UNIVERSITI TEKNOLOGI MARA

MICROBIAL TRANSFORMATION OF STEROIDS AND EVALUATION OF THEIR BIOTRANSFORMED PRODUCTS AS ANTI-PROLIFERATIVE AGENTS AND ACETYLCHOLINESTERASE INHIBITORS (*IN-VITRO* AND *IN-SILICO* STUDIES)

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PhD

March 2020

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.

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ABSTRACT

Natural products account for 60% of the total market, making them a major source of drug discovery. Some of these are sourced from the cultivation of microorganisms. Microbial transformation is an example of the application of cultivation of microorganisms. It is a method of modifying the chemical structure of compounds such as steroids by microorganisms. The diversity of the possible reactions types in microbial transformation includes the process of oxidation, hydroxylation, esterification, isomerization, reduction, acetylation, hydrogenation and glycosylation. Therefore, the present study aims to screen new microbial strains which can carry out steroid bioconversions, and isolate prospective biologically active biotransformed products. No report on biotransformation of medroxyprogesterone has been published to date although biotransformations of medroxyprogesterone acetate have been studied by several groups. To date, only one study has published information on the microbial transformation of ethynodiol diacetate (ED), which is achieved by *Cunninghamella elegans*. In this study, the biotransformations of steroids medroxyprogesterone, and ethynodiol diacetate by a series of fungi (ATCC, endophytes and Antarctic) have been investigated. The literature on manipulations of the psychrotolerant fungi, specifically the Antarctic fungi for biotransformations is almost non-existent. Screening experiments were performed in 100 ml conical flasks containing 40 ml media and were autoclaved at 121°C for 15 minutes. After 3 days of inoculation, substrates were introduced aseptically into fermented liquid media and further fermentations were allowed for 4-12 days. Metabolic changes were observed by comparing the HPLC chromatograms of starting compounds, control cultures and fermented extracts. For medroxyprogesterone, *Trichothecium roseum* ATCC 13411, R3-2 SP17, *Mucor plumbeus* ATCC 47400, and *Cunninghamella elegans* ATCC 36112 were found to be suitable for large scale productions. *Botrytis cinerea* ATCC 36112, *Trichothecium roseum* ATCC 13411, and R3-2 SP17 were selected to proceed with large scale fermentation of ethynodiol diacetate. After separation and purification of the mixtures, the complete spectrometric analysis was performed to verify the structures of transformed products. The products with satisfactory quantity along with their precursors were subjected to anti-proliferative assay and acetylcholinesterase inhibition assay. From the analysis of the above data, one of the IC_{50} of the biotransformed products (the new biotransformed product, **MP 2**) is close to the limits of the active cytotoxic limit of a pure compound defined by the American National Cancer Institute, which is 4 μg/mL or less. Modification of steroid has also revealed to improve its anti-acetylcholinesterase activity to some extent than its precursor specifically in the MP series. This is observed in **MP 7**, the new isolated biotransformed metabolite. The activities reported here deserve attention, and they are good examples in supporting the application of microbial transformation as a viable method of future development of anti-proliferative drug candidates, and acetylcholinesterase inhibitors.

ACKNOWLEDGEMENT

In the name of Allah, the Most Merciful, the Most Gracious. I am thankful to Allah, who supplied me with the courage, the guidance, and the love to complete this research.

Firstly, I would like to express my sincere gratitude to my advisor Associate. Prof Dr Sadia Sultan, for the continuous support of my PhD study, for her patience, motivation, and immense knowledge while granting me the room to work in my own way. Since my first day in graduate school, Dr Sadia believed in me like nobody else, and her guidance helped me in all the time of research and writing of this thesis.

Deepest gratitude is also due to Prof Dr Jean-Frédéric Faizal Weber Abdullah, my co-supervisor, who always gave me his comments to improve my defence preparation right from the proposal defence period. Special thanks go to my co-supervisor, Prof Dr Teh Lay Kek, who reviewed my conversion thesis, and counselled me on future directions for my project. I also wish to thank my co-supervisor, Dr Syed Adnan Ali Shah for his involvement in NMR experiments. In regards to bioassays and molecular docking studies in this thesis, I thank Prof Dr Mohd Ilham Adenan, and Dr Syahrul Imran Abu Bakar, respectively, for sharing their expertise with me. Thank you to Prof Dr Bohari M. Yamin, and Dr Humera Naz for the time they spared on assisting me with analysis of crystal data. Thanks to Dr Fatimah Salim for her advice on electronic configurations of selected biotransformed products.

A special thank you to Dr Anis Low Muhammad Low for her precious advice on my assays sample preparation/protocols, and entertaining my crazy monologues out loud. Not forgetting all staff in Analytical Chemistry Department, Faculty of Pharmacy, Universiti Teknologi Mara and Atta-ur-Rahman Institute for Natural Products Discovery. In my daily work, I have been blessed with my research team members: Fatmawati Lambuk, Normahanim Hassan, Siti Hajar Sadiran, Rohani Rahim, Nor Atiqah Jusril, Zetty Zulikha Hafiz, Shazwani Shariffudin, and Fatimah Bebe Mohamed Hussain. Thanks for helping me in numerous ways during the incredible whirlwind of ups and downs of various stages of my PhD.

Words fail to express my admiration to my father, Mr Wan Yusop whose love and confidence in me, has taken the load off my shoulder. I cannot count the sacrifices you have made for me. Thanks for everything Abah, I love you. I owe a special debt to my beloved family who has given me endless love, prayers, and patience to face come what may. Finally, I would like to thank everybody who was important to the successful realization of this thesis, as well as I am sorry that I could not mention personally one by one. Thank you very much.

TABLE OF CONTENT