INFLUENCE OF Β-CARYOPHYLLENE SESQUITERPENE ON THE STABILIZATION AND ANTIFUNGAL POTENTIAL OF NANOEMULSIONS BASED ON *Eugenia sulcata* Spring ex. Mart (Myrtaceae) ESSENTIAL OIL

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Introduction

The increasing demand for sustainable products and ecologically responsible alternatives has driven the search for technological innovations in the pharmaceutical sector. Natural products represent a rich source of pharmacologically relevant substances, particularly due to the bioactive metabolites found in their essential oils [1]. These compounds, primarily monoterpenes, sesquiterpenes, and phenylpropanoids, often exhibit instability under conditions of light, temperature, and other factors, resulting in a loss of efficacy. In this context, nanotechnology emerges as a promising strategy to enhance the performance of products derived from natural actives through nanoencapsulation, aimed at increasing stability, reducing potential toxic effects, and controlling the release of encapsulated substances [2]. *Eugenia sulcata* Spring ex. Mart, an endemic plant of Brazil found in the Jurubatiba Restinga National Park from *Myrtaceae* family, remains understudied despite its significant biotechnological potential [3, 4]. Its major component, β-caryophyllene (βC), is a widely distributed sesquiterpene known for its antimicrobial and pesticide effects [5]. Therefore, this work aimed to prepare and characterize the essential oil of *E. sulcata* and evaluate the influence of β-caryophyllene on the stabilization of its nanoemulsions and the antifungal activity of the essential oil.

Material and Methods

The fresh leaves (4950g) were collected in the Restinga de Jurubatiba National Park, Carapebus, Rio de Janeiro, Brasil (22º12.984'S 41º35.008'O e 22º13.050'S 41º35.121'O). SISBio/ICMBio: 13659-18 and SISGen: A491A56. The essential oil was extracted through hydrodistillation, utilizing a modified Clevenger apparatus. Nanoemulsions were produced using a low-energy method via homogenization in a vortex mixer with 90% deionized water and 10% oil phase, comprising either *E. sulcata* or βC combined with a nonionic surfactant (polysorbate 20 or 80 - P20/P80), and then observed macroscopically in vial-type containers. Droplet size was assessed using dynamic light scattering (DLS). The antifungal activity was tested with the inoculum of *Aspergillus flavus* (RC 2054) and *Aspergillus parasiticus* (NRRL 2999), incubated at 27.5ºC for 72 hours, with daily measurements of the colony diameter taken.

Results and Discussion

The extraction of fresh leaves yielded an essential oil with a content of 1.02% (w/w), displaying a transparent and clear appearance. Chemical analysis revealed the presence of 10 identified compounds, with sesquiterpenes accounting for the largest fraction at 66.2% of the total. β-Caryophyllene emerged as the main component, comprising 34.4%, followed by α-pinene (14.7%) and trans-calamenene (10.0%) [3, 4]. These results align with previous studies. However, Ramos and colleagues (2011) reported a different chemical profile, with a predominance of monoterpenes, where 1,8-cineole was the primary compound of this class (19.6%), accompanied by a high concentration of α-pinene (12.2%) [6]. The discrepancies in essential oil compositions can be attributed to intrinsic and extrinsic variables, such as geographical location, climatic conditions, harvesting time, extraction methods, and soil nutrients. Despite the variations observed across different studies, the consistent presence of β-caryophyllene as a major component in the essential oils of this species suggests that this compound may serve as a relevant phytochemical marker.

Formulations based on P20, with isolated β-caryophyllene and essential oil, exhibited an opaque appearance and phase separation after 24 hours. In contrast, the nanoformulations using P80 were more promising: as the surfactant-to-oil ratio (SOR) increased, they became more translucent and showed no phase separation for at least a week. For the essential oil, formulations with a lower SOR still displayed slight translucency, suggesting the potential for the addition of a co-surfactant. This difference may be attributed to the greater compatibility of β-caryophyllene with P80. When the hydrophilic-lipophilic balance (HLB) of an oil approaches the HLB of a surfactant, fine droplets are formed. Thus, the HLB of β-caryophyllene may be close to 15.0 (the HLB of P80), favoring the stabilization of the essential oil. Additionally, Ostwald ripening, which involves the transfer of hydrophilic compounds from smaller to larger droplets, is a mechanism of nanoemulsion destabilization. Since P20 is more hydrophilic than P80, it likely contributes to the solubilization of compounds, resulting in less stable nanoemulsions.

The dynamic light scattering analysis indicated average droplet sizes ranging from 47.6 to 80.9 nm for the nanosystems prepared with P80, which were selected for the antifungal assay. The inhibition of colonies of *A. parasiticus* and *A. flavus* indicates a fungistatic effect after 72 hours, but no fungicidal activity was observed. Nanoemulsions without active ingredients did not affect colony growth. After 24 hours, the essential oil did not inhibit *A. parasiticus* ($p<0.05$) but showed a fungistatic effect against *A. flavus* (21.50%; $p<0.01$). β-Caryophyllene inhibited both strains ($p<0.001$). After 48 hours, there was a significant increase in inhibition, which was maintained at 72 hours. No statistical difference was found between the essential oil and β-caryophyllene, suggesting that the sesquiterpene is not the only antifungal agent present in the chemical composition of the essential oil of *E. sulcata*. The nanoemulsions enhanced the fungistatic effect after 24 hours ($p<0.05$), proving effective at concentrations 100 times lower than the oil, likely due to the increased surface area and bioavailability.

Conclusion

It is concluded that β-caryophyllene plays a crucial role in the stabilization and biological effects of nanoemulsions derived from the *Eugenia sulcata* essential oil. However, it is not the only component responsible for the observed effects, highlighting the need for further investigations into the chemical composition of this oil. Additionally, this study contributes to the preservation of biodiversity and emphasizes the importance of researching native plants in Brazil, pointing to the feasibility of creating sustainable products that can help address public health issues.

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