Ocotea indecora (Schott) Mez ESSENTIAL OIL NANOEMULSION: A NOVEL ALTERNATIVE TO CONTROL *Biomphalaria glabrata*

Machado, F.P.[1*]; Rangel, L.S.[2]; Gomes K.F.[2,3], Santos, J.A.A.[2], Faria R.X.[2], Santos, M.G.[4], Fernandes, C.P[5]., Rocha, L[1].

[1]*Natural Products Technology Laboratory, LTPN, Department of Pharmaceutical Technology, Faculty of Pharmacy, Universidade Federal Fluminense, Rua, Mario Viana, 24241-000, Santa Rosa, Niterói, RJ, Brazil.*

[2]*Environmental Health Assessment and Promotion Laboratory (LAPSA), Oswaldo Cruz Institute, Rio de Janeiro, Brazil*

[3]*Graduate Program in Plant Biotechnology and Bioprocesses, Universidade Federal do Rio de Janeiro, 21941-599, Rio de Janeiro, RJ, Brazil.*

[4]*Department of Sciences, State University of Rio de Janeiro, 24435-005, São Gonçalo, Rio de Janeiro, Brazil.*

[5]*Phytopharmaceutical Nanobiotechnology Laboratory, Federal University of Amapá Foundation, Rodovia Juscelino Kubitscheck, KM-02, CEP 68903-419, Macapá, AP, Brazil.*

**fmachado@id.uff.br*

Introduction

Schistosomiasis is a neglected disease transmitted in Brazil by the trematode *Schistosoma mansoni*. The disease is associated with poor sanitary conditions in low-income communities, leading to major socio-economic impacts in tropical and subtropical developing countries. The World Health Organization (WHO) preconizes the use of niclosamide to control intermediate hosts, such as *Biomphalaria glabrata* mollusks, in endemic areas [1].

However, chemical pesticides are expensive and often toxic to humans and the environment. In this context, the search for new compounds of natural origin is in the spotlight for the development of new pesticides. Volatile oils have a complex chemical matrix, often with biological potential. They are consequential substances from plant-environment interaction, they often have repellent or biocidal action, suggesting that they are promising candidates for novel biopesticides. However, essential oils are constituted of lipophilic substances, such as monoterpenes, sesquiterpenes, and phenylpropanoids, which makes them difficult the application in aquatic environments [2].

Thus, essential oil nanoemulsification is a strategy to enable use in aquatic matrices, as well as rationalize actives and potentialize pharmacological effects due to increased permeability and other factors that can be associated with nanosized droplets. Nanoemulsions are thermodynamically stable systems, generally stabilized by surfactants, with average nanodroplts between 20 to 200 nm, that have been widely used in the pharmaceutical, food, and cosmetic industries [2].

Ocotea indecora, popularly known as “canela-sassafrás”, is a native plant that can be found in the Restinga de Jurubatiba National Park, in the north of Rio de Janeiro state. So far, the essential oil is poorly described in the literature, showing the insecticide potencial as the main bioactivity [2].

Therefore, this study aimed to evaluate the molluscicidal potential of the native and endemic plant *Ocotea indecora* from the Atlantic Forest in *Biomphalaria glabrata* mollusks.

Material and Methods

The *O. indecora* leaves were collected and submitted to hydrodistillation in a modified Clevenger apparatus type for 3h. Then, the essential oil was chemically characterized by gas chromatography coupled with mass spectrometry and a flame ionization detector. The chromatographic conditions used and data analysis were previously described by Machado et al. (2023) [2]. The nanoemulsion was prepared by low energy approach by phase inversion. The oil phase constituted 2 % (w/w) of essential oil, 2 % (w/w) of surfactants polysorbate 20 and sorbitan monooleate (1 : 4 proportion), and 96 % (w/w) of deionized water. The nanoformulation was characterized by its average droplet size (ADS) by dynamic light scattering (DLS), and zeta potential (ZP) by electrophoretic light scattering (ELS) in a Zetasizer

Advance Lab Blue (Malvern, UK) at room temperature (25 °C). The nanoemulsion stability was also evaluated after 15 days of preparation. The nanoemulsion was diluted (1 : 20) in deionized water. All analyses were realized in triplicate. The molluscicidal assay was realized as described by Santos et al., (2017) [3]. Adult mollusks with 10 to 12 mm were deposited in 24-well-plates and exposed to 2 mL of the nanoemulsion (100 – 10 µg/mL, expressed in essential oil), nanoemulsion blank (100 µg/mL), Dimethyl sulfoxide (DMSO) at 1 %, positive control (niclosamide 1 µg/mL), and negative control (deionized water), respectively. The mortality was observed after 24 hours of exposure. The median lethal concentration (LC_{50}) was estimated by probit with SAS software, and one-way ANOVA was realized in GraphPad prism (ver. 8) with a significance level of $p < 0.05$.

Results and Discussion

The essential oil yielded 0.79 % (w/w) with the sesquirosefuran (83.4 %) as a major metabolite, followed by (Z)-β-farnesene (3.6 %). Previously authors have described the *O. indecora* essential oil chemical profile, showing sesquirosefuran as a major metabolite, and in that way, corroborating with the actual study [2]. The nanoemulsion showed a typical bluish transparent aspect for colloidal systems, characteristic of the Tyndall effect. The ADS was 102.8 ± 0.754, with 0.243 ± 0.014 of Polydispersity index (PdI), and -34.66 ± 0.007 mV of ZP. These results are in accordance with those described in the literature for nanoemulsioned systems [2]. Also, the nanoemulsion maintained the physicochemical characteristics after 15 days of preparations showing ADS of 97.27 ± 0.310, 0.310 ± 0.038 of PdI, and -32.28 ± 0.574 of ZP.

The essential oil showed LC_{50} after 24h of 64.17 (52.6 - 74.2) µg/mL and 22.66 (18.50 – 27.02) µg/mL for the nanoemulsion, showing 3x potentiation of the essential oil mediated by the nanoemulsification. This can be explained by the nanometer droplet size, which may improve the permeability and disponibility of the active substances in the oil. The WHO established that molluscicidal agents must present LC_{50} less than 100 µg/mL to be considered a promissory agent [4]. In that context, both essential oil and nanoemulsion can be considered promisor molluscicidal agents. As the sesquirosefuran is the main component in the oil (± 80%) it suggests that may present a key role in the molluscicidal effects observed.

Conclusion

This work shows the potential of the essential oil of *Ocotea indecora* to assist in schistosomiasis control. Therefore, we describe a potential molluscicide nanoemulsion that was prepared by a green concept and can be of great interest for the prospection of novel molluscicidal systems.

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