UNIVERSITI TEKNOLOGI MARA

CYTOTOXIC AND ANTIMICROBIAL EFFECTS INDUCED BY ETHYL ACETATE EXTRACTS OF MALAYSIAN ENDOPHYTIC FUNGI (MKS 3, MPL 2 & MPRRW 2.3.1)

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ABSTRACT

Endophytes are microorganisms that live within healthy tissues of plants without causing any apparent symptoms of diseases. There is increasing evidence indicating endophytes as rich source of natural, novel bioactive compounds against cancers and infectious diseases. Capitalizing on the abundance of unexplored Malaysian endophytes that reside in marine plants, the present study was conducted to assess the cytotoxic and antimicrobial potential of ethyl acetate extracts of 3 endophytic fungi isolated from *Terminalia* sp. ("Ketapang" tree) and *Sonneratia* sp. ("Perepat" tree) plants. Another 2 marine fungi (SM 1.4 and SW 2.1 Plate 2) were also included for comparison purposes. For cytotoxic assay, HCT 116 (human colorectal carcinoma cells) were seeded at 2,500 cells/ 200µL and incubated overnight. The cancer cells were then treated with extracts (0.01 – 100 µg/mL) for 72 h. SRB assay was performed to generate dose-response curve from which the IC_{50} (Inhibitory concentration of the response is reduced by half) was determined. The anti-bacterial assay involved addition of pathogenic bacteria (Gram-positive and negative) into extracts (0.01 - 100µg/mL) prior to determination of the minimal inhibitory concentration (MIC). The present findings indicated the *Terminalia*-derived MKS 3 as the most potent endophytic fungal extracts against HCT 116 with IC_{50} observed at 2.5 µg/mL. Its cytotoxicity, however, was 5 times less potent when compared to that of 5-FU, the positive control. The *Sonneratia*-derived MPRRW 2.3.1, yet another endophytic fungal extract, emerged as the most potent antibacterial agent against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with MIC observed at 0.1 mg/mL. Its potency were 10 fold and 2.5 fold lower than that of gentamicin, the positive control against *P. aeruginosa* and *S. aureus*, respectively. Comparatively, both the marine fungal extracts elicited modest cytotoxic and antimicrobial activities. The present study has successfully identified MKS 3 and MPRRW 2.3.1 as potential cytotoxic and antimicrobial agents, respectively. These two lead extracts should be subjected to isolation of pure compound for in depth anticancer and antimicrobial studies in the future.

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CHAPTER 1
INTRODUCTION

Cancers, especially those at critical stage, have been known to cause death. At present, cancer that is of global concern is the colorectal cancer (CRC). CRC, also known as the bowel cancer, developes either in the colon or the rectum (Martinez, 2005). CRC occurs commonly in developing countries and is the third most common leading cancer with 1.23 million cases worldwide (Jemal et al., 2011). In 2006, the National Cancer Registry indicated 2,866 registered cases of CRC (representing 13.2% of all cases) in Malaysia (National Cancer Registry Malaysia, 2006). In Peninsular Malaysia, CRC is the second leading cancer, ranked after breast cancer. In terms of gender, CRC was ranked first among males and second among females.

One of the mode of treatments for cancer is chemotherapy. Chemotherapy kills rapid proliferating cells. Unfortunately, it also affects normal cells, giving rise to side effects (National Institute of Health, 2014). The first line treatment for CRC includes MAYO (bolus 5-FU/ folinic acid), the de Grammont (infusional 5-FU/ folinic acid) and FOLFOX (infusional 5-FU/ folinic acid plus oxaliplatin) clinic regimens. The efficacy of these regimens, however, is often compromised by side effects and drug resistance (Foubert, Matysiak-Budnik, & Touchefeu, 2014).

Infectious diseases can be defined as illnesses caused by infectious agents or their toxic products. These diseases can be transmitted by either direct or indirect contact through an intermediate animal or plant host, vector or inanimate environment, reservoir or infected person to a susceptible host (Shetty, Tang, & Andrews, 2009). Pathogenic bacteria that can cause infectious diseases in human body include *Staphylococcus aureus* and *Pseudomonas aeruginosa* amongst others. Various antimicrobial drugs are available for use against bacteria. Common antimicrobial agents include cephalosporin, vancomycin, penincillin, tetracycline and fluoroquinolone (Goldman & Schafer, 2011). In general, the mechanisms of action underlying these antimicrobial drug include adhesion,