

Title: Evaluation of antifungal activity of plumieride and plumieridine compounds against *Cryptococcus neoformans* and *Cryptococcus gattii*

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Abstract:

Cryptococcosis is an invasive fungal infection caused mainly by the pathogenic species *Cryptococcus neoformans* and *Cryptococcus gattii*. This disease affects approximately 1 million individuals annually worldwide with an estimated death rate of 60%. Currently, antifungal drugs show some limitations related to toxicity and resistance, which emphasizes the importance of search for new drugs. Our group identified the antifungal potential of two iridoids - plumieride and plumieridine - isolated from the aqueous extract of seeds of *Allamanda polyantha* (Apocynaceae) - plumieride and plumieridine - isolated from *Allamanda polyantha* (Apocynaceae). The objective of this study is to evaluate the biological activities of plumieride and plumieridine compounds against *C. neoformans* and *C. gattii*. Plumieride and plumieridine cause morphological change and decrease polysaccharide capsule in *C. neoformans*. Assays to evaluate the toxicity and cytotoxicity of plumieridine compounds were performed using BALB/c mice. Three concentrations (20 mg/kg, 30 mg/kg and 40 mg/kg) of plumieridine were tested in 20 day-old mice, with daily administration for 15 days. Mice survival was evaluated during this period and for 15 additional days post-treatment. These concentrations showed no toxicity for mice. Furthermore, we propose the use of a practical model to monitor the treatment efficacy of these compounds against *C. neoformans* and *C. gattii* infection. *C. neoformans* and *C. gattii* mutant strains that constitutively express the TurboFP635 reporter gene were constructed. Phenotypic analyses related to the major virulence factors (capsule, melanin and growth at 37 °C) were conducted with these mutant strains, showing no alterations in these traits. Our approach involves mice infection with *C. neoformans* and *C. gattii* fluorescent strains, followed by fluorescence-based imaging analyses in vivo to evaluate the infection progress during treatment with plumieride and plumieridine compounds. Our results may contribute for description and development of new antifungal compounds for cryptococcosis treatment.

Keywords: *Cryptococcus neoformans*, *Cryptococcus gattii*, plumieride, plumieridine fluorescence.

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