UNIVERSITI TEKNOLOGI MARA

DEVELOPMENT OF A MULTIFACETED (MECSUS) PROTOCOL IN THE SEARCH FOR NOVEL BIOACTIVE ENTITIES FROM MICROORGANISMS

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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 result of my own work, unless otherwise indicated or acknowledged as referenced work. This topic has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

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

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ABSTRACT

The advent of new chemical genetic tools and high-throughput screening technologies and platforms have already led and will continue to lead in accessing the natural product diversity of microorganisms. This thesis represents a continuation of the work on the investigation into means of making better use of the advantages of new technologies and the OSMAC approach (One Strain Many Compounds) in drug discovery that the Atta-ur-Rahman Research Institute of Natural Product Discovery (RiND) at MARA University of Technology has engaged in.
The result of this thesis is the MECSUS (**M**icrotiter plate, **E**licitors, **C**ombination, **S**olid phase extraction, **U**HPLC, **S**tatistical analysis) protocol. It involves miniaturized parallel fermentations in 96-well microtiter plate with up to ninety six different media, parallel extraction of the supernatant layer of the fungus via a polymer based solid phase extraction (SPE) plate, chromatographic assessment of the results via UHPLC, and multivariate analysis of the chromatograms.The aforementioned protocol introduces elements of incremental novelty in the natural product screening program by means of combining and harnessing existing ideas, techniques, and technologies into a protocol for the implementation of the OSMAC approach at micro-scale. Its main advantage is the decrease of the scale of operation with the use of the 96-well microtiter plates. It's benefits includ the possibility of overcoming few issues such as processing time and human resources that have somewhat hampered the implementation of the OSMAC approach and/or the systematic study of a large library of microorganisms. As a proof of concept for the MECSUS protocol, further evaluation on the metabolic potential of the already known fungus, *Aspergillus sp.* HAB10R12, was carried out through systematic alteration of the composition of the cultivation media by adding minerals and epigenetics elicitors. Computational analysis of the resulting 384 UHPLC chromatograms showed that the secondary metabolite production of *Aspergillus* HAB10R12 was altered by the use of sub-inhibitory concentrations of epigenetic elicitors. Various doses of the epigenetic modifiers suberoylanilide hydroxamic acid (SAHA), valproic acid, sodium butyrate, and SAHA + S-adenosylhomocysteine stimulated *Aspergillus* HAB10R12 secondary metabolite production.Chemical characterization of *Aspergillus* HAB10R12 extract, UV and Mass values, confirmed the identity of the series of peptides and pyrones previously identified, and revealed that the fungus extract is rich in compounds that potentially exceed the ones listed above. Cytotoxicity tests revealed the crude extract as well as the purified metabolites of *Aspergillus* HAB10R12 are potent cytotoxic compounds.

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