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EPIGENETIC MODIFIERS AS TOOLS FOR THE STUDY OF SECONDARY METABOLITES PRODUCED BY FUNGI FROM MALAYSIA AND POLAR REGIONS

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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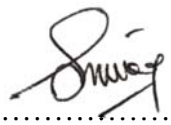
Signature of Student	:	 ……..
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ABSTRACT

Fungi produce a wide range of secondary metabolites that have various biological activities. Secondary metabolites production of fungi can be modified by different approaches, including culture-dependent methods, epigenetic modifiers, and genomic-based methods. In this study, secondary metabolite production was explored in the presence of epigenetic modifiers (suberoylanilide hydroxamic acid, S-adenosylhomocysteine, valproic acid, sodium butyrate, and 5-azacytidine) by applying an in-house protocol named MECSUS (Microtiter plate, Elicitors, Combination, Solid-phase extraction, UHPLC, Statistical analysis). The MECSUS protocol was modified, strengthen, and the procedure for culturing sporulating and non-sporulating fungi at a micro-scale level was successfully developed. This study included Malaysian (5) and polar fungi, which are Arctic (40) and Antarctic (10) fungi. A total of forty-one Arctic fungi were isolated from soil samples collected in Longyearbyen, Svalbard Island, Norway. Five fungi, namely *Geomyces* sp. D1D1, *Pleosporales* sp. B2C2, *Talaromyces aculeatus* B1-3*, Penicillium samsonianum* D2CD2-2, and *Aspergillus nomius* D1D1 were identified using microscopical, morphological, and molecular techniques. The different combinations and concentrations of epigenetic modifiers were added to the media of the fungi. All crude extracts were analysed using high-performance liquid chromatography (HPLC). Preliminary screening of the antimicrobial activity of the crude extracts using the MTT assay was evaluated against *Staphylococcus aureus*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Esterichia coli*, and *Candida albicans*. Six crude extracts (SHSF, A1C3, B2C2, B1-3, D1D1, and D2CD2-2) were exhibited antibacterial activity, however, three of them (A1C3, B1-3, and D1D1) did not demonstrate antifungal activities. Based on the antimicrobial activity and HPLC data analysis, three fungi were selected for further investigation which are one extract from Malaysian fungi (*Aspergillus longivesica* SHSF), and two extracts from Arctic fungi (*Pleosporales* sp. B2C2, and *Penicillium samsonianum* D2CD2-2). These extracts were fractionated using preparative HPLC and then purified by semi-preparative HPLC. Chemical structures of the isolated compounds were determined based on spectroscopic methods, including MS, NMR, and UV/Vis. An extract derived from *A. longivesica* was found to contain one major and one minor known compound, identified as avenaciolide-2 and avenaciolide-1, respectively, via comparison of their spectral data. Curvulin was isolated from *Pleosporales* sp. Extract and 2,3-dihydro-2-hydroxy-2,4-dimethyl-5-trans propenylfuran-3-one was identified from the extract *Penicillium samsonianum.* Based on the HPLC analysis, suberoylanilide hyroxamic acid (SAHA) and S-adenosylhomocysteine (SAHC) increased the production of secondary metabolites in the tested fungi. The usage of microtiter plate as massively parallel fermenters associated with robustly validated procedures in the MECSUS protocol and the addition of epigenetic modifiers allows screening a large number of fungi in various growth conditions for studying the production of secondary metabolites in short times and at a relatively low cost.

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