



METABOLITES OF *XYLARIA CUBENSIS* AND *COLLETOTRICHUM* SP., ENDOPHYTIC FUNGI FROM *EUGENIA* *BRASILIENSIS*

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Eugenia brasiliensis (Myrtaceae) is a Brazilian species that produces small edible fruits with a sweet cherry-like flavor. Its wood is used in carpentry and woodworking. This specie has also been used in traditional medicine as anti-reumatic, diuretic and anti-inflammatory [1]. As part of our studies on bioactive metabolites produced by endophytic fungi, we chose *E. brasiliensis* for studies. The endophytic fungi are microorganisms living asymptotically in the intercellular space of host plants and establish a mutualistic relationship with its host during a part or all their life [2]. Seventeen endophytic fungi were isolated from stem, leaves and ripe fruits using traditional methodology [3]. Among these, *Xylaria cubensis* and *Colletotrichum* sp. were chosen for detailed chemical investigation due to their anticholinesterasic and antifungal (*Cladosporium sphaerospermum*) activities. *Xylaria cubensis* and *Colletotrichum* sp. were cultivated on PDB medium (large scale) for 28 days at 25 °C. The broth was separated from the mycelium by filtration and the crude extracts were obtained by extraction with EtOAc (3 x 50% of the broth volume each) and dried in rotatory evaporator. The crude extracts were evaluated in TLC, HPLC-DAD and ¹H NMR and subjected to bioassays to evaluation of the antifungal, anticholinesterasic and cytotoxic activities. The crude EtOAc extracts were fractionated by column chromatography using reversed-phase silica gel (C18) and eluted with CH₃OH:H₂O gradient affording 6 fractions. The fraction Eb_Fr2 from *Xylaria cubensis* was submitted to HPLC preparative and resulted in the isolation of the 5-carboxy-6-hidroxy-3-methyl-3,4-dihydroisocoumarin, 7-hidroxy-mellein [4] and cytochalasin D [5]. The fraction Eb_Fr1 from *Colletotrichum* sp. was submitted to HPLC preparative and resulted in the isolation of the diketopiperazine *cycle*(Pro-Phe) [6]. The structures were elucidated by analysis of ¹H NMR and compared with the literature. These substances belong to important classes with several biological activities such as antioxidant, cytotoxic, antifungal and antibacterial [4,5,7]. These results reinforce the potential of these microorganisms as sources of bioactive natural products and corroborate the assumption of ecological interaction between the endophyte and its host plant. To our knowledge this is the first report of endophytic fungi from *Eugenia brasiliensis*.

References:

- [1] Morretes, B. L. Donato, A. M. 2007. Anatomia foliar de *Eugenia brasiliensis* Lam. (Myrtaceae) proveniente de áreas de restinga e de floresta. Brazilian Journal of Pharmacognosy. 17: 426-443.
- [2] Kusari, S.; Pandey, S. P.; Spiteller, M. 2013. Untapped mutualistic paradigms linking host plant and endophytic fungal production of similar bioactive secondary metabolites. Phytochemistry. 91: 81-87.
- [3] Helder, L. T. et al. 2005. Benzopyrans from *Curvularia* sp., an endophytic fungus associated with *Ocotea corymbosa* (Lauraceae). Phytochemistry. 66: 2363-2367.
- [4] Oliveira, C. M. et al. 2011. Dihydroisocoumarins produced by *Xylaria* sp. and *Penicillium* sp., endophytic fungi associated with *Piper aduncum* and *Alibertia macrophylla*. Phytochemistry Letters. 4: 93-96.
- [5] Cafeu, M. C. et al. 2005. Substâncias Antifúngicas de *Xylaria* sp., Um Fungo Endofítico Isolado de *Palicourea Marcgravii* (Rubiaceae). Quim. Nova. 28: 991-995.
- [6] Biasetto, C. R. 2011. Avaliação química e biológica do fungo endofítico *Schizophyllum commune* isolado de *Alchornea glandulosa*. 145f. Dissertação – Instituto de Química, Universidade Estadual Paulista, Araraquara.
- [7] Chapla, V. M.; Biasetto, C. R.; Araujo, A. R. 2013. Fungos Endofíticos: Uma Fonte Inexplorada e Sustentável de Novos e Bioativos Produtos Naturais. Rev. Virtual Quim. 5: 421-437.