PURIFICATION OF SECONDARY METABOLITES FROM THE FERMENT BROTH OF ENDOPHYTIC FUNGI OF *TAXUS BACCATA*

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Fungal endophytes are living in plant tissues without any harmful effects. Remarkable part of these microorganisms are able to produce secondary metabolites resulted from the high biochemical pressure of the host environment. Furthermore, these synthetized metabolites could be beneficial for host plants to facilitate their nutrient uptake, to take part in the systemic regulation, to keep the fitness at high level or to prevent the pathogen infections. Moreover, due to the various bioactivities of these secondary metabolites involving the antimicrobial effects, they could be potential candidates for the future applications in medical treatments against human pathogenic microorganisms.

In this study, the extracts of ferment broths originated from the endophytic isolates of *Taxus baccata* were screened for their antibacterial effects. Tests were carried out against selected bacteria including *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Micrococcus luteus,* and *Pseudomonas aeruginosa* on microtiter plates. For further examinations, the H1-3a-RB endophytic isolate (identified as *Penicillium sp*.) was selected based on the remarkable inhibitory effect of its ferment broth on *B. subtilis* and *E. coli.* For large scale purification it was cultivated in large volume and the secondary metabolites were extracted by ethyl-acetate. Pooled extracts were introduced into an activity guided multi-step chromatographic separation. The first step within the procedure was a flash chromatographic separation using normal phase cartridges and toluene/isopropanol as mobile phase. For the injection, the extract was directly evaporated onto the stationary phase. During the separation 70 fractions were collected with equal volumes; all of these were tested against *B. subtilis* on microtiter plates. The active fractions were further examined by a UHPLC-HRMS instrument using J'sphere ODS-H80 (250*2,1 mm, 4 µm) column and water/methanol gradient containing formic acid. Than the pooled crude fractions were subjected onto a reverse phase preparative column, where the collected fractions were tested again in the antibacterial

bioassay and analysed with UHPLC-MS. The recorded total ion chromatograms of both crude and partially purified extract were compared, and some constituents of the metabolite were identified.

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