UNIVERSITI TEKNOLOGI MARA

TOXIC VOLATILES AS TOOLS FOR THE SELECTIVE ISOLATION OF SOIL FUNGI AND MODULATING THEIR METABOLISM

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ABSTRACT

The fungal kingdom forms an important source for a broad range of secondary metabolites with widely different chemical structures, as well as diverse biological activities. In the present work, the main objective was to isolate fungi from local soil samples and identify bioactive metabolites that they could produce. To this end, soil samples were collected from a biological forest reserve, at UiTM's Puncak Alam Campus, Malaysia. The isolation from soils can proceed through many different techniques. Here, a new chemotechnique based on the use of five volatile compounds (dimethylsulphoxide, naphthalene, cineole, petroleum and formaldehyde) was used. As a result, a total of 82 fungi were isolated from soil samples taken at three different depths. The pure cultures were inoculated and fermented in the presence or in the absence of volatile compounds. A total of 100 crude extracts were analysed by HPLC and evaluated for antimicrobial activity against pathogenic microorganisms using the MTT assay. Twenty out of 100 crude extracts showed significant antibacterial activity against *Escherichia coli*, 17 extracts were active against *Enterococcus faecium*, 15 against *Pseudomonas aeruginosa* and 25 against *Staphylococcus aureus*. Twenty six crude extracts showed antifungal activity against *Candida albicans*. From the analysis of the above data, 8 out of the 82 fungal isolates were selected for further study. These include *Aspergillus nomius*, *A. terreus*, *Byssochlamys nivea*, *Talaromyces aculeatus*, *P. commune*, *Pseudallescheria minutispora*, *Trichoderma citrinoviride*, and *T. virens*, which were fully identified by morphological and genetic techniques. Their metabolites were purified by semi-preparative HPLC and identified by spectroscopic (MS, NMR, UV/Vis) and X-ray diffraction techniques. From *Aspergillus nomius*, four compounds were identified, namely kojic acid, aflatoxins B1 and G1, and 3-*O*-methylsterigmatocystin. Terrein was isolated from *A. terreus*. From the *Penicillium* and*Talaromyces* species, three compounds were identified, including penicillic acid, 6-methoxymellein and vermistatin. From *Pseudallescheria minutispora*, three rare pseurotins A, A1 and A2 were identified. From *Trichoderma citrinoviride*, hydroxyheptelidic acid and gliocladic acid were identified. Rare MK-8383 was obtained from *Byssochlamys nivea.* Finally, from *Trichoderma virens*, viridiol was identified, while the plane structure was established for a new metabolite named trichovirenic acid. Scanning electron microscopy (SEM) revealed marked effects when the *Talaromyces aculeatus* isolate was exposed to DMSO: (i) hyphae showing hairy spinulose appendages or nodules, (ii) hair-like appendages arising from penicillus phialides and long dense spines on the conidia, (iii) hyphae with wart-like growths, (iv) smooth conidia, (v) presence of mycelial coils, (vi) vesicle-like formations. Other less dramatic effects such as altered growth rate and discoloration were observed with some volatiles for some isolates. Significant changes in secondary metabolic profiles were observed with volatile exposure. Thus, the use of toxic volatile compounds presents a novel selective isolation procedure and allows for metabolic manipulation of fungi.

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CHAPTER ONE
INTRODUCTION

Soil has been one of the most highly researched microorganism biotopes for the discovery of new compounds because it carries a higher population of micro-organisms than any other habitat. Malaysia has diverse soil ecotypes, including lowland tropical forest soils, mangrove, and highland soils. One gram of soil may harbour up to 10 billion microorganisms of many different species (Rosselló-Mora and Amann, 2001) and it is estimated that less than 1% of the soil microorganisms have been characterised. These offer a great potential for the discovery of novel compounds, and hence opportunities for novel pharmaceuticals. In the past, the isolation of terrestrial microorganisms mainly relied on relatively few isolation procedures and this led to repeated isolations of the same species and - in the case of fungi - fast growing isolates.

The story of drugs from fungi started in 1928 with the serendipitous discovery by Fleming of penicillin, a potent antibiotic active against Gram-positive bacteria, from a *Penicillium*. The exceptional success of penicillin led to an intensive search for other antibiotic producing microorganisms. Drugs of fungal origin mainly come from fungi of temperate climates. Tropical regions, however, offer a greater wealth of biotopes and biodiversity, yet not much work has been carried out on tropical microbes in general.

Fungi are very diverse and produce an equivalent diversity of metabolites. Many secondary metabolites are bioactive and constitute the source of many important drugs to combat human and animal diseases. Important cholesterol lowering drugs, *i.e.* the satins, are produced from *Aspergillus* spp (Tobert, 2003).Transplantation of organs would not be possible without immunosuppressants of fungal origin such as cyclosporine and mycophenolic acid. The first multiple sclerosis drug fingolimod derives from a fungal product. The production of steroids for anti inflammatory drugs is dependent on fungal transformations.

It is hoped that using highly selective fungal isolation techniques from the soil of a tropical rain forest will lead to unusual strains, and thus offer a new tool for the discovery of novel drug lead compounds from local resources.