



***POTENTIAL OF MALAYSIAN SEAWEED EXTRACTS IN INHIBITING  
Ganoderma boninense AND IDENTIFICATION OF THEIR ANTIFUNGAL  
COMPOUNDS***

**SYAMIMI DIYANA BINTI ABDUL AZIZ**

**FBSB 2018 57**



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By

**SYAMIMI DIYANA BINTI ABDUL AZIZ**

**Thesis Submitted to School of Graduate Studies, Universiti Putra  
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Science**

**December 2018**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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**Chair : Zetty Norhana Balia Yusof, PhD**  
**Faculty : Biotechnology and Biomolecular Sciences**

Basal stem rot (BSR) disease is the most devastating disease in oil palm (*Elaeis guineensis*) caused by a fungal pathogen, *Ganoderma boninense*. Various control measures have been tested but to date the utilization of seaweeds as a source of control agent has not been explored. This study investigated the antifungal potential of Malaysian seaweed extracts against *G. boninense* and identifying the potential antifungal compounds. Polar and non-polar compounds were extracted from *Sargassum oligocystum*, *Caulerpa racemosa*, *Caulerpa racemosa* var. *lamourouxii* and *Halimeda macrophysa* using water, methanol, chloroform, dichloromethane and hexane. Methanolic extracts from all species displayed the highest yield with an average of 15.25 % compared to dry weight. At 0.25 mg/mL, *C. racemosa* var. *lamourouxii* dichloromethane extract recorded the highest growth reduction against *G. boninense* with 46.82 % followed by *C. racemosa* var. *lamourouxii* hexane extract with 36.43 % and *H. macrophysa* dichloromethane extract with 33.49 %. Statistical analysis from Kruskal-Wallis H test showed significant difference ( $H=77.23$ ,  $p=.00$ ) in growth reduction percentage between the different solvent. Dominant compounds detected via Gas Chromatography-Mass Spectrometry in extracts with antifungal potential were l-(+)-ascorbic acid 2,6-dihexadecanoate, phytol, tetradecanoic acid, isobutyl methylphosphonofluoridate and benzenamine,2-[2-(4-pyridinyl) ethyl]-. Further antifungal testing using standard phytol showed growth inhibition of *G. boninense* with 21 % inhibition and the amount of phytol in dichloromethane extracts were quantified via GC-MS. Dichloromethane extract of *C. racemosa* var. *lamourouxii*, *H. macrophysa*, *C. racemosa* and *S. oligocystum* contain 474, 117, 106 and 19 mg/L of phytol, respectively which were consistent with exhibited antifungal activities. These findings suggested that Malaysian seaweeds have potential as a source of natural and potent antifungal compounds for utilization in controlling the oil palm disease in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat of Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

**POTENSI EKSTRAK RUMPAI LAUT MALAYSIA DALAM PERENCATAN  
*Ganoderma boninense* DAN PENGENALPASTIAN SEBATIAN ANTIKULAT**

Oleh

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Penyakit reput pangkal batang ialah sejenis penyakit pokok kelapa sawit yang paling serius yang disebabkan oleh patogen kulat, *Ganoderma boninense*. Pelbagai kaedah pengurusan penyakit telah digunakan tetapi penggunaan rumput laut sebagai sumber agen kawalan masih belum diterokai. Kajian ini menyiasat potensi antikulat oleh ekstrak rumput laut Malaysia terhadap *G. boninense* dan pengenalpastian sebatian antikulat yang berpotensi. Sebatian berkutub dan tidak berkutub tersari daripada *Sargassum oligocystum*, *Caulerpa racemosa*, *Caulerpa racemosa* var. *lamourouxii* dan *Halimeda macrophysa* menggunakan akuas, metanol, kloroform, diklorometana dan heksana. Ekstrak metanol daripada semua spesies menunjukkan hasil tertinggi dengan purata sebanyak 15.25% berbanding berat kering. Pada kepekatan 0.25 mg/mL, ekstrak diklorometana *C. racemosa* var. *lamourouxii* merekodkan pengurangan pertumbuhan *G. boninense* yang tertinggi iaitu sebanyak 46.82 %, diikuti dengan ekstrak heksana *C. racemosa* dengan pengurangan pertumbuhan sebanyak 36.43 % dan ekstrak diklorometana *H. macrophysa* dengan pengurangan pertumbuhan sebanyak 33.49 %. Analisis statistik daripada ujian Kruskal-wallis menunjukkan perbezaan bererti ( $H=77.23$ ,  $p=.00$ ) dalam peratusan pengurangan pertumbuhan dengan perbezaan pelarut. Antara sebatian dominan yang dikesan melalui GC-MS di dalam ekstrak yang mempunyai potensi antikulat ialah "l-(+)-ascorbic acid 2,6-dihexadecanoate", fitol, "tetradecanoic acid", "isobutyl methylphosphonofluoridate" dan "benzenamine,2-[2-(4-pyridinyl) ethyl]-". Cerakinan antikulat menggunakan fitol piawai menunjukkan perencatan pertumbuhan terhadap *G. boninense* sehingga 21 % dan kandungan phytol di dalam ekstrak diklorometana dikuantifikasi menggunakan GC-MS. Ekstrak diklorometana *C. racemosa* var. *lamourouxii*, *H. macrophysa*, *C. racemosa* dan *S. oligocystum*, masing-masing mengandungi fitol sebanyak 474, 117, 106 and 19 mg/L bertekalan dengan dapatan aktiviti antikulat yang ditonjolkan. Dapatan ini mencadangkan

bahawa rumpai laut Malaysia merupakan sumber sebatian antikulat yang mempunyai potensi yang tinggi sebagai sumber yang asli dan sebatian antikulat yang poten untuk digunakan dalam pengawalan penyakit kelapa sawit di Malaysia.



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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

<i>et al.</i> ,	And friends
AA	L-(+)-Ascorbic acid 2,6-dihexadecanoate
BSR	Basal stem rot
Ca <sup>2+</sup>	Calcium ion
cm	Centimetre
cf	Latin: confer (compare)
Cu <sup>2+</sup>	Copper ion
°C	Degree celcius
DMSO	Dimethyl-sulfoxide
EDTA	Ethylenediamine-tetraacetic acid
EA	Ethyl iso-allocholate
eV	Electron volt
GCMS	Gas chromatography mass spectrometry
g	Gram
g/mL	Gram per millilitre
µA	Microampere
µL	Microlitre
µg	Microgram
µm	Micrometre
mg/mL	Miligram per millilitre
mL	Mililitre
mm	Milimetre
MF	Molecular formula
MW	Molecular weight
NIST	National Institute of Standard and Technology
N	Nitrogen
PKC	Palm kernel cake
PPM	Parts per million
%	Percent
min <sup>-1</sup>	Per minute
PDA	Potato dextrose agar

PRC	People's Republic of China
P	Phosphorus
P	Phytol
K	Potassium
R&D	Research and development
RT	Retention time
SA	Salicylic acid
sp.	Species (singular)
spp.	Species (plural)
SD	Standard deviation
SE	Standard error
TA	Tetradecanoic acid
USA	United states of America
USR	Upper stem rot

## CHAPTER 1

### INTRODUCTION

Malaysia is the world's major producer and exporter of palm oil after Indonesia by earnings of RM 64.58 billion (Din, 2017). As of 2017, Malaysia had 5.8 million hectares of total oil palm planted area (Malaysian Palm Oil Board, 2017). Oil palm is a major source of edible vegetable oil and biodiesel in Malaysia (Hushiarian *et al.*, 2015). Apart from that, palm oil has been broadly used in many industries such as in the production of toiletries, cosmetics, candles, pharmaceutical products as well as animal feed manufacturing (Choo, 2013; Hushiarian *et al.*, 2015; Zahazi & Alimon, 2004). While the biomass such as trunk and fronds were molded to be used in manufacturing of furniture, packaging, building and automobile industry (Basiron, 2015; Choo, 2013).

However, it is confronted with a devastating disease caused by a fungus, *Ganoderma boninense* that acts as the main factor of basal stem rot (BSR) disease (Hushiarian *et al.*, 2015). It is a soil borne pathogen which is hard to control in a way that no effective method available that can completely handle the disease (Chong *et al.*, 2017). Neglecting the disease will affect the yield of fresh fruit bunches which will trim down the production of crude palm oil. It was estimated that during 2020, 400 thousand hectares of oil palm plantations could be affected according to the annual growth rate of *G. boninense* (Roslan & Idris, 2012). Subsequently results in a huge loss in economy which was reported between RM225 million to RM1.5 billion per year (Arif *et al.*, 2011; Ommelna *et al.*, 2012). Many approaches to prevent this disease have been done such as sanitation but the most common method is by applying non-environmentally friendly fungicide which is also costly at the same time (Idris *et al.*, 2002).

Therefore, a more environmentally friendly and sustainable remedy to this disease would be worth exploring. Natural products are non-toxic and secure to be used. Seaweeds or also known as macrolagae are marine plants known to be rich with active compounds with various biological activities (Choi *et al.*, 2016; Fernando *et al.*, 2017; Park *et al.*, 2016; Sanjeeva *et al.*, 2017). The potential for fungicidal activity had been discovered in certain seaweeds extracts. For example, aqueous, methanol, ethyl acetate, chloroform and ethanol fraction of red alga *Solieria robusta* demonstrated antifungal activity against five fruit spoiling fungi namely *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *Penicillium funiculosum* and *Phytophthora infestans* (Khanzada *et al.*, 2007). In another study, five species of red alga *Laurencia* of various extracts were found to cause growth reduction of pathogenic fungi such as *Candida albicans*, *C. parapsilosis* and *Cryptococcus neoformans* (Stein *et al.*, 2011). More recent research by Rajasulochana and colleagues (2013) proposed the potential of *K. alvarezii* extracts in affecting the growth of fungal species. Result from this study reported that chloroform: methanol extract of *Kappaphycus alvarezii* actively against fungi *Aspergillus fumigates*,

*Microsporium canis*, *Epidermophyton* sp. and *C. albican* (Rajasulochana *et al.*, 2013). Seaweeds are abundant in Malaysia and most of their potential and advantages are underexplored. If *G. boninense* infection in oil palm affects the production of oil palm's yield, it is hypothesized that the application of potential seaweed extracts on the infected palms might suppressed the fungal attack. This study aimed to discover the possession of antifungal characteristics and identification of the bioactive compounds in the abundantly available Malaysian seaweed extracts which could be the answer to the quest of an environmentally friendly approach to control the BSR disease in oil palm. Thus, this is the first study in Malaysia providing information on the antifungal property of Malaysian seaweed extracts against *G. boninense*.

### 1.1 Objectives

The main goals of this study is to investigate the antifungal potential of selected Malaysian seaweed extracts against *G. boninense* and the identification of the compounds responsible for the antifungal activity. The specific objectives of this research are:

1. To screen the antifungal activity of selected Malaysian seaweeds extracts against *G. boninense*.
2. To identify the potential bioactive compounds from seaweed extracts with inhibitory activity against *G. boninense*.
3. To quantify potential bioactive compounds content in seaweed extracts with antifungal property against *G. boninense*.

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