10th Brazilian Conference on Natural Products
XXXVI RESEM
4-7 November 2025, Belo Horizonte, MG, Brazil

Section: 02

BIOASSAY-GUIDED FRACTIONATION OF POTENTIAL BLOCKERS OF SARS-COV-2 RBD :ACE2 INTERACTION FROM STINGLESS BEE (*Melipona mondury*) GEOPROPOLIS BY COUNTERCURRENT CHROMATOGRAPHY.

Cristiane C. dos Santos[1*], Rafael F. dos Santos[1], Suzana G. Leitão[3], Mariana F. Campos[4], Diego Allonso[3], Gilda G. Leitão[2], Rosane N. Castro[1]

ggleitao@yahoo.com.br

1- Instituto de Química, UFRRJ, Seropédica, Brazil. 2- Instituto de Pesquisas de Produtos Naturais, UFRJ, Brazil. 3- Fac. Farmácia, UFRJ, Brazil. 4-Prog. Biotecnol. Veg.e Bioproc., UFRJ, RJ, Brazil.

Geopropolis, a resinous bee product enriched with soil and clay and produced by stingless bees such as *Melipona mondury*, was investigated for its potential antiviral activity. Extracts were obtained via ultrasound-assisted extraction using 96°GL cereal alcohol (EEGP-U1) from geopropolis collected at Barra da Tijuca Forest (Rio de Janeiro, Brazil). The dried extracts were reconstituted in a methanol:water mixture (7:3, v/v) and subsequently partitioned with hexane (FrH) and dichloromethane (FrDCM). The inhibitory effect of these extracts on the interaction between the SARS-CoV-2 receptor-binding domain (RBD) and the human ACE2 receptor was evaluated using an *in vitro* bioluminescence assay (Lumit®-Promega). The EEGP-U1 extract showed 60.90% inhibition, while FrH, FrDCM, and the MeOH:H_2O extract exhibited 38.20%, 79.23%, and 75.71% inhibition, respectively. Based on these results, FrDCM was selected for further fractionation using countercurrent chromatography (CCC), a liquid–liquid partition technique that enables purification without solid supports. Solvent system optimization for CCC was performed using shake-flask experiments, in which 2 mg of FrDCM was tested in various proportions of petroleum ether, ethyl acetate, methanol (or ethanol), and water. The system composed of petroleum ether–ethyl acetate–methanol–water (1:0.8:1.1:0.6, v/v) was selected for the fractionation of 123 mg of FrDCM in normal elution mode (2.0mL/min, 3mL fraction), dissolved in 5 mL of both phases and injected into a 112 mL column of an HT Prep, yielding 80 fractions. CCC fractionation successfully led to the isolation and purification of vanillin (2.1 mg, 70% purity by HPLC-DAD), demethoxymatteucinol (8.8 mg, 91% purity), and cryptostrobin (3 mg, 77% purity), along with a mixture of 40:60 *C*-methylated flavanones, strobopinin and cryptostrobin, respectively (14.3 mg,).These results demonstrate the efficiency of CCC in separating bioactive constituents from *M. mondury* geopropolis and provide access to highly purified compounds for future evaluation of their antiviral activity.

Keywords: *C-methylated flavanones, countercurrent chromatography, coronavirus*

Acknowledgments: *to CAPES, FASPERJ and CNPq*