UNIVERSITI TEKNOLOGI MARA

SECONDARY METABOLITES FROM SELECTED FUNGAL ENDOPHYTES FROM *UNCARIA* SP. AND SVALBARD PLANTS

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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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ABSTRACT

Endophytes are microorganisms that inhabit host plants asymptomatically and have a fascinating potential as a source of new drug leads in many sectors. Studying microorganisms from a variety of biotopes increases the chances to isolate structurally diverse metabolites. In this respect, psychrophiles from the Arctic fulfil the criteria of an interesting source of potential new drugs. However, endophytes from tropical regions should be able to resist stronger competition through the production of various antimicrobial agents. This study included 31 endophytic fungi that were isolated from ten plants from Longyearbyen, Svalbard Island, Norway, and 54 endophytic fungi obtained from three plants belonging to the *Uncaria* family growing in UiTM Puncak Alam's biological reserve, Malaysia. The objective of this study was to isolate secondary metabolites from endophytic fungi whose extracts demonstrated antimicrobial properties. Ethyl acetate extracts of all the isolates were analysed by HPLC and evaluated for preliminary screening of antimicrobial activity against S. aureus, *E. faecium*, *P. aeruginosa*, *E. coli*, and *C. albicans*, using the MTT assay. From the analysis of the above data, 4 out of 85 (3 Malaysian and 1 Arctic) endophytic fungal isolates were selected for further investigation. These include *Nigrospora oryzae*, *Diaporthe phaseolorum*, *Trichoderma virens*, and *Poaceicola* sp., which were identified by morphological and genetic techniques. The crude extracts were fractionated, and their components purified by semi-preparative HPLC. Chemical structures were determined based on spectroscopic methods including MS, NMR, UV/Vis, ECD and X-ray diffraction techniques. The *Nigrospora oryzae* extract included two major and three minor compounds. They were subsequently identified as pestalopyrone, hydroxypestalopyrone, 4-dehydroxyaltersolanol A, macrosporin, and altersolanol B, respectively, via comparison of their spectral data. The absolute configuration of 4-dehydroxyaltersolanol A was unambiguously confirmed by X-ray crystallography, ECD spectral analysis in combination with quantum chemistry simulations. An extract derived from *Diaporthe phaseolorum* was found to contain cytochalasins H and J. From the *Trichoderma virens* culture, two compounds were identified, namely gliocladic and heptelidic acids. Finally, from the culture of *Poaceicola* sp. one known compound was identified, annularin D. Five new polyketides, including (-)-cleanarol C ($C_{13}H_{14}O_4$), (-)-3,8-dihydroxy-3-hydroxymethyl-6-methoxy-4,5-dimethyl-isochroman-1-one ($C_{13}H_{16}O_6$) and svalbardines A ($C_{16}H_{14}O_6$), B ($C_{32}H_{28}O_{12}$) and C ($C_{10}H_{14}O_4$) had their structures established. Svalbardine A was determined as a new pyranochromene, while svalbardine B was assigned a new carbon skeleton based on a spiro chromone-oxanaphthalene frame. Svalbardine C was determined as a hydroxylated derivative of annularin D, while the remaining two compounds were new enantiomers of known structures. Most of the known compounds mentioned above were previously recognised for their antimicrobial properties and can be regarded as responsible for the overall activity of the crude extracts during the MTT screening, thus vindicating the initial hypothesis. It is therefore believed that this work could be the basis for a more targeted search of novel antimicrobials.

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