Semester grant and grant for short-term stays of the "Aktion" Austria-Czech Republic

SCHOLARSHIP REPORT

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Grant period: winter term 2021 (3 months)

The project has been carried out in a research laboratory at the Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, supervised by prof. Franz Bucar. I have been working on natural compounds with antimicrobial properties to expand the range of antimicrobial drugs and prevent the spread of multi-resistant strains of bacteria. More than 30% of mortality caused by resistant strains is due to tuberculosis [l]. The uprising number of multidrug-resistant strains of M. tuberculosis, and non-tuberculous mycobacterial strains, has become a critical global health concern [2]. An urgent need for new antibacterial agents is attracting the attention of the scientific community.

The antimicrobial activity of prenylated and geranylated flavonoids isolated from Paulownia tomentosa Steud. (Paulowniaceae) and Morus alba L. (Moraceae) are perspective options for new antibiotics. Both of these timber trees are used in traditional Chinese medicine for their various properties and represent a rich source of secondary metabolites such as prenylated flavonoids and other prenylated compounds. These properties have demonstrated antiviral, anti-inflammatory, antioxidative, and antimicrobial activity. Antibacterial activity against Staphylococcus aureus, including methicillin-resistant strains, has been reported recently, but the antimicrobial properties of these compounds against mycobacteria have not been described in detail, yet.

We have evaluated an antimicrobial activity of twelve compounds du ring the grant period against three strains of mycobacteria. At first, a wild type of Mycobacterium smegmatis was examined. Minimum inhibitory concentration (MIC) was discovered by microdilution method according to the protocol. The MIC was set as the lowest concentration of a tested sample, which still inhibited the growth of bacteria. Considering valuable results and low MIC values, selected compounds were also measured against different strains of mycobacterium including M. fortuitum and M. phlei.

There is no evidence that bacteria can develop resistance against natural products. Thus, we want to understand the mechanism of antimicrobial activity against bacteria. Ways which natural compounds kill bacteria are mostly multiple. One of the mechanisms is based on disruption of the membrane of bacteria, so we pursue to the membrane integrity assay. ln order to evaluate the impact on membrane permeabilization of M. smegmatis we tested the effect at sub-inhibitory concentration (1/4 of MIC) using Live/Dead bacterial viability kits. A heat-treated culture served as a negative control, an untreated culture as a positive control. Fluorescence quenching of SYTO9 by propidium iodide was measured for six compounds and measurements were performed in triplicate. According to the results we evaluated the effect on membrane permeability at the same concentration as MIC, as well. Three compounds showed unreasonable results, so we tried to explain the discrepancy by measuring the fluorescence in the environment with dead bacterial culture and without bacteria. The

effect of the temperature on the natural compound in conditions comparable to the assay of establishing the MIC was measured specifically.

Eventually, the set of compounds revealed promising results for a development of new, perspective antibiotics for tuberculosis. Regarding MIC of prenylated flavonoids and Diels-Alder adducts the set of compounds will be measured against tuberculous type of mycobacterium by one of prof. Bucar' s colleagues over Europe.

Due to the traineeship the cooperation between Masaryk University in Brno, Czech Republic and University of Graz, Austria has launched. Prof. Bucar is a distinguished researcher and I appreciate the opportunity to become a part of his team for a grant period. During the traineeship I experienced new methodology and techniques applied in evaluation of the antimicrobial activity of natural products against mycobacteria that I haven't been working with before. 1 learnt to describe and establish one of the mechanisms that natural antibiotics effect bacteria. Based on the valuable experience I will applicate the methodology learned abroad at my home Department of Natural Drugs at Masaryk University in Brno and I pian to test natural compounds against different strains of bacteria.

Thanks to OeAD-GmbH and AKTION financed by the Aktion Austria-Czech Republic the traineeship at the University of Graz supervised by prof. Franz Bucar provided me an opportunity to improve my laboratory skills and gain precious contact that enrich my future research career and international background experience.

[1] Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., ... & Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. lnfection and drug resistance, 11, .1645.

[2] Šimunovic, K., Solnier, J., Alperth, F., Kunert, O., Smole Možina, S., Bucar, F. (2021). Efflux Pump Inhibition and Resistance Modulation in Mycobacterium smegmatis by Peucedanum ostruthium and Its Coumarins. Antibiotics, 10(9), 1075.