UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL CONSTITUENTS AND BIOASSAY-GUIDED ISOLATION OF AN ANTICANDIDAL COMPOUND FROM Albizia myriophylla Benth

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By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Master of Science

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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.

PHYTOCHEMICAL CONSTITUENTS AND BIOASSAY-GUIDEDISOLATION OF AN ANTICANDIDAL COMPOUND FROM*Albizia myriophylla* Benth.

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Chair: **Professor Khozirah Binti Shaari PhD**
Faculty: **Science**

Albizia myriophylla Benth is used as a remedy for cough, as an antidiabetic agent and as a starter culture in the preparation of rice beer. This study used a bioassay guided approach to investigate the anti-candidal properties of *A. myriophylla* and identify compound(s) responsible for the anti-candidal activity. In addition, other phytochemical constituents of the plant were also investigated.

Wood extract of *A. myriophylla* was screened for anti-candidal activity against four *Candida* species human pathogens: *C. albicans*, *C. krussei*, *C. parapsilosis,* and *C. glabrata*. Solvent fractionation technique was used to achieve a partition of the extract into four different fractions. Disc diffusion assay was used to determine the presence of anti-candidal activity in the fractions. Bioassay-guided column chromatography separation was used to retain anti-candidal fractions while separating out non-active fractions. Thin layer chromatography (TLC) bioautographic assay was used in an attempt to directly localize the anti-candidal compound(s) in the active fractions. Following this, separation and purification using preparative thin layer chromatography of the active fraction led to the isolation of an anti-candidal compound. Nuclear magnetic resonance spectroscopy and mass spectroscopy were used for the characterization of the isolated compounds.

The result showed that an anti-candidal compound was isolated from the chloroform fraction of the aqueous methanol extract of *A. myriophylla*. The compound, identified to be 4-hydroxy-3,5-dimethoxybenzaldehyde or syringaldehyde, had MIC and MFC values of 0.000056 and 0.020000µg/µL respectively on *C. parapsilosis*- which was the most susceptible species. Meanwhile, chromatographic work up of the ethyl acetate fraction afforded five flavonoids, three of which were stereoisomeric flavans identified as (2*R*,3*R*,4*R*)-2-(3,5-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,4,7-triol, (2*R*,3*S*,4*S*)-2-(3,5-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,4,7-triol and (2*R*,3*R*,4*R*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,4,7-triol; a flavanone, 2,3-dihydro-7-hydroxy-2-(3,5-dihydroxyphenyl)-4H-1-benzopyran-4-one; and a flavone, 7-hydroxy-3-methoxy-2-(3,4-dihydroxyphenyl)-4H-1-benzopyran-4-one.

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

SEBATIAN FITOKIMIA DAN PENGASINGAN BERPANDUKAN BIOCERAKIN SEBATIAN ANTI-KANDIDA DARIPADA*Albiziamyriophylla*Benth.

Oleh

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Pengerusi: **Professor Khozirah Shaari Binti PhD**
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Albiziamyriophylla Benth digunakan sebagaipenawar kepada batuk, sebagai agen antidiabetik dan sebagai satu kulturpermulaan dalam penyediaan arak beras. Kajianinimenggunakan pendekatan bioassai berpandu untuk mengkajisifat anti-kandida*A. myriophylla* danmengenalpastisebatian yang bertanggungjawabkeatas aktiviti anti-kandidal. Selainitu, jujukanfitokimia lain tumbuhanini juga dikaji.

Ekstrakkayu*A.myriophylla* telah disaring untuk aktiviti anti-kandidal terhadapempat spesis *Kandida*patogen manusia: *C. albicans, C. krusei, C. parapsilosis*, dan *C. glabrata*. Teknikpemecahanpelarut digunakan untuk ekstrak kepada empat pecahan yang berbeza.Analisis cakeraresapantelah digunakan untuk menentukankehadiran aktivitianti-kandida dalam fraksi.Pengasingan menggunakan kromatografi turus berpandukan biocerakin digunakan untuk mengekalkanpecahan anti-kandida disamping memisahkanpecah antidakaktif. Kromatografi lapisannipis (TLC) biocerakin bioautografi kromatografi lapisan tipistelahdigunakan dalam usaha untuk menyetempatkanterus sebatianantikandida dalam pecahanaktif. Berikutanitu, pemisahan dan penulenan menggunakankromatografi lapisannipis yang aktifmembawa kepada pengasingansebatian anti-kandida.Nuklear spektroskopi resonans magnet dan spektroskopi jisimtelah digunakan untuk pengenalpastian sebatianterpencil struktur molekul.

Hasilnyamenunjukkanbahawa sebatian anti-kandida telahdiasingkandaripada pecahan kloroform dari ekstrak methanol akueus*A.myriophylla*. Sebatian itudikenalpasti sebagai 4-hidroksi-3,5-dimetoksibenzaldehid atau siringaldehid, mempunyainilai MIC dan MFC 0.000056 dan 0.020000μg/μL, masing-masing, pada *C.parapsilosis* - spesis yang paling mudah dipengaruhi. Sementaraitu, hasil kerja kromatografi ke atas pecahanetilasetatmemberikan lima flavonoid, tiga daripa nya adalah sebatianflavanstereoisomerik dikenalpastisebagai (2*R*,3*R*,4*R*)-2-(3,5-dihidroksifenil)-3,4-dihidro-2H-kromin-3,4,7-triol, (2*R*,3*S*,4*S*)-2-(3,5-dihidroksifenil)-3,4-dihidro-2H-kromin-3,4,7-triol dan (2*R*,3*R*,4*R*)-2-(3,4-dihidroksifenil)-3,4-dihidro-2H-kromin-3,4,7-triol; satu sebatian flavanon, 2,3-dihidro-7-hidroksi-2-(3,5-dihidroksifenil)-4H-1-

benzopiran-4-on; satu sebatian flavon, 7-hidroksi-3-metoksi-2-(3,4-dihidroksifenil)-4H-1-benzopiran-4-on.

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APPROVAL

I certify that a Thesis Examination Committee has met on 2 July 2015 to conduct the final examination of Adegoke Damilola Samuel on his thesis entitled "Phytochemical Constituents and Bioassay-Guided Isolation of an Anticandidal compound from Albizia myriophylla Benth" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

AMW:	*Albizia myriophylla* wood
amwea:	*Albizia myriophylla* wood ethyl acetate
ATCC:	American type culture collection
BLP:	Butanol lower phase
BUP:	Butanol lower phase
BSI:	Blood stream infection
^{13}CNMR:	Carbon thirteen nuclear magnetic resonance
CDC:	Centre for disease control
CD_3OD:	Deuterated methanol
$CDCl_3$:	Deuterated chloroform
$CHCl_3$:	Chloroform
COSY:	Correlation spectroscopy
DMSO:	Dimethyl sulphoxide
EtOAc:	Ethyl acetate
EA:	Ethyl acetate
GCMS:	Gas chromatography mass spectrometry
^{1}HNMR:	Proton nuclear magnetic resonance
Hex:	Hexane
HPLC:	High performance liquid chromatography
HSQC:	Heteronuclear single quantum coherence spectroscopy
HMBC:	Heteronuclear multiple bond correlation spectroscopy
INT:	Iodonitrotetrazolium
MeOH:	Methanol
MHz:	Mega hertz
MIC:	Minimum inhibition concentration
MFC:	Minimum fungicidal concentration
m/z:	Mass/charge
NMR:	Nuclear magnetic resonance
NNIS:	National Nosocomial Infections
R*f*:	Retardation faction
rpm:	Revolution per minute
SDA:	Sabouraud Dextrose agar
SDB:	Sabouraud Dextrose broth
spp:	Species
TLC:	Thin layer chromatography
UV:	Ultra violet
VVC:	Vulvovaginal candidiasis

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Diseases caused by pathogenic microorganisms such as bacteria, protozoa, fungi and viruses, have still a high rate of mortality and this is despite the availability of many antibiotics (Coelho et al., 2004; Courtney, 2012). Amongst fungi, *Candida* species have been identified as a significant cause of infections in humans and as the commonest species that colonized and caused severe symptoms in immunocompromised patients (Erköse et al., 2007). This has increased the number of patients with compromised immune system due to cancer treatment, AIDS and immune suppressive therapies, with a consequent worry in the medical field(Miceli et al., 2011).

Currently, the azoles, the echinocandins, and the polyenes (eg amphotericin B) are the only three classes of antifungal agents available to treat serious *Candida* infection (World, 2014). However, the antifungal compound, amphotericin B, which is the standard drug for the treatment of fungal diseases, has toxicity issues associated with it (David et al., 2001). Besides, the azoles are less effective on some *Candida* species(Pfaller et al., 2007; Klevay et al., 2008), while the prolong use of fluconazole has made some*Candida* species resistant. Graeme et al.(2008) observed that the administration of fluconazole over a specified long period of time could not cause any observed difference in the prevalence of *Candida* infection amongst patients. As a result of the increasing occurrence of resistant microbes, the WHO has warned that "we may be heading towards a post antibiotic era in which common infections and minor injuries can kill" (World, 2014). This underscores a dire need to develop newer antifungal drugs, free from toxicity, and belonging to classes of compounds different from the available ones.

Plants'secondary metabolites may deliver the much needed, more effective and less toxic antifungal drug. They offer a diverse class of chemical compounds which may exhibit a wider range of mode of action. Scientific investigations have revealed that the abundant pharmacological properties possessed by plants were apparently as a result of specific chemical components which could be isolated and characterized (Heinrich et al., 2012). Leslie (1996) reports that "There are at least a hundred and twenty distinct chemical substances derived from plants that are considered important drugs and are currently in use in one or more countries of the world". It is expected that the number will increase as medicinal plants research is being explored even more.

As plants pharmacological properties have been shown to be the result of specific chemical component(s) present in them (Heinrich et al., 2012),the specific chemical compound(s) which give(s) *A. myriophylla*which was found to inhibit the growth of tested *Candida* species at an MIC range value to all *Candida* species of 100-500 μgmL^{-1} (Rukayadi et al., 2008) will be investigated. Therefore, thefocus of this research is to identify the specific chemical component(s) that give(s) *A. myriophylla* its anti-candidal activity, isolate and characterize it/them.

Other medicinal properties reported in the genus *Albizia* include *A. amara's antiulcer* activity (Rajkumar et al., 2011), *A. lebbeck's* anti-tumor and anti-inflammatory activity(Liang et al., 2005; Babu et al.,2009; Lam et al., 2011).*A. odoratissima's*

antidiabetic activity (Kumar et al., 2011), and antimalarial activity (Ovenden et al., 2002).

1.2 Objectives

The main objective of this study is to investigate *A.myriophylla's* potential as an anti-candidal plant by using a bioassay-guided approach to screen its aqueous methanol extract for anti-candidal activity against four *Candida* species, namely *C.albicans*, *C.krussei*,*C.parapsilosis* and *C. glabrata*. Fractions which possess activity are screened using TLC bioautography, a method aimed at the rapid identification of the chemical compounds responsible for the activity.

The specific objectives include:
1. To isolate the compound(s) that is/are responsible for *A.myriophylla's* anti-candidal activity.
2. To characterize the anti-candidal compound(s) using spectroscopic methods.

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