BIOACTIVITY OF *Myrcia amazonica* DC. ESSENTIAL OIL NANOEMULSION ON *Schistosoma mansoni* CERCARIAE

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Introduction

Schistosomiasis is caused by trematodes of the genus Schistosoma, transmitted by snails, their intermediate hosts, of the genus Biomphalaria [1]. The World Health Organization (WHO) estimates that around 258 million people in 78 countries worldwide are infected with the disease, with 52 of these countries being associated with moderate and high transmission, resulting in around 200,000 deaths per annum [2]. The WHO recommends using the synthetic molluscicide Niclosamide (Bayluscide®) as a strategy to combat schistosomiasis. However, this molluscicide has secondary effects, such as bioaccumulation, resistance of the *Biomphalaria glabrata* species, and high toxicity in non-target animals [3]. Due to the aforementioned scenario, the search for more effective, low-cost molluscicides with less environmental impact began. The species *Myrcia amazonica* DC. (Myrtaceae) is known for its medicinal properties. According to previous studies, the oil from the leaves of this species has anti-inflammatory, antimicrobial, and antioxidant properties [4]. The objective of this study was to evaluate the activity of the nanoemulsion of essential oil from *Myrcia amazonica* leaves on *Schistosoma mansoni* cercariae.

Material and Methods

Myrcia amazonica leaves were collected in the Restinga de Jurubatiba National Park, Rio de Janeiro, Brazil (22° 12.594'S - 41° 35.118'W and 22° 13.461'S - 41° 35.100'W). It was identified by botanist Dr. Marcelo Guerra. The essential oil was prepared using fresh leaves of the species *M. amazonica* (3791 g) and swirled with distilled water. After this step, the plant material was immediately placed in a 5 L round-bottom flask and subjected to hydrodistillation for 4 h in a Clevenger-type apparatus. Then, the essential oil was dried using anhydrous sodium sulfate and stored in an amber glass bottle. The oil was chemically characterized using a GC–MS QP2010 gas chromatograph (Shimadzu) equipped with a mass spectrometer and a GC-2014 gas chromatograph (Shimadzu) equipped with a flame ionization detector (FID). The nanoemulsions were obtained using the low-energy input phase inversion method [5]. The constitution of the oily phase consisted of 5% (m/m) essential oil and 5% (m/m) surfactants. Then, the oil phase was homogenized for 30 minutes under magnetic stirring (700 rpm) at a room temperature of 25°C. After this period, the aqueous phase (90% distilled water) was added, dripping through a syringe under magnetic stirring (700 rpm) for 60 min.

In the Cercaricidal activity experiment, 1 mL of the nanoemulsion from *Myrcia amazonica* leaves was placed at concentrations of 20, 40, 60, 80, and 100 mg/L. After this step, 1 mL of the Cercariae suspension was added to each well. Then, 40 µL of Trypan Blue dye is added. The experiment was read every 1 hour, for a period of 4 hours, where each hour, the number of stained, that is, dead, cercariae was counted. As a

positive control, niclosamide will be used and a negative control will be distilled water and dimethyl sulfoxide (1% DMSO). To obtain the result, the number of dead Cercariae in each well was compared with the estimate of the Cercariae in 1 mL of suspension mentioned at the beginning of the experiment, with 100% mortality being admitted when the number of dead Cercariae reached the value of the estimate counted in the start of the experiment.

Results and Discussion

M. amazonica essential oil yielded 0.48%. The chemical characterization of the essential oil by GC-MS and FID allowed the identification of 67 substances, with the sesquiterpenes class being predominant. The majority compound identified was σ-cadinene with 11.7%. In the Cercaricidal activity bioassay, it was possible to observe that the nanoemulsion of *M. amazonica* leaves showed 100% mortality at concentrations of 100, 80 and 60 ppm. It was possible to calculate the lethal concentrations 50 and 90, 35.5 and 65.4 ppm, respectively.

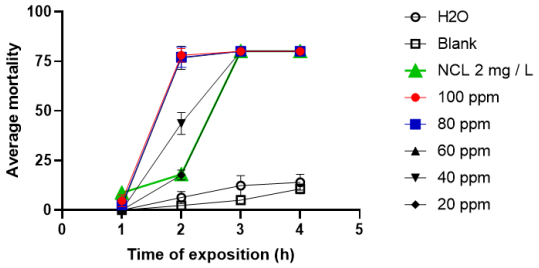

Figure 1: Dose-response relationship between mortality (percentage) of cercariae *Schistosoma mansoni* strain BH, in relation to the negative controls water and 1% dimethyl sulfoxide and positive niclosamide, after 48 h of experiment. Dose-response relationship between mortality (percentage) of *Schistosoma mansoni* cercariae strain BH, in relation to the concentrations of *Myrcia amazonica* nanoemulsion after 4 h of experiment. This experiment was carried out in triplicate on at least 3 different days.

Conclusion

This study showed the potential of nanoemulsion of *M. amazonica* essential oil on *S. mansoni* cercariae, being a way of controlling and reducing the transmission of schistosomiasis, potentially having a direct impact on regions with occurrences of this endemic disease.

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