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CIRCADIAN VARIATION AND ANTILEISHMANIAL POTENTIAL OF THE VOLATILE COMPOUNDS OF *SCHINUS TEREBINTHIFOLIUS* RADDI

Idalina Cândido^{1*}, Felipe Sales¹, André Gustavo Tempone², João Henrique Ghilardi Lago¹

i.candido@ufabc.edu.br

1 -Center for Natural and Human Sciences, Federal University of ABC, Santo André, SP, Brazil.

2 -Laboratory of Pathophysiology, Butantan Institute, São Paulo, SP, Brazil

Schinus terebinthifolius Raddi (Anacardiaceae), commonly known as the Brazilian pepper tree, has several well-documented therapeutic applications and properties, including anti-inflammatory, wound-healing, and antimicrobial activities. The essential oils from this plant displayed selective activity against cancer cells as well as allelopathic potential. As part of our ongoing research on antileishmanial agents derived from Brazilian flora, this study aimed to analyze the chemical composition of essential oils extracted from the leaves of *S. terebinthifolius* collected in Santo André, São Paulo, Brazil, at four different periods (6h, 12h, 18h, and 24 h) on December 15th, 2024, to assess a possible circadian variation, and to evaluate the potential of each oil and main compounds against promastigote forms of *Leishmania infantum*. During each period, the aerial parts were collected, and the essential oils were obtained individually by hydrodistillation using a Clevenger apparatus. The respective yields, calculated based on the fresh weight of the plant material, were 0,51% (6 h), 0,71% (12 h), 0,57% (18 h), and 0,55% (24 h). Chemically, the oils were found to consist mainly by non-oxygenated monoterpenes and sesquiterpenes. Quantitatively, minor variations were observed in each oil. The highest concentration of monoterpenes was observed at 6 h (58.0%), followed by a reduction in the subsequent time periods (49.9% at 12 h, 41.6% at 18 h, and 43.4% at 24 h). The proportion of the main monoterpenes, α - and β -pinene, changed from 28.4% and 12.9%, respectively, at 6 h, to 23.9% and 9.7% at 12 h, 19.7% and 9.7% at 18 h, and 22.0% and 8.6% at 24 h. Similarly, the amount of sesquiterpenes changed little over time, with higher amounts detected at 18 h (52.7%) and 24 h (49.1%). Among the main compounds identified were germacrene D and germacrene B, at 17.0% and 6.4% at 6 h, 16.6% and 9.8% at 12 h, 19.8% and 9.9% at 18 h, and 13.9% and 7.6% at 24 h, respectively. The effectiveness of each crude oil against promastigote forms of *Leishmania infantum* was determined *in vitro*, and all of the oils exhibited potency, resulting in 100% parasite death at a concentration of 200 mg/mL. To evaluate the effects of main compounds in bioactivity, a pool of the four oils was prepared and this material was subjected to column chromatography over $\text{SiO}_2/\text{AgNO}_3$, yielding α -pinene, β -pinene, α -copaene, germacrene D, and germacrene B in pure forms. These compounds were tested individually against *L. infantum* promastigotes and displayed EC₅₀ values of 15.5, 12.3, 18.1, 25.6, and 46.5 mg/mL, respectively, with reduced toxicity against NCTC cells (CC₅₀ > 200 mg/mL). These results indicate that the proportion of main compounds does not change over the course of a day and that these metabolites may be responsible for the antileishmanial effect of the crude oils (CNPq, FAPESP, CAPES).

Keywords: Volatile compounds; circadian variation; *Schinus terebinthifolius*; antileishmanial.

