SECONDARY METABOLITES FROM THREE SPECIES OF ANNONACEAE PLANTS AND THEIR BIOLOGICAL ACTIVITIES

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

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ABSTRACT

The study aimed to investigate the chemical constituents from *Polyalthia lateriflora*, *Alphonsea elliptica* and *Alphonsea cylindrica* as well as their cytotoxic, antioxidant and xanthine oxidase inhibitory activities. These samples were collected from Peninsular Malaysia and then extracted sequentially using hexane, dichloromethane and methanol. The chemical compounds were isolated and purified by various chromatographic techniques and their structures were elucidated *via* spectroscopic method especially Nuclear Magnetic Resonance, Mass Spectrometry, Infrared, Ultraviolet and comparison with literature. Cytotoxic activity was analysed on MCF-7 human breast cancer cells while antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Study on *P. lateriflora* has yielded six compounds which were lupeol, stigmasterol, *O*-methylmoschotaline, atherospermidine, lysicamine and liriodenine. Meanwhile, five compounds namely kinabaline, atherospermidine, liriodenine, *N*-methylouregidione and cepharadione A were obtained from the barks of *A. elliptica*. In addition, study on the barks of *A. cylindrica* has found eleven compounds in which two are new namely iraqiine and kareemine. Other compounds were identified as isoursuline, cyathocaline, kinabaline, muniranine, isooncodine, atherospermidine, *O*-methylmoschatoline, *N*-methylouregidione, and stigmasterol. Antioxidant activity was tested on six compounds in which iraqiine, muniranine and kinabaline showed the highest activity with IC_{50} values of 48.77, 44.51 and 64.28 mg/ml, respectively. Thirteen compounds were tested against MCF-7 human breast cancer cells of which isoursuline possessed the most potent inhibitory activity with IC_{50} value of 33 µg/ml. Ten compounds displayed moderate xanthine oxidase inhibitory activity. As a conclusion, 22 compounds were successfully isolated from three plant species and two of them are new derivatives of oxoaporphine and bisbenzylisoquinoline alkaloids. Some compounds have been identified as potentially cytotoxic, antioxidant and xanthine oxidase inhibitor. The findings of this study can enhance the understanding of the chemotaxonomy aspect and potential of the Annonaceae family in traditional and modern medicine.

METABOLIT SEKUNDER DARIPADA TIGA SPESIES TUMBUHAN ANNONACEAE DAN KEAKTIFAN BIOLOGINYA

ABSTRAK

Kajian bertujuan untuk mengkaji sebatian kimia daripada spesies *Polyalthia lateriflora*, *Alphonsea elliptica* dan *Alphonsea cylindrica* dan juga aktiviti sitotoksik, antioksidan dan rencatan xantina oksidase. Sampel ini telah dikumpulkan daripada Semenanjung Malaysia dan kemudiannya diekstrak secara berturutan menggunakan heksana, diklorometana dan metanol. Sebatian kimia diasing dan ditulenkan melalui pelbagai teknik kromatografi dan struktur sebatian dikenalpasti melalui kaedah spektroskopi terutamanya Resonans Magnet Nukleus, Spektrometri Jisim, Infra Merah, Ultra Lembayung dan menerusi perbandingan literatur. Aktiviti sitotoksik telah dijalankan ke atas sel kanser payudara manusia MCF-7 manakala aktiviti antioksidan telah diukur menggunakan 2,2-difenil-1-pikrilhidrazil (DPPH). Kajian ke atas *P. lateriflora* telah menghasilkan enam sebatian kimia iaitu lupeol, stigmasterol, *O*-metilmoskotalina, aterospermidina, lisikamina dan liriodenina. Sementara itu, lima sebatian iaitu kinabalina, aterospermidina, liriodenina, *N*-metilorigidiona dan sepharadiona A diperoleh daripada bahagian kulit batang *A. elliptica*. Sebagai tambahan, kajian ke atas kulit batang *A. cylindrica* telah menemui sebelas sebatian yang mana dua daripadanya adalah baharu iaitu iraqiina dan kareemina. Sebatian lain telah dikenalpasti sebagai isoursulina, siatokalina, kinabalina, muniranina, isoonkodina, aterospermidina, *O*-metilmoskotalina, *N*-metilorigidiona, dan stigmasterol. Aktiviti antioksidan telah dijalankan ke atas enam sebatian di mana iraqiina, muniranina dan kinabalina menunjukkan aktiviti tertinggi dengan nilai IC_{50} masing-masingnya adalah 48.77, 44.51 dan 64.28 mg/ml. Tiga belas sebatian telah dianalisa ke atas sel kanser payudara manusia MCF-7 yang mana isoursulina menunjukkan aktiviti perencatan yang paling tinggi dengan nilai IC_{50} adalah 33 μg/ml. Sepuluh sebatian menunjukkan perencatan sederhana terhadap aktiviti rencatan xantina oksidase. Kesimpulannya, 22 sebatian telah berjaya diasingkan daripada tiga spesies tumbuhan dan dua daripadanya merupakan terbitan baharu alkaloid oksoapofina dan bisbenzilisokuinolina. Beberapa sebatian telah dikenalpasti berpotensi sebagai sitotoksik, antioksidan dan perencat xantina oksidase. Penemuan kajian ini dapat meningkatkan pemahaman tentang aspek kemotaksonomi dan potensi tumbuhan daripada keluarga Annonaceae dalam perubatan tradisional dan moden.

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LIST OF SCHEME

LIST OF ABBREVIATIONS

α	Alpha
β	Beta
br	Broad
δ	Delta value (chemical shift) in ppm
CC	Column Chromatography
$CDCl_3$	Deuterated chloroform
CH_3	Methyl group
$CHCl_3$	Chloroform
CH_2Cl_2/ DCM	Dichloromethane
C_6H_{12}	Hexane
COSY	H-H Correlation Spectroscopy
DEPT	Distortioness Enhancement by Polarization Transfer
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EA	Ethyl acetate
FTIR	Fourier Transformation Infra Red
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
Hz	Hertz
IC_{50}	Inhibitory concentration at 50%
IR	Infrared

J	Coupling Constant (Hz)
LCMS	Liquid Chromatography Mass Spectrometry
m	Multiplet
MeOH/CH_3OH	Methanol
MHz	Mega Hertz
MS	Mass Spectrum
m/z	Mass per charge
NH_3	Ammonia
nm	Nanometer
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser effect spectroscopy
OH	Hydroxyl group
OCH_3	Methoxyl group
OCH_2O	Methylenedioxy group
ppm	Part per million
s	Singlet
t	Triplet
TLC	Thin Layer Chromatography
UV	Ultraviolet
λmax	Maximum wavelength
°C	Degree Celsius
^1H	Proton

^{13}C	13 Carbon
1D NMR	One dimensional Nuclear Magnetic Resonance
2D NMR	Two-dimensional Nuclear Magnetic Resonance

CHAPTER 1

INTRODUCTION

1.0 Introduction

Phytochemicals or secondary metabolites, or alternately known as natural products, are produced from various natural resources such as marine organisms, microorganisms and plants. They act as a protective mechanism against predators and herbivores to survive in their surroundings. A variety of fruits, vegetables, seeds, and herbs have phytochemicals such as alkaloids, flavanoids, phenolic, and terpenes (Young et al., 2005). The largest class of secondary metabolites with more than 6,500 known compounds is alkaloids (Schafer & Wink, 2009). They are biosynthesized based on nitrogen molecules which are mainly formed from amino acids such as

arginine, lysine, phenylalanine, and tryptophan. In human folklore history, particularly among the indigenous peoples and traditional healers, plants with medicinal properties and herbs were used to treat different symptoms of diseases (Lee et al., 2008). The first documentation of plants used for medicinal purposes such as *Glycyrrhiza glabra* (licorice) and *Papaver somniferum* (poppy juice), were recorded on clay tablets during the Mesopotamia period which dated back to about 2600 BC. Meanwhile, in year 500 AD, Greek physician, Hippocrates, acknowledged a mixture of various elements from willow bark which evolved in the discovery of aspirin which currently being used to treat pain, fevers, inflammation and reduce the risk of heart attack and stroke by preventing the accumulation of platelets (Bailey et al., 2010). While in 1816, morphine, an analgesic agent was separated from opium poppy, or scientifically known as *Papaver somnifrum* (McChesney et al., 2007).

In recent times, there has been a growing interest in the area of bioactive secondary metabolites. Based on a survey that was developed between 1981 and 2002 on the origin of drugs, about 63% of the marketed medicines that were sold are direct or synthetic lead compounds from natural products such as vegetation, microorganisms and marine organisms (Donald et al., 2006, Newman et al., 2003). Meanwhile, in the United States between 2005 until 2007, 13 clinical drugs were approved and derived from natural products (Li & Vederas, 2009, Krueger, 2011). Nitisinone is derived from *Callistemon citrinus* and was used for genetic tyrosinemia type 1 (HT-1) treatment while galantamine hydrobromide is an alkaloid of Amaryllidaceae which derived from *Galanthus nivalis* (Kaya et al., 2004, Wang,

2008). Additionally, drugs such as paclitaxel which act as anticancer agent had been isolated from *Taxus brevifolia* while salicin,

an analgesic agent, had been isolated from the willow tree (Yaw. et al., 2005). Taxol, morphine, quinine, caffeine, atropine and reserpine derived from higher plants and are among the one-fourth of the drugs being used in the world today (Balandrin et al., 1993). These drugs had proven that natural products are vital sources for new healing agents due to their significant contributions as lead compounds in the production of medicinal drugs. (Skytte et al., 2010, Lajimia et al., 2010). Besides plants, other chemical compounds with biological properties such as bacteria, fungi and marine organisms also contributed to the production of new drugs (Alonso et al., 2005, Leena et al., 2007).

Studies on phytochemicals are limitless and interest in this field has been picked up and increased considerably. This is due to the latest development and inventions of technology to facilitate various procedures such as advanced bioassays, spectroscopic techniques, and separation methods. Over the years, natural compounds from plants has revealed the potentials to be formed and manufactured into modern therapeutic drugs. The development of conventional medicines from bioactive compounds such as plants or herbs has becoming more marketed in comparison to modern medicine around the world. World Health Organization (WHO) had reported that more than 80% of the people in emerging and developing countries are

continuously depending on plants for traditional medicines and cure of common illnesses and diseases. At present, many users around the world use plants for traditional medicines for the care of daily well-being. It is undeniable that with deep comprehension of the biological actions and active compound mechanisms, natural products have consistently become cherished sources for new therapeutic agents. As of 2004, about one percent of tropical species have been filtered and researched due to their possession of pharmaceutical properties against various illnesses such as cancer, cardiovascular disease, and diabetes (Jachak & Saklani, 2004). There were increasing demand for drugs and nutraceuticals from plants and their extracts and derivatives due to their major contributions in pharmacy and medicine (Shahidi, 2005). Hence, it is undeniable that plants will remain and continue to be the main source of new discovery for therapeutic agents due to their pharmaceutical and medicinal features.

1.1 Problem statement

Natural products have been study as sources of traditional and modern drugs for years. The interest on this field remains relevant as new and novel potential therapeutic drugs have been emerging continuously.

Annonaceae family has long been used as traditional medicines to treat diarrhea, dysentery, fever and rheumatism (Bele et al., 2011, Moghadamtousi et al., 2015). Besides that, Annonaceae plants are also being used to treat snakebite, respiratory infections, malaria and pneumonia (Okhale et al., 2016, Mustapha, 2013). Scientific studies had demonstrated that several species of this family exhibited various actions such as antiplasmodial (Ameyaw et al., 2018, Boyom et al., 2011), antioxidant, antidiabetic (Simo et al., 2018, Novaes et al., 2018), anti-inflammatory (Yasmen et al., 2018, Pompermaier et al., 2018), cytotoxic (Rajca et al., 2018, Alencar et al., 2018), insecticidal (Negbenebor et al., 2018) and antimicrobial (Ebelle et al., 2018, Amin et al., 2018, Ononiwu et al., 2017). Chemistry of Annonaceae family showed various group of chemical constituents of which the alkaloids dominated that include aporphinoids (Silva et al., 2018), oxoaporphines (Wijeratne et al., 1996) and phenanthrenes (Rivera & Álvarez, 2018). Therefore, it is important to investigate the phytochemical constituents of genus of this family.

Polyalthia genus belong to Annonaceae family has been investigated as source of many potential compounds and was reported to have biological activities such as anticancer (Nahata, 2017), antibacterial (Ram and Singh, 2017, Negi and Sharma, 2010), antifungal (Barman et al., 2016, Sant et al., 2016) and antioxidant (Adaramola et al., 2017, Jothy et al., 2015, Yang et al., 2016). There has not been any report in the phytochemical and biological activity of *Polyalthia lateriflora.* Hence, there is a possibility to isolate new chemical compounds which may have various biological activities such as anticancer and antimicrobial.

Alphonsea genus belongs to the Annonaceae family. Based on author extensive searches on *Alphonsea* species, the chemical and biological activities of *A. elliptica* have yet to be established and remains to be investigated, whereas the fruits of *Alphonsea* species were traditionally used as emmanogogue, diarrhea and fever treatments (Bakri et al., 2017). Moreover, previous studies had reported that *Alphonsea* species portray various purposes such as antifungal (Suman et al., 2017), antioxidant (Dsd et al., 2018), anticancer (Dsd et al., 2018, Doddapaneni et al., 2018), anti-inflammatory (Attiq et al., 2017) and antibacterial (Talip et al., 2017). Literature study revealed that there was no report on phytochemicals from *A. elliptica*, thus requiring more research. Moreover the phytochemical study on the bark of *A. cylindrica* (Talip et al., 2017) led to isolated new compound which is make this species as a target to find new chemical agent to treat various diseases.

Therefore, there is a need to study the chemical compounds from natural source such as plants that have medicinal properties to discover the new therapeutic drug. Additionally, natural sources were reported to have fewer side effects, affordable, better patience tolerance and renewable in nature.

1.2 Research Objectives

This research was conducted to:

1. Extract and isolate compounds from barks of *P. lateriflora*, *A. elliptica* and *A. cylindrica* using different chromatographic techniques.
2. Identify the chemical structures of the isolated compounds using spectroscopy techniques (NMR, IR, MS and UV).
3. Investigate the anticancer, antioxidant and xanthine oxidase inhibitory activities of the crude extract and isolated compounds from barks of *P. lateriflora*, *A. elliptica* and *A. cylindrica*.

1.3 Significance of study

This study is significant to the research development by expanding the information and knowledge base about the chemical characterization of the phytochemical studies of *P. lateriflora*, *A. elliptica* and *A. cylindrica* barks. The outcomes of this study will serve as future references and be resources for knowledge on therapeutic discovery that can lead to the development of potential and safe drugs in medical field.