

**UNIVERSITI TEKNOLOGI MARA**

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

**RASHA SAAD ABD ELGADIR SOLIMAN**

Thesis submitted in fulfilment  
of the requirement for the degree of  
**Doctor of Philosophy**

**Faculty of Pharmacy**

**June 2013**

## 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, 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.


Name of Student : Rasha Saad Abdelgader Soliman

Student ID No. : 2008704419

Programme : Doctor of Philosophy (PhD)

Faculty : Faculty of Pharmacy

Thesis Title : Development of A Multifaceted (MECSUS)  
Protocol in the Search for Novel Bioactive  
Entities from Microorganisms

Signature of Student :  .....

Date : June 2013

## 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 (Microtiter plate, Elicitors, Combination, Solid phase extraction, UHPLC, Statistical 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. Its benefits include 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.

## TABLE OF CONTENTS

	<b>Page</b>
<b>AUTHORS DECLARATION</b>	ii
<b>ABSTRACT</b>	iii
<b>ACKNOWLEDGEMENTS</b>	iv
<b>TABLE OF CONTENTS</b>	v
<b>LIST OF TABLES</b>	xi
<b>LIST OF FIGURES</b>	xii
<b>LIST OF PLATES</b>	xvi
<b>LIST OF SCHEMES</b>	xvii
<b>LIST OF GRAPHS</b>	xviii
<b>LIST OF SYMBOLS</b>	xix
<b>LIST OF ABBREVIATIONS</b>	xx
<b>CHAPTER ONE: INTRODUCTION</b>	<b>1</b>
<b>CHAPTER TWO: LITERATURE REVIEW</b>	<b>4</b>
2.1 <i>Aspergillus</i> Species and their Bioactive Metabolites	4
2.2 <i>Aspergillus</i> HAB10R12 Secondary Metabolites	9
2.3 Bioactivity of <i>Aspergillus</i> HAB10R12 and <i>Aspergillus</i> NF00659 Extracts and Compounds	11
2.4 OSMAC Approach and Production of Microbial Secondary Bioactive Metabolites	14
2.5 Fermentations and Extraction at Micro-scale	17
2.6 Solid Phase Extraction (SPE)	21
2.6.1 Overview of the Main SPE Strategies	22
2.6.1 [a] Selective Extraction	22
2.6.1 [b] Selective Washing	22
2.6.1 [c] Selective Elution	23
2.6.2 Solid Phase Extraction Modes	23

2.6.2 [a] Normal Phase SPE	24
2.6.2 [b] Reversed Phase SPE	24
2.6.2 [c] Ion- exchange SPE	24
2.6.3 SPE in Microbial Metabolite Research	25
2.6.4 General Concepts on Method Validation	26
2.7 High Speed Analysis Using Ultra High Performance Liquid Chromatography (UHPLC)	27
2.8 Data Mining – Chemometric Analysis	28
2.9 Conclusion	31
<b>CHAPTER THREE: <i>ASPERGILLUS</i> HAB10R12 CHEMISTRY</b>	<b>32</b>
3.1 Introduction	32
3.2 Material and Methods	33
3.2.1 <i>Aspergillus</i> HAB10R12 Standard Extract Preparation	33
3.2.1 [a] Reagents and Instruments	33
3.2.1 [b] Procedure: Culture of <i>Aspergillus</i> HAB10R12 Standard Extract	34
3.2.1 [c] Procedure: Extraction of The Fermentation Product of <i>Aspergillus</i> HAB10R1	34
3.2.1 [d] Procedure: Analysis of The Fungi Extract by Analytical HPLC-DAD	37
3.2.2 <i>Aspergillus</i> HAB10R12 Extract Analysis	38
3.2.2 [a] Reagents and Instruments	38
3.2.2 [b] Procedure: Small-scale Purification via HPLC Fraction collector	40
3.2.2 [c] Procedure: Chemical Characterization via HRESIMS	41
3.2.2 [d] Procedure: Fractionation via Gel Filtration Chromatography (GFC)	41
3.2.2 [e] Procedure: Large-scale Purification via Semi-prep HPLC	42
3.2.2 [f] Procedure: Cytotoxicity Tests	44
3.3 Results and discussion	45