UNIVERSITI PUTRA MALAYSIA

ANTINOCICEPTIVE ACTIVITIES OF SENDUDUK (MELASTOMA MALABATHRICUM L.) LEAVES METHANOLIC EXTRACT AND ITS PETROLEUM ETHER FRACTIONS

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By

ERMAN SHAH JAIOS

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements 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

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Chairperson : Associate Professor Zainul Amiruddin Zakaria, PhD
Faculty : Medicine and Health Sciences

Natural products that obtained from the extraction process of medicinal plants are being studied scientifically and endeavor to discover new potential therapeutic agents with less, or no side effect. *Melastoma malabathricum* L. is one of the medicinally important plants belonging to the family Melastomaceae, commonly known as "Senduduk" in Malay culture. Traditionally, leaves are claimed to relieve diverse pain-related ailments. Therefore, the objective of the present study was to examine the antinociceptive activities of *M. malabathricum* L. leaves methanolic extract (MEMM) and its petroleum ether (PEMM) fraction by using the *in vivo* models of nociception in both thermal- and chemical-induced pain tests. The dose of extracts (100, 250, and 500 mg/kg) was administered via orally 60 minutes (min) prior to subjection of the respective test in the volume of 10 mL/kg. Throughout this study, rats and mice (n=6) were pre-treated with the drugs or extract per group. The study was designed as a preventive method and the potential of MEMM and PEMM against nociception has never been reported. In the first stage, we were attempted to evaluate the extract antinociceptive activities, the *in vivo* thermal (hot plate test; HT), chemicals (acetic acid-induced abdominal constriction; ACT and formalin-induced paw licking test; FT) models of nociception were used. In order to elucidate the mechanisms of action involved, the role of opioid, vanilloid receptors (capsaicin), glutamate system (glutamatergic) and nitric-oxide/cyclic guanosine phosphate (NO/cGMP) pathway in modulation of the extract antinociceptive activities were determined. In the second stage, MEMM was partitioned into three fractions: petroleum ether (PEMM), ethyl acetate (EAMM), and aqueous (AQMM). Nevertheless, our objective in this second stage was to investigate the most potent fraction among the three extracts. Therefore, the experiment ED_{50} (effective dose producing a 50% reduction in relative to control value) and its 95% confidence intervals (CI) values were conducted to determine the most potent fraction and the ACT was used to screen the antinociceptive effect. From the calculation, PEMM is the most effective fraction was further used to assess the antinociceptive properties

using the *in vivo* models of nociception. Moreover, all the extracts (MEMM, PEMM, EAMM and AQMM) underwent the phytochemical screening such as Flavanoids Test, Saponins Test, Tannins and Polyphenolic Compounds Test, Steroids / Triterpenes Test, and Alkaloids Test were recorded. Analysis and identification of phytochemical constituents with the aid of High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC-MS) technique were performed. In the first stage, MEMM significantly ($P < 0.05$) was exhibited antinociceptive activities in all the chemically- and thermally-induced nociception models. Naloxone (5 mg/kg), a non-selective opioid antagonist, significantly ($P < 0.05$) was failed to affect the antinociceptive activity of MEMM. Moreover, MEMM antinociception significantly ($P < 0.05$) was reversed the capsaicin- and glutamate-induced paw licking test. Whereas, L-arginine (a nitric oxide precursor), L-NAME (an inhibitor of NO synthase), methylene blue, MB (an inhibitor of cGMP), or their combination significantly ($P < 0.05$) was failed to change the intensity of MEMM antinociception. In the second stage, it was shown the verified screening of the antinociceptive effect of PEMM, EAMM and AQMM fractions assessed by ACT. Likewise, the PEMM and EAMM had similar efficacy to produce antinociceptive effect [max. inhibitions of 24.17±1.33 (70.94%) and 18.83±0.91 (77.36%)] at the dose 500 mg/kg, respectively. As a result, the PEMM was more effective than the EAMM with the calculation of ED_{50} values [with 95% confidence interval (C.I)] of 119.5 mg/kg (97.03 – 147.1 mg/kg) and 125.9 mg/kg (109.9 – 144.1 mg/kg), respectively. PEMM significantly ($P < 0.05$) was exhibited antinociceptive activity in all the chemically- and thermally-induced nociception models. Naloxone (5 mg/kg), a non-selective opioid antagonist, significantly ($P < 0.05$) was failed to reverse the antinociceptive effect of PEMM assessed using the HT and FT. PEMM antinociception significantly ($P < 0.05$) was reversed the capsaicin- and glutamate-induced paw licking test. Furthermore, L-arginine, L-NAME, MB, or their combination significantly ($P < 0.05$) was also failed to interfere the PEMM antinociception effect. The phytochemical analysis was screened for all the extracts, and presence of flavonoids, tannins, saponins, triterpenes and steroids, but no alkaloids. In addition, the HPLC analysis of MEMM and PEMM were demonstrated the presence of flavonoids as its major constituents. In the GC-MS analysis, the phytoconstituents were screened and majority of these identified compounds are palmitic acid, terpene, diterpene, α-Linolenic acid and fatty acid ester. Together, these results indicate that the MEMM produced dose-dependent antinociception in the *in vivo* nociception models of chemical and thermal with the aids of the phytoconstituents, whereby, the PEMM was considered to have the best activity of antinociceptive activities among the fractions, which warrants further investigation.

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

AKTIVITI ANTINOSISEPTIF OLEH EKSTRAK METANOL DARI DAUN SENDUDUK (*Melastoma malabathricum* L.) DAN PECAHAN PETROLEUM ETER

Oleh

ERMAN SHAH JAIOS

November 2016

Pengerusi : Professor Madya Zainul Amiruddin Zakaria, PhD
Fakuti : Perubatan dan Sains Kesihatan

Produk semulajadi yang diperolehi daripada tumbuhan perubatan melalui proses pengekstrakan sedang giat dikaji secara saintifik dan berusaha untuk menemui agen terapeutik baru yang berpontensi mempunyai kurang atau tidak kesan sampingan. *Melastoma malabathricum* L. merupakan salah satu tumbuhan perubatan yang berpontesi dan penting di dalam keluarga Melastomaceae dan kebiasannya dikenali sebagai Senduduk dikalangan kaum Melayu. Secara tradisional, daunnya banyak digunakan sebagai rawatan untuk menghilangkan masalah kesihatan atau penyakit berkaitan kesakitan. Objektif kajian ini adalah untuk menilai aktiviti antinosiseptif ekstrak metanol dari daun *M. malabathricum* L. (MEMM) dan pecahan petroleum eter (PEMM) menggunakan ujian model *in vivo* nosiseptif secara haba- dan bahan kimia-penghambatan kesakitan. Dos ekstrak (100, 250, and 500 mg/kg) dimasukkan melalui mulut 60 minit awal sebelum dikenakan ujian dengan menggunakan kiraan isipadu 10mL/kg. Sepanjang kajian ini, bilangan (n = 6) bagi tikus dan mencit telah digunakan untuk rawatan bagi ekstrak/dadah per satu kumpulan rawatan. Kajian ini menggunakan kaedah pencengahan dan tiada sebarang laporan berkaitan MEMM dan PEMM melawan nosiseptif direkodkan. Pada peringkat yang pertama, penilaian akitiviti MEMM antinosiseptif menggunakan model ujian nosiseptif teknik *in vivo* secara haba (ujian plat panas, UPP) dan kimia (ujian asid asetik-penghambatan pengeliatan perut; UAA dan ujian formalin-penghambatan penjilatan tapak kaki; UF). Penjelasan lebih lanjut mengenai tindakan mekanisma yang terlibat telah dilakukan dengan menilai fungsi reseptor opiat, vaniloid (capsaicin) sistem glutamat (glutamatergik) dan laluan nitrik-oksida/siklik-guanosin fosfat (NO/cGMP) dalam modulasi aktiviti ekstrak antinosiseptif. Pada peringkat yang kedua, MEMM telah dipecahkan kepada tiga pecahan iaitu petroleum eter (PEMM), etil esetat (EAMM) dan akueus (AQMM). Namun begitu, objektif kami pada peringkat kedua ini adalah untuk menyiasat pecahan yang paling poten diantara tiga pecahan tersebut. Oleh itu, ujian ED_{50} (dos efektif menghasilkan pengurangan 50% relatif mengawal nilai kawalan) dan 95% selang keyakinan (CI) telah digunapakai untuk menentukan dos yang paling efektif diantara tiga pecahan, dan ujian UAA telah digunapakai bagi

saringan aktiviti antinosiseptif tersebut. Daripada pengiraan tersebut, pecahan PEMM adalah yang paling berkesan dan susulan ujian telah dilakukan untuk mendapatkan profil antinosiseptif menggunakan model teknik *in vivo* nosiseptif. Tambahan lagi, semua ekstrak (MEMM, PEMM, EAMM dan AQMM) telah menjalani ujian saringan fitokimia seperti penentuan flavonoid, saponins, tannins dan sebatian polifenol, steroid/tritepen, dan alkaloid. Analisis dan identifikasi juzuk - juzuk fitokimia telah dilakukan dengan penggunaan teknik kromatografi cecair berprestasi tinggi (HPLC) serta kromatografi gas (GC-MS). Di peringkat pertama, MEMM secara signifikasi ($P < 0.05$) mempamerkan aktiviti antinosiseptif dalam semua *in vivo* model nosiseptif melibatkan ujian secara kimia- dan haba-penghambatan. Naloxon (5 mg/kg), antagonis opiad-tidak terpilih, secara signifikasi ($P < 0.05$) telah gagal memberi kesan kepada aktiviti MEMM antinosiseptif. Selain itu, MEMM antinosiseptif secara signifikasi ($P < 0.05$) membalikkan aktiviti capsaicin dan glutamat di dalam ujian penghambatan penjilatan tapak kaki, UF. Manakala, L-argina (pencetus nitrik oksida, NO), L-NAME (perencat NO sintase), metilena biru, MB (perencat cGMP), atau gabungannya secara signifikasi ($P < 0.05$) telah gagal mengubah intensiti MEMM antinosiseptif. Pada peringkat kedua pula, penilaian menggunakan UAA telah mengesahkan bahawa terdapat kesan antinosiseptif daripada semua pecahan (PEMM, EAMM dan AQMM) tersebut. Walaubagaimanapun, pecahan PEMM dan EAMM mempunyai tahap keberkesanan yang serupa untuk menghasilkan kesan antinosiseptif **[**mak. kekangan daripada 24.17±1.33 (70.94%) dan 18.83±0.91 (77.36%)**]** pada setiap dos 500 mg/kg. Keputusannya, secara respektif, pecahan PEMM adalah lebih berkesan atau poten daripada pecahan EAMM dengan nilai kiraan ED_{50} (paras keyakinan 95%) iaitu 119.5 mg/kg (97.03 – 147.1 mg/kg) dan 125.9 mg/kg (109.9 – 144.1 mg/kg). PEMM secara signifikasi ($P < 0.05$) mempamerkan aktiviti antinosiseptif di dalam semua model ujian nosiseptif teknik *in vivo* secara kimia- dan haba-penghambatan. Penilaian menggunakan antagonis opiat-tidak terpilih (naloxon (5 mg/kg), secara signifikasi ($P < 0.05$) gagal untuk membalikkan kesan antinosiseptif daripada PEMM melalui ujian UPP dan UF. PEMM antinosiseptif secara signifikasi ($P < 0.05$) membalikkan aktiviti capsaicin dan glutamat di dalam ujian penghambatan penjilatan kaki. Tambahan lagi, L-arginina, L-NAME, MB, atau gabungannya juga secara signifikasi ($P < 0.05$) telah gagal untuk mengganggu kesan PEMM antinosiseptif. Penilaian saringan analisis fitokimia bagi semua ekstrak mendapati, kehadiran flavonoid, tannin, saponin, triterpen and steroid, kecuali alkaloid. Tamabahan lagi, analisis HPLC bagi MEMM dan PEMM telah merekodkan dan menunjukkan kehadiran flavonoid sebagai juzuk utamanya dan di dalam analisis GC-MS pula, saringan fitokomponen telah dilakukan dan majoriti sebatian yang dikenalpasti adalah sebatian asid palmitik, terpene, diterpene, asid α-Linolenik dan asid lemak ester. Sebagai kesimpulan, keputusan ini menunjukkan bahawa MEMM menghasilkan dos-hubungan antinosiseptif di dalam model nosiseptif ujian teknik *in vivo* melalui secara kimia dan haba, dan mencadangkan PEMM menunjukkan aktiviti antinosiseptif yang terbaik di antara pecahan - pecahan serta memerlukan siasatan atau kajian lebih lanjut.

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

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of the thesis was under my supervision;
- supervision and responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012 – 2013) are adhered to

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MEMM	Methanolic crude extract *Melastoma malabathricum* L. leaves
PEMM	Petroleum ether semi-purified extract of *M. malabathricum* L. leaves
EAMM	Ethyl acetate semi-purified extract of *M. malabathricum* L. leaves
AQMM	Aqueous semi-purified extract of *M. malabathricum* L. leaves
MeOH	Methanol
PE	Petroleum ether
EA	Ethyl acetate
AQ	Aqueous
dH_2O	Distilled water
NaCL	Normal saline
p.o	Orally
i.p	Intraperintoneally
i.pl	Intraplantarly
s.c	Subcutaneously
HPT	Hot-Plate Test
ACT	Acetic Acid-Abdominal Constriction Test
FT	Formalin Test
NO	Nitric oxide
cGMP	Cyclic Guanosine Monophospate
DMSO	Dimethyl sulfoxide
ASA	Acetyl salicylic acid
NLX	Naloxone
Capz	Capsazepine
L-arg	L-arginine
L-NAME	N^G-nitro-L-arginine methyl esters
MB	methylene blue
mL	Milliliter
L	Liter
kg	Kilogram
gm	Gram
°C	Degree Celsius
min	Minute
sec	Second(s)
µL	Microliter
µmol	Micromole
mM	Milimoles
cm	centimeter
µM	Micrometer
h	Hour(s)
ID_{50}	Effective dose producing a 50% reduction in relative to control value
CI	Confidence Interval
ANOVA	Analysis of variance
S.E.M	Standard error mean
USA	United States of America

WHO	World Health Organization
UPM	Universiti Putra Malaysia
IIUM	International Islamic University Malaysia
IASP	International Association for the Study of Pain
BC	Before Century
p	P-value
NSAIDs	Non-steroidal anti-inflammatory drugs
%	Percent
GIT	Gastrointestinal tract
COX	Cyclooxygenase
LOX	Lipooxygenase
TRPA1	Transient receptor potentially A1
TRPV1	Transient receptor potential cation channel subfamily V member 1
CNS	Central nervous system
PNS	Peripheral nervous system
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
NMDA	N-methyl-D-aspartic acid receptor
PGE_2	Prostaglandin E2
sGC	Soluble guanylate cyclase
GC	Guanylate cyclase
HPLC	High Performance Liquid Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
MPLC	Medium-Pressure Liquid Chromatography
LC-MS	Liquid chromatography–mass spectrometry
SFE	Supercritical Fluid Extraction
IR	Infrared
NMR	Nuclear Magnetic Resonance
RP	Reversed-phase
TPC	Total phenolic content
DPPH	2, 2-diphenyl-1- picrylhydrazyl
SOD	Superoxide anion radical scavenging
ORAC	Oxygen radical absorbance capacity
PMS-NADH	Phenazine methosulphate - nicotinamide adenine dinucleotide
NBT	Nitroblue tetrazolium
AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
UV-Vis	Ultra Violet-Visible Spectrophometer
NSIT	National Institute Standard and Technology
R_T	Retention time
nm	Nanometer
TE	Trolox Equivalent
GAE	Gallic Acid Equivalent
PKC	Protein Kinace C
K^+	Potassium ion
Ca_2^+	Calcium ion
Na^+	Sodium ion
CO_2	Carbon dioxide
ADME	Absorption, Distribution, Metabolism and Excretion
EAA	Excitatory amino acids
5HT	Serotonin

NE	Norepinephrine

CHAPTER 1

INTRODUCTION

Pain is the most common symptom of ailments, which refers to an individual experience that accompanies us from childhood. It is a protective mechanism or warning signal to which the body responds to harmful stimulus (Swieboda P *et al.*, 2013). According to the International Association for the Study of Pain (IASP), pain is an unpleasant sensory and emotional experience associated with potential or actual tissue damaged and/or reaction of the body to harmful stimuli or indicates a protective early signal/warning to the body system (MH Mohd Sani *et al.*, 2012). However, the experience of pain depends on the individual strength of the stimulus, tendency and resistance to pain. Nevertheless, the way of pain perception is differ from time to time in the same individual, and also depending on the several factors such as arousal, attention, distraction and expectations (Swieboda P *et al.*, 2013). Therefore, no one patient with pain experience can be treated with exactly the same methods or medications as another patient.

Subsequently, several reports have claimed that, pain experience is the most common reason for any individual to seek for health medication (Hui Ming Ong *et al.*, 2010). For example, in year 2008, the Ministry of Health Malaysia (MOH) recognized Pain as the fifth vital sign among the other major ailments, which giving emphasis and serious observation in their strategy to improve pain management in the hospitals (MOH Malaysia, 2013). Furthermore, according to the World Health Organization (WHO) estimated that approximately 80% of the world population has either no or insufficient access for treatment of pain (Jaganath IB and LT Ng, 2002). In addition, every year tens of millions of people around the world are suffering from pain without treatment (MOH Malaysia, 2013). Therefore, relieve from pain is desirable when the duration and intensity of pain alters the ability of a subject to function efficiently. In such situation, analgesics drugs are useful due to these agents could relieve pain without producing a loss of consciousness. Commonly, the treatment of pain is using non-opioid analgesic drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) that act to reduce the generation process of the pain mediators at the site tissue damaged, despite several of the opioid analgesic drugs are also have some effects within the central nervous system (CNS). Meaning that, the opioid analgesic drugs are unique, which not only block the incoming nociceptive signals to the brain, however, they are also capable to control the affective components of the pain, which act at higher brain centres (Sanda P Welch and Billy R Martin, 1997).

Considering, various ailments have been detected recently, use and practice alternative medicinal treatment, which approach the uses of medicinal plants due to minimum or no adverse side effect. Moreover, the use of plant-related natural products relatively, has indicates lower incidence of adverse side effect reaction in comparing to modern or conventional pharmaceutical products, as well as linked with their reduced cost and encouraging effort from both public consumer and health

care, which highlighted medicinal plant as an alternatives approach to replace the synthetic drugs.Therefore, the provision and preparation for a range of ailments, that using medicinal plants for the treatment are commonly experienced by contemporary community of people such as pain and inflammation (SK Raghav *et al*., 2006; HP Rang *et al*., 2011). Such that approaches, one of the medicinal plants, which possess medicinal values, and being used by the various communities is called *Melastoma malabathricum* L. known as 'Senduduk' of the family Melastomataceae. This plant has attribute valuable healing properties to relieve fever (antipyretics), pain reducer (analgesics), treating vaginal discharge (leucorrhoea), reduction of inflammation, excessive menstrual bleeding, and treating burns or bleeding, inflammation of the walls of blood vessels with blood clots in the vessels (Dalimartha, 2000). It also has been used extensively in traditional medicine both locally and abroad.

Generally, the *M. malabathricum* L. can be described as a small shrub, which commonly can be found in waste places, previously cleared land and along roadside throughout the Southeast Asian countries including Malaysia (JLCH Van Valkenberg and N Bunyapraphatsara, 2001). In Malaysia, the plant commonly growth in the lowland and slope mountain forests, especially in open places. It is also native to tropical and temperate in Asia and Pacific Islands (KH Ling *et al.,* 2009). Interestingly, it has different vernacular names depending on the location/countries (e.g., Malaysia, Indonesia, China and India), and the communities or tribes (e.g., Malay, Chinese and Indian), which traditionally used for medicinal purposes (FA Abdul Majid and LY Ting, 2011). Moreover, parts of the *M. malabathricum* L. plant have also been widely used in traditional remedies. Commonly, every part of the plant including roots can be used as the biomaterial resources for the preparation of traditional medicine, and as natural food colourants due to the presence of anthocyanins (Janna *et al*., 2006). Furthermore, Jaganath IB and LT Ng, 2002 has also reported that, puerperal disease and infectious diarrhea could be treated by eating the raw 'Senduduk' leaves. It was also proven to have anti-inflammation and antinociceptive effect on mice (Sulaiman *et al.,* 2004; ZA Zakaria *et al.,* 2006; Zakaria ZA *et al.,* 2008). On the other hand, various parts of the *M. malabathricum* L. have been claimed to possess medicinal values, which is supported particularly by the Malay and Indian traditional uses of the plants in the treatment of a number of ailments as described earlier. Therefore, a systematic scientific research of the *M. malabathricum* L. was prepared as extracts using different types of solvents and tested using a range of *in vitro* and *in vivo* test models, which was demonstrated various pharmacological findings that required in-depth studies. Such that plant, regardless of the parts used has been shown to exert anti-bacterial, anti-viral, anti-parasitic, antioxidant, cytotoxicity, anti-coagulant, platelet-activating factor inhibitory, wound healing, anti-ulcer, anti-diarrheal, anti-venom, anti-inflammatory, antinociceptive and anti-pyretic activities at different doses or concentrations (Sulaiman *et al*., 2004; ZA Zakaria *et al.,* 2006; Zakaria ZA *et al.,* 2008; S Mohd Joffry *et al*, 2012).

Problem statement

Generally, response of pain could be the first treatment with the non-opioid analgesics drugs (NSAIDs) such as acetylsalicylic acid (ASA), which commonly useful for treatment of pain, fever, and inflammation but less effective than the opioids to relieve pain (mild-to-moderate). Impressively, opioid analgesic drugs such as morphine known as narcotic analgesics could relieve severe pain (moderate-to-severe) by selective acting on CNS to reduce the pain reaction, at the same time do not dissipate the function of peripheral nerves, which means it is capable of inhibiting pain of any origin (Sanda P Welch and Billy, 1997). However, consumption of these drugs associated with their prolong used to treat pain has several adverse side effects and most commonly are nausea, vomiting, dry mouth, constipation, urinary retention, and bring to mental confusion (Sanda P Welch and Billy R Martin, 1997). Moreover, the most serious adverse side effect that associated with chronic use of opioid analgesic drugs, which cause physical dependency and development of patience (Henry Hitners and Barbara Nagle, 1999). Based on the report from Ministry of Health Malaysia 2014, Malaysian statistics on medicine 2009 & 2010 indicates the total opioid consumption in Malaysia that used for pain control, which was recorded 0.3643 DDD/1000 population/day in year 2010 and in year 2009 was 0.3174 DDD/1000 population/day, respectively showing an increase of 15% in year 2010 compared to year 2009, and morphine remains as the most commonly used strong opioid, with an increasing trend over the years approximately 73.2% of all strong opioids in 2009 and 86.8% in 2010, as compared to 65% in 2008. Moreover, the report is also mentioned the increase in expenditure on medicines every year is an indication of the increasing burden of diseases whereby the commitment and responsibility of the healthcare industries in the country to treat the population and to fight against the emerging diseases is essential. Hence, there is need to find alternative agents with less or possibly no side effects, lower cost and medicinal plant is one of source of these agents.

Justification for studying the antinociceptive potential of *M. malabahricum* L. leaves

It is time demanding to explore and develop potential new drugs from natural resources as many ailments are continuously arising. Furthermore, due to unpleasant side effects, limitation on dosage consumption as well as high cost of available drugs, many synthetic drugs are withdrawn years after their introduction into the market. Therefore, the bioactive compounds, which discovered in medicinal plants as an alternative medicine for treating of ailment related to pain as a substitute for available or current drugs that have less or no side effect, and considered cheaper as well as widely available. In addition, the number of researchers, which studied the potential plant extracts, produce the antinociceptive and anti-inflammatory agents have been increased, and the interest has been enhanced lately (Ferguson *et al.*, 2003; Bighetti *et al*., 2005; Orhan *et al*., 2007), thus, in this study, we aimed to discover the potential antinociceptive activities of the *M. malabathricum* leaves that might add to

another candidates to the list, which also support with the various evidences on the traditional application or ethno-medicinal uses of *M. malabathricum* leaves to treat pain-related ailments (Sharma *et al.*, 2001; Zakaria ZA *et al.*, 2008; Umali-Stuart and Stiuart-Santiago, 2010). Subsequently, scientific studies, reported that *M. malabathricum* leaves possess antinociceptive activities (Sulaiman *et al.*, 2004; ZA Zakaria *et al.*, 2006; Zakaria ZA *et al.*, 2008). However, the three researches have reported the use of ethanol, aqueous and chloroform extracts as their source of antinociceptive study, and was administered systemically either by the intraperitoneal or subcutaneous routes. In contrast to those reports, our present study used methanolic extract that was administered orally, which represent the traditional way of consuming the plant's extract. Furthermore, this plant is considered one of the most common weeds that grow wildly and available in open places such as lowland and mountain forests especially in the moist areas (S Mohd Joffry *et al.*, 2012). In consideration of the ethno-medicinal and scientific reports together with the identification of phytoconstituents through the phytochemical screening and analysis via chromatography, therefore, this study is expected to discover the capacity of *M. malabathricum* leaves for antinociceptive activity.

Hypothesis

Methanolic extract of *M. malabathricum* L. (MEMM) leaves possess the antinociceptive activity in thermal- and chemicals-induced nociception assays, and petroleum ether (PEMM) fraction is expected to exert antinociceptive effects induced by thermal and chemicals in animal models.

General objectives:

To determine the antinociceptive activities of methanolic extract of *Melastoma malabathricum* leaves and its petroleum ether fractions in animal models.

Specific objectives:

1. To determine safety of methanolic extract of *M. malabathricum* leaves (MEMM) using the single high-dose acute toxicity model.

2. To determine the antinociceptive profile of MEMM using various animal models.

3. To elucidate the possible mechanisms of action that takes part in the antinociceptive of MEMM.

4. To determine the most effective fraction from MEMM using acetic acid-induced abdominal constriction test.

5. To elucidate the possible mechanisms of action that takes part in the antinociceptive of petroleum ether (PEMM) fraction.

6. To screen and identify the possible bioactive compounds that present in the MEMM and its fractions triggered antinociception using the phytochemical screening test, HPLC and GC-MS analysis.

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LIST OF PUBLICATIONS

E.S. Jaios., Abdul, Rahman, S., Ching, S.M., Arifah, A.K., Desa, M.N., Zakaria, Z.A. (2016). Possible mechanisms of antinociception of methanol extract of *Melastoma malabathricum* Leaves. *Braz J Pharmacognocy*. doi.org/10.1016/j.bjp.2016.01.011.

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Presentations

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