UNIVERSITI TEKNOLOGI MARA

CYTOTOXIC AND ANTIMICROBIAL EFFECTS INDUCED BY ETHYL ACETATE EXTRACTS OF MALAYSIAN ENDOPHYTIC FUNGI

(MBS 3.2, MDBS 1.1 and MBL 1.2)

NUR SYAZWANI BINTI ABDUL RASHID

SUPERVISOR:

A/ PROF. DR. KALAVATHY RAMASAMY

Dissertation submitted in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy

BACHELOR OF PHARMACY (Hons.)

2015

ABSTRACT

It is known that plants host endophytic microorganisms which are rich sources of bioactive metabolites. Endophytic bioactive compounds are progressively becoming significant in discoveries of novel drugs given the diversity of their biological activities which include antimicrobial and anticancer effects. Capitalising on the abundance of unexplored Malaysian endophytes that reside within marine plants, this study was undertaken to assess the cytotoxic and antimicrobial profiles of ethyl acetate extracts of endophytic fungi (MBS 3.2, MDBS 1.1 and MBL 1.2) originated from *Sonneratia* sp. ("Berembang"), *Thespesia* sp. ("Daun Baru") and *Avicennia* sp. ("Bakau"). Another two marine fungi (SM 1.4 PLATE 1 and SW 4.1) originated from sea mud and sea water were also included for comparison purposes. For cytotoxic assay, HCT116 (human colorectal carcinoma cell line) were treated with the ethyl acetate fungal extracts (0.01 - 100 µg/mL) for 72 h. SRB assay was performed to generate data from which the IC_{50} (the concentration required to achieve half maximal inhibition.) was determined. The antimicrobial assay was carried out using the Minimum Inhibitory Concentration (MIC) Method. Both Gram-positive (*Staphylococcus aureus*) and negative (*Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*) bacteria were exposed to ethyl acetate fungal extracts (0.01 - 100µg/mL) for 24 h after which MIC was determined. The present findings found MBS 3.2 to be the most potent extract with IC_{50} observed at 0.16µg/mL Interestingly, its IC_{50} is approximately 3-fold more potent than that of 5-FU, the positive control. MBS 3.2 also demonstrated potential antimicrobial activity against *S. aureus*, *P. aeruginosa* and *S. typhimurium* (MIC = 0.1mg/mL, 0.7mg/mL and 1mg/mL, respectively). Its MIC against *S. typhimurium*, in particular, was comparable to that of gentamicin, the positive control. MBS 3.2 has emerged as the lead ethyl acetate extract and the promising results warrant in-depth cytotoxic and antimicrobial studies using its pure compounds.

ACKNOWLEDGEMENTS

First of all, I am grateful to the Almighty Allah in giving me strength and patience to complete this final year project as partial fulfillment of the subject Research II (PHC 567).

I wish to express my warm appreciation to my supervisors, A/ Prof. Dr. Kalavathy Ramasamy and Dr. Lim Siong Meng for their continuous support, guidance and assistance throughout the course of my final year project. For without them, I would not have completed my research work as planned.

I also would like to dedicate my gratitude to all Collaborative Drug Discovery Research (CDDR) Laboratory members, especially Miss Nur Syafiqah Rahim, Miss Siti Aisyah Sayadi and Mr. Mohd Zaki Ramli who were generous in sharing their knowledge and information. I deeply valued their kind assistance.

Many thanks to my group members, Siti Syazana Hasni, Nurfaizura Mohd Tahir and Syaidatul Farhana Shamsul Bahrin who have been a great help to me.

Last but not least, I would like to thank my family especially my father (Abdul Rashid Bin Ismail) and mother for all their love and supports

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

Cancers are diseases in which normal cells undergo abnormal proliferation, differentiation and growth. There are many possible causes to cancers and they include genetic, lifestyle, tobacco usage, certain types of infections and environmental exposure (ACS, 2014). Cancers are responsible for one of the top ten causes of hospitalisation and one of the top five causes of death in both Ministry of Health (MOH) and private hospitals in Malaysia (CARIF, 2013). Colorectal cancer (CRC) refers to cancer originating from the colon and the rectum. It is the third most common cause of cancer deaths in Malaysia (Radiology Malaysia Administrator, 2014). The high mortality rate is due to the fact that CRC is often diagnosed at late stage. Given that the colon is located deep within the abdominal cavity, it is common for a developing cancer to go undetected until significant symptoms develop. CRC can derive from either inherited or somatic genetic alterations that develop over the course of a lifetime (Yeatman, 2001).

Chemotherapy is one of the common approaches to treat cancer. It involves the use of drugs or chemical agents to interfere with the growth of cancerous cells. Unfortunately, chemotherapeutic agents target both rapidly dividing cancer and normal cells, resulting in numerous side effects (Alison, 2001). Besides, cancers that survive after chemotherapy can repopulate into a resistant tumour (Rebucci & Michiels, 2013). Several possible reasons for development of chemotherapy resistance are gene amplification, change in drug export which cancer cells pump the drug out of