ISOLATION OF CHEMICAL CONSTITUENTS FROM ALPHONSEA SP AND THEIR IN SILICO XANTHINE OXIDASE INHIBITORY ACTIVITIES

M.NUR SIDIK

SULTAN IDRIS EDUCATION UNIVERSITY

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ISOLATION OF CHEMICAL CONSTITUENTS FROM ALPHONSEA SP AND THEIR IN SILICO XANTHINE OXIDASE INHIBITORY ACTIVITIES

M.NUR SIDIK

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DR YUHANIS MHD BAKRI
SENIOR LECTURER
DEPARTMENT OF CHEMISTRY
FACULTY OF SCIENCE & MATHEMATICS
SULTAN IDRIS EDUCATION UNIVERSITY
35900, TANJONG MALIM
PERAK

Signature of Supervisor

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ISOLATION OF CHEMICALS CONSTITUENTS FROM ALPHONSEA SP AND THEIR INSILICO XANTHINE OXIDASE INHIBITORY ACTIVITIES

ABSTRACT

The aim of this study was to identify the chemical constituents from two species of *Alphonsea sp*., which were *Alphonsea cylindrica* and *Alphonsea elliptica* and their in silico xanthine oxidase inhibitory activities. The samples were dried, then extracted sequentially using hexane, dichloromethane, and methanol. Chemical constituents were isolated and purified through chromatographic techniques. The structure of the compounds were elucidated through spectroscopic data and comparison with literature. In silico based on molecular docking using YASARA program was carried out to study the inhibition mechanism and interactions of compounds against xanthine oxidase. Six chemical constituents were isolated; stigmasterol, atherospermidine, kinabaline, muniranine, methyl 4-hydroxy-3,5-dimethoxybenzoate and 3- hydroxy-4-methoxy-benzoic acid. Both methyl 4-hydroxy-3,5-dimethoxybenzoate and 3- hydroxy-4-methoxy-benzoic acid were firstly reported from *Alphonsea sp.* The results of the molecular docking study revealed that atherospermidine bind to active sites located in the FAD domain of xanthine oxidase which suggest it is a competitive inhibitor. Meanwhile, other compounds isolated are non-competitive inhibitors. In conclusion, six chemical compounds were isolated from *Alphonsea sp* and all interactions of the compounds with xanthine oxidase in silico were investigated. The findings of this study reveal the potential of *Alphonsea sp.* as remedy for gout.

ABSTRAK

Tujuan kajian ini adalah untuk mengenal pasti sebatian kimia daripada dua spesies *Alphonsea sp*., iaitu *Alphonsea cylindrica* dan *Alphonsea elliptica* dan aktiviti perencatan in siliko enzim xantina oksidase. Sampel telah dikeringkan, kemudian diekstrakkan secara berurutan menggunakan heksana, diklorometana, dan metanol. Komponen kimia diasingkan dan ditulenkan melalui teknik kromatografi. Struktur sebatian ditentukan melalui data spektroskopi dan perbandingan dengan literatur. In siliko berdasarkan dok molekul menggunakan program YASARA dilakukan untuk mengkaji mekanisme perencatan dan interaksi sebatian terhadap xanthine oxidase. Enam sebatian kimia telah diasingkan; stigmasterol, aterospermidina, kinabalina, muniranina, metil 4-hidroksi-3,5- dimetoksibenzoat dan asid 3-hidroksi-4-metoksi-benzoik. Kedua-dua metil 4-hidroksi-3,5- dimetoksibenzoat dan asid 3-hidroksi-4-metoksi-benzoik adalah pertama kali dilaporkan daripada *Alphonsea sp*. Hasil kajian dok molekul menunjukkan bahawa aterospermidina mengikat ke tapak aktif yang terletak di domain FAD xantina oksidase yang menunjukkan bahawa ia adalah perencat kompetitif. Sementara itu, sebatian lain yang diasingkan adalah perencat tidak kompetitif. Kesimpulannya, enam sebatian kimia diasingkan dari *Alphonsea sp* dan semua interaksi sebatian dengan xanthine oxidase in siliko telah dikaji. Dapatan kajian menunjukkan potensi *Alphonsea sp.* sebagai ubat untuk gout.

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LIST OFABBREVIATIONS

°C	Degree Celsius
^{13}C	13 Carbon
1D NMR	One dimensional Nuclear Magnetic Resonance
^{1}H	Proton
2D NMR	Two-dimensional Nuclear Magnetic Resonance
Å	Amstrong
br	Broad
C_6H_{12}	Hexane
CC	Column Chromatography
$CDCl_3$	Deuterated chloroform
CH_2Cl_2/ DCM	Dichloromethane
CH_3	Methyl group
$CHCl_3$	Chloroform
COSY	H-H Correlation Spectroscopy
d	*Doublet*
dd	*Doublet of doublet*
DEPT	*Distortioness Enhancement by Polarization Transfer*
EA	Ethyl acetate
FAD	*Flavin Adenine Dinucleotide*
FTIR	*Fourier Transformation Infra Red*

GC-MS	*Gas Chromatography - Mass Spectroscopy*
HMBC	*Heteronuclear Multiple Bond Correlation*
HMQC	*Heteronuclear Multiple Quantum Correlation*
Hz	*Hertz*
IC_{50}	Inhibitory concentration at 50%
IR	Infrared
J	Coupling Constant (Hz)
m	Metre
m	*Multiplet*
m/z	Mass per charge
MeOH/CH_3OH	Methanol
mg/ml^{-1}	Microgram per mililitre
MHz	Mega Hertz
MS	Mass Spectrum
NH_3	Ammonia
nm	Nanometer
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser effect spectroscopy
OCH_2O	Methylenedioxy group
OCH_3	Methoxyl group
OH	Hydroxyl group
ppm	Part per million

s	*Singlet*
t	*Triplet*
TLC	*Thin Layer Chromatography*
UV	Ultraviolet
α	Alpha
β	Beta
δ	Delta value (chemical shift) in ppm
λmax	Maximum wavelength

APPENDIX LIST

1.	Title	:	Methyl syringate and isovanilic acid from the bark of *Alphonsea elliptica*
	Author	:	Sidik M. Nur, Mhd Bakri Yuhanis, Syed Abdul Azziz Saripah Salbiah, Wong Chee Fah, Ibrahim Mastura
	Journal	:	*Research Journal of Chemistry and Environment*
2.	Title	:	In silico xanthine oxidase inhibitory activities of alkaloids isolated from *Alphonsea sp.*
	Author	:	M. Nur Sidik, Mhd Bakri Yuhanis, Syed Abdul Azziz Saripah Salbiah, Ahmeed Kareem Obaid Aldulaimi, Wong Chee Fah, Ibrahim Mastura
	Journal	:	*South African Journal of Botany*

CHAPTER 1

INTRODUCTIONS

1.1 Introduction

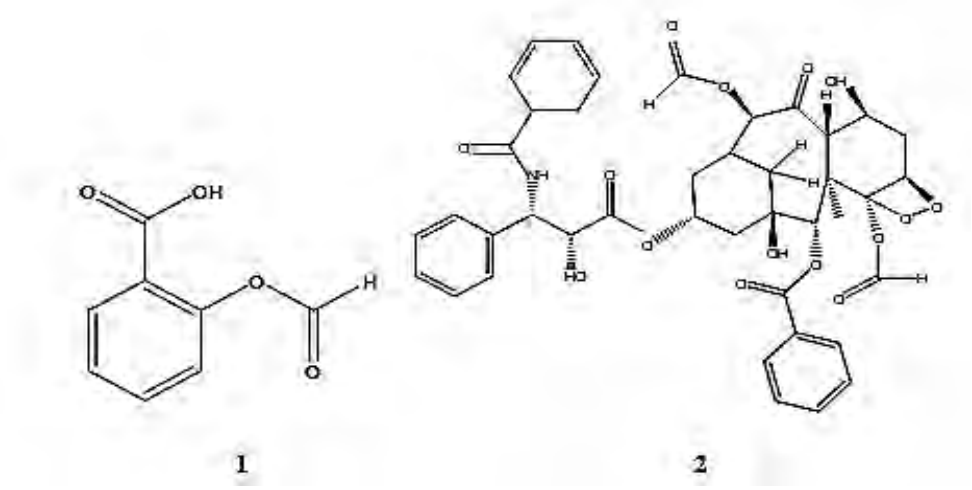
Natural products chemistry continues to be significance research in finding solutions for diseases. History has proven that natural products are important source of drug leads that will be potential to be used as medicines. Even more so that ASEAN countries including Malaysia and Indonesia are rich in biodiversity which some are untapped and limitedly studied. According to National Biodiversity Index, Malaysia is one of the twelve countries recognized as the world's biological 'mega-diversity' (Abdul Latieff

Mohamad, 2015). In fact, the forest area of Peninsular Malaysia has an area of 11 million hectares which is confirmed to be a habitat for various types of flora and fauna that are interesting to be explored and studied (Nor 'Asyikin Mat Hayin, 2018).

Natural products chemistry research involves investigating and finding bioactive compounds from natural sources including plants, animals, microorganisms and marine organisms. This field was pioneered by previous and ancient generations who used many plants for the many purposes including body care and healing diseases. The belief that plants are very effective and nutritious in solving various problems of life are passed down through generations without scientific evidence. Hence, the study of natural products will affirm the traditional knowledge by identifying the chemical composition of plants and investigate their pharmacological activity. The results of this research are important and fundamental for the discovery and development of more modern medicine effective and safe (Kuppusamy et al., 2015).

Many drugs available on the market today have been found from natural resources (Nurraihana Hamzah, 2015). Aspirin (**1**) which shows analgesic activity is one of the most well-known and popular drugs in world. It is obtained from the plant species *Salix sp*. (willow tree) and *Populus sp*. (poplar tree) (Lumintang, Wuisan and Worwor, 2015). In addition, paclitaxel or taxol (**2**) which has shown anticancer activity was first discovered from the bark of the *Taxus brevifolia* (Taxaceae) or The Sumatran Yew tree (Silmi Qurrotu Aini, 2018). Also, two the chemical compounds which display potential as antivirals including HIV, hypericin (**3**) and pseudohypericin (**4**) were isolated from microorganisms *Hypericum perforatum*

(Guttiferae) (Sanna et al.,2018).



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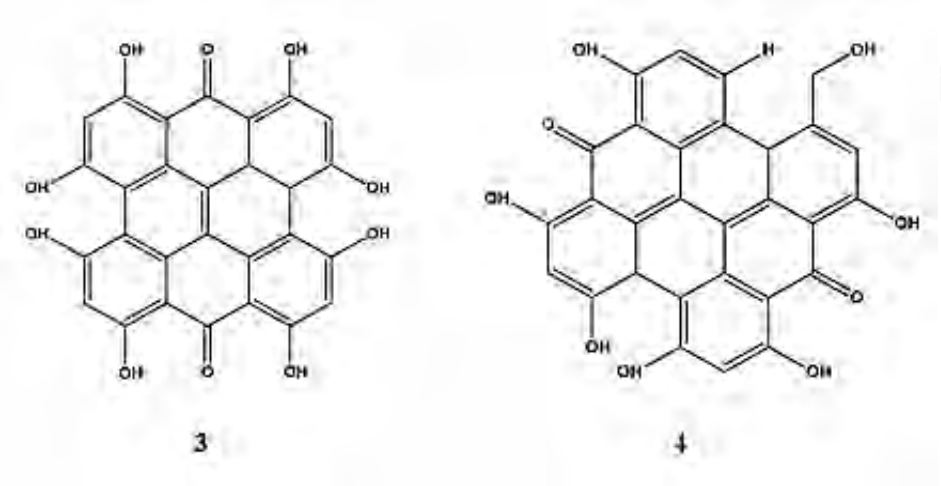

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In fact, a recent review by Newman and Cragg in 2020 which covers almost 39 years of natural products as source of new drugs revealed that they are still and very relevant as precursor of new drugs (Figure 1.1).

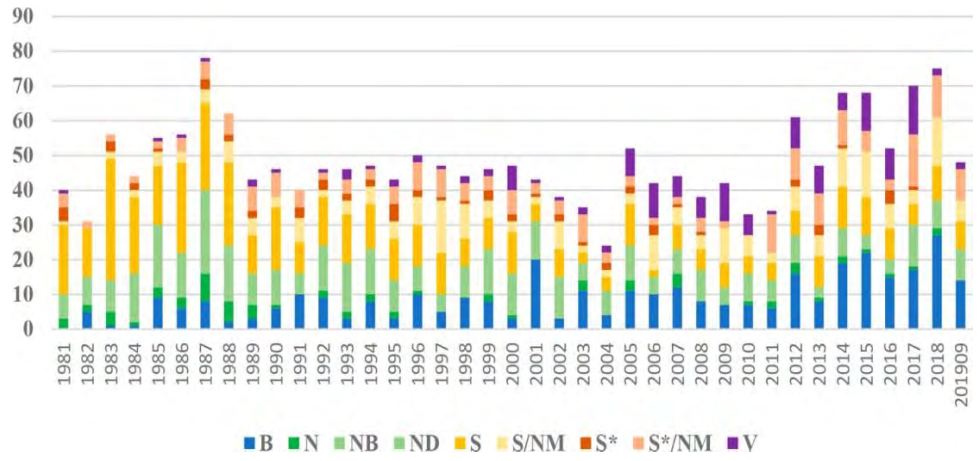

Figure 1.1. Approved drugs by source (B: biological macromolecule, N: unaltered natural product, NB: botanical drug (defined mixture), ND: natural product derivative, S: synthetic drug, S/NM: synthetic drug/mimic of natural product, S*/NM: synthetic drug (pharmacophore)/mimic of natural product, V: vaccine) according to year. (Adopted from Newman and Cragg, 2020).

History shows that modern medicines which are based on natural products have developed into the important medicine of today. However, only 6% of plant species have been studied for their pharmacological activities while less than 20% of investigations were carried out to investigate their phytochemistry (Arumugam, Swamy and Sinniah, 2016). Hence, there is needs to increase the discovery of new drugs from natural resources to aid in facing various chronic diseases and increasingly challenging health problems.

Plants contain various valuable bioactive compounds. These naturally derive compounds, or natural products are widely used in both traditional and modern therapy to enhance human health with few or no side effects (Kuppusamy et al. 2015). Medicinal plants have always been the most appropriate choice in almost all cultures of civilization because medicinal plants are considered as a traditional medicinal resource which is rich in benefits and will produce modern medicine (Dar, Shahnawaz, and Qazi, 2017). Various medicinal plants have been well explored

worldwide and new molecules were discovered from plants to cure diseases (Arumugam, Swamy and Sinniah, 2016). Unfortunately, most medicinal plants are understudied (Havva and Turkmen, 2019).

Many natural products have been reported as enzyme inhibitors (Abdur Rauf and Noor Jehan, 2017). Discoveries and developments in the fields of natural products chemistry, biochemistry, pharmacognosy and pharmacology allow the potential enzyme inhibitors to develop into new drugs. According to Shapiro and Vallee (1991), drugs developed through enzyme inhibition are commonly mediated by its specificity and effectiveness, hence, the drugs will have fewer side effects and has lower toxicity.

1.2 Problem Statement

Xanthine oxidase is an enzyme responsible for the build-up of excess uric acid that causes gout, one of the most common inflammatory arthritis found in Malaysia. Currently, allopurinol which acts to inhibit xantine oxidase has been used as a drug to treat gout. However, allopurinol has side effects. Natural products chemistry are proven to result in potential bioactive compounds that will further aid in discovering new drugs. Therefore, it is necessary to study the potential natural compounds as an alternative to allopurinol. In fact, current gout treatments also tend to be expensive, therefore natural compounds without these disadvantages offer great opportunities. The results of this study are expected to yield additional knowledge about natural

xanthine oxidase inhibitors and understand the interaction between active constituent and the enzyme.

1.3 Research objective

This research was conducted to:

1. Isolate chemical constituents from bark extracts of *Alphonsea cylindrical* dan *Alphonsea elliptica* using different chromatographic techniques
2. Elucidate the structure of chemical constituents using various spectroscopy techniques
3. Identify active site of chemical constituents from *Alphonsea sp.* through in silico study of xanthine oxidase
4. Design modification of derivative of active constituent from *Alphonsea sp.*
5. Determine in silico xanthine oxidase inhibitory activities of tentative active constituent derivatives.

1.4 Significance of study

The results of this study are expected to expand the knowledge on xanthine oxidase inhibitors from natural products. In addition, it is hopeful that through this research, interaction between active compounds and the enzyme will be understood. Hence,

the study will be a good starting point for conducting further research in finding an alternative medicines and cure for gout.