

UNIVERSITI TEKNOLOGI MARA

**MALAYSIAN FUNGAL ENDOPHYTES AS A
SOURCE OF BIOACTIVE METABOLITES**

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ABSTRACT

Endophytes are microorganisms that reside within plants and appear as an enormous yet relatively untapped source of natural product. The objectives of this study include isolation of endophytes from medicinal plants, determination of antimicrobial and antitumour activity of cultures extracts, identification of the bioactive endophytes through morphological, biochemical and genetic characterization. A total number of 348 endophytes were isolated through surface sterilization from 24 local medicinal plants of the Kuala Pilah Rain Forest, Malaysia. For the antimicrobial test, a disc diffusion assay was employed and the endophytic crude extracts were tested against bacteria (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus* and *Escherichia coli*) and fungi (*Candida albicans*, *Aspergillus niger*, and *Saccharomyces cerevisiae*). The preliminary results showed 20 % of all endophytic crude extracts possessed antimicrobial activity with an inhibition zone ranging from 10 to 24 mm in diameter. The antimicrobial activity of four samples of interests [HAB 2(R1), HAB 10(R12), HAB 11(R3) and HAB 21(F25)] were further investigated and compared with commercial antibacterials (ampicillin, ceftriaxone, cephalixin and gentamicin) and antifungal (amphotericin B) agents. The HAB 10(R12) exhibited good antibacterial activity and significantly better than cephalixin and gentamicin against *B. subtilis* and *E. coli* respectively. The antifungal activity of HAB 2(R1) was significantly better than amphotericin B against *Aspergillus niger*. The MTT assay was employed to detect the cytotoxic activity against tumour type (breast adenocarcinoma, MCF7 and colonic carcinoma HCT116) and non tumour type (embryonic liver WRL68 and Chang liver) cell lines. Preliminary screening showed that 39 % of the collected endophytes were active ($IC_{50} < 20 \mu\text{g/ml}$) against a murine leukaemic (P388) cell. Interestingly, eight samples were extremely cytotoxic [(HAB 10 (R12), HAB 13(R21), HAB 14(R3), HAB 15(L10), HAB 21(F7), HAB 21(F22) and HAB 21 (F25)] as their IC_{50} values were less than $0.1 \mu\text{g/ml}$ against P388 cell line. The extracts [HAB 10(R12) and HAB 21(F25)] were generally more selective towards tumor type, HCT116 and MCF7 than normal cell line (Chang). However, extract HAB 11(R3) did not show any selectivity between normal and tumour cells. Morphological and genetic studies showed both HAB 10 (R12) and HAB 21(F25) to be *Aspergillus* sp. Studies also showed that 22 known compounds and 15 possibly new compounds were isolated from the collected endophytes. Endophytes show promise as a source for new bioactive compounds and warrant further exploration.

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CHAPTER I

INTRODUCTION

The need for new antimicrobial and antitumor agents arises from the fact that pathogenic microbes and cancerous cells become more and more resistant to existing drugs. Bacteria have developed resistance to all classes of antibiotics discovered to date (Alanis, 2005). Furthermore, the number of developed antibiotics has declined tremendously during the last decade as a consequence of diverse factors, including the lack of interest of industry in these fields. Many tumours are dose-dependent in which higher doses of antitumor agents are required to kill more cancer cells. However, patients may suffer from toxic effects such as nausea, vomiting and bone marrow suppression (Hardman & Limbird, 2001). As a result, new agents that are more effective and less toxic than the existing drugs are required. Intensive research for such therapeutic agents has led to the exploration of new sources of natural products.

Natural products are an important source of drug leads against both infectious diseases and cancer. Seventy out of 90 (77 %) antibiotics marketed in the years 1982-2002 originated from natural products (Newman et al., 2003). Recent trends, however, show that the discovery rate of novel chemical entities is declining (Lam, 2007). Therefore, there is a need to bioprospect new sources and if possible from less explored regions and habitats to discover novel bioactive metabolites. Plants have been one of the main sources of drugs, however, the endophytic microorganisms which live within these plant tissues, including bacteria and fungi, have not been widely explored thus far for therapeutic properties. Almost all plants have from a few to several hundred endophytes residing within them (Zinniel et al., 2002). A single endophyte may be able to produce not one but several bioactive