INHIBITORY EFFECTS OF THE CRUDE EXTRACT FROM Phomopsis sp. ON SEVERAL PATHOGENIC FUNGI

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INHIBITORY EFFECTS OF THE CRUDE EXTRACT FROM *Phomopsis* sp. ON SEVERAL PATHOGENIC FUNGI

BY

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DEDICATION

My beloved family;

Rasidan Saidi,

Rosidah Ahmad,

Razin Hazeeq Rasidan,

Rashidi Husaini Rasidan,

Nur Rasyiqa Abdullah,

Miko and Taro.

also

My supervisor:

Prof. Dr. Rozi Mohamed,

Lab members in Forest Biotech Laboratory,

And

all my friends.

Thank you for your encouragement, support and help.

ABSTRACT

Endophytic fungi inhabiting plant hosts may contain fungitoxic properties and can be useful for biological control agents. An isolate of *Phomopsis* sp. from the mangrove tree has been determined if it has possessed antifungal properties. In this study, *Phomopsis* sp. was cultured in Potato Dextrose Broth (PDB) for 14 day. After which the mycelia were removed, the filtered culture was subjected to liquid-liquid extraction using ethyl acetate (EtOAc) as the solvent. Then, it was purified through a chromatography column using various solvents. Two eluents, hexane (PH) and the mixture of hexane + methanol (PMH), when tested against the pathogenic fungus (*Ceratocystis fimbriata*) gave 25% inhibitory effect. These findings indicate extraction of secondary metabolite from *Phomopsis* sp. have the antifungal ability as it can be used as biological control agent.

ABSTRAK

Kulat endofitik yang terdapat pada tumbuhan mungkin mengandungi sifat antikulat dan boleh menjadi agen kawalan biologi yang berguna. Pengambilan *Phomopsis* sp. dari pokok bakau dikaji adakah ia bersifat antikulat. Dalam kajian ini, *Phomopsis* sp. telah dikulturkan dalam air kaldu dekstrosa kentang (PDB) selama 14 hari. Selepas itu, miselium dikeluarkan. Kultur yang ditapis digunakan untuk pengekstrakan cecair-cecair menggunakan etil asetat (EtOAc) sebagai pelarut. Kemudian, ia didilarutkan melalui kolumn kromatografi menggunakan berbagai pelarut. Dua eluen, heksana (PH) dan campuran heksana + metanol (PMH), apabila diuji terhadap kulat patogenik (*Ceratocystis fimbriata*) memberikan 25% kesan perencatan pertumbuhan. Penemuan ini menunjukkan pengambilan metabolit sekunder dari Phomopsis sp. mempunyai keupayaan antikulat, ia boleh digunakan sebagai agen kawalan biologi.

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APPROVAL SHEET

I certify that this research project report entitled "**Inhibitory Effects of the Crude Extracts from *Phomopsis* sp. on Several Pathogenic Fungi**" by Rasyiffa' Hayati binti Rasidan had been examined and approved as a partial fulfillment of the requirements for the Degree of Bachelor of Forestry Science in the Faculty of Forestry, Universiti Putra Malaysia.

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˚C	Degree Celsius
mm	Millimetre
mL	millilitre
µL	Microliter
g	gram
mg	milligram
MeOH	Methanol
CH_2Cl_2	Dichloromethane
C_6H_{14}	Hexane
EtoAC	Ethyl acetate
SMs	Secondary metabolite
PDA	Potato dextrose agar
PDB	Potato dextrose broth
UPM	Universiti Putra Malaysia
MMFR	Matang Mangrove Forest Reserve
DNA	Deoxyribonucleic acid
LLE	Liquid-liquid extraction

CHAPTER ONE

INTRODUCTION

1.1 General Background

Malaysia is a tropical rainforest consisting a diversity of species in it. In addition, wood products in Malaysia are also a major revenue for this country. Therefore, the initiative to build a forest plantation has been devised. Forest plantation are built on a large scale over a long period of depending on the species grown (Cossalter & Pye-smith, 2003). Therefore, any disease that is capable of reducing yield quality is of great concern to forest planters. Plant disease is a very crucial thing to cater in plantation and sometimes can occur in natural habitat. The plant disease can make a lot of loss to the farmers and the owner (Hamzah et al., 2018). Mostly, the disease was caused by the pathogenic fungal that infect the plant. So, synthetic fungicide is always being used. But this practice is anthropogenic. It can harm the environment, animal and human being(Jing et al, 2011; Luo et al., 2016).

Therefore, a lot of alternatives for the substitution of synthetic fungicide were formulated. Natural fungicide has less impact on the environment but possesses the effectiveness as good as the synthetic fungicides. This is because consumers are very concerned with the safety of the environment and some of the certifications require entrepreneurs to use a more natural approach. This approach enables the product to get a higher price offer and increase the productivity of the product more widely. Above and beyond, some

researches prove that there are antifungal properties in second metabolite from the endophytic fungal. (Suresh & Nelson, 2016)

Phomopsis is one of the endophytic fungus that can be found in mangrove plant (*Rhizophora mucronate*). Previous studies prove that *Phomopsis* have antagonistic behaviour on several pathogenic fungi (Hamzah et al., 2018), which means the Phomopsis can reduce the infection of pathogenic fungi on tree in natural way. The specific pathogenic fungi can be eradicated from the tree without harming other organisms. Thus, a research on inhibitory effects of crude extracts from *Phomopsis* towards several pathogenic fungi will be conducted to prove the antifungal properties in the *Phomopsis*.

1.2 Problem statement

Synthetic fungicide is widely used to eradicate pathogen, but it can also be harmful (Doehlemann et al., 2017). Alternatively, natural fungicides from endophytic fungi can be useful to control plant pathogens (Suresh & Nelson, 2016). Recently, a study has shown that the endophyte *Phomopsis* sp. inhibiting mangrove tree has antifungal effect (Hamzah et al., 2018). Specific elution fraction from *Phomopsis* crude extract might have differences in its inhibitory effects to the pathogenic fungi (Jing et al., 2011). Thus, the active fraction may be isolated to be used as biocontrol (BCA) agent in order to control fungal plant diseases.

1.3 Objectives

This research was conducted to find the inhibitory effects of crude extracts from *Phomopsis* sp. towards several pathogenic fungi.

i. To evaluate the effect of the crude extract from an isolate of *Phomopsis* sp. against several pathogenic fungi.

ii. To identify purified eluents from the crude extract with antifungal effect against selected pathogenic fungi.

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