UNIVERSITI TEKNOLOGI MARA

ANTICANCER ACTIVITY OF MARINE ENDOPHYTIC FUNGAL EXTRACTS FROM MALAYSIAN SEAWEEDS AGAINST HEPG2 CELLS

HARMAYUMI BINTI WAHID

MSc

November 2021

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 results of my own work unless otherwise indicated or acknowledged as reference work. This thesis has not been submitted to any other academic institution or non-academic institution for any 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	: Harmayumi Binti Wahid
Student ID No	: 2014240774
Programme	: Master of Science (Microbiology)-PH756
Faculty	: Pharmacy
Thesis Title	: Anticancer Activity of Marine Endophytic Fungal Extracts from Malaysian Seaweeds against HepG2 Cells.
Signature of student	: 
Date	: November 2021

ABSTRACT

Marine endophytes fungi are known to be the important resource of bioactive metabolites in drug development for the pharmaceutical industry. This bioactive has the potential to treat diseases such as cancer, as an alternative to synthetic drug use in chemotherapy. This study aims to determine the potent cytotoxicity of five marine endophytic fungal extracts coded as (CN, MV, ED, CS, and UF) isolated from seaweed; *Gracilaria coronopifolia*, *Gracilaria arcuata Zanardini*, *Acanthophora picifera (M.Vahl) Borgesen*, *Caulerpa sertularioides*, and *Chaemotorpha minima* respectively, against human hepatocellular carcinoma cells (HepG2) and normal human liver cells (Chang), and evaluate their potential to induce apoptosis and antioxidant activity. All five marine endophytic fungi were grown in different salinity (1% and 3%) of artificial sea salt (ASS) potatoes dextrose agar (PDA). Initial cytotoxicity screening showed that 2 out of 10 marine endophytic fungal extracts (CN 1% and MV 1%) showed potential cytotoxic effect against HepG2 at 24, 48, and 72 hours incubation time with IC_{50} values detected for CN 1% (35.2 – 53.3 µg/ml) and MV 1% (41.8 – 50.0 µg/ml), which are <60 µg/ml. Both extracts were determined as non-toxic effect to Chang (IC_{50} value > 60 µg/ml). Further with cytotoxic quick screening after fractionation, CN 1% and MV 1% itself displayed higher cytotoxicity effect against HepG2 (35.5 % and 37.4 % respectively) compared to 10 selective fractions at concentration 20 µg/ml (concentration based on National Cancer Institute guidelines). Moreover, the morphological studies indicated that CN 1% and MV 1% induced apoptosis with apoptosis features such as membrane loss, condensation of cell nuclei, nuclear fragmentation, and apoptotic body formation. Furthermore, the Annexin-V assay was done to further confirm apoptosis. Data revealed, by treating with IC_{50} doses exhibited significant increases in early apoptosis (CN 1%= 18.7 %, MV 1%= 20.7%) and late apoptosis (CN 1%= 38.4%, MV 1%= 54.4%) entering from the viable cell, with less than 1% necrosis. Interestingly, both extracts exhibited a dose-dependent trend in early apoptosis (CN 1%= 70.9%, MV 1%= 33%) after HepG2 cells were treated with IC_{70} doses. Similarly, both extracts displayed ABTS radical scavenging activity with IC_{50} values (CN1% = 43.6 µg/ml, MV 1% = 53.5 µg/ml). Thus, the conclusion is CN 1% and MV 1% possess cell death mechanism through apoptosis induction against HepG2 at non-toxic in Chang cells, with antioxidant properties. This potential extract should be further explored in the future for marine endophytic fungus discovery for pharmaceutical application.

ACKNOWLEDGEMENT

Alhamdulillah, firstly I praised Allah SWT, the Most Gracious and Most Merciful, for His blessing, I'm able to complete this thesis. It is impossible for me to complete my thesis alone. There were many people officially and unofficially being involved in my study journey to accomplish my study. They do deserve this appreciation.

My gratitude and many thanks go to my supervisor Dr. Siti Alwani Binti Ariffin for her continuous and non-stop encouragement, patience, guidance, and ideas in assisting me to complete this project and thesis. She did her job very well as a supervisor, besides motivating, sharing experiences and knowledge to develop me as a positive person in my master study journey. I also would like to express my gratitude to SIRIM Berhad, for sponsoring my study and providing the facilities. My next gratitude goes to my General Manager (Dr. Ahmad Hazri Ab Rashid), my Programme Head (Mdm Sarifah Rejab) and all my colleagues for their concern and consideration in assisting me as part-time student/staff. Many thanks are also express to all staff in the Faculty of Pharmacy for helping me during my study.

Special and many thanks to my husband, and my two sons for understanding my multiple tasks as wife/mom/student. Special thanks also to my lovely parents and siblings for keeping du'as and encouraging me.

"May Allah SWT bless their life here and here after".

TABLE OF CONTENTS