UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL CONSTITUENTS OF Hymenophyllum javanicum SPRENG., Huperzia carinata (DESV. EX POIR.) TREVIS AND Huperzia nummolarifolia (BLUME) JERMY

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PHYTOCHEMICAL CONSTITUENTS OF *Hymenophyllum javanicum* SPRENG., *Huperzia carinata* (DESV. EX POIR.) TREVIS AND *Huperzia nummolarifolia* (BLUME) JERMY

By

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

PHYTOCHEMICAL CONSTITUENTS OF *Hymenophyllum javanicum* SPRENG., *Huperzia carinata* (DESV. EX POIR.) TREVIS AND *Huperzia nummolarifolia* (BLUME) JERMY

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November 2015

Chairman : Professor Khozirah Shaari, PhD
Institute : Bioscience

Huperzia carinata and *Huperzia nummolarifolia* were chosen to be studied along with *Hymenophyllum javanicum* due to their relation in their phylogeny. These plants have been used traditionally for the treatment of contusions, strains, swellings, schizophrenia, myasthenia gravis and organophosphate poisoning. The purpose of this study is to isolate and purify the chemical constituents for these species and elucidate the structures of the compounds isolated. The isolation procedures were carried out by using a few separation methods such as column chromatography, Sephadex and SPE. As a result, four compounds have been isolated from both *Huperzia* species. They are 14β,21α-dihydroxyserrat-3β-yl acetate **(S4)**, 14β,21β-dihydroxyserrat-3β-yl acetate **(S6)**, serratenediol **(N2211-1-4)** and 14β,21β-trihydroxyserrat-3β-yl-p-dihydrocaffeate **(E377)**. Whereas, four compounds have been isolated from *Hymenophyllum javanicum* which are, Hymenoside W **(KS-5)**, [6-(3,4-dihydroxy-2-methylenebutoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)methyl-2-(3,4 dihydroxyphenyl)acetate] **(KS-10-2)** [6-(3,4-dihydroxy-2-methylenebutoxy)-2-{[2-(3,4-dihydroxyphenyl)acetoxy]methyl}-4,5-dihydroxy-tetrahydro-2*H*-pyran-3-yl-2-phenylacetate] **(KS-8-10-4)** and [6-(3,4-dihydroxy-2-methylenebutoxy)-5-hydroxy-2-{(2-phenylacetoxy)methyl}tetrahydro-2H-pyran-3,4-diyl-bis{2-(3,4-dihydroxyphenyl)acetate}] **(KS-8-10-6)**. The structures of isolated compounds were identified based on spectroscopic data including 1D and 2D NMR and comparison with the literature.

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

KANDUNGAN KIMIA *Hymenophyllum javanicum* SPRENG., *Huperzia carinata* (DESV. EX POIR.) TREVIS DAN *Huperzia nummolarifolia* (BLUME) JERMY

Oleh

HAZWANI BINTI MOHD RANI

November 2015

Pengerusi : Profesor Khozirah Shaari, PhD
Institut : Biosains

Huperzia carinata dan *Huperzia nummolarifolia* dipilih untuk kajian bersama *Hymenophyllum javanicum* disebabkan oleh hubungan antara filogeni. Tumbuhan ini digunakan secara tradisional untuk merawat lebam, strain, bengkak, skizofrenia, myasthenia gravis, dan keracunan organofosfat. Tujuan kajian adalah untuk mengasingkan dan menulenkan juzuk kimia serta menjelaskan sebatian kimia yang diasingkan. Hasilnya, empat sebatian telah diasingkan daripada kedua-dua spesis Huperzia. Kaedah pengasingkan yang dijalankan termasuklah kromatografi kolum, Sephadex dan SPE. Antaranya 14β,21β-dihidroksiserrat-3β-yl acetate **(S4)**, 14β,21β-dihidroksiserrat-3β-yl acetate **(S6)**, serratenediol **(N2211-1-4)** dan 14β,21β-trihidroksiserrat-3β-yl-p-dihidrocaffeate **(E377)**. Manakala, empat sebatian telah diasingkan daripada Hymenophyllum javanicum iaitu Hymenosid W **(KS-5)**, [6-(3,4-dihidroksi-2-metilenebutoksi)-3,4,5-trihidroksitetrahidro-2H-piran-2-il)metil-2-(3,4-dihidroksifenil)asetik] **(KS-10-2)**, [6-(3,4-dihidroksi-2-methilenebutoksi)-2-{[2-(3,4-dihidroxifenil)acetoxy]methil}-4,5-dihidroksi-tetrahidro-2*H*-pyran-3-il-2-fenilasetik] **(KS-8-10-4)** dan [6-(3,4-dihidroksi-2-metilenebutoksi)-5-hidroksi-2-{(2-fenilasetoksi)methil}tetrahidro-2H-pyran-3,4-diyl-bis{2-(3,4-dihidroksifenil)asetik}] **(KS-8-10-6)**. Semua struktur yang diasingkan dikenal pasti berdasarkan kaedah spektroskopi termasuklah 1D dan 2D NMR serta perbandingan dengan literatur.

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I certify that a Thesis Examination Committee has met on 13 November 2015 to conduct the final examination of Hazwani binti Mohd Rani on her thesis entitled "Phytochemical Constituents of *Hymenophyllum javanicum* Spreng., *Huperzia carinata* (Desv. ex Poir.) Trevis and *Huperzia nummulariifolia* (Blume) Jermy" 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.

Members of the Thesis Examination Committee were as follows:

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

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LIST OF ABBREVIATIONS

δ	Chemical shift in ppm
br	Broad
^{13}C	Carbon-13
COSY	Correlation Spectroscopy
d	Doublet
dd	Doublet of doublets
ddd	Doublet od doublets of doublets
dt	Doublet of triplets
^{1}H	Proton
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single-Quantum Coherence
EIMS	Electron Impact Mass Spectrum
Hz	Hertz
J	Coupling constant in Hz
m	Multiplet
m/z	Mass per charge
MHz	Megahertz
nm	Nanometer
NMR	Nuclear Magnetic Resonance
s	Singlet
t	Triplet

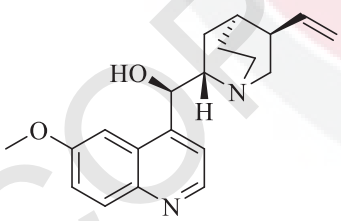
CHAPTER 1

INTRODUCTION

1.1 Medicinal importance of natural products

Natural products chemistry is a chemistry branch dealing with chemical compounds produced by living organisms. It has been a source of food, clothing, cosmetics, constructions of shelters and traps, tools and weapons, poisons for game and fish, medicine or crop protection agents for the past thousands of years. In particular, it has played a major role in early medicine and contributed immensely in drug discovery and the development of modern medicine. It has provided alleviations for many of the world major diseases such as cancer, AIDS and infectious diseases. According to Newman and his co-workers (2012), from 1940s to date, of the 175 anticancer lead molecules, about 49% of them are natural products or derived from natural products (Newman *et al*., 2012).

Natural products will have great importance of in future drug discovery as biochemical probes for the discovery of biochemical and pharmacological processes (Clark, 1996). Natural products may also serve as novel pharmacophores for chemical modification and drug design, which have great potential to generate clinically useful drugs. It is anticipated that biologically active natural products will continue to serve as lead compounds for drug development (Lahlou, 2013). Some important examples of clinically used drugs derived from natural products include the cornerstone discoveries of the antimalarial drugs quinine **(1)** and artemisinin **(2)**, and the anticancer drugs etoposide **(3)** and paclitaxel **(4)**.



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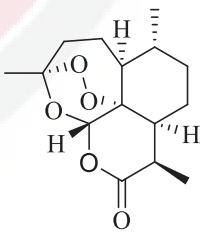

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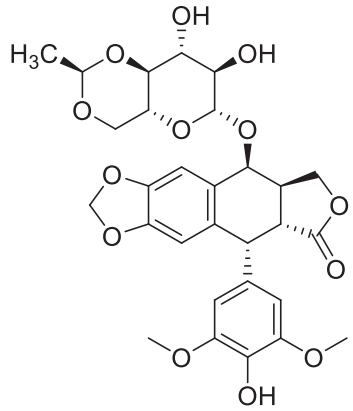

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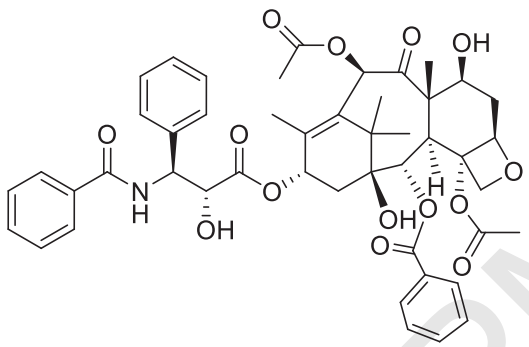

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The search for new drug leads from natural resources is based on several approaches. The most popular approach has been based on the biological and ecological rationale. This is because natural resources such as plants, microorganisms and marine species produce biologically active compounds for their defence from attack against external threats and predators (Strobel *et al*., 2004). For instance, plants that coexisting with animals and microorganisms develop defence strategies to ensure their survival in such a competitive environment. Productions of toxic and other bioactive traits (bitter tasting, pungent and irritant) are common strategies in providing such defences and protection against predators. Often times, these compounds will find some important use or applications in combating human diseases.

1.2 Ferns and lycophytes as a source of useful natural products

Ferns and lycophytes are groups of green plants that lack in flower. They reproduced by means of spores rather than seeds. Ferns cannot be defined by a single character as one group is easily recognised by its highly divided fronds with branching veins and sporangia on the margins or undersides while the other group is not as leafy, and contains members that look quite dissimilar from each other. On the other hand, sporangia of lycophytes are on the upper surface of small leaves with unbranched veins with leaves spirally arranged around the stem. Ferns and lycophyes share similar reproductive cycle where mature plants produce fertile fronds with sori on their edges or undersides. They also reproduce by non-sexual or vegetative. http://www.teara.govt.nz/en/ferns-and-lycophytes/page-1(accessed 30 January 2014).

In the last decade, a variety of natural compounds have been identified from different species of ferns during last decades such as bioactive flavonoids like chalcones, dihydrochalcones, avanones, dihydro-flavonols, flavones, flavonols, and bisflavonoids as being reviewed by Wollenweber and Schneider (2000). As for

lycophytes especially in various species of *huperzia*, several chemical compounds like triterpenes, flavones, phenolic acids and alkaloids have been discovered (Ma and Gang, 2004).

1.3 Problem statement

Due to the side effects of most the synthetic drugs available in the market in treating several diseases, the researchers are trying to find alternative medicines from natural resources. The difficulty in the access to the medicines because of the high cost is also reason to find cheap source from plants. The increasing demand for natural medicines has resulted in huge commercial values of these medicines. This has led to the growing interest in searching potent natural drugs and testing their benefits. Ferns and lycophytes present an interesting family of plants for the discovery of new molecules that may have important biological properties. These plants have been used in different systems of medicine such as the Unani, Ayurveda, Homeopathy and Traditional Chinese Medicine. Considering the used of ferns and lycophytes species in many traditional preparation in Malaysia, not many compounds are isolated from these plants to be separately tested on several bioactivities. There exist a huge body of literature on the taxonomy, ecology and distribution of ferns and its other allies. However, compared to other forest plants, ferns and lycophytes have not received enough attention in terms of their utilization despite that they could have equal economic importance as food or medicine. The phytochemistry of many genera within this class of plants has yet to be studied. In fact, very little literature exists for many of the species found in Malaysia. These include the interesting filmy fern species *Hymenophyllum javanicum* and various *Huperzia* species that thrives in the tropical rainforests of Malaysia.

1.4 Objectives of the research

In a continuous effort of enriching the phytochemical knowledge on tropical rainforest plants including ferns and lycophytes, in search of novel and useful natural product compounds, the present study was initiated with the following objectives:

1) To isolate the chemical constituents of the filmy fern, *Hymenophyllum javanicum*
2) To isolate the chemical constituents of two lycophytes i.e, *Huperzia nummularifolia* and *H. carinata*
3) To elucidate the chemical structures of the isolated compounds using spectroscopic methods.

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