UNIVERSITI PUTRA MALAYSIA

STRUCTURAL ELUCIDATION OF CHEMICAL COMPOUNDS FROM Dysoxylum acutangulum Miq. EXTRACT AND THEIR ANTIFUNGAL PROPERTIES AGAINST FILAMENTOUS FUNGI

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Chairman : Associate Professor Yaya Rukayadi, PhD
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The search for chemical compounds from plants still needs to be done, because there are still many plants that have not known much about chemical compounds and also the biological activity of the chemical compound that plant. One of native Malaysia's native plants that has not much been explored much is *Dysoxylum acutangulum* Miq. was known as *Bekak* for Malaysian people. The diversity of secondary metabolites from this plant promises a tone of novel finding in various classes of compounds as well as its bioactivities. Fewer studies conducted on antimicrobial activities of *Dysoxylum* spp., and to the best of our knowledge less studies conducted on its antifungal activities against filamentous fungi. The aim of this study was to elucidate the active compounds from *D. acutangulum* Miq. bark extract, and also to determine the antifungal properties of each active compounds against filamentous fungi, such as *Aspergillus flavus* ATTC22546, *Aspergillus niger* ATTC9029, *Beuveria bassiana* UPMC28, *Cunninghamella echinulata* UPMC24, *Mucor plembeus* UPMC26 and *Mucor rouxii* UPMC23. Methanol extract of this plant was portioned using chloroform and further fractioned using open column chromatography with different solvent system. The profile of chemical constituent in the fraction was monitored via Thin Layer Chromatography profiling. The isolated compound was further purified using multiple Preparative Thin Layer Chromatography with suitable solvent system. Four compounds successfully isolated and elucidated, they are scopoletin DYC 61 (3.7 mg), 2-hydroxyl-2oxa-cycloarta-23-one-28-oic acid DYC 62 (21.9 mg), 4,29-dihydroxy-31-methyl-3,4-*seco*-cycloart-23-one-3-oate DYC 81 (92.7mg), and 4-hydroxy-3,4-*seco*-cycloart-23-one-28-oic acid DYC 42 (3.3 mg). The elucidation of each compound were established using few spectroscopy analysis, such as ^{1}H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (125 MHz, $CDCl_3$), ^{1}H-^{1}H COSY ($CDCl_3$) NMR, HSQC NMR ($CDCl_3$), HMBC NMR ($CDCl_3$), FTIR and HREIMS. The confirmation of the structure was justified by comparing the spectral data with the similar structure of the literature review. Some of the compound didn't have any FTIR and HREIMS data due to the small amount yield after the isolation process. Four structure

compound were being elucidated using the spectroscopy method listed before. One coumarine type compound was isolated and known as Scopoletin, and three of the compounds were belong to cycloartane triterpenoids of secondary metabolites. Two of the cycloartane compound was 3,4- *seco*-cycloartane (ring A opened) and onther one compound was cycloartane compound with ring A closed. Two compounds, DYC62 and DYC81, were tested for antifungal activity. The results showed that 1% DYC62 reduced 54.55 %, 28.11 %, 64.71 %, 24.35 %, 50.00% and 36.36 % colony size of *A. flavus, A. niger, B. bassiana, C. echinulate, M. plembeus*, and *M. rouxii*, rescepectively. Moreover, 1% DYC81 reduced the colony size growth of *A. flavus, A. niger, B. bassiana, C. echinulate, M. plembeus,* and *M. rouxii*, with 44.10 %, 18.89 %, 52.94 %, 29.74 %, 30.00 % and 9.09 %, respectively. The conidia growth of *A. flavus, A. niger, B. bassiana, C. echinulate, M. plembeus,* and *M. rouxii* can be inhibited with compound DYC 62 with minimum inhibition concentration (MIC) of 2.50 mg/ml, 1.25 mg/ml, 5.00 mg/ml, 2.50 mg/ml, 5.00 mg/ml and 5.00 mg/mL, respectively and can be killed completely with minimum fungicidal activity (MFC) of 2.50 mg/ml, 2.50 mg/ml, 5.00 mg/ml, 2.50 mg/ml, 5.00 mg/ml and 5.00 mg/ml, respectively. Meanwhile MICs and MFCs of compound DYC 81 against conidia of those filamentous fungi ranged between 1.20 mg/ml to >5.00 mg/mL. In conclusion, both compounds, DYC 62 and DYC 81 exhibit antifungal activity against filamentous fungi and have potential to be developed and natural antifungal agents.

Keywords: *D. acutangulum* Miq., cycloartane triterpenoid, 3,4-*seco*-cycloartane, antifungal activity, filamentous fungi.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

ELUSIDASI STRUKTUR KOMPOUN KIMIA DARIPADA EKSTRAK *Dysoxylum acutangulum* Miq. DAN SIFAT ANTIFUNGALNYA TERHADAP KULAT BERFILAMEN

Oleh

MOHD AZUAR HAMIZAN BIN RAHMAN

Disenber 2018

Pengerusi : Profesor Madya Yaya Rukayadi,PhD
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Pencarian sebatian kimia daripada tumbuh-tumbuhan masih perlu giat dijalankan, ini kerana masih banyak tumbuh-tumbuhan yang tidak diketahui secara menyeluruh tentang sebatian kimia dan aktiviti biologinya. Salah satu tumbuhan asli yang masih belum diterokai terdapat di Malaysia ialah *Dysoxylum acutangulum* Miq. dan ianya dikenali sebagai Bekak oleh rakyat tempatan Malaysia. Kepelbagaian metabolit sekunder daripada tumbuhan ini menjanjikan penemuan baru yang banyak dari segi kepelbagian kelas sebatian dan aktiviti biologinya. Terdapat hanyan sedikit kajian dijalankan terhadap *Dysoxylum* spp. tentang antimikrobialnya, dan juga pada pengetahuan kami terlalu kurang kajian dijalankan terhadap antikulatnya. Tujuan utama pembelajaran ini adalah untuk menjalankan elusidasi kompoun aktif daripada ekstrak kulit pokok *D. acutangulum*, dan juga mengenal pasti sifat antikulat bagi setiap kompoun aktif tersebut terhadap kulat berfilamen seperti *Aspergillus flavus* ATTC22546, *Aspergillus niger* ATTC9029, *Beuveria bassiana* UPMC28, *Cunninghamella echinulata* UPMC24, *Mucor plembeus* UPMC26 and *Mucor rouxii* UPMC23. Ekstrak metanol daripada tumbuhan ini telah dibahagikan menggunakan kloroform dan diperkecilkan lagi dengan kromatografi turus secara terbuka menggunakan sistem cecair yang perlbagai. Profil komposisi kimia didalam pecahanan sebatian dipantau melalui kromatografi lapisan nipis. Sebatian yang telah terasing dimurnikan lagi dengan penyediaan kromatografi lapisan nipis menggunakan sistem secair yang bersesuaian. Empat kompoun telah berjaya di isolasi dan elusidasi, ataranya adalah Scopoletin (3.7 mg), 2-hydroxyl-2oxa-cycloarta-23-one-28-oic acid DYC 62 (21.9mg), 4,29-dihydroxy-31-methyl-3,4-*seco*-cycloart-23-one-3-oate DYC 81 (92.7mg), and 4-hydroxy-3,4-*seco*-cycloart-23-one-28-oic acid DYC 42 (3.3 mg). Elusidasi bagi setiap kompoun telah di kukuhkan dengan menggunakan analisis spektrokopi, seperti ^{1}H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (125 MHz, $CDCl_3$), ^{1}H-^{1}H COSY ($CDCl_3$) NMR, HSQC NMR ($CDCl_3$), HMBC NMR ($CDCl_3$), FTIR dan HREIMS. Pembuktian struktur telah dibuktikan dengan perbandingan data spektra dengan kompoun yang memiliki struktur yang sama. Beberapa sebatian tidak dapat

menjalani analisis spektoskopi FTIR dan HREIMS kerana mempunyai jumlah yang sedikit selepas proses pemencilan. Empat struktur sebatian berhasil di elusidasi dengan mengunakan teknik spektroskopi yang telah dinyatakan. Satu jenis kompoun *Coumarine* berjaya di elusidasi dan dikenali sebagai *scopoletin* dan tiga lagi kompoun merupakan kompoun daripada kumpulan metabolit skunder jenis *cycloartane triterpenoids*. Dua daripada kompoun *cycloartane triterpenoids* itu merupakan *3,4 seco cycloartane* (*ring* A terbuka) dan struktur cycloartane yang terakhir adalah stuktur yang mempunyai *ring* A tertutup. Dua sebatian, iaitu DYC 62 dan DYC 81 telah menjalani ujikaji terhadap aktiviti antikulatnya. Keputusan terhadap 1% DYC62 telah mengurangkan sebanyak 54.55 %, 28.11 %, 64.71 %, 24.35 %, 50.00% and 36.36 % saiz koloni *A. niger, B. bassiana, C. echinulate, M. plembeus,* dan *M. rouxii*. Selain itu, 1% DYC 81 telah mengurangkan saiz koloni *A. flavus, A. niger, B. bassiana, C. echinulate, M. plembeus,* dan *M. rouxii* dengan 44.10 %, 18.89 %, 52.94 %, 29.74 %, 30.00 % dan 9.09 %. Tumbusaran konida *A. flavus, A. niger, B. bassiana, C. echinulate, M. plembeus,* dan *M. rouxii* boleh direncatkan menggunakan sebatian DYC 62 dengan kepekatan perencatan minima (MIC) sebanyak 2.50 mg/ml, 1.25 mg/ml, 5.00 mg/ml, 2.50 mg/ml, 5.00 mg/ml dan 5.00 mg/mL, dan juga boleh dibunuh secara menyeluruh dengan kadar kepekatan membunuh minima (MFC) 2.50 mg/ml, 2.50 mg/ml, 5.00 mg/ml, 2.50 mg/ml, 5.00 mg/ml dan 5.00 mg/ml selain itu kepekatan perencatan dan membunuh minima daripada sebatian DYC 81 terhadap kulat berfilament yang digunakan adalah pada jarak nilai diantara 1.20 mg/ml keapda >5.00 mg/mL. Secara kesimpulannya, kedua-dua sebatian DYC 62 dan DYC 81 mempamerkan aktiviti antikulat terhadap kulat berfilamen dan mempunyai potensi dijadikan sebagai agen antikulat semulajadi.

Kata kunci: *D. acutangulum* Miq., *cycloartane triterpenoid*, *3,4-seco-cycloartane*, aktiviti antikulat, kulat berfilamen.

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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 Science. The members of the Supervisory Committee were as follows:

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Signature: _____

Name of Member of Supervisory Committee: Professor Dr. Khozirah Shaari

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%	Percent
NMR	Nuclear Magnetic Resonance
sp.	Species
FT-IR	Fourier transform infrared
HREIMS	High Resolution Electron Impact Mass Spectroscopy
eV	Electronvolt
^{1}H	Proton
^{13}C	Carbon-thirteen
δ	Chemical shift
TMS	Tetramethylsilane
s	Singlet
d	Doublet
t	Triplet
q	Quartet
m	Multiplet
COSY	Homonuclear correlation spectroscopy
NOESY	Nuclear Overhauser Effect Spectroscopy
HSQC	Heteronuclear single quantum coherence spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
UNiCC	Microbial Culture Collection Unit
°C	Degree Celsius
MIC	Minimum inhibitory concentration

MFC	Minimum fungicidal concentration
µl	Microliter.
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
-OH	Hydroxy
C=O	Carbonyl (Ketone group)

CHAPTER 1

INTRODUCTION

1.1 Background

Since the ancient time, plant has been used traditionally as a remedy for various illnesses and now the demand for further scientific studies on the plant was increasing as it becomes the source of novel drug lead (Atanasov et al., 2015). Traditional plant can be described as a complimentary medicine or alternative medicine, becomes the primary medicine to major population across the world (Khatun et al., 2011). The report from the World Health Organization (WHO, 2003) state that traditional plant mainly used to prevent or treat diseases and chronic illness. An analysis conducted at the source of novel lead drug from the plant over the period 1981 to 2002 shows that 877 small molecule derived from the natural product are able to synthesize and 16.4% of its containing pharmacological values (Fakim, 2006).

Dysoxylum is a tropical plant genus come from the *Maliaceae* family, this plant have been reported to be a good source of terpene (Mabberley, 2011). *Dysoxylum acutangulum* Miq. come from *Meliaceace* family, previously known as *Dysoxylum schultzii* Miq. and it also known as Bekak among Malaysian people (Lazim et al., 2013). The woods from these plants have a faint fragrance make it suitable for cabinet work and also furniture. As reported from isolation of *D. acutangulum* Miq. previous studies, these plant rich with terpenoid and alkaloids. Several new compound from these plant are managed to be isolated and reported as a novel compound with bioactivity properties. Traditional folks use variety parts of these plants as a remedy for skin irritation and also to cure the sexual (Lakshimi et al., 2009).

There are about 300 fungal species that are infectious and can cause a variety of diseases (Khan et al., 2017). Approximately 2 million people in the USA were suffering from bacterial and fungal infections each year, and 65% of the patients faces a problem to antimicrobial resistant pathogens (Sobel et al., 2011). Antifungal resistance and drastically increases on number of fungal infection due to a compressed immune system were the recent global medical challenge. The impact from the resistance to common antifungal, million people around the world suffer from the various fungal infection and the rate of mortality was increased (Perea and Patterson, 2002; Vandeputte et al., 2012; Xie et al., 2014; Ribas et al., 2016).

In 2001, the US National Institute for Health recommended the continuation of development of novel antifungal drugs, which belong to classes other than existing ones and possess a different mode of action (Scorzoni et al., 2007). Therefore, this present study was conducted to isolate and elucidate the new compound come from *D.*

acutangulum Miq. and aslso evaluate the antifungal activity of *D. acutangulum* Miq. extract againts few selected filamentous fungi.

1.2 Problem statement

The plant from the *Meliaceae* family were known to be a good source of various terpene-type metabolites. Previous studies conducted on various plant extract belonging to *Meliaceace* family reported a diverse class of secondary metabolite able to be isolated with a promising bioactivity such as limonoid, meliacin-type compound (antifungal), dammarane triterpenoids (antivirus) and spermidine alkaloids (Connolly et al., 1979; Bordoli et al., 1993; Inada et al., 1993; Gunning et al., 1994; Tzourous et al., 2004). Recent study conducted by Ismail et al. (2009a) two novel triterpene were able to elucidate from *D. acutangulum* Miq. leaves methanol extract, which are Acutaxylines A and Acutaxylines B. Acutaxylines B. Both isolated compound show some moderate activity *in vitro* cytotoxic activity on human blood premyelocytic leukemia. Two new compound, Cumingianoside A and C was isolated from *Dysoxylum cumingianum* Miq. was show a potent cytotoxic activity against leukemia cell (Kurimoto et al., 2011). The isolation of secondary metabolite from the *Meliaceae* family yield to a ton of promising compound with a various bioactivity.

Nuclear magnetic resonance (NMR) spectroscopy is one of useful technique for identification of structure of isolated unknown compound from the plant extract. Metabolite fingerprinting by NMR is a fast, convenient, and effective tool for discriminating between groups of related samples and it identifies the most important regions of the spectrum for further analysis (Krishnan et al., 2005). With help the data from NMR spectrum, the elucidation process to determine the unknown compound become easier. The success in isolation method and structure elucidation of single compound from the plant has greatly influenced the increment of bioactivity examination of that specific single compound of the plant origin (Scorzoni et al., 2007). Therefore, the elucidation of isolated chemical compound from the extract of *Dysoxylum acutangulum* Miq. were carried out using various spectroscopy method.

The extract from *Meliaceae* family such as *Dysoxylum ramiflorum* (bark), *Chisocheton macranthus* (leaf) and *Aglaia affinis* (bark) show moderate (10.0 to 14.9 mm inhibition zone) antibacterial activities against *Staphylococcus aureus,* the leaves part of *D. ramiflorum* Miq. was reported to have an antibacterial activities with less than 9.5 mm inhibition zone against *Enterococcus faecalis* (Chung et al., 2008). There is few number of studies conducted on the antimicrobial activities of *Dysoxylum* spp against pathogenic microorganisms, to the best of our knowledge less studies had been conducted on antifungal activities against filamentous fungi. Hence, studies to determine the antifungal activities of pure compound from *Dysoxylum acutangulum* Miq. extract against filamentous fungi were conducted.

1.3 Objectives

The objectives of this study are:

1. To elucidate the chemical structures of isolated pure compound from *D. acutangulum* Miq. extract using various spectroscopic method.
2. To evaluate antifungal activities in term of percentage growth reduction, minimum inhibitory concentration and minimum fungicidal concentration of pure compound from *D. acutangulum* Miq.

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