**Anti Mycobacterial Activity of Aspergilic acid derivatives from Marine-derived Fungi *Aspergillus ostianus***

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**Abstract**

**Background and aims.** Tuberculosis remains a global problem due to the ability of this bacterium to form a dormant state that is tolerant to first-line drugs. This ability is one of the reasons why tuberculosis therapy requires a long and complex time. This lengthy therapy is a factor in the failure of tuberculosis therapy, leading to the high number of cases of drug-resistant tuberculosis. Therefore, it is necessary to search for alternative compounds that are capable of killing both active and dormant forms in the hope of shortening the duration of tuberculosis therapy in the future. Meanwhile, the marine environment is home to macro- and microorganisms with high biodiversity and has been recognized as a potential source of beneficial bioactive compounds. Therefore, this study aims to isolate the active compounds from Marine-derived Fungi *Aspergillus ostianus* with antimycobacterial activity in active and dormant state and to investigate the mechanism of their action.

**Methods.** This study included large-scale fermentation of A. ostianus (FSPL 3). Subsequently, extraction, fractionation, sub-fractionation, and purification of active compounds were performed using various extraction and chromatographic techniques, applying a bioassay-guided separation approach under both aerobic and hypoxic conditions against *Mycobacterium smegmatis* as a surrogate model. The antimycobacterial activity is determined using the microdilution method and visualization by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The isolated compounds were identified using mass spectrometry (MS) and one- and two-dimensional nuclear magnetic resonance (NMR) spectroscopy. The mechanism of action studies was performed by time-kill curve determination.

**Results.** Isolation of active compounds from fermentation medium (EM) and biomass (EB) revealed two active compounds from EM and one from EB. Analysis of MS and 1D and 2D NMR identified these three compounds as neohydroxyaspergillic acid (NHAA), hydroxyaspergillic acid (HAA), and neoaspergillic acid (NAA). All three compounds exhibited antimycobacterial activity under both aerobic (active) and hypoxic (dormant) conditions, with MIC values of 1.56 μg/mL for NHAA and 3.125 μg/mL for both HAA and NAA. Time-kill curve analysis demonstrated that all three compounds exhibited bactericidal activity under both aerobic and hypoxic test conditions, within a concentration range of 8x MIC and an incubation period of ≥ 48 hours.

**Conclusion.** Aspergillic acid derivatives, namely neohydroxyaspergillic acid (NHAA), hydroxyaspergillic acid (HAA), and neoaspergillic acid (NAA), from *A. ostianus* exhibited potent antimycobacterium in active and dormant state.

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