In vitro evaluation of inhibitory activity of some species of *Croton* and *Piper* essential oils in secreted proteases of *Pseudallescheria boydii*

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Pseudallescheria boydii is a fungal organism known as a soil and water natural inhabitant and decaying vegetation, which has become an increasingly recognized pathogen among immunocompromised individuals, including HIV infected patients. It is related to cause madura foot and in severe cases, invasive infection of various organs (central nervous, cardiovascular, respiratory systems). One potential drug target for the pharmaceutical industry is proteases. Proteases regulate the fate, localization, and activity of many proteins, modulate protein-protein interactions, create new bioactive molecules, contribute to the processing of cellular information, and generate, transduce, and amplify molecular signals. The aim of this study was to evaluate the inhibitory activity of some species of *Croton* and *Piper* essential oils (EOs) in *P. boydii* secreted proteases. The EOs of 3 species of *Croton* plants: *C*. *tricolor* (sacatinga), *C*. *pulegioides* (velandinho) and *C*. *blanchetianus* (marmeleiro); and 4 species of *Piper* plants: *P*. *marginatum* (capeba-cheirosa), *P*. *tuberculatum* (pimenta darta), *P*. *hispidum* (matico-falso) and *Piper* sp. were obtained by hydrodistillation using a Clevenger-type apparatus for 4 h. The identification of EO was performed by GC/FID and GC/MS in an Agilent 6890N and an Agilent 5973N systems with HP-5MS fused silica capillary columns (30 m X 0.25 mm X 0.25 µm). Hydrogen was used as carrier gas for GC/FID and helium for GC/MS, both with a flow rate of 1.0 mL min^{-1}.Oven temperature was raised from 60 to 240 °C at 3 °C min^{-1}. Mass detector was operated in electronic ionization mode at 70 eV. The minimum inhibitory concentration (MIC) was evaluated in triplicate according standard method from the Clinical and Laboratory Standards Institute (CLSi). To evaluate the activity of *Croton* and *Piper* EOs in secreted proteases of *P. boydii* it was performed assays of proteolytic activity inhibition according to Buroker-Kilgore and Wang. Cell-free supernatants of *P. boydii* grown in RPMI broth, were incubated with bovine serum albumin (0.1 mg mL^{-1}), as proteic substrate, and some pH buffers. The calculated MIC were >1250 µg mL^{-1}.The preliminary results showed that the extracellular peptidases were able tohydrolizate the proteic substrate on pH 6 in RPMI broth. The proteolytic inhibition obtained ranging of 29 % for *P. tuberculatum* and 100 % for *C*. *pulegioides* and *C.tricolor* (48 µL mL^{-1}) in RPMI supernatant of *P. boydii*. These results showed promising activity and suggests a possible target that justifies the antifungal activity of *Croton* and *Piper* EOs.

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