UNIVERSITI TEKNOLOGI MARA

MICROBIAL TRANSFORMATION OF STEROID

NURULHUDA BINTI MAZLAN

Dissertation submitted in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons)

Faculty of Pharmacy

November 2008

ACKNOWLEDGEMENT

Alhamdulillah, all praise to Allah S.W.T. With His help and blessing, this study has been done successfully. First of all I would like to thank the Director of Institute of Chemistry and Herbal Remedies (iKUS), Professor Dr. Jean-Frédéric Faizal Weber Abdullah for giving me a chance to conduct my thesis. I would like to express my deep and sincere gratitude to my respectful supervisor, Dr Sadia Sultan. Her wide knowledge and her logical way of thinking have been a great value for me. Her understanding, encouraging and personal guidance have provided a good basis for the present thesis. Special thanks to my co-supervisor, Dr Syed Adnan Ali Shah, the coordinator of this course, Dr Zainul Amiruddin Zakaria for support and advice and not forgotten Dr Kalavathy Ramasamy for providing me the endophytes.

I would also like to thank all the rest of the academic and support staff of Institute of Chemistry and Herbal Remedies (iKUS) laboratory especially to Siti Hajar binti Sadiran for providing me the beneficial knowledge about the equipment in the laboratory and exact way to use the equipment. Much respect and greatest thanks to my lovely fellows, Nor Azra and Syuhaida for putting me up and work together with me all the time during this study. I owe my loving thanks to my parents, brothers and sisters. Without their encouragement and understanding it would have been impossible for me to finish this work. Finally, my special gratitude is due to my friends for their loving support and always being there every time when I need them for help.

TABLE OF CONTENTS

		·	Page
TITI	LE PAG	E	
APP	ROVAL	FORM	
ACKNOWLEDGEMENT			ii
TABLE OF CONTENT			iii
LIST OF FIGURES			v
LIST OF TABLES			vii
LIST OF ABBREVIATIONS			viii
ABSTRACT			ix
СНА	PTER (ONE (INTRODUCTION)	1
СНА	PTER 7	ΓWO (LITERATURE REVIEW)	
2.1	Overv	view of Microbial Transformation	3
2.2	Thera	peutic Uses of Steroids	4
2.3	Micro	obial Transformation of Steroids	9
СНА	PTER T	THREE (MATERIALS AND METHODS)	
3.1	Mate	Materials	
3.2	Methods		
	3.2.1	Preparation of media	15
	3.2.2	Preparation of seed flask	17
	3.2.3	Inoculation of substrate	17
	3.2.4	Extraction	18
	3.2.5	Analyzing of the fractions	18
	326	Preparative Thin Laver Chromatography (PTLC)	10

ABSTRACT

The main goal of this project is to study the ability of three different endophytes: Aspergillus niger (HAB 10 R12), Coniothyrium lunata (HAB 2 R1) and Berkholderia (HAB 11 R3) to transform pregnenolene acetate in three different media without causing harm to environment, animals or even humans. The extracts after one and two weeks of incubation period on a shake table were analyzed by Thin Layer Chromatography (TLC) to determine the significant compound(s). Isolation process by using Preparative TLC was done to purify the interested compound(s). Purified compound(s) was further identified by using Nuclear Magnetic Resonance (NMR) spectrometer.

CHAPTER 1

INTRODUCTION

"Biotransformation" refers to any structural modifications brought by enzymes, microorganisms, animals or plant cell cultures. In microbial transformation, a substance (substrate) will be incubated with microorganism to produce another (product). Microbial transformation also called "bioconversion" or "microbial conversion".

Microbial transformation is the design, development and implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and the environment. Microbial transformation is an innovative, non-regulatory, economically driven approach toward sustainability. Microbial transformation increased performance and value while concern about human health and the environment (Manley *et al.*, 2008).

Microbial transformation involved chemical synthesis steps leads to large production of natural or modified analogues. The latter are currently favored due to some advantages, such as an increased potency, longer half-lives in the blood stream, simpler delivery methods and reduced side effects (Fernandes *et al.*, 2003 and He *et al.*, 2006). The aim of microbial transformation lies in improvement in the yields of desired metabolites as well as preparation of novel compound that are difficult to synthesize by chemical