Faculty of Resource Science and Technology

Phytochemical Study of *Belalai Gajah* (*Clinacanthus nutans* Lindau) as Analgesic Agent Using Pharmacophore Modelling and Biological Assay

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DECLARATION

I declare the work in this thesis was carried out in accordance with the regulations of University Malaysia Sarawak. It is original and is the result of my work, unless otherwise indicated or acknowledged as reference work. The thesis has not been accepted for any degree and is not concurrently submitted in candidature for any other degree.

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Signature

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ABSTRACT

Pain is a global health problem that impairs life quality. The rapid advancement in drug study is still relying on herbal plants as the source for bioactive compounds. First documented by Nicolaas Laurens Burman in 1984, *Belalai gajah* (*Clinacanthus nutans* Lindau) is a herbal plant from Acanthaceae family that is well-known in Asia as a herbal remedy for snake bites, herpes infection, skin infection, cancer, dysentery and diabetes. In recent years, phytochemical screening of *C. nutans* revealed the presence of bioactive metabolites which potentially contributes to its antiviral, antimicrobial, antioxidant and anti-inflammatory properties as reported in previous studies. However, it is difficult to determine the safe dosage of *C. nutans* when it is used in different ways. Much of the studies were conducted through *in vivo* method which provided little information on biomolecular interaction. Therefore, this study aimed to extract, isolate and elucidate compounds from *C. nutans.* The potential of *C. nutans* phytochemicals as analgesic was assessed via ligand-based and structure-based pharmacophore modelling. Antioxidant assay, toxicity assay and analgesic assay of extracts, partitions and isolated compounds of *C. nutans* were conducted in this study. *C. nutans* leaves was extracted and proceeded to compound isolation using column chromatography technique. Using spectroscopy technique, three compounds were elucidated as 3,7,11,15-tetramethyl-2-hexadecen-1-ol, (2S)-1-O-linolenoyl-3-O-*β*-D-galactopyranosyl glycerol and 8-*β*-D-glucopyranosyl-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one. These compounds were described as EA-1, EA-2 and MEOH-1, respectively. Pharmacophore analysis of isolated and reported compounds were conducted using LigandScout 4.2 software. Pharmacophore model was generated from training set comprised of analgesic drugs. Test set was aligned to pharmacophore model to obtain fit value and common pharmacophore features. Crystal structure of cyclooxygenase-2 (COX-

2) with bound ibuprofen was the selected target complex. The interaction of test set to amino acid residue in binding site and score were obtained. 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant, toxicity and *in vivo* analgesic assay were conducted to evaluate biological activity of extract and isolated compounds. Pharmacophore analysis showed MEOH-1 and EA-2 had good fit value (53.59, 52.85), five interactions with pharmacophore model but EA-1 was scored low (43.64). Isoorientin and isovitexin were scored best with six pharmacophore interactions compared to other test set. MEOH-1 had better alignment score to COX-2 active site than EA-1 and EA-2 with value 45.45, 45.36 and 40.18, respectively. Orientin and isomollupentin scored the best among all test set. MEOH-1 satisfied all Lipinski's parameter, while EA-2 and EA-1 did not satisfy molecular weight and *Log P* value. MeOH partition had the best antioxidant activity of IC_{50} 15.73 µg/mL while MEOH-1 was IC_{50} 19.27 µg/mL. *C. nutans* was concluded to be less toxic (LC_{50} < 1000 µg/mL) with hexane partition had the highest LC_{50} value of 128.4 µg/mL. LD_{50} of extracts and all partitions were determined to be greater than 2000 mg/kg and classified closely to Category 5. Both analgesic tests revealed MeOH extract to have better capability to prolong latency response than partitions but did not show significant analgesic effect as compared to ibuprofen ($p<0.05$) at concentration 200 mg/kg. To conclude, *C. nutans* leaves extract had mild analgesic effect, possess bioactive compounds suitable as analgesic agent based on pharmacophore interaction and *in vivo* assay. *C. nutans* extracts and partitions are not toxic, and safe for consumption.

Keywords: *Belalai gajah, Clinacanthus nutans*, Nicolaas Laurens Burman, pharmacophore analysis, antioxidant, analgesic agent, spectroscopy technique, chromatographic technique.

Kajian Fitokimia ke atas Belalai Gajah (Clinacanthus nutans* Lindau*) sebagai Agen Analgesik Melalui Analisa Farmakofor dan Kaedah Biologi

ABSTRAK

Rasa sakit ialah masalah kesihatan global yang menjejaskan kualiti kehidupan. Perkembangan pesat kajian perubatan masih bergantung kepada tumbuhan herba sebagai sumber sebatian aktif. Pertama kali didokumenkan oleh Nicolaas Laurens Burman pada tahun 1984, belalai gajah (*Clinacanthus nutans* Lindau) *adalah tumbuhan daripada keluarga Acanthaceae yang terkenal di Asia sebagai herba untuk gigitan ular, jangkitan herpes dan kulit, kanser, keradangan perut dan diabetes. Penyaringan fitokimia C. nutans menunjukkan potensi metabolit aktif terhadap sifat antiviral, antimikrobial, antioksida dan anti-radang. Namun, dos selamat untuk C. nutans sukar ditentukan kerana perbezaan cara penggunaan. Terdahulu, kajian in vivo kekurangan maklumat berkenaan interaksi biomolekul. Kajian ini bertujuan untuk mengekstrak, menulen dan mengenal pasti sebatian dari C. nutans. Potensi analgesik fitokimia ditentukan melalui analisa farmakofor berdasarkan ligan dan struktur. Kajian antioksida, toksisiti dan analgesik dilakukan pada ekstrak, fraksi dan sebatian tulen dari C. nutans. Daun C. nutans telah diekstrak dan melalui penulenan sebatian menggunakan teknik kromatografi. Melalui teknik spektroskopi, tiga sebatian tulen telah dikenalpasti sebagai 3,7,11,15-tetramethyl-2-hexadecen-1-ol, (2S)-1-O-linolenoyl-3-O-β-D-galactopyranosyl glycerol, dan 8-β-D-glucopyranosyl-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one. Sebatian tulen ini dijelaskan sebagai EA-1, EA-2 dan MEOH-1. Analisa farmakofor sebatian tulen dan yang pernah dilaporkan dilakukan menggunakan perisian LigandScout 4.2. Model farmakofor dijana menggunakan set cadangan yang terdiri daripada ubat analgesik. Set ujian diselaraskan pada model farmakofor untuk mendapatkan nilai kesesuaian dan persamaan sifat farmakofor. Struktur*

kristal cyclooxygenase-2 (COX-2) dengan ikatan ibuprofen adalah kompleks sasaran. Interaksi set ujian dengan asid amino di dalam tapak ikatan dan skor kelarasan telah diperoleh. Ujian antioksida 2,2-diphenyl-1-picrylhydrazyl (DPPH), ujian toksisiti, dan ujian analgesik in vivo dilakukan untuk menilai kesan biologi dari ekstrak dan sebatian tulen. Analisa farmakofor menunjukkan MEOH-1 dan EA-2 mempunyai nilai kesesuaian bagus (53.59, 52.85), lima interaksi terhadap model farmakofor tetapi EA-1 mempunyai nilai kesesuaian rendah (43.64). Isoorientin dan isovitexin mempunyai nilai kesesuaian terbaik dan enam interaksi berbanding yang lain. MEOH-1 mempunyai skor keselarasan lebih baik terhadap tapak ikatan COX-2 berbanding EA-2 dan EA-1 dengan nilai 45.45, 45.36 dan 40.18 masing-masingnya. Orientin dan isomollupentin mempunyai nilai keselarasan terbaik berbanding yang lain. MEOH-1 melepasi semua parameter Lipinski, sementara EA-2 dan EA-1 tidak melepasi parameter berat molekul dan nilai Log P. Fraksi metanol mempunyai aktiviti antioksida terbaik iaitu IC_{50} 15.73 µg/mL manakala MEOH-1 ialah IC_{50} 19.27 µg/mL. C. nutans dikenalpasti sebagai kurang toksik (LC_{50} < 1000 µg/mL) dan fraksi hexana mempunyai nilai LC_{50} tertinggi iaitu 128.4 µg/mL. LD_{50} ekstrak dan fraksi melebihi 2000 mg/kg, dikelaskan dalam kategori 5. Kedua-dua ujian analgesik menunjukkan ekstrak metanol mampu memanjangkan kesan latensi berbanding fraksi tetapi tiada kesan analgesik yang ketara berbanding ibuprofen ($p<0.05$) pada 200mg/kg. Kesimpulannya, ekstrak daun C. nutans mempunyai kesan analgesik yang sederhana, mempunyai sebatian aktif sesuai sebagai agen analgesik berdasarkan interaksi farmakofor dan kajian in vivo. Ekstrak dan fraksi dari C. nutans adalah tidak toksik dan selamat untuk pemakanan.

Kata kunci: *Belalai gajah, Clinacanthus nutans*, Nicolaas Laurens Burman, *analisa farmakofor*, *antioksida, agen analgesik*, *teknik spektroskopi, teknik kromatografi*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Ar	Aromatic
CADD	Computer aided drug design
$CHCl_3$	Chloroform
cm	Centimeter
^{13}C-NMR	Carbon-13 nuclear magnetic resonance
COX	Cyclooxygenase enzyme
DCM	Dichloromethane
δ_C	Chemical shift of carbon
δ_H	Chemical shift of proton
DPPH	2,2-diphenyl-1-picrylhydrazyl
EtOAc	Ethyl acetate
EtOH	Ethanol
FTIR	Fourier-transform infra-red
GABA	Gamma aminobutyric acid
HBA	Hydrogen bond donor
HBD	Hydrogen bond acceptor
HMBC	Heteronuclear multiple bond correlation
^{1}H-NMR	Proton nuclear magnetic resonance
Hy	Hydrophobic
Hz	Hertz
IUPAC	International Union of Pure and Applied Science
Log P	Lipophilicity
MeOH	Methanol
mL	Mililiter

mm	Milimeter
M.W	Molecular weight
NI	Negative ionizable area
NSAIDs	Non-steroidal anti-inflammatory drugs
OECD	Organisation for Economic Co-Operation and Development
PDB	Protein Data Bank
PI	Positive ionizable area
RCSB	Research Collaboratory for Structural Bioinformatics
s	second
TLC	Thin layer chromatography
TMS	Tetramethylsilane
UV-Vis	Ultra-violet visible spectrophotometry

CHAPTER 1

INTRODUCTION

1.1 Background of the Research

Plant has been a great source of medicine since the early time from the medicine records of Chinese, Hindu and Mediterranean civilization. *Belalai gajah* (*Clinacanthus nutans* Lindau) is one of well recognized herbs from Acanthaceae family commonly found in Southeast Asia countries, where it thrives in tropical climate and grows well in the wild (Fongod et al., 2013). The first record of *C. nutans* was by a dutch botanist named Nicolaas Laurens Burman dated back to 1894. He recorded the specimen as *Clinacanthus nutans* (Burm. f.) Lindau, which was later accepted as the species name by the International Plant Names Index in 1899 (Kamarudin et al., 2017).

Leaves is the only part of *C. nutans* that is utilized as herbs. Traditionally, it is consumed as tea, juice or *ulam* as it is believed to cure diabetes, cancer and gout. It is used as poultice to relieve skin inflammation due to rashes, lesion and burns, insects and snake bites. Nowadays, it is commercialized as health products in the form of cream for topical application and capsules especially in Thailand. In recent years, several pharmacological properties of *C. nutans* have been tested based on the therapeutic claims and some interesting findings were highlighted. The anti-inflammatory response was found to be dose dependent by inhibiting elastase and proinflammatory cytokines release in mice (Thongrakard & Tencomnao, 2010; Wanikiat et al., 2008). The antiviral property is associated with its moderate activity against dengue virus type-2 and inhibition of late stage herpex simplex virus multiplication using alcoholic extract, containing monogalactosyl and digalactosyl diglyceride (Yong et al., 2013). The aqueous extract is potentially an antidiabetic as it was

found to inhibit α-glucosidase at 88 % activity (50 mg/mL) (Wong et al., 2014). Also, anticancer activity was observed against cervical cancer cells and melanoma cells (Fong et al., 2016; Yong et al., 2013). *C. nutans* extract was suggested to be an effective complementary approach for cancer prevention and treatment as it exerted antitumor and antioxidant activity against mouse model with no toxicity effect (Nik et al., 2019). The antioxidant activity is most likely due to the presence of flavonoids in the leaves extract. Previous studies reported various compounds from *C. nutans* in the form of steroids, triterpenoids, flavonoids, phenolic acid, glycosides and chlorophyll derivative (Irchhaiya et al., 2015).

However, herbal medicine is to be consumed moderately as it may bring adverse effect to those suffering chronic diseases. Traditional Chinese medicine highlighted that incompatible herbs mixture will impair the overall efficacy of drug prescription. The transition of herbs to synthetic drugs was accelerated during world war as there were pressing needs for better surgical technique and more effective drug to treat severe infections (Dailey, 2018). Pharmaceutical companies were pushed to develop more effective drugs at industrial scale. Despite this, isolated active compounds from herbs are still useful as lead compounds and have been modified into derivatives to improve their side effects.

1.2 Analgesic Agent Discovery

Opioids and non-steroidal anti-inflammation drugs have remained as the main treatment in chronic pain. The development of analgesics for acute and chronic pain relieve is an ongoing discovery since pain is a pervasive health problem related to cancer, inflammation and nervous system injury (Decosterd & Woolf, 2000). The four largest causes of pain are cancer, arthritis, operations and injuries, and spinal problems, making the etiology of pain a complex, transdisciplinary affair (Goldberg & McGee, 2011). Following these

years, drug development research is driven by advanced instruments, databank resources available online and *in silico* approach which complements *in vivo* approach.

Pharmacophore modelling is one of computer-aided approach to enable study of compound interaction with or without 3-dimensional (3D) macromolecular target. Softwares are developed with multilevel search filter to assist in screening of bioactive compounds. Pharmacophore application is becoming the main tool in drug discover as its application been extended to lead optimization, evaluate pharmacokinetic properties and optimize derivatives based on structural information (Thiel, 2004).

1.3 Problem Statement

The analgesic drugs could be useful over wide range of conditions but they are not uniformly effective and may cause adverse effects due to long consumption. Consequently, the ongoing drug discovery focus is to find novel analgesics with improved properties. Traditional medicine is easily obtained from nature but its long-term use could not be easily assessed without much understanding of the active components. The study of medicinal plant incorporated with *in silico* method is still uncommon. Through chromatography technique, isolation of pure compounds could be achieved with the use of suitable solvent and monitored by thin layer chromatography. *In silico* method using pharmacophore modelling allows the bioactivity of *C. nutans* compounds to be assessed at biomolecular level. It provides analysis from wider spectrum, faster time and to support findings from *in vivo* test. The existing studies of *C. nutans* has been mainly on antioxidant and anti-cancer properties. Thus, the study of its analgesic effect could further increase the medicinal value of *C. nutans* without overlooking the risk of its toxicity.