UNIVERSITI TEKNOLOGI MARA

PROCEDURE FOR CULTURING NON-SPORULATING FUNGI AT MICROSCALE LEVEL

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TABLE OF CONTENTS

ABSTRACT

Filamentous fungi provide unique compounds as a source for drug discovery. Conventional screening method is not practical to screen the bioactive compounds produced by thousands fungi. In order to increase the output, there is need to decrease the scale and increase the output. Using in-house MECSUS protocol with slight modification, a procedure for culturing non-sporulating fungi at microscale level using microtiter plate was developed by studying 11 fungi from Atta-ur-Rahman Institute's collection. The procedure consists of four major stages including growth study, culturing process, solid phase extraction process and HPLC chromatographic screening, profiling and analysis. Eight fungi were characterized as fast growing fungi while three fungi were medium growing fungi. The fungal inoculum preparation was established based on growth kinetic study by preparing mycelium suspension. The fungal mycelium suspensions were cultured in microtiter plate and incubated according to growth study. The fungi were extracted using parallel solid phase extraction based on in-house MECSUS protocol. The fungal extracts were analyzed using HPLC. The profiling showed consistency and reproducible results. The procedure was found effective and reliable. The procedure will be included as the part of in-house MECSUS protocol.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Isolation of fungi in various environments such as from plants, aquatic, soil and others results in hundreds and even thousands of them. Some of them are sporulating and others are non-sporulating in standard culture conditions. Non-sporulating filamentous fungi are fungi that do not produce spores but only mycelium. These fungi vary in growth kinetics and are somehow difficult to control (Bills et. al., 2008). However, non-sporulating fungi have been proven as the one of the sources for drugs and antibiotics (Ho et al., 2003).

Conventional secondary metabolites screening method always uses Petri dishes and Erlenmeyer flasks as bioreactors for the non-sporulating fungi, followed by liquid-liquid extraction and HPLC analysis which is time consuming, and costly. Hence, Atta-ur-Rahman Institute of Natural Product Discovery, Universiti Teknologi MARA (UiTM), developed an integrated protocol for screening fungal secondary metabolites at microscale level named MECSUS, (Microtiter plate, Elicitors, in Combination, Solid Phase Extraction, UHPLC and Statistical Analysis). At the moment, MECSUS is already developed and integrated for sporulating fungi and