West-Central region of Brazil. Bioreus Technol. 2009;100(24):6647-50.
10. Narciso JOA, Soares ROA, Mallet JRS, Guimaraes AE, Chaves COM, Barbosa-Filho JM, et al. Burchellin: study of bioactivity against Aedes aegypti. Parasit Vectors. 2014;7(172):1-10.
11. Santos SRL, Melo MA, Cardoso AV, Santos RLC, Sousa DP, Cavalcanti SCH. Structure-activity relationship of larvicidal monoterpenes and derivates against Aedes aegypti Linn. Chemosphere. 2011;84(1):150-3.
12. Musau JK, Mbaria JM, Nguta JM, Mathiu M, Kiama SG. Phytochemical composition and larvicidal properties of plants used for mosquito control in Kwale County, Kenya. Int J Mosq Res. 2016;3(3):12-7.
13. Lima EP, Oliveira-Filho AM, Lima JW0, Ramos AN, Cavalcanti LPG, Pontes RJS. Resistência do Aedes aegypti ao Temefós em Municípios do Estado do Ceará. Rev Soc Bras Med Trop. 2006;39(3):259-63.
14. Fujiwara GM, Annies V, de Oliveira CF, Lara RA, Gabriel MM, Betim FCM, et al. Evaluation of larvicidal activity and ecotoxicity of linalool, methyl cinnamate and methyl cinnamate/linalool in combination against Aedes aegypti. Ecotoxicol Environ Saf. 2017;139:238-44.
15. Braga IA, Valle D. Aedes aegypti: histórico do controle no Brasil. Epidemiol Serv Saude. 2007;16(2):113-8.