ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF THAI TRADITIONAL REMEDY CALLED YA-HOM KAE-LOM-WING-WIEN AND ITS INGREDIENTS

BY

MISS NATTANIDA JANTARACH

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF MASTER OF
SCIENCE IN APPLIED THAI TRADITIONAL MEDICINE
FACULTY OF MEDICINE
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ENTITLED

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Thesis Title	ANTIOXIDANT AND ANTI- INFLAMMATORY ACTIVITIES OF THAI TRADITIONAL REMEDY CALLED YA-HOM KAE-LOM-WING-WIEN AND ITS INGREDIENTS
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ABSTRACT

Ya- Hom KAE- LOM- WING- WIEN remedy (KLWW) has been used for treatment of dizziness, fatigue, and sleepiness. It is registered to be a remedy on the Thai National List of Essential Medicines. A.D. 2013. Ya-Hom is nice-smelling herbal remedy and consists of various proportions of twenty-three medicinal ingredients. There are *Glycyrrhiza glabra* L. (root), *Myristica fragrans* Houtt. (heartwood), *Syzygium aromaticum* Merr. et Perry (flower-bud), *Angelica sinensis* (Oliv.) Diels (root), *Ligusticum sinense* Oliv. cv. Chuanxiong Hort (rhizome), *Vetiveria zizanioides* (L.) Nash ex Small (root), *Nelumbo nucifera* Gaertn. (pollen), *Cinnamomum bejolghota* (Buch.-Ham.) Sweet (bark), *Cinnamomum loureirii* Nees. (bark), *Cinnamomum verum* J. Presl. (bark), *Aquilaria crassna* Pierre ex Lecomte. (wood), *Euphorbia antiquorum* L. (heartwood), *Artemisia annua* L. (aerial part), *Terminalia chebula* Retz. var chebula (gall), *Alyxia reinwardtii* Blume var. lucida Markgr. (bark), borneo camphor, *Mimusops elengi* L. (wood), *Mesua ferrea* Linn. (flower), *Mimusops elengi* L. (flower), *Mammea siamensis* Kosterm (flower), *Ecdysanthera rosea* Hook. & Arn. (vine), sodium borate ,and *Dracaena loureiri* Gagnep. (heartwood). The aim of this study was to investigate the antioxidant and anti-inflammatory activities of KLWW and its ingredients against super

oxide anion, malondialdehyde, and nitric oxide release for preventing and treating chronic diseases.

The KLWW consists of four extractions. The ethanolic extracts were extracted by maceration in 95% and 70% ethanol. The aqueous extract was extracted by decoction. The hydrolyzed extract was obtained from aqueous extract, which was extracted similar to physiology of gastro-intestinal tract. The results of percentage of yield the 70% ethanolic extracts had the highest percentage of yield (%yield = 20.86). The ingredients were extracted by maceration in 95% ethanol.

The raw materials of plant ingredients were controlled quality following Thai Herbal Pharmacopoeia (THP) standard. All of plant ingredients were investigated quality control methods except borneo camphor and sodium borate. However, KLWW following THP criteria showed loss on drying value of 6.40±0.20%, total ash value of 6.77±0.48%, and acid insoluble ash value of 0.91±0.11%.

For antioxidant activity, the extracts of KLWW and its ingredients were investigated by nitroblue tetrazolium (NBT) dye reduction assay in HL-60 cell lines. The 95% ethanolic extract of *Mammea siamensis* Kosterm showed the highest inhibition by IC_{50} value of 13.51±2.20 µg/ml, followed by the 95% ethanolic extract of *Ecdysanthera rosea*. Hook. & Arn. and *Terminalia chebula* Retz. var chebula with IC_{50} values of 25.68±2.26 and 32.02±2.36 µg/ml, respectively.

Antioxidant activity, the extracts of KLWW and its ingredients were also investigated by thiobarbituric acid reactive substances (TBARS) assay. The hydrolyzed extract of KLWW showed stronger inhibition of TBARS formation by MDA value of 7.72±0.91 nM than propyl gallate (positive control) with MDA value of 10.42±0.66 nM. The 95% ethanolic extract of *Mimusops elengi* L. showed the highest inhibition of TBARS formation of ingredients with MDA value of (-1.64)±3.22 nM.

Anti-inflammatory activity, the extracts of KLWW and its ingredients were investigated by nitric oxide (NO) inhibitory assay in RAW 264.7 cell lines. The 95% extract of KLWW exhibited NO production with IC_{50} value of 19.53±1.95 µg/ml and the highest inhibition NO of ingredients was the 95% ethanolic extract of *Mammea siamensis* Kosterm showing IC_{50} value of 17.13±0.84 µg/ml.

Gas chromatography-mass spectrometry (GC-MS) (Thermo Focus GC + polaris Q + auto injector) with column thermo TG-5silms, KLWW of the hydrolyzed, 70% ethanolic, and aqueous extracts showed eugenol as a main compound (42.02, 34.89, and 28.80 % area, respectively) and the 95% ethanolic extract showed that borneol as a main compound (33.31 % area). These results are new knowledge about specific fingerprint profiles and quantification of characteristic compounds in KLWW.

For stability study, the 95% ethanolic extract of KLWW was tested by anti-inflammatory activity using NO inhibitory assay. The 95% ethanolic extract of KLWW was stable at least two months because there was significant difference between control and the IC_{50} values of extract with p-value < 0.05. It lost anti-inflammatory activity within two months but after that it was stable until day 180. However, it has less anti-inflammatory activity than day 0 one time.

In conclusion, the hydrolyzed extract of KAE-LOM-WING-WIEN remedy had antioxidant activity by TBARS assay, and the 95% extract of KLWW had anti-inflammatory activity by NO inhibitory assay. All results supported the use of KLWW for reducing free radicals and inflammation. Moreover, KLWW has a strong protective role against oxidative stress and reducing risk of progress in chronic diseases. Therefore, its ethanolic and aqueous extracts should be continuously studied *in vitro* and *in vivo* biological activities and using eugenol and borneol as markers to develop further.

Keywords: Ya-Hom KAE-LOM-WING-WIEN remedy, Thai Traditional Remedy, Antioxidant, Anti-inflammation

หัวข้อวิทยานิพนธ์	ฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ต้านการอักเสบของตำรับยาหอมแก้ลมวิงเวียนและสมุนไพรในตำรับ
ชื่อผู้เขียน	นางสาวนาฏนิดา จันทราช
ชื่อปริญญา	วิทยาศาสตรมหาบัณฑิต
สาขาวิชา/คณะ/มหาวิทยาลัย	สาขาวิชาการแพทย์แผนไทยประยุกต์ คณะแพทยศาสตร์/มหาวิทยาลัยธรรมศาสตร์
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ปีการศึกษา	2560

บทคัดย่อ

ยาหอมแก้ลมวิงเวียนสรรพคุณแก้ลมวิงเวียน อ่อนเพลีย และนอนไม่หลับ เป็นหนึ่งในบัญชียาหลักแห่งชาติ พ.ศ.2556 ยาหอมกลุ่มยาพื้นฐานเป็นสมุนไพรที่มีกลิ่นหอม ประกอบด้วยสมุนไพรอัตราส่วนที่หลากหลายมากถึง 23 ชนิด ชะเอมเทศ (*Glycyrrhiza glabra* L.), จันทน์เทศ (*Myristica fragrans* Houtt.), กานพลู (*Syzygium aromaticum* Merr. et Perry), โกฐเชียง (*Angelica sinensis* (Oliv.) Diels), โกฐหัวบัว (*Ligusticum sinense* Oliv. cv.Chuanxiong Hort), แฝกหอม (*Vetiveria zizanioides* (L.) Nash ex Small), เกสรบัวหลวง (*Nelumbo nucifera* Gaertn), สมุลแว้ง (*Cinnamomum bejolghota* (Buch.-Ham.) Sweet), อบเชยญวณ (*Cinnamomum loureirii* Nees.), อบเชยเทศ (*Cinnamomum verum* J. Presl.), กฤษณา (*Aquilaria crassna* Pierre ex Lecomte.), กระลำพัก (*Euphorbia antiquorum* L.), โกฐจุฬาลัมพา (*Artemisia annua* L.), โกฐพุงปลา (*Terminalia chebula* Retz. var chebula), ชะลูด (*Alyxia reinwardtii* Blume var. lucida Markgr.), พิมเสน (borneo camphor), ขอนดอก (*Mimusops elengi* L.), บุนนาค (*Mesua ferrea* Linn.), พิกุล (*Mimusops elengi* L.), สารภี (*Mammea siamensis* Kosterm), มวกแดง (*Ecdysanthera rosea* Hook. & Arn.), น้ำประสานทองสะตุ (sodium borate) และจันทน์แดง (*Dracaena loureiri* Gagnep.) วัตถุประสงค์ของการวิจัยครั้งนี้เพื่อศึกษาฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ต้านอักเสบของตำรับยาหอมแก้ลมวิงเวียนและสมุนไพรในตำรับ เพื่อใช้ในการต่อต้านอนุมูลอิสระชนิดซุปเปอร์ออกไซด์ แอนไอออน ($O_2^{\bullet-}$) มาลอนไดแอลดีไฮด์และไนตริก ออกไซด์ ในการชะลอความก้าวหน้าของโรคเรื้อรัง

การสกัดตำรับประกอบด้วย 4 ชั้น สารสกัดชั้นแอลกอฮอล์โดยการหมักกับ 95% และ 70% เอทานอล ส่วนสารสกัดชั้นน้ำโดยการต้มน้ำ และสารสกัดชั้นไฮโดรไลซ์โดยการนำสารสกัดชั้น

น้ำมาสกัดโดยเลียนแบบสรีรวิทยาของระบบทางเดินอาหาร พบว่าสารสกัดชั้น 70% เอทานอลให้เปอร์เซนต์สารสกัดมากที่สุดเท่ากับ 20.86% สมุนไพรในตำรับสกัดด้วยการหมัก 95% เอทานอล

สมุนไพรในตำรับควบคุมคุณภาพวัตถุดิบตามตำรามาตรฐานยาสมุนไพรไทย ส่วนประกอบของสมุนไพรในตำรับทั้งหมดผ่านการทดสอบการควบคุมคุณภาพ ยกเว้นพิมเสนและน้ำประสานทอง อย่างไรก็ตาม เมื่อทดสอบรวมเป็นตำรับแก้ลมวิงเวียน พบว่า ผ่านเกณฑ์ตามที่มาตรฐานกำหนด โดยมีค่าความชื้น เท่ากับ 6.40 ± 0.20%, ปริมาณเถ้ารวม เท่ากับ 6.77 ± 0.48% และปริมาณเถ้าที่ไม่ละลายในกรด เท่ากับ 0.91 ± 0.11%

การทดสอบฤทธิ์ต้านอนุมูลอิสระของตำรับและสมุนไพรในตำรับโดยวิธี NBT dye reduction assay พบว่าสารสกัดสารภีชั้น 95% เอทานอล มีฤทธิ์ต้านอนุมูลอิสระสูงที่สุด มีค่า IC_{50} เท่ากับ 13.51 ± 2.20 ไมโครกรัมต่อมิลลิลิตร รองลงมาคือ มวกแดงและโกฐพุงปลามีค่า IC_{50} เท่ากับ 25.68 ± 2.26 และ32.02 ± 2.36 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ

การทดสอบฤทธิ์ต้านอนุมูลอิสระของตำรับและสมุนไพรในตำรับโดยวิธี TBARS assay พบว่าสารสกัดตำรับชั้นไฮโดรไลซ์มีฤทธิ์ยับยั้งสาร TBARS โดยมีค่า MDA เท่ากับ 7.71 ± 0.02 นาโนมาลาร์ สูงกว่าโพรพิลแกลเลตซึ่งมีค่า MDA เท่ากับ 10.42 ± 0.66 นาโนมาลาร์ ในส่วนของสารสกัดพิกุลชั้น 95% เอทานอลมีฤทธิ์ยับยั้ง TBARS ดีที่สุด มีค่า MDA เท่ากับ (-1.64) ± 3.22 นาโนมาลาร์

การทดสอบฤทธิ์ต้านการอักเสบของตำรับและสมุนไพรในตำรับโดยวิธี NO inhibitory assay พบว่าสารสกัดตำรับชั้น 95% เอทานอล มีฤทธิ์ยับยั้งการสร้างไนตริกออกไซด์มีค่า IC_{50} เท่ากับ 19.53 ± 1.95 ไมโครกรัมต่อมิลลิลิตร และสารสกัดสารภีชั้น 95% เอทานอลมีฤทธิ์ต้านการอักเสบดีที่สุด โดยมีค่า IC_{50} เท่ากับ 17.13 ± 0.84 ไมโครกรัมต่อมิลลิลิตร

การวิเคราะห์หาชนิดและปริมาณสารในตำรับด้วยเครื่องแก๊สโครมาโตกราฟ-แมสสเปกโทรมิเตอร์ (Thermo Focus GC + polaris Q + auto injector)) โดยใช้คอลัมน์เทอร์โม TG-5silms สารสกัดตำรับชั้นไฮโดรไลซ์, 70% เอทานอลและชั้นน้ำ พบว่า eugenol เป็นสารหลักที่มีปริมาณมากที่สุด (ร้อยละของพื้นที่ใต้พีกสัมพัทธ์ เท่ากับ 42.02, 34.89 และ 28.80 ตามลำดับ) และสารสกัดตำรับชั้น 95% เอทานอล พบ borneol เป็นสารหลักที่มีปริมาณมากที่สุด (ร้อยละของพื้นที่ใต้พีกสัมพัทธ์ เท่ากับ 33.31) จากผลการศึกษาที่ได้เป็นความรู้ใหม่เกี่ยวกับสารสกัดตำรับมาตรวจรูปแบบพิมพ์ลายนิ้วมือ (fingerprint) และปริมาณสารสำคัญในสารสกัดตำรับยาหอมแก้ลมวิงเวียน

การทดสอบความคงตัวของสารสกัดตำรับชั้น 95% เอทานอล โดยนำมาทดสอบฤทธิ์ต้านการอักเสบโดยวิธี NO inhibitory assay ฤทธิ์การยับยั้งการสร้างไนตริกออกไซด์ พบว่าสารสกัดตำรับมีความคงตัวได้ประมาณ 2 เดือนเมื่อทดสอบฤทธิ์ต้านการอักเสบผลที่ได้มีความแตกต่างอย่างมี

นัยสำคัญเมื่อเปรียบเทียบวันที่ 0 และค่า IC_{50} โดยมีค่า p-value < 0.05 สรุปได้ว่า สารสกัดตำรับชั้น 95% เอทานอลแม้ฤทธิ์ต้านการอักเสบจะลดลงเมื่อเทียบกับวันที่ 0 เพียงหนึ่งครั้ง แต่อย่างไรก็ตามหลังจากนั้นฤทธิ์มีค่าคงที่จนถึงวันที่ 180

จากผลการทดลอง พบว่าสารสกัดตำรับยาหอมแก้ลมวิงเวียนมีฤทธิ์ต้านอนุมูลอิสระโดยวิธี NBT dye reduction assay และTBARS assay และมีฤทธิ์ต้านการอักเสบโดยวิธี NO inhibitory assay จากผลการทดลองทั้งหมด สรุปว่าสนับสนุนการใช้ตำรับยาหอมในการลดอนุมูลอิสระและการอักเสบ อีกทั้งตำรับยาหอมยังมีบทบาทในการช่วยป้องกันความเครียดออกซิเดชันและลดความเสี่ยงในการดำเนินของโรคเรื้อรังได้ สุดท้ายนี้ตำรับยาหอมควรมีการศึกษาฤทธิ์ทางชีวภาพด้านอื่นทั้งในหลอดทดลองและสัตว์ทดลอง รวมถึงการใช้ eugenol และborneol เป็น marker ในการพัฒนาต่อไป

คำสำคัญ: ตำรับยาหอมแก้ลมวิงเวียน, ตำรับยาแผนไทย, ฤทธิ์ต้านอนุมูลอิสระ, ฤทธิ์ต้านการอักเสบ

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Miss Nattanida Jantarach

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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
/	Per
%	Percent
>	More than
<	Less than
=	Equal
µg	Microgram
µg/ml	Microgram per milliliter
µl	Microliter
µm	Micrometer
^{o}C	Degree celsius
ATCC	American type culture collection
cm	Centimeter
CO_2	Carbon dioxide
DMSO	Dimethylsulfoxide
et al.	Et alii, and others
Fe^{2+}	ferrous
FBS	Fetal bovine serum
g	Gram
g/kg	Gram per kilogram
HCl	Hydrochloric acid
HL-60	Human promyelocytic leukemia cell line
IC_{50}	Concentration causing 50% inhibition effect
LPS	Lipopolysaccharide
m	Meter
mg	Milligram
mg/kg	Milligram per kilogram
mg/ml	Milligram per millimeter
ml	Milliliter
mm	Millimeter
mM	Millimolar

LIST OF ABBREVIATIONS (CONTINUED)

Symbols/Abbreviations	Terms
MTT	Thiazolyl blue tetrazolium bromide or 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide
NaOH	Sodium hydroxide
ng	Nanogram
nM	Nanomolar
nm	Nanometer
NBT	Nitroblue tetrazolium
NO	Nitric oxide
PBS	Phosphate buffer saline
pH	Potential of hydrogen ion
PMA	Phorbol 12-myristate 13-acetate
P/S	Penicillin/Streptomycin solution
RAW 264.7	Murine macrophage leukemia
RH	Relative humidity
rpm	Revolution per minute
RPMI 1640	Roswell Park Memorial Institute 1640
SEM	Standard error of mean
THP	Thai Herbal Pharmacopoeia
w/v	Weight by volume

CHAPTER 1
INTRODUCTION

1.1 Introduction

Free radicals are unstable molecules with unpaired electrons. The sources of free radicals and oxidants are both external and internal. When they overload, they cause damage to many body tissues and gradually accumulate to the generation of a phenomenon known as oxidative stress. This process is one important cause of development of chronic degenerative disease (Pham-Huy LA, He, and Pham-Huy C, 2008).

There are three types of free radicals (reactive species), namely: reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species (RCS). Important reactive species in biological system are examples of superoxide ($O_2^{\bullet -}$), nitric oxide, ($NO^{\bullet}$), and atomic chlorine ($Cl^{\bullet}$) derivatives also called oxidants. Functions of free radicals are cell physiological processes at low and moderate concentrations, whereas at high concentrations, they produce adverse modifications to cell components (Birben, Sahiner, Sackesen, Erzurum, and Kalayci, 2012).

Inflammation is commonly a complex reaction as a protective response. However, unregulated prolonged inflammatory process can induce tissue damage leading to exaggerated oxidative stress and is the cause of many chronic diseases (Biswas, 2016). Oxidative stress is an imbalance between the production of reactive oxygen species (free radicals) and antioxidant defenses (Betteridge, 2000) and is the cause of chronic diseases such as atherosclerosis, chronic inflammation, aging, cardiovascular diseases, and other degenerative diseases in humans (Uttara, Sindh, Zamboni, and Mahajan, 2009). A large number of researchers all over the world have investigated whether antioxidants are capable of preventing diseases (Biswas, 2016). Therefore, the intake of antioxidants might help prevent and reduce the risk of chronic diseases.

Ya-Hom is a traditional herbal remedy used widely and for a long time in Thailand. It has been used for treatment of dizziness, fatigue, fainting, abdominal discomfort etc. It is also used as a cardiotonic agent and adjustment of the wind element for healthy circulation in Thai traditional medicine. Several Ya-Hom preparations are registered as traditional medicines by the Thai Food and Drug Administration. There are only five Ya-Hom (Tip-Osot, Tepajit, Navakote, Kae-Lom-Wing-Wien, Intajak) are registered on List of Herbal Medicinal Products in Thai National List of Essential Medicine A.D. 2013 which considers ethnopharmacological evidence and efficacy. There are scientific data of plant ingredients of Ya-Hom showing effect on antioxidant and anti-inflammatory activities such as radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Suantawee *et al*., 2015; Teh *et al*., 2013; Kotan *et.al*., 2010) and inhibitory activity on nitric oxide (NO) production (Visavadiya, Soni, and Dalwad, 2009; Itharat, Makchuchit, and Tewtrakul, 2009).

However, the previous research didn't study on Ya-Hom KAE-LOM-WING-WIEN remedy. Ya-Hom KAE-LOM-WING-WIEN remedy consists of twenty-three medicinal plants. There are *Glycyrrhiza glabra* L. (root), *Myristica fragrans* Houtt. (heartwood), *Syzygium aromaticum* Merr. et Perry (flower-bud), *Angelica sinensis* (Oliv.) Diels (root), *Ligusticum sinense* Oliv. cv.Chuanxiong Hort (rhizome), *Vetiveria zizanioides* (L.) Nash ex Small (root), *Nelumbo nucifera* Gaertn. (pollen), *Cinnamomum bejolghota* (Buch.-Ham.) Sweet (bark), *Cinnamomum loureirii* Nees. (bark), *Cinnamomum verum* J. Presl. (bark), *Aquilaria crassna* Pierre ex Lecomte. (wood), *Euphorbia antiquorum* L. (heartwood), *Artemisia annua* L. (aerial part), *Terminalia chebula* Retz. var chebula (gall), *Alyxia reinwardtii* Blume var. lucida Markgr. (bark), borneo camphor, *Mimusops elengi* L. (wood), *Mesua ferrea* Linn. (flower), *Mimusops elengi* L. (flower), *Mammea siamensis* Kosterm (flower), *Ecdysanthera rosea* Hook. & Arn. (vine), sodium borate ,and *Dracaena loureiri* Gagnep.(heartwood).

Antioxidant activities by nitroblue tetrazolium (NBT) dye reduction assay and thiobarbituric acid reactive substances (TBARS) assay have been studied on some plant ingredients namely: *Glycyrrhiza glabra* L. , *Myristica fragrans* Houtt. , *Cinnamomum verum* J. Presl., *Artemisia annua* L., *Alyxia reinwardtii* Blume var. lucida

Markgr., *Mesua ferrea* Linn., and *Mammea siamensis* Kosterm. Anti-inflammatory activity by nitric oxide (NO) inhibitory assay had been studied on *Glycyrrhiza glabra* L., *Myristica fragrans* Houtt., *Angelica sinensis* (Oliv.) Diels, *Ligusticum sinense* Oliv. cv.Chuanxiong Hort, *Vetiveria zizanioides* (L.) Nash ex Small, *Nelumbo nucifera* Gaertn., *Cinnamomum verum* J. Presl., *Mesua ferrea* Linn., and *Mammea siamensis* Kosterm. The aim of this study was to study antioxidant and anti-inflammatory activities of Ya-Hom KAE-LOM-WING-WIEN extract and its ingredients. The results are likely to support using Ya-Hom KAE-LOM-WING-WIEN remedy against the superoxide anion, malondialdehyde, and nitric oxide to slow down the progress of chronic diseases.

1.2 Aims of this study

1.2.1 Overall aims

The overall aims of this research are to study the antioxidant and anti-inflammatory activities of ethanolic, aqueous, and hydrolyzed extracts of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients that prevent and slow down the progress of chronic diseases.

1.2.2 Specific aims

1.2.2.1 To study antioxidant activity by nitroblue tetrazolium (NBT) dye reduction assay of the extracts of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients.

1.2.2.2 To study antioxidant activity by thiobarbituric acid reactive substances (TBARS) assay of the extracts of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients.

1.2.2.3 To study anti-inflammatory activity by nitric oxide (NO) inhibitory assay of the extracts of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients.

1.2.2.4 To study the chemical fingerprint identification of the extracts of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography mass spectrometry (GC-MS).

1.2.2.5 To study the stability of Ya-Hom KAE-LOM-WING-WIEN remedy extract.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Free radicals

A free radical is any molecular species that contains an unpaired electron in an atomic and molecular orbital (Halliwell and Whiteman, 2004). Because unpaired electrons are unstable and highly reactive, they have a strong tendency to form pair to become stable. Therefore, a radical might donate its unpaired electron to another molecule or it might steal an electron from another molecule in order to form a pair. However, if a radical gives one electron to another molecule or takes one from another molecule, that other molecule itself becomes a radical (Biswas, 2016). Thus, an important characteristic of free radical reaction tends to proceed as chain reaction leading to cell damage and homeostatic disruption (Halliwell, 1989; Lobo, Phatak, and Chandra, 2010)

Three types of reactive species (RS) in biology and medicine consist of reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chlorine species (RCS). Structure of reactive species may be a free radical or a nonradical (Halliwell and Whiteman, 2004). Examples of radical and nonradical ROS are superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), respectively. The collective term RNS includes like radicals (nitric oxide, $NO^{\bullet}$) and nonradicals (peroxynitrite, $ONOO^-$), RCS includes also radicals (atomic chlorine, $Cl^{\bullet}$) and nonradicals (hypochlorous acid, HOCl) (Halliwell, 2006). A list of important reactive species in biological systems is shown in **Table2-1** (Halliwell, 2006).

Table 2-1 Important reactive species in biological systems (Halliwell, 2006)

Free radicals	Nonradicals
Reactive oxygen species (ROS)	
Superoxide, $O_2^{\cdot -}$	Hydrogen peroxide, H_2O_2
Hydroxyl, $OH^{\cdot}$	Singlet oxygen, $O_2\ ^1\Delta g$
Peroxyl, $RO_2^{\cdot}$	Organic peroxides, ROOH
Alkoxyl, $RO^{\cdot}$	Peroxynitrite, $ONOO^-$
Carbonate, $CO_3^{\cdot -}$	Peroxynitrous acid, ONOOH
Reactive nitrogen species (RNS)	
Atomic chlorine, $Cl^{\cdot}$	Hypochlorous acid, HOCl
	Chlorine gas, Cl_2
	Nitryl (nitronium) chloride, NO_2Cl
Reactive chlorine species (RCS)	
Nitric oxide, $NO^{\cdot}$	Nitrous acid, HNO_2
itrogen dioxide, $NO_2^{\cdot}$	Nitrosyl cation, NO^+
	Nitroxyl anion, NO^-
	Dinitrogen tetroxide, N_2O_4
	Dinitrogen trioxide, N_2O_3
	Peroxynitrous acid, ONOOH
	Alkyl peroxynitrites, ROONO

Free radicals and other ROS are derived either from endogenous sources, which are normal essential metabolic processes in the human body, or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals (Bagchi and Puri, 1998). At low or moderate concentrations, ROS and RNS exert beneficial effects on cellular responses and immune function. At high concentrations, they generate a phenomenon known as oxidative stress, a deleterious process that can damage all cell structures (Pham-Huy LA, He, and Pham-Huy C, 2008).

2.1.1 Important reactive species

Phagocytic cells, such as macrophages and neutrophils are also prominent sources of $O_2^{\bullet -}$. In the presence of invading pathogens like bacteria, phagocytic cells become activated and they generate $O_2^{\bullet -}$ which attacks the invading pathogens as a part of the inflammatory response (Victor, Rocha, and De la Fuente, 2004). Superoxide anion is important in the body because it generates other free radicals capable of causing cell injury (Cadenas, 2004; Pacher, Beckman, and Liaudet, 2007).

NO is synthesized through the enzymatic conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS), which exists in three known forms, namely: endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS) which can be up-regulated under certain conditions to induce oxidetive stress (Ishikawa, Kondo, Goda, Fujisawa, Androl, 2005). Inducible NOS (iNOS) is up-regulated by oxidative stress, producing a burst of NO that far exceeds basal levels which can cause significant cellular injury via different mechanisms (Aprioku, 2013).

2.2 Inflammation

Inflammation is a commonly complex reaction in the body's response to exogenous and endogenous stimuli. The ultimate goal of this protective response is to eliminate the organism of both the initial cause of cell injury (e.g., microbes, toxins) and the consequences of such injury (e.g., necrotic cells and tissues) (Collins, 1999). At the site of inflammation, the activated inflammatory cells release many enzymes (neutral proteases, elastase, collagenase, acid hydrolases, phosphatases, lipases, etc.), reactive species (superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid, etc.), and chemical mediators (eicosanoids, complement components, cytokines, chemokines, nitric oxide, etc.) and thereby induce tissue damage and oxidative stress (Collins, 1999).

2.3 Oxidative stress

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (free radicals) and antioxidant defenses (Betteridge, 2000). Under oxidative stress, cellular systems can produce a higher amount of ROS in the body, which may change DNA structure, result in modification of proteins and lipids, activation of several stress- induced transcription factors, and the production of proinflammatory and anti-inflammatory cytokines. (Birben, Sahiner, Sackesen, Erzurum, and Kalayci, 2012).

2.4 The relationship between inflammation and oxidative stress

Numerous studies support an interdependent relationship between inflammation and oxidative stress, as reviewed in (Castellani, Balza, and Rubartelli, 2014; Mittal, Siddiqui, Tran, Reddy, and Malik, 2014). During the inflammatory process the activated phagocytic cells like neutrophils and macrophages produce large amounts of ROS, RNS, and RCS including superoxide, hydrogen peroxide, hydroxyl free radical, nitric oxide, peroxynitrite, and hypochlorous acid to kill the invading agents (Fialkow, Wang, and Downey, 2007). Under conditions of pathological inflammation there may be exaggerated generation of reactive species and some of these reactive species diffuse out of the phagocytic cells and thus induce localized oxidative stress and tissue injury (Fialkow, Wang, and Downey, 2007). As oxidative stress and inflammation are closely related on the pathophysiological processes, one can be easily induced by the other. Oxidative stress has also been implicated in the cause of chronic diseases (Biswas, 2016).

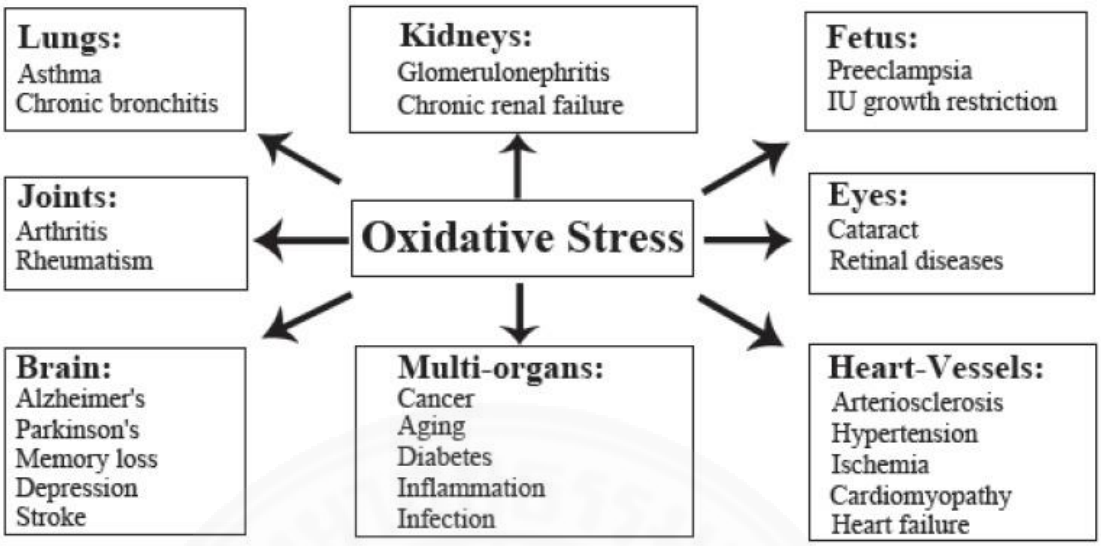

Figure 2-1 Human diseases induced by oxidative stress (Pham-Huy LA, He, and Pham-Huy C, 2008)

2.5 Lipid peroxidation

Lipid peroxidation is a process initiated when free radicals attack lipids containing carbon- carbon double bond(s) , especially polyunsaturated fatty acids (PUFAs). The overall three steps of lipid peroxidation process are initiation, propagation and termination (Yin, Xu, and Porter, 2011; Girotti, 1998, Kanner, German, and Kinsella, 1987) . Lipid peroxidation produces various oxidation products. The main primary products of lipid peroxidation are lipid hydroperoxides (LOOH) . The secondary lipid peroxidation products are many different aldehydes like malondialdehyde (MDA). MDA has been widely used for many years as a convenient biomarker for lipid peroxidation because of its simple reaction with thiobarbituric acid (TBA) (Ayala, Munoz, and Arguelles, 2014).

2.6 Free radicals and inflammation in Thai traditional medicine

2.6.1 Pathogenesis of free radicals and inflammation in Thai traditional medicine

The theory of Thai medicine is that the human body consists of four basic elements: earth (Pathavi), water (Apo), fire (Techo), and wind (Wayo). Causes of illness from free radicals and inflammation have many factors including food, bodily movement, excessive work, the effect of hot and cold weather, lack of sleep-food-water, suppression of defecation and micturition, grief and sorrow, and violent temper in Thai medicine. In this case, there are four factors inducing Pitta (one of Tri Dosha) consisting of food, excessive work, lack of sleep-food-water, and violent temper, and two factors inducing Vata (one of Tri Dosha) consisting of bodily movement and excessive work. Both free radicals and inflammation are one of increasing fire (Techo) element and the effect of imbalance on four elements. These effects result in fire element (santappakkhi, parithaihakkhi, chiranakkhi, parinamakkhi), wind element (utthangkhamawata, otthakhamawata, angkhamangkhanusariwata), earth element (naharu, atthi), and water element (lohitang). First symptom of fire element affects inflammatory tissue damage and an abnormal increase in the body's basal metabolic rate. Secondary symptom of wind and earth element affect tendon stiffness and an abnormal increase and decrease air which circulates throughout the body. Last symptom of water element affects blood disorders when fire and wind element are induced too much of an abnormality. Treatment should use mild-tasting drugs for relieving wind and fire elements. Ya-Hom KAE-LOM-WING-WIEN remedy can adjust wind element for healthy circulation and relieving fire element. In addition, it nourishes the heart. The mechanism concept is shown in **Figure2-2.**

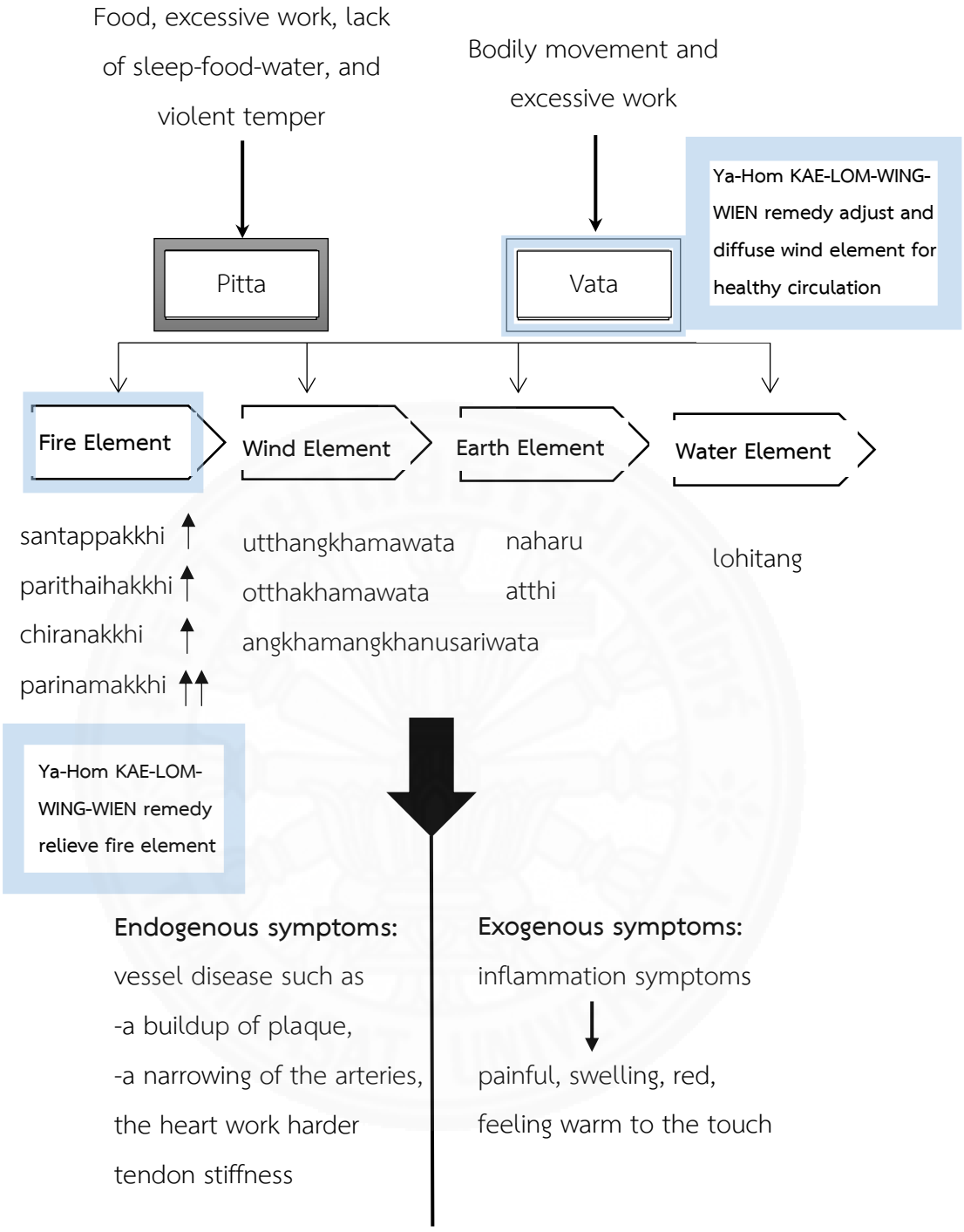

Figure 2-2 The mechanism concept of free radicals and inflammation in Thai traditional medicine

2.7 Ya-Hom KAE-LOM-WING-WIEN remedy and ingredients

2.7.1 Ya-Hom KAE-LOM-WING-WIEN remedy

Ya-Hom KAE-LOM-WING-WIEN remedy (KLWW) is used for treatment of dizziness, fatigue, and sleepiness which is registered on the Thai National List of Essential Medicines. A.D. 2013. There are twenty-three medicinal ingredients consist of *Glycyrrhiza glabra* L. (root), *Myristica fragrans* Houtt. (heartwood), *Syzygium aromaticum* Merr. et Perry (flower-bud), *Angelica sinensis* (Oliv.) Diels (root), *Ligusticum sinense* Oliv. cv. Chuanxiong Hort (rhizome), *Vetiveria zizanioides* (L.) Nash ex Small (root), *Nelumbo nucifera* Gaertn. (pollen), *Cinnamomum* bejolghota (Buch.-Ham.) Sweet (bark), *Cinnamomum loureirii* Nees. (bark), *Cinnamomum verum* J. Presl. (bark), *Aquilaria crassna* Pierre ex Lecomte. (wood), *Euphorbia antiquorum* L. (heartwood), *Artemisia annua* L. (aerial part), *Terminalia chebula* Retz. var chebula (gall), *Alyxia reinwardtii* Blume var. lucida Markgr. (bark), borneo camphor, *Mimusops elengi* L. (wood), *Mesua ferrea* Linn. (flower), *Mimusops elengi* L. (flower), *Mammea siamensis* Kosterm (flower), *Ecdysanthera rosea* Hook. & Arn. (vine), sodium borate ,and *Dracaena loureiri* Gagnep. (heartwood). This remedy has cool, fragrant, and sweet taste. In Thai traditional medicine believed that these tastes of drugs which can help relieving and adjustment wind and fire elements and be able to return to their regular work. Moreover, they nourish the heart.

2.7.2 Medicinal ingredients

2.7.2.1 *Artemisia annua* L. (COMPOSITAE)

Figure 2-3 *Artemisia annua* L.

Common names: Kot chula lampa (Thai), Mugwort tribe, Qinghao, Sagebrush tribe, Tribe anthemis

Family: COMPOSITAE

Botanical characteristics

Sweet wormwood is a single-stemmed, hairless, sweetly aromatic annual growing up to 1 m high (2 m in cultivation). The stem is erect, ribbed and brownish or violet-brown.

Part used: Aerial parts

Chemical constituents

Thirty - two components were identified in the oil, representing 96.76 % of the total composition. The major components of the essential oil were camphor (48.00 %), 1, 8 - cineole (9.39 %), camphene (6.98 %) and spathulenol (4.695 %). The oil consists of 24 monoterpenoids (83.72%) and 7 sesquiterpenoids (12.59 %). The essential oil of the dried flowering aerial parts of *A. annua* was rich in monoterpenoids (Verdian-rizi, 2009).

Traditional use: Medicinal, important antimalarial, essential oil used in cosmetics, cultivated as an ornamental in Indonesia

2.7.2.2 *Angelica sinensis* (Oliv.) Diels (UMBELLIFERAE)

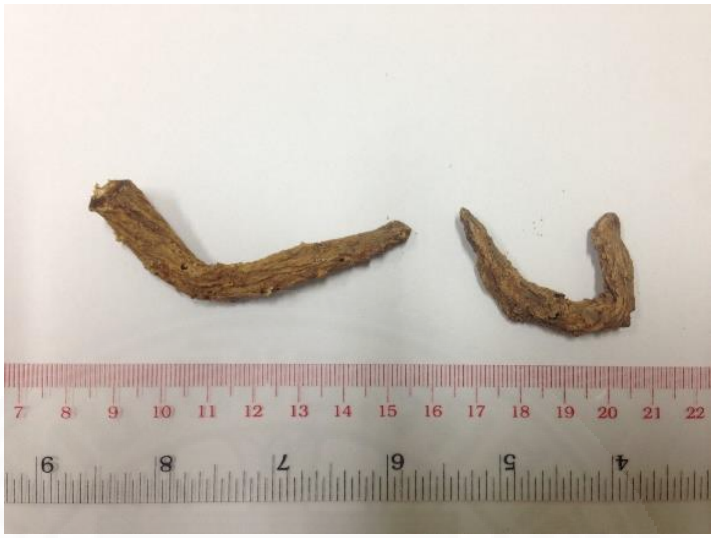

Figure 2-4 *Angelica sinensis* (Oliv.) Diels

Common names: Kot chiang (Thai), Dong quai (Chinese)

Family: UMBELLIFERAE

Botanical characteristics

Angelica sinensis grows in cool high-altitude mountains in China, Japan, and Korea. The yellowish-brown root of the plant is harvested in fall and is a well-known Chinese medicine used over thousands of years.

Part used: Root

Chemical constituents

The plant's chemical constituents include phytosterols, polysaccharides, ligustilit, b-butyl phtalit, cnidilit, isoenidilit, p-cymen, ferulate, and flavonoids. When isolated from the plant, one of the chemicals, angelica polysaccharide sulfate, has *in vitro* antioxidant activity (Jia *et al*., 2007).

Traditional use: The heartwood is used as a haemostatic, anti-inflammation, blood tonic and astringent.

2.7.2.3 *Aquilaria crassna* Pierre ex Lecomte. (THYMELAEACEAE)

Figure 2-5 *Aquilaria crassna* Pierre ex Lecomte.

Common names: Krit sana (Thai), Eagle wood

Family: THYMELAEACEAE

Botanical characteristics

Aquilaria crassna Pierre ex Lecomte. is an evergreen tree with an open crown; usually growing 15 - 20 meters tall but with exceptional specimens to 30 meters. The bole is 40 – 60 cm in diameter, exceptionally to 100 cm (Jensen and Meilby, 2012).

Part used: Wood

Chemical constituents

The major compounds in *Aquilaria* species are sesquiterpenes and 2-(2-phenylethyl) chromone derivatives (Naef, 2011).

Traditional use: Treatment of weakness, stomachache, fever, body pain, rheumatism, women's disease, and dropsy

2.7.2.4 *Alyxia reinwardtii* Blume var. lucida Markgr. (APOCYNACEAE)

Figure 2-6 *Alyxia reinwardtii* Blume var. lucida Markgr.

Common names: Cha lud (Thai)

Family: APOCYNACEAE

Botanical characteristics

Alyxia reinwardtii is a slender climbing or scandent, evergreen shrub producing stems up to 3 meters long that can twine into the surrounding vegetation (Missouri Botanical Garden Press, 1989).

Part used: Bark

Chemical constituents

Iridoids, coumarins and lignans were isolated from the stems, bark, leaves and inner bark of *A. reinwardtii*. The presence of compounds 2, 6 and 7, especially zhebeiresinol showed good potential antioxidant activity with fairly low IC_{50} values in antioxidant tests (Rattanapan, Sichaem, and Tip-pyang, 2012).

Traditional use: The leaves and fruits of this plant can be used to reduce fever, the flowers are effective in treating mental confusion and hallucination associated with high fever, and the stems are used to treat fainting, heart failure and abdominal discomforts due to gaseous distention or other unspecified causes.

2.7.2.5 Borneo camphor

Figure 2-7 Borneo camphor

Common names: Phim sen (Thai)

Family:

Characteristics

Borneol is a bicyclic organic compound and a terpene derivative. The hydroxyl group in this compound is placed in an endo position. There are two different enantiomers of borneol. Both d- (+)-borneol and l- (−)-borneol are found in nature. Appearance is colorless to white lumps.

Part used:

Chemical constituents

Chemical formula is $C_{10}H_{18}O$. Bornyl is a univalent radical $C_{10}H_{17}$ derived from borneol by removal of hydroxyl and is also known as 2-bornyl. Isobornyl is the univalent radical $C_{10}H_{17}$ that is derived from isoborneol.

Traditional use: Treatment for flatulence and dizziness

2.7.2.6 *Cinnamomum bejolghota* (Buch.-Ham.) Sweet (LAURACEAE)

Figure 2-8 *Cinnamomum bejolghota* (Buch.-Ham.) Sweet

Common names: Sa mul lawang (Thai)

Family: LAURACEAE

Botanical characteristics

Cinnamomum bejolghota (Buch.-Ham.) Sweet is perennial plant in 7 m high evergreen forest. There are the opposite tri-and triplinerved leaves.

Part used: Bark

Chemical constituents

0.08% Essential oils consist of **α**-terpineol, (E) – nerolidol, 1, 8 cineole.

Traditional use: Treatment for dizziness and carminative

2.7.2.7 *Cinnamomum loureirii* Nees. (LAURACEAE)

Figure 2-9 *Cinnamomum loureirii* Nees.

Common names: Op choei yawn (Thai), China cinnamon, Saigon cassia, Saigon cinnamon

Family: LAURACEAE

Botanical characteristics

Cinnamomum loureirii Nees. is an evergreen tree indigenous to mainland Southeast Asia and can grow 15 - 20 meters tall (McNeill *et al*., 2012).

Part used: Bark

Chemical constituents

C. loureirii has 1-5% essential oil in content and 25% cinnamaldehyde in essential oil, which is the highest of all the cinnamon species. It contains the highest amount of coumarin of all the four *Cinnamomum* species sold as cinnamon, with one study detecting 6.97 g/kg in an authenticated sample (McNeill *et al*., 2012).

The bark contains around 2.5% essential oil, which is particularly rich in cinnamic acid (Doung, 1993).

Traditional use: The dried bark is aromatic, astringent, carminative, stimulant and stomachic.

2.7.2.8 *Cinnamomum verum* J. Presl. (LAURACEAE)

Figure 2-10 *Cinnamomum verum* J. Presl.

Common names: Op choei thet (Thai), Cinnamon tree, Ceylon cinnamon

Family: LAURACEAE

Botanical characteristics

Cinnamomum verum J. Presl. is an evergreen tree that reaches a height of 8-17 m in the wild. In an unharvested state, the trunk is stout, 30-60 cm in diameter, with a thick, grey bark and the branches set low down (Orwa *et al.,* 2009).

Part used: Bark

Chemical constituents

Cinnamon bark contains an aromatic-smelling essential oil ("cinnamon oil") with cinnamaldehyde as the major component; also, catechin tannins and caffeic acid derivatives (Horvath, Molnar, and Bencsik, 2013).

Traditional use: Used to aid in digestion, relieve from headache, gastrointestinal and respiratory disorders, and as an aphrodisiac

2.7.2.9 *Dracaena loureiri* Gagnep. (DRACAENACEAE)

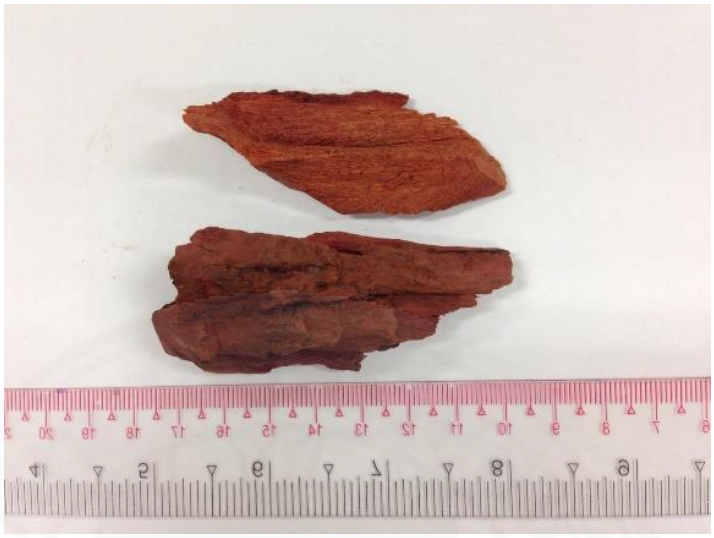

Figure 2-11 *Dracaena loureiri* Gagnep.

Common names: Chan daeng, Chan par (Thai), Dragon's blood tree

Family: DRACAENACEAE

Botanical characteristics

It is a shrub or slender many-branched tree (Garder *et al*., 2000). When the plant becomes old, it has a red core in the stem. The stem gradually becomes until all cores become red, this core wood is called ChanDaeng. Most of plant grows in the high mountains, but it can be found in any parts of Thailand (Thai Traditional Medicine Association, 1964; Pongbunrod, 1979).

Part used: Heartwood

Chemical constituents

Fifteen flavonoid derivatives have been isolated from the chloroform fraction, they were (7S, 12bR)-10-hydroxy-11-methoxy-dracaenone; (7R, 12bR)-7, 10-dihydroxy-11-methoxy-dracaenone; (3S)-7,4'-dihydroxy-3-(4-hydroxybenzyl)-chromane; loureirin A, B, C and D; 7,4'-dihydroxyflavone; (2S)-7-hydroxyflavanone; (2S)-pinocembrin; (2S)-7,4'-dihydroxy-5-methoxyflavone; 4,4'-dihydroxy-2'-methoxychalcone; (2R)-7,4'-dihydroxyflavan; (2R)-4'-hydroxy-7-methoxyflavan and (3R)-eucomol (Meksuriyen and Cordell, 1988).

Traditional use: Reduce fever, scurvy, and abscess

2.7.2.10 *Euphorbia antiquorum* L. (EUPHORBIACEAE)

Figure 2-12 *Euphorbia antiquorum* L.

Common names: Kra lumphak (Thai), Malayan spurge tree, Triangular spurge

Family: EUPHORBIACEAE

Botanical characteristics

A large shrub or small, spinous tree, 4.5-9 m high, with white latex; branches numerous curving upwards, stout, fleshy, green, jointed, with 3-5 wide thick sinuate wings. Leaves small, 6-13 mm long, subsessile, obovate-oblong, fleshy, soon deciduous; stipular spines short, divaricate. Involucres hemispherical, yellow, 3-nate, forming small pedunculate cymes in the sinuses, the central flower sessile, female.

Part used: Heartwood

Chemical constituents

Latex present in branches contains β-amyrin, cycloartenol, euphol, euphadienol and euphorbol. Juice contains diterpene esters, euphorbin. Stem-bark and latex contain triterpenoids, taraxerol and taraxerone, friedelanol and epi-friedelanol, euphol. Roots also contain taraxerol (Ghani, 2003, Rastogi and Mehrotra, 1990).

Traditional use: Inflammation, arthritis, wounds, stomach ache, antioxidant activity, cutaneous infection, diabetes, and as purgative

2.7.2.11 *Ecdysanthera rosea* Hook. & Arn. (APOCYNACEAE)

Figure 2-13 *Ecdysanthera rosea* Hook. & Arn.

Common names: Mwak daeng (Thai)

Family: APOCYNACEAE

Botanical characteristics

Ecdysanthera rosea is a large climbing shrub. The *Ecdysanthera* comprises 15 species, of which *E. rosea* is mainly distributed in tropical and subtropical areas of Asia (Song *et al*., 2014).

Part used: Vine

Chemical constituents

E. rosea contains terpenoids, flavonoids, phenolic glycosides, steroid and glycosides.

Most of the pregnane glycosides isolated from the stem of *E. rosea* have a straight sugar chain which consists of glucose and cymarose units. In addition, all the aglycones of these compounds have the same skeleton of C-21 pregnane (Song *et al*., 2014).

Traditional use: Treatment of traumatic injury, sore throat, and chronic nephritis

2.7.2.12 *Glycyrrhiza glab*ra L. (LEGUMINOSAE (FABACEAE))

Figure 2-14 *Glycyrrhiza glab*ra L.

Common names: Cha aim thet (Thai), Common licorice, Licorice, Russian licorice, Spanish licorice

Family: LEGUMINOSAE (FABACEAE)

Botanical characteristics

Glycyrrhiza glabra is native to Eurasia, northern Africa and western Asia, where it grows up to 1,200 m above sea level. It has also been introduced to many countries, for example the USA where it is a weed of moist roadside sites. Liquorice is also cultivated as a crop plant, particularly in Russia, Spain and the Middle East. A sticky, perennial herb with underground stems (rhizomes). The hairy stems are upright, growing to about 1 m tall.

Part used: Root

Chemical constituents

A number of components have been isolated from the roots of *G. glabra*, including a water- soluble, biologically active complex that accounts for 40–50 % of total dry material weight. This complex is composed of triterpene, saponin, flavo-noids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, aspara- gines, essential oil, fat, female hormone estrogen, gums, mucilage (rhizome), protein, resins,

starches, sterols, volatile oils, tannins, glycosides, and various other substances. Glycyrrhizin, a triterpenoid compound, accounts for the sweet taste of licorice root. This compound represents a mixture of potassium- calcium- magnesium salts of glycyrrhizic acid that varies within a 2–25 % range. Among the natural saponins and glycyrrhizic acid is a molecule composed of a hydro-philic part, two molecules of glucuronic acid, and a hydrophobic fragment, glycyrrhetic acid. The yellow color of licorice is due to the flavonoid content of the plant, which includes liquiritin, isoliquiritin (a chalcone) and other compounds. The isoflavones, glabridin and hispaglabridins A and B have significant antioxidant activity, and both glabridin and glabrene possess estrogen-like activity (Sharma, Katiyar, and Agrawal, 2016).

Traditional use: Confectionery, medicine, beverages

2.7.2.13 *Ligusticum sinense* Oliv. cv. Chuanxiong Hort (UMBELLIFERAE)

Figure 2-15 *Ligusticum sinense* Oliv. cv. Chuanxiong Hort

Common names: Kot hau bua (Thai), Tousenkyu (Japanese), Chuan xiong (Chinese), Szechuan lovage (English)

Family: UMBELLIFERAE

Botanical characteristics

Ligusticum sinense Oliv. cv. Chuanxiong Hort is a perennial plant with massive fist-like rhizomes of brown colour and irregular shape. Its stems are quite thin and tender, but they can reach from about 1 m in height. The leaves look like carrot or parsley, feather-like shape and multi-pinnate leaflets. The small flowers gathered in umbels, bloom during July and August, and are pollinated by insects (MakChuchit, Itharat, Tewtrakul, 2010).

Part used: Rhizome

Chemical constituents

L. sinense is also known as Chinese lovage, which has been employed as a traditional Chinese medicine in folk remedies for a long time. Twenty compounds have been identified in the essential oil from *L. chuanxiong*, and the major compounds are phenolics. As a consequence, it is widely applied in food preparation as an

antioxidant. Moreover, *L. chuanxiong* oils (LCO) have been found to possess insecticidal activity against maize weevils, *Sitophilus zeamais* (Coleoptera: Curculionidae) (Zhang, Liu, He, Ma, and Zhang, 2016).

Traditional use: Prevention and treatment of inflammatory and cardiovascular disease

2.7.2.14 *Mesua ferrea* Linn. (GUTTIFERAE)

Figure 2-16 *Mesua ferrea* Linn.

Common names: Bunnak (Thai), Iron wood (English)

Family: GUTTIFERAE

Botanical characteristics

Mesua ferrea Linn. is a tree up to 15-20 m tall. The young shoots are red or white. Leaves are simple, opposite, lanceolate or oblong-leanceolate, 2-4 cm wide, 7-12 cm long, coriaceous. Flower is solitary, terminal or leaf axils, fragrant with numerous stamens, yellow. Fruit is an ellipsoid drupe (MakChuchit, Itharat, Tewtrakul, 2010).

Part used: Flower

Chemical constituents

From the stem bark of *Mesua ferrea* L. two main components, betulinic acid and 1,8-dihydroxy-3-methoxy-6-methyl-anthraquinone were found in all crude extracts. In addition, stigmasterol and sitosterol were also isolated from the methanol extract. The fresh blossoms of *M. ferrea* gave Lup-20(29)-en-3β-ol, long chain hydrocarbons and carboxylic acids as major compounds (Kim, 2006).

Traditional use: The flowers of *M. ferrea* are used as astringent, carminative, blood tonic and cardiac tonic.

2.7.2.15 *Mimusops elengi* Linn. (SOPOTACEAE)

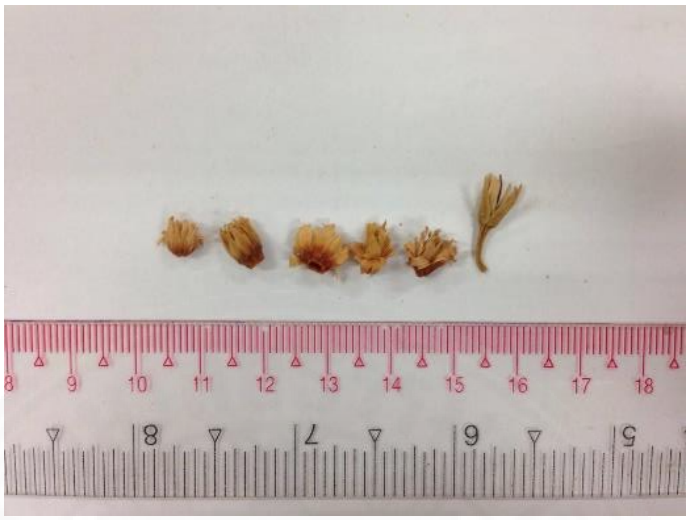

Figure 2-17 *Mimusops elengi* Linn.

Common names: Phikul (Central), Sang dong (Lampang), Phikul khao or Pkikul thuean (Nakhon Si Thammarat) and Bullet Wood (English)

Family: SOPOTACEAE

Botanical characteristics

A small to large evergreen tree growing up to 15 m high. Generally characterized by a short, dark and very rough trunk and wide spreading, the ends of which tend to rise and forms a thick globular head to the tree. The bark is dark grey, occurs in pieces of 15-25 cm long and 10 - 15 cm broad. Externally rough due to the presence of vertical lenticels, cracks, and longitudinal fissures. The dried bark is thin and occurs in quills. Berry is ovoid, 2.5 cm long with. It turns yellow and it tastes astringent and sweet. Fruition occurs in the rainy season and when ripe contains 1, rarely 2 seeds. Seeds are grayish brown, solitary, ovoid, compressed, shining. The leaves are glossy and are dark green when old measring 6.3-10 cm long and 3.2-5 cm wide. The new leaves mostly appear in February when the trees often appear bright vivid green. Leaves are variable, elliptic, oblong or oblanceolae, short or long acuminate, margin undulate, closely but faintly veined. Petioles 1.2 - 2.5 cm long (Kadam, Yadav, Deoda, Shivatare, and Patil, 2012).

Part used: Flower

Chemical constituents

The flowers contain volatile oil (Kadam, Yadav, Deoda, Shivatare, and Patil, 2012).

Traditional use: This flower is an ingredient in Ya-Hom used for the treatment of cardiac tonic, sores and muscular pain.

2.7.2.16 *Mimusops elengi* L. (SOPOTACEAE)

Figure 2-18 *Mimusops elengi* L.

Common names: Khon dok (Thai)

Family: SOPOTACEAE

Botanical characteristics

Khon dok is wood from *Mimusops elengi* L. or *Lagerstroemia floribunda* Jack with mold. Wood is dark brown, white spots, and fragrant. Inner wood is full of small cavities.

Part used: Wood

Chemical constituents

Bark of *M. elengi* contains tannin, some caoutchouc, wax, coloring matter, starch and ash forming inorganic salts (Gami, Pathak, and Parabia, 2012).

Traditional use: Antinociceptive, diuretic effects, gastroprotective, antibacterial, antifungal, anticariogenic, free radical scavenging, antihyperglycemic etc.

2.7.2.17 *Myristica fragrans* Houtt. (MYRISTICACEAE)

Figure 2-19 *Myristica fragrans* Houtt.

Common names: Chan thet (Thai), Nutmeg tree, Mace

Family: MYRISTICACEAE

Botanical characteristics

Myristica fragrans Houtt. is a spreading aromatic evergreen tree usually growing to around 5 to 13 meters high, occasionally 20 meters. Its bark contains watery pink or red sap. The pointed dark green leaves (5 to 15 cm × 2 to 7 cm) are arranged alternately along the branches and are borne on leaf stems about 1 cm long. Upper leaf surfaces are shiny. Flowers are usually single sexed; occasionally male and female flowers are found on the same tree. Female flowers arise in groups of 1 to 3; males in groups of 1 to 10. Flowers are pale yellow, waxy, fleshy and bell-shaped. Male flowers are 5 to 7 mm long; female flowers are up to 1 cm long. The fruits are fleshy, drooping, yellow, smooth, 6 to 9 cm long with a longitudinal ridge. When ripe, the succulent yellow fruit coat splits into 2 valves revealing a purplish-brown, shiny seed (nutmeg) surrounded by a red aril (mace). Seeds (nutmegs) are broadly ovoid (2 to 3 cm long), firm, fleshy, whitish and transversed by red-brown veins. When fresh, the aril (mace) is bright scarlet becoming hornier, brittle and a yellowish-brown color when dried (Orwa *et al*., 2009).

Part used: Heartwood

Chemical constituents

The major components in *M. fragrans* are terpenes, terpene alcohols, and phenolic ethers. The major phenolic ether is myristicin (4-methoxy-6-(2-propenyl)-1,3-benzadioxole) accompanied by safrole (5-(2-propenyl)-3-benzodioxole) and eugenol methyl ether (3,4,-dimethoxy-(2-propenyl)-benzene). Myristicin had 2.12 to 2.88% of the total weight of the nutmeg whereas safrole accounts for 0.27 to 0.39%. The volatile oil content of nutmeg depends on the geographical origin and length of storage. Chemical analysis has shown that even though there is a real variability between the quality (differences in composition) and quantity of nutmeg oil from various samples of nutmegs oil accounts for 84 to 95% of the total aromatic fraction of the volatile oil from all the samples tested. In the samples, myristicin, safrole, and elemicin accounted for 3.86 to 12.7%, 0.53 to 3.42% and 0.02 to 2.36%, respectively of the nutmeg oil samples. Early work on myristicin used myristicin distilled from nutmeg oil. It has been subsequently proven that myristicin extracted from nutmeg via this method is not elemicin free and therefore the effects reported may be due to either substance found in the extract.

Traditional use: Reduce fever, use for carminative

2.7.2.18 *Mammea siamensis* Kosterm (GUTTIFERAE)

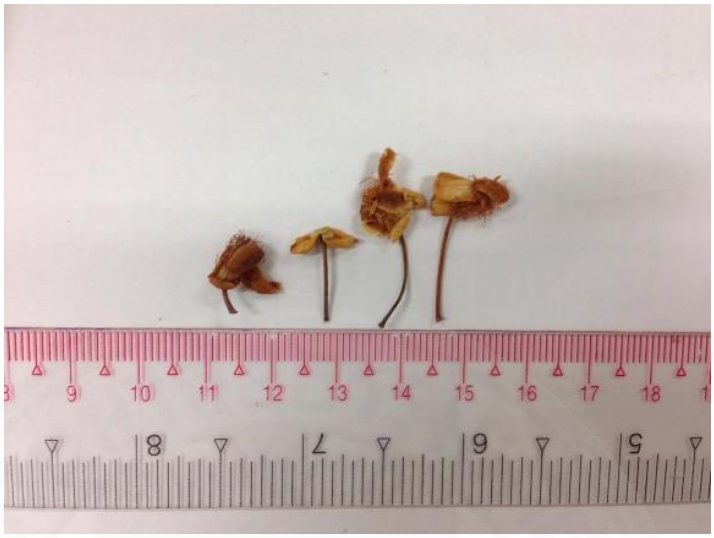

Figure 2-20 *Mammea siamensis* Kosterm

Common names: Saraphi (Thai)

Family: GUTTIFERAE

Botanical characteristics

Mammea siamensis Kosterm is a small evergreen, which grows up to 15 cm tall. Leaves simple, opposite, oblong-oblong-obovate, 4-5 cm wide, 10-15 cm long, coriaceous, glabrous. Flowers are solitary or few-flowered fascicle, ramiflorous or cauliflorous, white, fragrant, stamens numerous and yellow. Fruit is drupe, ellipsoid, ellipsoid, 1-seeded (Subhadhirasakul and Pechpong, 2004).

Part used: Flower

Chemical constituents

Four new mammea coumarins, mammea E/BA cyclo D, mammea E/BC cyclo D, mammea E/BD cyclo D, and mammea E/AC cyclo D, were isolated from the flowers of *M. siamensis* (Mahidol, Kaweetripob, Prawat, and Ruchirawat, 2002).

Traditional use: The flowers have traditionally been used as a heart tonic, reducing of fever, and enhancement of appetite.

2.7.2.19 *Nelumbo nucifera* Gaertn. (NELUMBONACEAE)

Figure 2-21 *Nelumbo nucifera* Gaertn.

Common names: Bua luang (Thai), Lotus, East Indian lotus (English)

Family: NELUMBONACEAE

Botanical characteristics

Nelumbo nucifera is a large aquatic rhizomatous herb consisting of slender, elongated, creeping stem with nodal roots. Lotus is a perennial plant with both aerial and floating orbicular leaves. Aerial leaves are cup shaped and floating leaves have a flat shape. Its petioles are considerably long and rough with distinct prickles. Flowers vary in color from white to rosy and are pleasantly sweet- scented, solitary, and hermaphrodite. Flower average diameter is 10–25 cm, and it is ovoid and glabrous. Fruit which contains seeds are black in color and are hard and ovoid are arranged in whorls; seeds ripen and are released as a result of bending down of the pod to the water. Tuberous roots are 8 inches long and 2 inches in diameter. Smooth outer skin of the lotus root is green in color (Paudel and Panth, 2015).

Part used: Pollen

Chemical constituents

Several flavonoids have been identified in the stamens of *N. nucifera*. These include kaempferol and seven of its glycosides: kaempferol 3- O- β- D-

galactopyranoside, kaempferol 3-O-β-D-glucopyranoside, kaempferol 7-O-β-D-glucopyranoside, kaempferol 3-O-a-L-rhamnopyranosyl-(1-6)-β-D-glucopyranoside, kaempferol 3-O-α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranoside, kaempferol 3-O-α-L-rhamnopyranosyl-(1-2)-β-D-lucuronopyranoside,kaempferol 3-O-β-D-glucuronopyranoside, kaempferol 3-O-β-D-glucuronopyranosyl methylester, myricetin3',5'-dimethylether 3-O-β-D-glucopyranoside, quercetin 3-O-β-D-glucopyranoside, nelumboroside A and nelumboroside B. It also contains two isorhamnetin glycosides: isorhamnetin 3-O-β-D-glucopyranoside and isorhamnetin 3-O-a-L-rhamnopyranosyl-(1-6)-β-D-glucopyranoside. Some non-flavonoid compounds, including adenine, myo-inositol, arbutin and β-sitosterol glucopyranoside, have also been identified in stamen extract (Mukherjee *et al*., 2009).

Traditional use: The pollen treats vertigo, faintness, and is a cardiac tonic.

2.7.2.20 *Syzygium aromaticum* Merr. & L.M. Perry (MYRTACEAE)

Figure 2-22 *Syzygium aromaticum* Merr. & L.M. Perry

Common names: Kan phlu (Thai), Clove tree

Family: MYRTACEAE

Botanical characteristics

A bushy, evergreen tree with a medium-sized crown, growing 8-20 meters tall. Several parts of the tree are aromatic, including the leaves and bark, but it is most valued for the aromatic flower buds, which are usually harvested by hand.

Part used: Flower

Chemical constituents

Clove represents one of the major vegetal sources of phenolic compounds such as flavonoids, hidroxibenzoic acids, hidroxicinamic acids and hidroxiphenyl propens. Eugenol is the main bioactive compound of clove, which is found in concentrations ranging from 9 381.70 to 14 650.00 mg per 100 g of fresh plant material (Neveu *et al*., 2010).

With regard to the phenolic acids, gallic acid is the compound found in higher concentration (783.50 mg/100 g fresh weight). However, other gallic acid derivates as hidrolizable tannins are present in higher concentrations (2375.8 mg/100 g). Other phenolic acids found in clove are the caffeic, ferulic, elagic and salicylic acids.

Flavonoids as kaempferol, quercetin and its derivates (glycosilated) are also found in clove in lower concentrations (Shan, Cai, Sun, Corke, 2005).

Concentrations up to 18% of essential oil can be found in the clove flower buds. Roughly, 89% of the clove essential oil is eugenol and 5% to 15% is eugenol acetate and β-cariofileno (Jirovetz *et al*., 2006). Another important compound found in the essential oil of clove in concentrations up to 2.1% is α-humulen. Other volatile compounds present in lower concentrations in clove essential oil are β-pinene, limonene, farnesol, benzaldehyde, 2-heptanone and ethyl hexanoate (Cortes-Rojas, Souza, and Oliveira, 2014).

Traditional use: Culinary, medicinal, dentistry, perfumery

2.7.2.21 Sodium borate

Figure 2-23 Sodium borate

Common names: Nam phra santoeng (Thai)

Family:

Characteristics

Borax, also known as sodium borate, sodium tetraborate, or disodium tetraborate, is an important boron compound, a mineral, and a salt of boric acid. Powdered borax is white, consisting of soft colorless crystals that dissolve easily in water.

Part used:

Chemical constituents

Borax is generally described as $Na_2B_4O_7{\cdot}10H_2O$. However, it is better formulated as $Na_2\ [B_4O_5(OH)_4]{\cdot}8H_2O$, since borax contains the $[B_4O_5(OH)_4]^{2-}$ ion. In this structure, there are two four-coordinate boron atoms (two BO_4 tetrahedra) and two three-coordinate boron atoms (two BO_3 triangles) (Mendham, Denney, Barnes, and Thomas, 2000).

Traditional use: Used as an ingredient in foods and food preservation

2.7.2.22 *Terminalia chebula* Retz. var chebula (COMBRETACEAE)

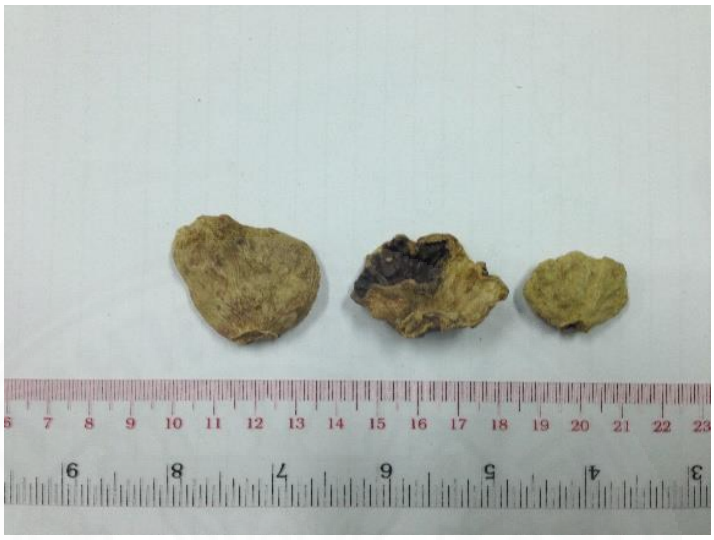

Figure 2-24 *Terminalia chebula* Retz. var chebula

Common names: Kot phung pla (Thai), Terminalia gall, Myrobalan gall, Murobalan wood

Family: COMBRETACEAE

Botanical characteristics

Kot phung pla is a wart born on *Terminalia chebula* Retz. called terminalia gall. It is caused by drilling of insects on leaves and twigs of *T. chebula*, and then laying eggs. After that, *T. chebula* will create the warts to cover those insect eggs. When they are dry, they look like a flat bag and are hollow.

Part used: Gall

Chemical constituents

The main component is tannin, tannic acid, gallic acid and derivatives.

Traditional use: Reducing fever, wound healing

2.7.2.23 *Vetiveria zizanioides* (L.) Nash ex Small (GRAMINEAE)

Figure 2-25 *Vetiveria zizanioides* (L.) Nash ex Small

Common names: Faek hom (Thai), Vetiver grass (English)

Family: GRAMINEAE

Botanical characteristics

Vetiveria zizanioides (L.) Nash ex Small is a densely tufted grass with the culms arising from an aromatic rhizome up to 2 m tall; the roots are stout, dense and aromatic; leaves are narrow, erect, keeled with scabrid margins; inflorescence is a panicle, up to 15-45 cm long of numerous slender racemes in whorls on a central axis; spikelets are grey to purplish, 4-6 mm long, in pairs, one sessile the other pedicelled; 2-flowered; the lower floret is reduced to a lemma, upper bisexual in sessile, male in the pedicelled spikelet; glumes are armed with stout, tubercle-based spines, lemmas awnless, palea minute (Singh *et al*., 2013).

Part used: Root

Chemical constituents

The chemical constituents identified as β- vetivenene, khusimol, vetiselinenol, isovalencenol, vetivenic acid, α- vetivone and β- vetivone in CH_2Cl_2 fractions from roots of *V. zizanioides* (Bhuiyan *et al.,* 2014).

Traditional use: Treatment for diuretic and carminative

Table 2-2 Biological activities of plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy

Scientific Name	Activities	Part used/Bioactive compounds	Detail on biological activities	References
Artemisia annua L. (COMPOSITAE)	Antioxidant	Aerial parts	The ethanolic extract showed IC_{50} >200 µg/ml by DPPH assay.	Klinthong *et al*., 2015
Angelica sinensis (Oliv.) Diels (UMBELLIFERAE)	Anti-inflammatory	Root	The ethyl acetate extract inhibited NO production with IC_{50} = 3.88 ± 0.50 µM.	Chao *et a*l., 2010
		Root	The ethanolic extract inhibited NO production with IC_{50} = 12.52 µg/ml.	Makchuchit *et a*l., 2010
Aquilaria crassna Pierre ex Lecomte. (THYMELAEACEAE)	Anti-inflammatory	Heartwood	Ethyl acetate extract (1. 5 mg/ ml) significantly reduced the TNF-α level.	Kumphune *et al*., 2011
	Antioxidant	Wood	β-Caryophyllene from the essential oil showed IC_{50} = 1.25 ± 0.06 µM by DPPH assay.	Dahham *et al*., 2015

Table 2-2 Biological activities of plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy (Continued)

Scientific Name	Activities	Part used/Bioactive compounds	Detail on biological activities	References
Alyxia reinwardtii Blume var. lucida Markgr. (APOCYNACEAE)	Antioxidant	Heartwood	Ethyl acetate extract of compound 7 showed IC_{50} = 0.19 ± 0.02 mM by DPPH assay and IC_{50} = 2.08 ± 0.06 mM by TBARS assay.	Rattanapan, Sichaem, and Tippyang, 2012
Cinnamomum loureirii Nees. (LAURACEAE)	Anti-inflammatory	Bark	The methanolic extract had %inhibition of iNOS and COX-2 = 28.5 and 85.2, respectively.	Hong, 2002
Cinnamomum verum J. Presl. (LAURACEAE)	Anti-inflammatory	Bark	The sequential extraction of E-cinnamaldehyde and O-methoxycinnamaldehyde inhibited NO production with IC_{50} = 7.3 ± 1.2 and 5.7 ± 1.5 µg/ml, respectively.	Gunawardena *et al*., 2015

Table 2-2 Biological activities of plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy (Continued)

Scientific Name	Activities	Part used/Bioactive compounds	Detail on biological activities	References
Dracaena loureiri Gagnep. (DRACAENACEAE)	Anti-inflammatory	Resin	The methanolic extract (5, 10, 25, and 50 µg/ml) had %inhibition of TNF-α = 26, 54, 74, and 77%, respectively.	Gupta *et al*., 2014
	Antioxidant	Heartwood	The ethanolic extract showed IC_{50} = 98.67 ± 1.52 µg/ml by DPPH assay.	Sakunpak *et al*., 2014
Euphorbia antiquorum L. (EUPHORBIACEAE)	Anti-inflammatory	-	Aqueous and alcoholic extracts at 200 and 400 mg/ kg produced significant inhibition of carrageenan induced rat paw edema.	Kumar and Saikia, 2016

Table 2-2 Biological activities of plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy (Continued)

Scientific Name	Activities	Part used/Bioactive compounds	Detail on biological activities	References
	Antioxidant	Leaf	Aqueous extract exhibited significant antioxidant activity (at 20, 40, 60, 80, and 100 µg/ml) by hydroxyl and superoxide anion radical scavenging activities.	Kumar and Saikia, 2016
Glycyrrhiza glabra L. (LEGUMINOSAE (FABACEAE))	Anti-inflammatory	Root	The ethanolic extract inhibited NO production with IC_{50} = 62.1 µg/ml.	Visavadiya, Soni, and Dalwad, 2009
	Antioxidant	Rhizome and Root	The methanolic/ water extract showed EC_{50} = 0.24 ± 0.01 µg/ml by TBARS assay.	Martins *et al*., 2015
Ligusticum sinense Oliv. cv.Chuanxiong Hort (UMBELLIFERAE)	Anti-inflammatory	Ligustilides	The ethanolic extract of compound 5 (50 µM) had %inhibitory rate = 69.46. by NO assay.	Huang *et al*., 2013

Table 2-2 Biological activities of plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy (Continued)

Scientific Name	Activities	Part used/Bioactive compounds	Detail on biological activities	References
Mesua ferrea Linn. (GUTTIFERAE)	Anti-inflammatory	Flower	Crude extracts caused significant reduction in paw edema from the second hour at the 200 and 400 mg/kg dose level.	Tiwari and Nandy, 2012
		Flower	The ethanolic extract inhibited NO production with IC_{50} = 60.10 ± 0.65 µg/ml.	Inprasit, 2014
	Antioxidant	Flower	The ethanolic extract showed IC_{50} = 55.56 ± 2.75 µg/ml by NBT assay.	Inprasit, 2014
Mimusops elengi L. (SOPOTACEAE)	Anti-inflammatory	Flower	Methanolic extracts at the 100 and 200 mg/kg was 65.57% and 78.68% at 3 hours, respectively by carrageenan induced paw edema assay.	Zahid *et al*., 2016

Table 2-2 Biological activities of plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy (Continued)

Scientific Name	Activities	Part used/Bioactive compounds	Detail on biological activities	References
Mimusops elengi L.* (SOPOTACEAE)	Anti-inflammatory	Bark	The ethanolic extract (200 mg/ kg body weight) significantly inhibited the carrageenan- induced paw edema at 3rd and 4^{th} in cotton pellet model.	Gupta, 2013
	Antioxidant	Leaf	Alcoholic extract exhibited dose dependent free radical scavenging property in peroxynitrite, superoxide and hypochlorous acid models with IC_{50} = (205.53 ± 2.30), (60.5±2.3), (202.4±5.3) µg/ml, respectively.	Kar *et al*., 2012
		Leaf	The methanolic extract showed IC_{50} = 43.26 µg/ml by DPPH assay.	Saha *et al*., 2008

Table 2-2 Biological activities of plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy (Continued)

Scientific Name	Activities	Part used/Bioactive compounds	Detail on biological activities	References
Myristica fragrans Houtt. (MYRISTICACEAE)	Anti-inflammatory	Heartwood	The ethanolic extract inhibited NO production with IC_{50} = 25.14 µg/ml.	Itharat, Makchuchit, and Tewtrakul, 2009
	Antioxidant	Nutmeg	The concentration of 5% methanolic extract showed reducing range = 30-40% by TBARS assay in beef treated.	Zakaria, Abas, and Rukayadi, 2015
Mammea siamensis Kosterm (GUTTIFERAE)	Anti-inflammatory	Flower	The ethanolic extract inhibited NO production with IC_{50} = 74.62 µg/ml.	Makchuchit *et al*., 2010
		Flower	The methanolic extract and ethyl acetate- soluble fraction inhibited NO production with = 28.9 and 8.3 µg/ml, respectively.	Morikawa *et al*., 2012

Table 2-2 Biological activities of plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy (Continued)

Scientific Name	Activities	Part used/Bioactive compounds	Detail on biological activities	References
		Flower	The ethanolic extract inhibited NO production with IC_{50} = 7.67 ± 1.90 µg/ml.	Inprasit, 2014
	Antioxidant	Heartwood	The compounds (kayeassamin A, surangin C, and theraphin B) showed IC_{50} = 11.86, 13.29, and 13.65 µM, respectively by NBT assay.	Tung *et al*., 2013
		Flower	The ethanolic extract showed IC_{50} = 29.07 ± 1.31 µg/ml by NBT assay.	Inprasit, 2014
Nelumbo nucifera Gaertn. (NELUMBONACEAE)	Anti-inflammatory	Pollen	The hydro alcoholic extract inhibited NO production with IC_{50} = 84.86 µg/ml.	Rai *et al*., 2006
	Antioxidant	Pollen	The methanolic extract showed EC_{50} = 9.77 ± 1.67 µg/ml by DPPH assay.	Teh *et al*., 2013

Table 2-2 Biological activities of plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy (Continued)

Scientific Name	Activities	Part used/Bioactive compounds	Detail on biological activities	References
Syzygium aromaticum Merr. & L.M.Perry (MYRTACEAE)	Anti-inflammatory	Flower	Ethanol extract (50 ml/ kg) had % inhibition of writhing = 75 in mice.	Tanko *et al*., 2008
	Antioxidant	Flower	The aqueous extract of clove showed IC_{50} = 0.29 ± 0.01 mg/ml by DPPH assay.	Suantawee *et al*., 2015
Terminalia chebula Retz. var chebula (COMBRETACEAE)	Antioxidant	Leaf gall	Ethanolic extract showed higher free radical scavenging potential than super oxide radical scavenging, hydroxyl scavenging and ferric reducing power (FRAP) methods.	Eshwarappa *et al*., 2015
		Gall	The CW (cold aqueous process) extract (0.1 mg/ml) had %inhibition = 84.64 ± 2.22% by DPPH assay.	Manosroi *et al*., 2010

Table 2-2 Biological activities of plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy (Continued)

Scientific Name	Activities	Part used/Bioactive compounds	Detail on biological activities	References
Vetiveria zizanioides (L.) Nash ex Small (GRAMINEAE)	Anti-inflammatory	Root	Essential oil (12. 51 g/ ml) showed reducing NO production = 26.5%.	Chou *et al*., 2012
	Antioxidant	Root	The methanolic of vetiver oil (10 µg/ml) had % inhibition = 93 by DPPH assay.	Kim *et al*., 2005

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Materials

3.1.1 Laboratory chemicals and reagents

3.1.1.1 Extraction

Table 3-1 List of chemicals and reagents used in extraction

Name	Source
95% ethanol (commercial grade)	C.M.J Anchor company, Thailand
Distilled water	Milford, USA

3.1.1.2 Quality control

(1) Acid insoluble ash

Table 3-2 List of chemicals and reagents used in acid insoluble ash

Name	Source
10% Hydrochloric acid (HCl)	Merck, Germany
Distilled water	Milford, USA

(2) Extractive value

Table 3-3 List of chemicals and reagents used in extractive value

Name	Source
95% ethanol (commercial grade)	C.M.J Anchor company, Thailand
Chloroform ($CHCl_3$) (analytical grade)	RCL labscan, Thailand
Distilled water	Milford, USA

3.1.1.3 Antioxidant activities

(1) Nitroblue tetrazolium (NBT) dye reduction assay

Table 3-4 List of chemicals and reagents used in nitroblue tetrazolium (NBT) dye reduction assay

Name	Source
3-[4, 5-Dimethyl-2-thiazolyl]-2, 5-dipheyl-2H-tetrazalium bromide or Thiazolyl blue tetrazolium bromide (MTT)	Sigma, USA
Dimethyl sulfoxide $(CH_3)_2SO$ (DMSO)	RCL Labscan, Thailand
Distilled water	Milford, USA
Fetal Bovine Serum (FBS)	Biochem, Germany
Hanks' Balanced Salt Solution	Sigma, Germany
Hydrochloric acid (HCl)	Univar, Australia
Nitroblue tetrazolium chloride (NBT)	Sigma, Germany
Penicilin-Streptomycin (P/S)	Sigma, USA
Phorbol myristate acetate (PMA)	Sigma, USA
RPMI medium 1640	Gibco, USA
Sodium bicarbonate ($NaHCO_3$)	BHD, English
Trypan blue stain 0.4%	Gibco, USA

(2) Thiobarbituric acid reactive substances (TBARS) assay

Table 3-5 List of chemicals and reagents used in thiobarbituric acid reactive substances (TBARS) assay

Name	Source
1,1,3,3 tetramethoxypropane	Sigma-Aldrich, USA
Absolute ethanol	QREC, New zealand
Distilled water	Milford, USA
Ethylenediaminetetraacetic acid (EDTA)	Merck, Germany
Ferrous sulphate	Merck, Germany
Hydrochloric acid (HCl)	Univar, Australia
Phosphate buffered saline (PBS)	Amresco, USA
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich, USA
Thiobarbituric acid (TBA)	Sigma, USA
Trichloroacetic acid	Merck, Germany
Tris [hydroxymethyl] aminoethane, $[NH_2C[CH_2OH]_3]$	Sigma-Aldrich, USA

3.1.1.4 Anti-inflammatory activity

(1) Nitric oxide (NO) inhibitory assay

Table 3-6 List of chemicals and reagents used in nitric oxide (NO) inhibitory assay

Name	Source
3-[4, 5-Dimethyl-2-thiazolyl]-2, 5-dipheyl-2H-tetrazalium bromide or Thiazolyl blue tetrazolium bromide (MTT)	Sigma, USA
Dimethyl sulfoxide [$(CH_3)_2SO$)] (DMSO)	RCL Labscan, Thailand
Fetal Bovine Serum (FBS)	Biochem, Germany
Hydrochloric acid (HCl)	Univar, Australia
N-(1-Naphthyl) ethylenediamine dihydrochloride	Sigma, USA
Penicillin-Streptomycin(P/S)	Gibco, USA
Phosphate buffered saline (PBS)	Amresco, USA
Phosphoric acid solution	Sigma, USA
RPMI medium 1640	Gibco, USA
Sodium bicarbonate ($NaHCO_3$)	BHD, England
Sodium hydroxide (NaOH)	Univar, Australia
Sulfanylamide	Sigma, USA
Trypan blue stain 0.4%	Gibco, USA
Trypsin-EDTA	Gibco, USA

3.1.2 Equipment

Table 3-7 List of equipment, plastics and glass wares used in this study

Name	Source
96-well plate flat bottom with lid	Costar Corning, USA
96-well plate flat bottom without lid	Costar Corning, USA
Autoclave	Hirayama, Japan
Balance 0.01 mg - 41 g	Mettler-Toledo, Swizerland
Balance 0.01 g - 220 g	Precica, Swizerland
Balance 0.5 mg - 3100 g	Mettler-Toledo, Swizerland
Buchner funnel	Schott Duran, Germany
Cell culture flask, canted neck 25, 75 cm^3	Corning, USA
Centrifuge machine	Boeco, Germany
Centrifuge tube 15, 50 ml	Costar Corning, USA
CO_2 humidified incubator	Forma, USA
Crucibles	Coorstex, USA
Disposable pipette 25 ml	Corning, USA
Erlenmeyer flask	Schott Duran, Germany
Eppendorf	Costar Corning, USA
Examination gloves	Sritrang gloves, Thailand
Filter paper no.1 (125 mm, diameter)	Whatman, USA
Filter paper no.40 (125 mm, diameter)	Whatman, USA
Freezer	Sanyo, Japan
Glass bottle (50, 250, 500, 1000 ml)	Schott Duran, Germany
Glassware (10, 25, 50, 100, 250, 600, 1000 ml)	Schott Duran, Germany
Haemocytometer	Boeco, Germany
Hot air oven	Memmert, Germany
Hot plate	Thermolyne, USA
Incubated tabletop orbital shaker	Thermo Scientific, USA
Inverted microscope	Nikon, Japan

Table 3-7 List of equipment, plastics and glass wares used in this study (Continued)

Name	Source
Laminar air flow	Boss tech, Thailand
Lyophilizer	Telster, Spain
Micropipettes 1-20, 20-200, 100-1000 µl	Gilson, USA
Microplate reader	Bio Tek, USA
Moisture analyzer	Scaltec instrument, Germany
Muffle furnace	Nabertherm, Germany
Multi-channels pipette	Costar Corning, USA
pH buffer	Thermo Scientific, USA
pH meter	WTW inolab, Germany
Pipette tips	Costar Corning, USA
Pipette boy	Brand, USA
Reagent reservoir (Sterile)	Costar Corning, USA
Refrigerator (4°C)	Sharp, Japan
Refrigerator (-20°C)	Sanyo, Japan
Rotary evaporator	Buchi, Swizerland
Shaking incubator	Vision Scientific, Korea
Stability incubator	Termarks, Norway
Sonicator	Elma, Germany
Syringes	Nipro, Thailand
Vacuum desiccator	Simax, USA
Vacuum pump	Rocker, Taiwan
Vortex mixer	Scientific industries, USA
Water bath	Memmert, Germany
Water purification machine	Elga, UK

3.1.3 Medicinal ingredients

Ya- Hom KAE- LOM- WING- WIEN remedy consists of twenty- three medicinal ingredients which were purchased from Tai An Jan drug store. The voucher specimens were identified by the herbarium of Southern Center of Thai Medicinal

Plants at Faculty of Pharmaceutical Sciences, Prince of Songkhla University, Songkhla Province, Thailand. The ingredients are shown in **Table 3-8.**

Table 3-8 List of ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy

Scientific Name	Family Name	Thai name	Places for specimen collection	Part of plant used	Voucher specimen number	Ratio in remedy
Artemisia annua L.	COMPOSITAE	Kot chula lampa	India	Aerial parts	SKP051010101	3.56
Angelica sinensis (Oliv.) Diels	UMBELLIFERAE	Kot chiang	China	Root	SKP199011901	5.33
Aquilaria crassna Pierre ex Lecomte.	THYMELAEACEAE	Krit sana	Nakhon Ratchasima	Wood	SKP193010301	3.56
Alyxia reinwardtii Blume var. lucida Markgr.	APOCYNACEAE	Cha lud	Surin	Bark	SKP013011801	3.56
Borneo camphor	-	Phim sen	China	-	-	2.67
Cinnamomum bejolghota (Buch.-Ham.) Sweet	LAURACEAE	Sa mul lawang	Surin, Nakhon Si Thammarat	Bark	SKP096030201	4.44
Cinnamomum loureirii Nees.	LAURACEAE	Op choei ywan	China, Vietnam	Bark	SKP096031201	3.56
Cinnamomum verum J. Presl.	LAURACEAE	Op choei thet	Indonesia	Bark	SKP096032201	3.56
Dracaena loureiri Gagnep.	DRACAENACEAE	Chan daeng	Nakhon Ratchasima	Heartwood	SKP005041201	2.22
Euphorbia antiquorum L.	EUPHORBIACEAE	Kra lumphak	Nakhon Ratchasima	Heartwood	SKP071050101	3.56
Ecdysanthera rosea Hook. & Arn.	APOCYNACEAE	Mwak daeng	Nakhon Ratchasima	Vine	SKP013051801	2.22
Glycyrrhiza glabra L.	LEGUMINOSAE	Cha aim thet	China	Root	SKP072070701	14.22

Table 3-8 List of ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy (Continued)

Scientific Name	Family Name	Thai name	Places for specimen collection	Part of plant used	Voucher specimen number	Ratio in remedy
Ligusticum sinense Oliv. cv. Chuanxiong Hort	UMBELLIFERAE	Kot hau bua	China	Rhizome	SKP199121901	5.33
Mesua ferrea Linn.	GUTTIFERAE	Bunnak	Phetchabun	Flower	SKP083130601	2.67
Mimusops elengi Linn.	SOPOTACEAE	Phikul	Angthong, Singburi	Flower	SKP171130501	2.67
Mimusops elengi Linn.	SOPOTACEAE	Khon dok	Nakhon Ratchasima	Wood	SKP171130501	2.67
Myristica fragrans Houtt.	MYRISTICACEAE	Chan thet	Australia	Heartwood	SKP121130601	10.67
Mammea siamensis Kosterm	GUTTIFERAE	Saraphi	Ratchaburi, Singburi	Flower	SKP083131901	2.67
Nelumbo nucifera Gaertn.	NELUMBONACEAE	Bua luang	Nakhon Sawan	Pollen	SKP125141401	5.33
Syzygium aromaticum Merr. & L.M. Perry	MYRTACEAE	Kan phlu	Indonesia	Flower	SKP123190101	5.33
Sodium borate	-	Nam phra santoeng	America	-	-	2.22
Terminalia chebula Retz. var chebula	COMBRETACEAE	Kot phung pla	India	Gall	SKP0459200301	3.56

Table 3-8 List of ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy (Continued)

Scientific Name	Family Name	Thai name	Places for specimen collection	Part of plant used	Voucher specimen number	Ratio in remedy
Vetiveria zizanioides (L.) Nash ex Small	GRAMINEAE	Faek hom	Maha Sarakham	Root	SKP081222601	5.33

3.2 Methods

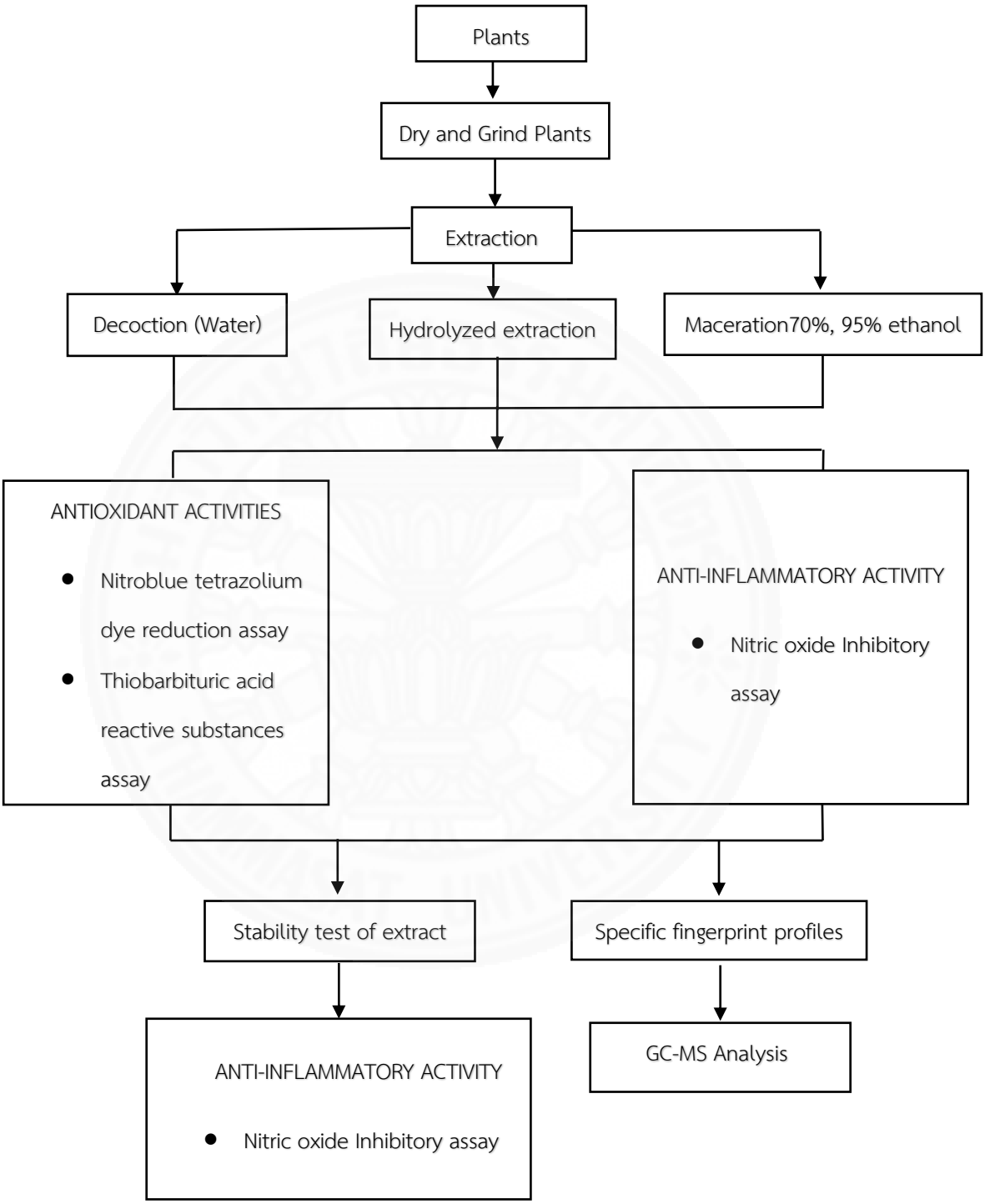

Figure 3-1 Conceptual framework of thesis

3.2.1 Preparation of Crude extracts

Plant ingredients of Ya-Hom KAE-LOM-WING-WIEN remedy were washed, sliced into small pieces and dried in a hot air oven at 50ºC. These were ground to powders. These plant ingredients were weighed and mixed to the Ya-Hom KAE-LOM-WING-WIEN remedy. The preparation of crude extracts was in 70%, 95% ethanolic, hydrolyzed extraction, and decoction.

3.2.1.1 Maceration

Crude powders of the remedy were extracted with 70% and 95% ethanol by macerated for 3 days and filtered through a Whatman No. 1 filter paper. The filtrate was dried using rotary evaporator. The maceration was repeated twice on the residue. Percentage yields of all extracts were calculated.

3.2.1.2 Hydrolyzed extraction

The aqueous extracts (20 g.) were dissolved in 0.01 N HCl (pH 2.0 gastric pH, fasting state) and extracted using by reflux for 30 minutes. After, the solvent fractions were mixed with chloroform (1: 1) in separatory funnel for three times. The chloroform fractions were filtered and dried by rotary evaporator.

3.2.1.3 Decoction

Crude powders of the remedy were extracted with distilled water by boiling for 15 minutes and filtered through a Whatman No.1 filter paper. The decoction was repeated twice on the residue and dried by lyophilizer. The yields of the above extracts were calculated from the following equation:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dry extract}}{\text{Weight of the dry plant material (powder)}} \times 100$$

Crude extracts were stored at -20 ºC until use.

3.2.2 Quality control of Ya-Hom remedy and its plant ingredients

Quality control methods were investigated following Thai Herbal Pharmacopoeia. The study includes loss on drying, extractive value, total ash, and acid insoluble ash. These methods were tested in triplicate.

3.2.2.1 Loss on drying

Moisture analyzers provide a rapid and accurate method for moisture content and dry weight analysis of ingredients. The method analyzed by electronic moisture analyzer of loss on drying. Two grams of each sample were put into the moisture analyzer at 105 °C. The weight of the dried sample was displayed and moisture content was calculated from the following equation:

$$\text{Moisture content (\%)} = \frac{\text{Weight of start sample – weight dried sample (g)}}{\text{Weight of start sample (g)}} \times 100$$

3.2.2.2 Extractive value

(1) Ethanol-soluble extractive value

Five grams of dried plant powders were macerated in 100 ml of 95% ethanol in erlenmeyer flask with foil for 24 hours, shaking frequently during the first 6 hours and then allowing to stand for 18 hours. The plant extract was filtered and 20 ml of filtrate on to an evaporating dish. After that, the extract was dried at 105°C until constant weight.

(2) Water-soluble extractive value

The procedure for determination of water-soluble extractive values is similarly to the method for ethanol-soluble extractive value but using 0.25% chloroform in water instead of ethanol. The percentage of ethanol-soluble extractive and water-soluble extractive values were calculated from the following equation:

$$\text{Ethanol (water) extractive value (\%)} = \frac{\text{Weight of the extract (g)}}{\text{Weight of dried powder (g)}} \times 100 \times 5$$

3.2.2.3 Total ash

The total ash method measures the total amount of material remaining after ignition. This includes both physiologic ash and non-physiologic ash. Preparation of crucible was by drying in a hot air oven at 105°C until the weight of crucible was stable. Two grams of sample was weighed in crucible and the crucible burned using muffle furnace at 450°C for 9 hours. The crucible was cooled in a desiccator, then put in the muffle furnace at 450°C for 5 hours and placed in desiccator to cool down. This procedure was repeated until the weight was stable. Total ash was calculated from the following equation:

$$\text{Total ash (\%)} = \frac{\text{Stable weight after burning (g)}}{\text{Weight before burning (g)}} \times 100$$

3.2.2.4 Acid insoluble ash

This method was continued from the previous method. The total ash was added to twenty-five ml of 10% hydrochloric acid (HCl), boiled on hot plate for 5 minutes and filtered using Whatman paper no. 42. The ashless on filter paper was washed by distilled water until pH 7. Next, residue on filter paper was put in the crucible and burned in muffle furnace at 500°C for 9 hours and repeated until the weight of crucible was constant. Acid insoluble ash was calculated from the following equation:

$$\text{Acid insoluble ash (\%)} = \frac{\text{Stable weight after burning (g)}}{\text{Weight before burning (g)}} \times 100$$

3.2.3 Antioxidant activities

3.2.3.1 Nitroblue tetrazolium (NBT) dye reduction assay

3.2.3.1.1 Human cell lines

HL-60 is a human promyelocytic leukemia cell line, which was cultured in RPMI 1640 medium supplemented with 10% heated fetal bovine serum (FBS), 50 IU/ml penicillin, and 50 µg/ml streptomycin. The cells were grown and maintained at 37 °C in an incubator with 5% CO_2 and 95% humidity. The HL-60 cells in RPMI 1640 were induced to myeloid differentiation with 1.3% DMSO for 6 days.

3.2.3.1.2 Preparation of sample solution

The ethanolic and hydrolyzed samples were dissolved in sterile dimethyl sulfoxide (DMSO) to a final stock concentration of 50 mg/ml and the aqueous samples were dissolved in sterile distilled water and filtered through 0.22 µm sterile filter (Millipore) to a final stock concentration of 10 mg/ml. Each sample was diluted by RPMI 1640 medium to obtain final concentrations of 0.01-100 µg/ml.

3.2.3.1.3 Nitroblue tetrazolium (NBT) dye reduction assay (Makishima *et al*., 1996, Srisawat *et al*., 2010)

The intracellular superoxide anion formation ($O_2^{\bullet-}$) was determined by nitroblue tetrazolium (NBT) dye reduction assay. Briefly, HL-60 cells (1×10^6 cells/ml) were incubated with various dilutions of the extracts and dissolved in 200 µl of Hanks' balanced salt solution (HBSS) incubated for 15 minutes at 37°C in CO_2 incubator. Next, the cells were incubated with 250 ng/ml of Phorbol 12-myristate 13-acetate (PMA) and 1.25 mg/ml of NBT solution for another 60 minutes. At the end of incubation, 2 ml of ice-cold 1M HCl were added. After centrifugation at 4000 rpm for 10 minutes, the supernatants were removed. The insoluble formazan precipitate was dissolved in 300 µl of DMSO. Then, 100 µl of sample solution was added into 96 well-micro plates. The absorbance was measured at 572 nm using a microplate reader (Power Wave XS, Bio Tek) and compared with the control (without the extract). This assay was repeated in triplicate. Inhibition of each concentration of the extract against superoxide anion formation ($O_2^{\bullet-}$) was calculated from the following equation:

$$\text{NBT reduction (\%)} = \frac{(\text{ODcontrol-ODblank})-(\text{ODsample-ODblank})}{(\text{ODcontrol-ODblank})} \times 100$$

Where: OD control is the absorbance of a reaction mixture containing only PMA, OD blank is neither PMA nor extract and OD sample is both PMA and extract. The scavenging activity of the extracts against superoxide is expressed as IC_{50} (µg/ml).

3.2.3.1.4 Cell Viability with 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay (Tewtrakul and Itharat, 2007)

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay is a reliable method for colorimetric determination. This method continued from NBT dye reduction assay as shown above. After incubation for 15 minutes at 37°C in CO_2 incubator, the cells were incubated with 250 ng/ml of PMA solution in HBSS and 250 µl of HBSS for 60 minutes and centrifuged at 1,500 rpm for 5 minutes. The supernatants were removed and were added 1,000 µl and 100 µl of HBSS and MTT, respectively and incubated for 2 hours. After centrifugation at 4000 rpm for 10 minutes, the supernatants were removed. The insoluble formazan precipitate was dissolved in 300 µl of DMSO. Then, 100 µl of sample solution was added into 96 well-micro plates. The absorbance was measured at 570 nm using a microplate reader (Power Wave XS, BioTek). The test sample was considered to be cytotoxic when the density of the sample-treated group was more than 30%, compared with control group. Cytotoxicity was calculated from the following equation:

$$\text{Toxicity (\%)} = \frac{(\text{ODcontrol-ODsample})}{\text{ODcontrol}} \times 100$$

3.2.3.2 Thiobarbituric acid reactive substances (TBARS) assay

3.2.3.2.1 Preparation of brain homogenates

Whole porcine brain tissue was homogenized in buffer (10 mM Tris-HCl, pH 7.5, 1/10 (w/v)) and centrifuged at 4,000 rpm for 10 minutes at 4°C.

3.2.3.2.2 Preparation of sample solution

The ethanolic and hydrolyzed samples were dissolved in absolute ethanol and the aqueous samples were dissolved in distilled water, all to a final stock concentration of 50 mg/ml. Each sample was diluted absolute ethanol for the ethanolic and hydrolyzed samples and distilled water for the aqueous samples to obtain final concentrations of 5-500 µg/ml.

3.2.3.2.3 Thiobarbituric acid reactive substances (TBARS) assay (Ayyappan, Palayyan, Gopalan, 2016, Alinezhad *et al*., 2012)

Briefly, 200 µl of each fraction was mixed with 100 µl of extraction solvent and 40 µl of 100 µM Fe^{2+} /EDTA. This mixture was incubated at 37°C for 1 hour. Color reaction was developed by adding 200 µl of 8.1% sodium dodecyl sulphate (SDS) to the reaction mixture followed by 500 µl of 2.8% trichloroacetic acid (TCA) and 0.6% thiobarbituric acid (TBA), respectively. The mixture was heated at 100 °C for 1 hour. After cooling 10 minutes, TBARS production were measured using a microplate reader at 532 nm (Power Wave XS, Bio Tek) and the absorbance was compared with the standard curve using 1, 1, 3, 3 - tetramethoxypropane. Blank contained all the reagents except samples and neurotoxic agents. Total malondialdehyde content is expressed as nM malondialdehyde, obtained from a calibration curve of 1, 1, 3, 3 – tetramethoxypropane standard solutions. This assay was repeated in triplicate.

3.2.4 Anti-inflammatory activity

3.2.4.1 Animal cell lines

The murine leukemia macrophage cell line (RAW 264.7), the most commonly used mouse macrophage cell line in medical research, was obtained from American Type Culture Collection (ATTC TIB-71). RAW 264.7 cells were cultured in RPMI 1640 medium supplemented with 10% heated fetal bovine serum (FBS), penicillin and streptomycin stored at 37 °C in an incubator with 5% CO_2 and 95% humidity.

3.2.4.2 Preparation of sample solution

The ethanolic and hydrolyzed samples were dissolved in sterile dimethyl sulfoxide (DMSO) to a final stock concentration of 50 mg/ml and the aqueous samples were dissolved in sterile distilled water and filtered through 0.22 µm sterile filter (Millipore) to a final stock concentration of 10 mg/ml. Each sample was diluted by RPMI 1640 medium to obtain final concentrations of 1-100 µg/ml.

3.2.4.3 Nitric oxide (NO) inhibitory assay (Tewtrakul and Subhadhirasakul, 2008, Tewtrakul and Itharat, 2007)

Inhibitory activity on nitric oxide (NO) production in RAW 264.7 cell lines was evaluated by the following method. RAW 264.7 cells were cultured in RPMI 1640 medium containing 10% FBS, 100 units/ml penicillin and 100 units/ml streptomycin. Cells were washed with PBS, followed by 0.25% trysin-EDTA, and fresh medium added. Centrifugation of cells was at 1,500 rpm for 5 minutes, removed and replaced with 10 ml of fresh medium. Counting of viable cells was by using trypan blue. Seeding cells to the given final concentration of $1x10^5$ cells/well was in 96 well microplates which were incubated at 37 °C in 5% CO_2 for 24 hours. Then, the medium was removed and fresh medium added with 100 µl containing 2 ng/ml of lipopolysaccharide (LPS) together with sample solution at various concentrations. The extracts were dissolved with DMSO, diluted in medium according to the desired concentration, and 100 µl/well added to the microplate. After that, the cells were incubated at 37 °C in 5% CO_2 for 24 hours. Nitric oxide (NO) production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess

reagent (0.1% naphthalene diamine dihydrochloride, 1% sulfanilamide in 5% H_2SO_4) 100 µl/well. The absorbance was measured with a microplate reader (Power Wave XS, BioTek) at 570 nm. Inhibition (%) was calculated by using the following equation and IC_{50} values was calculated by the Prism program.

$$\text{Inhibition (\%)} = \frac{(\text{ODcontrol-ODsample})}{\text{ODcontrol}} \times 100$$

ODcontrol: Mean of control (+LPS) – Mean of control (-LPS)

ODsample: Mean of sample (+LPS) – Mean of sample (-LPS)

3.2.4.4 Cell Viability with 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay (Tewtrakul and Itharat, 2007)

Cytotoxicity was also tested to ensure that nitric oxide production was not produced by destroying the cell membrane (% cytotoxicity less than 30%). The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay is a reliable method for colorimetric determination. This procedure continued from NO assay as shown above. After incubation for 24 hours at 37°C in CO_2 incubator, MTT solution (10 µl, 5 mg/ml in PBS) was added to each well of the 96-well plate and incubated again for 2 hours. The supernatants were removed and 100 µl of isopropanol added containing 0.04 M HCl for dissolving the formazan production. The absorbance was measured at 570 nm using a microplate reader (Power Wave XS, BioTek). The test sample was considered to be cytotoxic when the optical density of the sample-treated group was more than 30%, compared with control group. Cytotoxicity was calculated from the following equation:

$$\text{Toxicity (\%)} = \frac{(\text{ODcontrol-ODsample})}{\text{ODcontrol}} \times 100$$

3.2.5 The stability test of Ya-Hom KAE-LOM-WING-WIEN remedy extract (Grimm, 1998)

The stability testing was modified method from Grimm (1998), carried out in triplicate. The 95% ethanolic extract of Ya-Hom KAE-LOM-WING-WIEN remedy was stored over a six-month period, under 40 ± 2 ℃ with 75 ± 5% RH as accelerated conditions. Samples were taken at days 0 (control sample), 15, 30, 60, 90, 120, 150 and 180 and results were compared with control. The purpose of this investigation was implied that the extract is stable when kept in a closed container protected from light and stored at room temperature for at least two years. After testing, the samples were evaluated the anti-inflammatory activity by nitric oxide (NO) inhibitory assay.

3.2.6 Phytochemical screening of the extracts of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS)

50 mg of 70%, 95% ethanolic, aqueous, and hydrolyzed extracts of Ya-Hom KAE-LOM-WING-WIEN remedy were transferred into 10 ml volumetric flask and diluted to volume with methanol. The solutions were analyzed by using gas chromatography-mass spectrometry (GC-MS) with Thermo Focus GC, polaris Q, and auto injector (column thermo TG-5silms). Helium gas (He) was carrier with flow rate 1.0 ml/minutes. The initial temperature of column oven was programmed 60^{o}C, and then heated to 300^{o}C with a rate of 5^{o}C/minute and kept constant at 300^{o}C for 5 minutes. Each peak was recorded on the mass spectrum. Identification of chemical components was analyzed by Herb and Thai Traditional Medicine Development Division, BIOTEC Pilot Plant, Thailand Science Park.

3.2.7 Statistical analysis

All determinations were repeated in triplicate. Different parameter values were expressed as the mean ± standard error of mean. Statistical analysis was performed with SPSS statistical software.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Preparation of Crude extracts

4.1.1 Percentage yield

4.1.1.1 Percentage yields of Ya-Hom KAE-LOM-WING-WIEN remedy

The ethanolic, aqueous, and hydrolyzed extracts of Ya-Hom KAE-LOM-WING-WIEN remedy were prepared by maceration with 70% and 95% ethanol, decoction, and hydrolyzed extraction as described in Chapter 3 **(section 3.2.1)**. The highest percentage yield of Ya-Hom remedy extracts was the 70% ethanolic extract (20.86%) and the lowest percentage yield of Ya-Hom remedy extracts was the hydrolyzed extract (1.46%). The percentage yields of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients are shown as percentage by weight in **Table 4-1**.

4.1.1.2 Percentage yield of plant ingredients

The ethanolic extracts of plant ingredients were prepared by maceration with 95% ethanol. The percentage yields of all ethanolic extracts ranged from 3.22% to 35.44%. The highest percentage yield of the ethanolic extracts was *Terminalia chebula* Retz. var chebula and the lowest percentage yield of the ethanolic extracts was *Alyxia reinwardtii* Blume var. lucida Markgr.

Table 4-1 The percentage yields of the ethanolic, aqueous, and hydrolyzed extracts of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients

Sample	Thai name	Extraction	Code	%Yield
Artemisia annua L.	โกฐจุฬาลัมภา	Ethanolic(95%)	AAE95	4.11
Angelica sinensis (Oliv.) Diels	โกฐเชียง	Ethanolic(95%)	ASE95	3.12
Aquilaria crassna Pierre ex Lecomte.	กฤษณา	Ethanolic(95%)	ACE95	2.71
Alyxia reinwardtii Blume var. lucida Markgr.	ชะลูด	Ethanolic(95%)	ARE95	3.22
Borneo camphor	พิมเสน	Ethanolic(95%)	BSE95	-
Cinnamomum bejolghota (Buch.-Ham.) Sweet	สมุลแว้ง	Ethanolic(95%)	CBE95	16.13
Cinnamomum loureirii Nees.	อบเชยญวณ	Ethanolic(95%)	CLE95	3.85
Cinnamomum verum J. Presl.	อบเชยเทศ	Ethanolic(95%)	CVE95	4.25
Dracaena loureiri Gagnep.	จันทน์แดง	Ethanolic(95%)	DLE95	19.27
Euphorbia antiquorum L.	กระลำพัก	Ethanolic(95%)	EAE95	24.09
Ecdysanthera rosea Hook. & Arn.	มวกแดง	Ethanolic(95%)	ERE95	4.80
Glycyrrhiza glabra L.	ชะเอมเทศ	Ethanolic(95%)	GGE95	5.64
Ligusticum sinense Oliv. cv. Chuanxiong Hort	โกฐหัวบัว	Ethanolic(95%)	LSE95	6.16
Mesua ferrea Linn.	บุนนาค	Ethanolic(95%)	MLE95	17.38
Mimusops elengi Linn.	พิกุล	Ethanolic(95%)	MEE95	12.09
Mimusops elengi Linn. *	ขอนดอก	Ethanolic(95%)	MKE95	6.65
Myristica fragrans Houtt.	จันทน์เทศ	Ethanolic(95%)	MFE95	6.27
Mammea siamensis Kosterm	สารภี	Ethanolic(95%)	MSE95	16.41
Nelumbo nucifera Gaertn.	บัวหลวง	Ethanolic(95%)	NCE95	7.58
Syzygium aromaticum Merr. & L.M. Perry	กานพลู	Ethanolic(95%)	SAE95	14.72
Sodium borate	น้ำประสานทอง	Ethanolic(95%)	SBE95	-
Terminalia chebula Retz. var chebula	โกฐพุงปลา	Ethanolic(95%)	TCE95	35.44
Vetiveria zizanioides (L.) Nash ex Small	แฝกหอม	Ethanolic(95%)	VZE95	11.58

Asterisk **(*)** indicates wood of *Mimusops elengi* Linn. known as Khon dok.

Table 4-1 The percentage yields of the ethanolic, aqueous, and hydrolyzed extracts of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (Continued)

Sample	Thai name	Extraction	Code	%Yield
Ya-Hom KAE-LOM-WING-WIEN remedy	ยาหอมแก้ลมวิงเวียน	Ethanolic(70%)	KLWWE70	20.86
		Ethanolic(95%)	KLWW95	10.30
		Aqueous	KLWWA	18.76
		Hydrolyzed	KLWWH	1.46

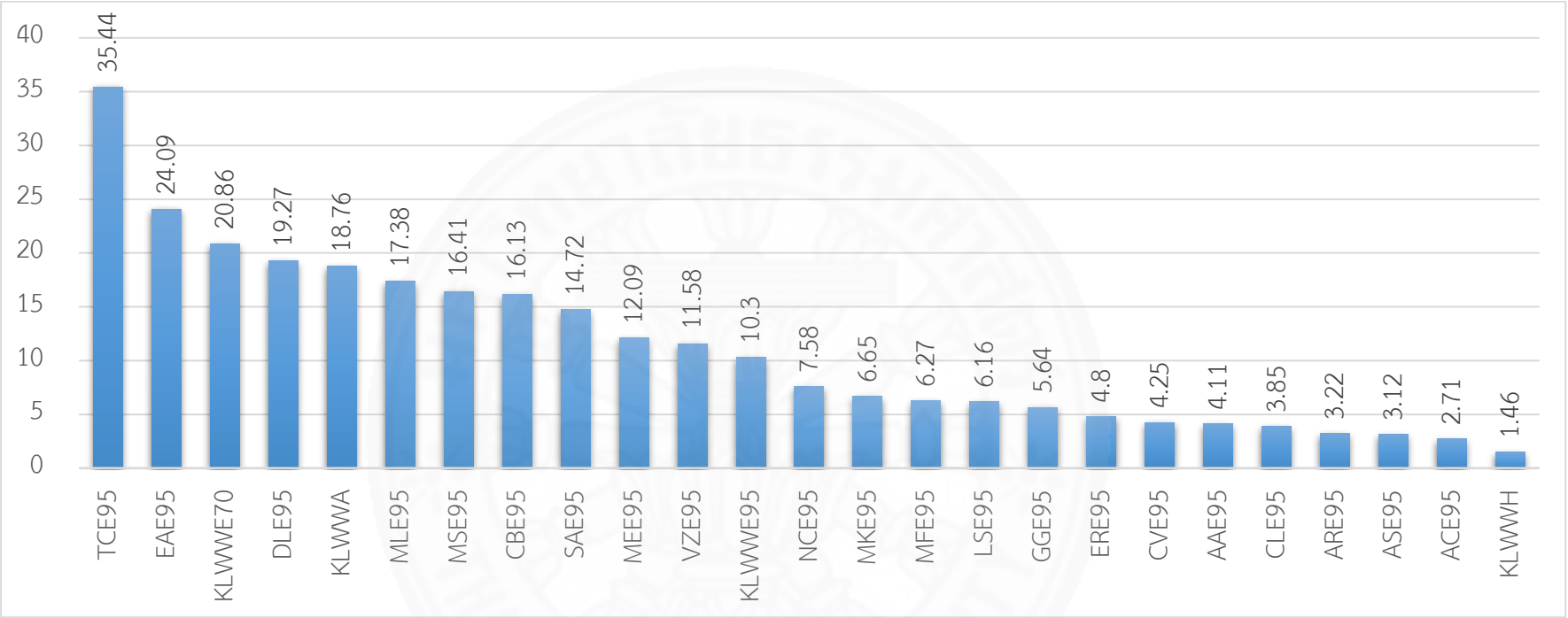

Figure 4-1 The percentage yields of the ethanolic, aqueous, and hydrolyzed extracts of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients

4.2 Quality control

Quality control methods of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients include loss on drying, extractive value, total ash, and acid insoluble ash. These methods were investigated following the standard values given in Thai Herbal Pharmacopoeia.

4.2.1 Loss on drying

The standard quality value of Thai Herbal Pharmacopoeia represented by loss on drying is not more than 10%. The highest percentage of loss on drying was *Mimusops elengi* Linn. (8.72±0.37%) and the lowest percentage of loss on drying was *Angelica sinensis* (Oliv.) Diels (3.47±0.29%). The percentage loss on drying of Ya-Hom KAE-LOM-WING-WIEN remedy was 6.40%±0.12%. Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients were within the standard value of Thai Herbal Pharmacopoeia. The results of loss on drying of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients are shown in **Table 4-2**.

4.2.2 Extractive value

Extractive value method consists of ethanol-soluble extractive and water-soluble extractive. The highest percentage of ethanol-soluble extractive value was *Euphorbia antiquorum* L. (24.02±1.25%) and the lowest percentage of ethanol-soluble extractive value was *Alyxia reinwardtii* Blume var. lucida Markgr. (1.28±0.09%). The highest percentage of water-soluble extractive value was *Angelica sinensis* (Oliv.) Diels (48.56±4.47%) and the lowest percentage of water-soluble extractive value was *Myristica fragrans* Houtt. (1.52±0.04%). The percentages of ethanol-soluble extractive and water-soluble extractive values of Ya-Hom KAE-LOM-WING-WIEN remedy were 7.64±0.33% and 18.37±0.74%, respectively. The water-soluble extractive value of Ya-Hom KAE-LOM-WING-WIEN remedy was higher than the ethanol-soluble extractive value which was related with the percentage yields. The results of extractive value of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients are shown in **Table 4-2**.

4.2.3 Total ash

The standard quality control of Thai Herbal Pharmacopoeia represented by total ash is not more than 10%. The highest percentage of total ash was *Artemisia annua* L. (13.18±0.15 %) and the lowest percentage of total ash was *Aquilaria crassna* Pierre ex Lecomte. (0.74±0.01%). The percentage total ash of Ya-Hom KAE-LOM-WING-WIEN remedy was 6.77±0.48%. Although the percentage total ash of *Artemisia annua* L. was more than 10%, Ya-Hom KAE-LOM-WING-WIEN remedy was within the standard value of Thai Herbal Pharmacopoeia. The results of total ash of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients are shown in **Table 4-2**.

4.2.4 Acid insoluble ash

The standard quality control of Thai Herbal Pharmacopoeia represented by acid insoluble ash is not more than 2%. The highest percentage of acid insoluble ash was *Angelica sinensis* (Oliv.) Diels (2.44±0.34 %) and the lowest percentage of acid insoluble ash was *Cinnamomum bejolghota* (Buch.-Ham.) Sweet and *Cinnamomum verum* J. Presl. (0.06±0.00%, 0.06±0.00%). The percentage acid insoluble ash of Ya-Hom KAE-LOM-WING-WIEN remedy was 0.91±0.11%. Although the percentage acid insoluble ash of *Angelica sinensis* (Oliv.) Diels was more than 2%, Ya-Hom KAE-LOM-WING-WIEN remedy was within the standard value of Thai Herbal Pharmacopoeia. The results of acid insoluble ash of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients are shown in **Table 4-2**.

Table 4-2 The results of quality control of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients (mean±SEM), (n=3)

Sample	Code	%Loss on drying	%Extractive value		%Ash content	
			Ethanol-soluble	Water-soluble	Total ash	Acid insoluble ash
Artemisia annua L.	AA	8.08±0.23	5.10±0.25	19.09±0.24	13.18±0.15	0.43±0.01
Angelica sinensis (Oliv.) Diels	AS	3.47±0.29	6.36±0.62	48.56±4.47	8.65±0.37	2.44±0.34
Aquilaria crassna Pierre ex Lecomte.	AC	6.92±0.10	3.62±0.14	2.74±0.02	0.74±0.01	0.08±0.01
Alyxia reinwardtii Blume var. lucida Markgr.	AR	6.30±0.34	1.28±0.09	15.99±0.23	8.20±0.17	0.08±0.02
Cinnamomum bejolghota (Buch.-Ham.) Sweet	CB	8.01±0.35	20.37±2.37	13.48±0.22	1.30±0.02	0.06±0.00
Cinnamomum loureirii Nees.	CL	6.27±0.50	2.35±0.25	5.89±0.09	2.98±0.02	0.09±0.01
Cinnamomum verum J. Presl.	CV	4.47±0.32	2.47±0.34	9.65±0.20	4.10±0.07	0.06±0.00
Dracaena loureiri Gagnep.	DL	4.40±0.22	18.73±0.18	2.61±1.04	5.62±0.23	0.37±0.08
Euphorbia antiquorum L.	EA	4.53±0.18	24.02±1.25	2.05±0.21	4.20±0.10	0.12±0.01
Ecdysanthera rosea Hook. & Arn.	ER	5.81±0.28	2.43±0.30	10.14±0.80	4.14±0.38	0.14±0.03

Table 4-2 The results of quality control of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients (mean±SEM), (n=3) (Continued)

Sample	Code	%Loss on drying	%Extractive value		%Ash content	
			Ethanol-soluble	Water-soluble	Total ash	Acid insoluble ash
Glycyrrhiza glabra L.	GG	6.73±0.62	6.99±0.41	16.33±0.50	8.83±0.39	1.32±0.18
Ligusticum sinense Oliv. cv. Chuanxiong Hort	LS	8.68±0.43	9.71±0.57	21.61±2.66	4.20±0.12	0.36±0.01
Mesua ferrea Linn.	ML	7.13±0.22	15.77±0.53	9.39±1.21	4.59±0.35	1.54±0.12
Mimusops elengi Linn.	ME	8.72±0.38	8.06±0.17	11.96±0.05	5.67±0.23	1.12±0.18
Mimusops elengi Linn.*	MK	5.68±0.21	5.02±0.04	5.09±0.02	1.02±0.03	0.26±0.01
Myristica fragrans Houtt.	MF	6.48±0.44	6.61±0.34	1.52±0.04	7.78±0.02	1.68±1.08
Mammea siamensis Kosterm	MS	8.01±0.20	15.08±0.20	25.43±0.31	8.12±0.05	0.39±0.01
Nelumbo nucifera Gaertn.	NC	7.82±0.29	6.05±0.07	7.62±0.55	7.89±0.18	1.77±0.02
Syzygium aromaticum Merr. & L.M. Perry	SA	5.78±0.49	6.43±0.16	20.74±0.44	5.71±0.13	0.19±0.01
Terminalia chebula Retz. var chebula	TC	5.23±0.37	21.56±2.05	45.14±0.82	3.72±0.10	0.08±0.03

Asterisk (*) indicates wood of *Mimusops elengi* Linn. known as Khon dok.

Table 4-2 The results of quality control of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients (mean±SEM), (n=3) (Continued)

Sample	Code	%Loss on drying	%Extractive value		%Ash content	
			Ethanol-soluble	Water-soluble	Total ash	Acid insoluble ash
Vetiveria zizanioides (L.) Nash ex Small	VZ	7.91±0.44	10.21±0.51	40.10±0.24	9.54±0.07	1.85±0.07
Ya-Hom KAE-LOM-WING-WIEN remedy	KLWW	6.40±0.12	7.64±0.33	18.37±0.74	6.77±0.48	0.91±0.11

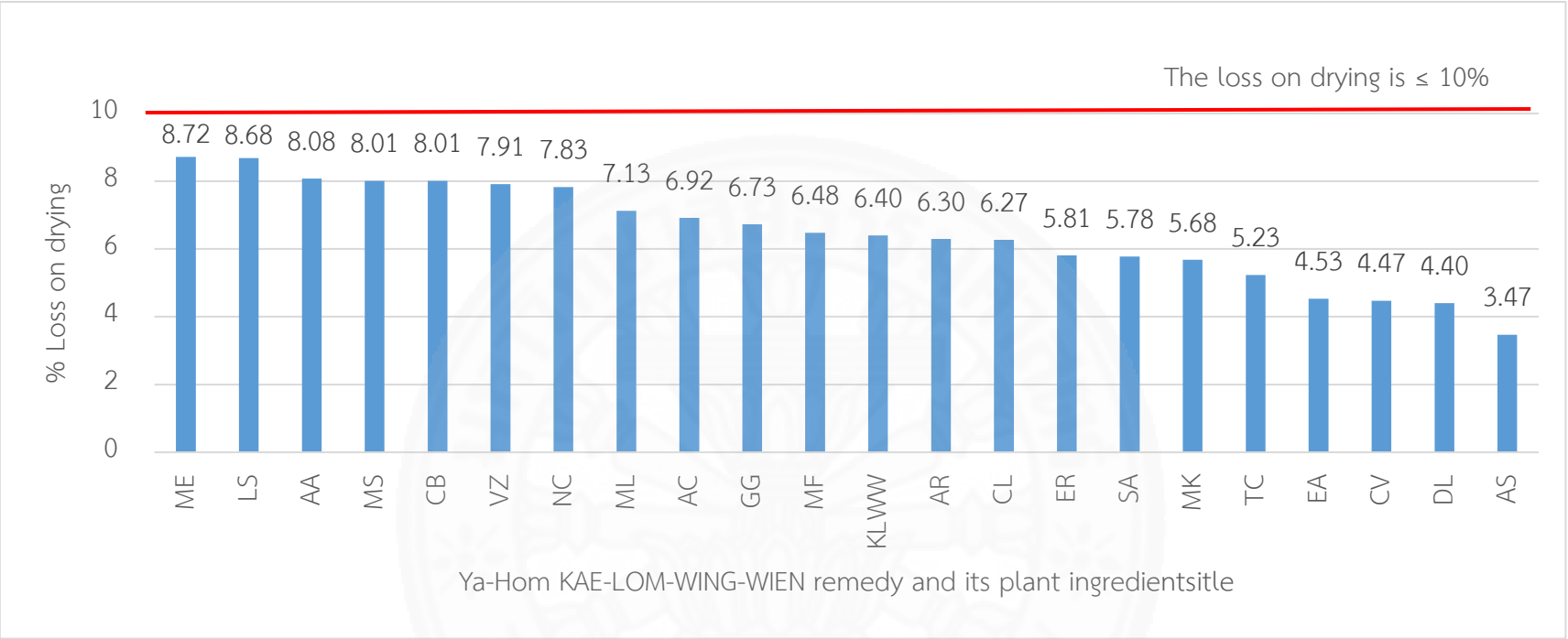

Figure 4-2 The percentage loss on drying of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients

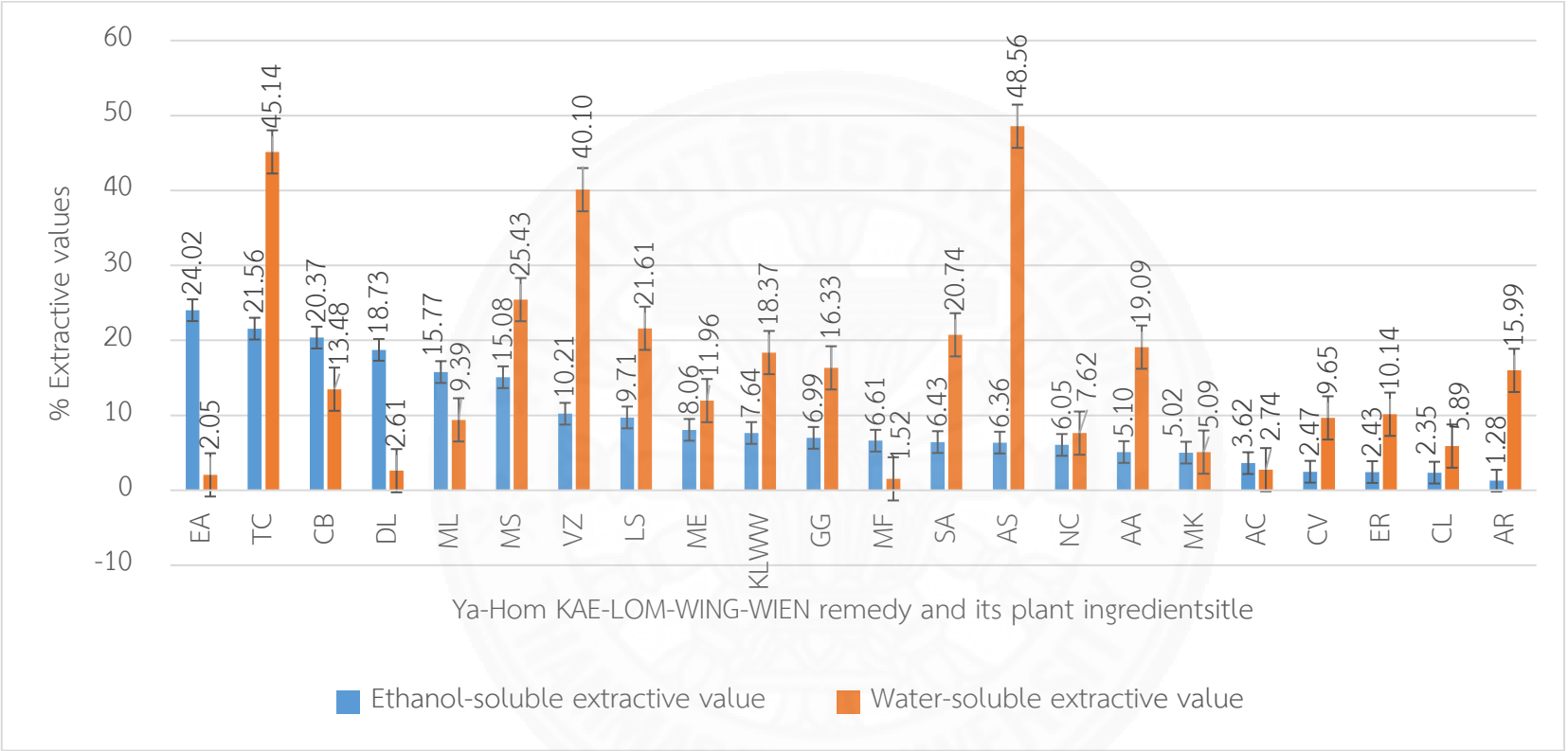

Figure 4-3 The percentage extractive values of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients

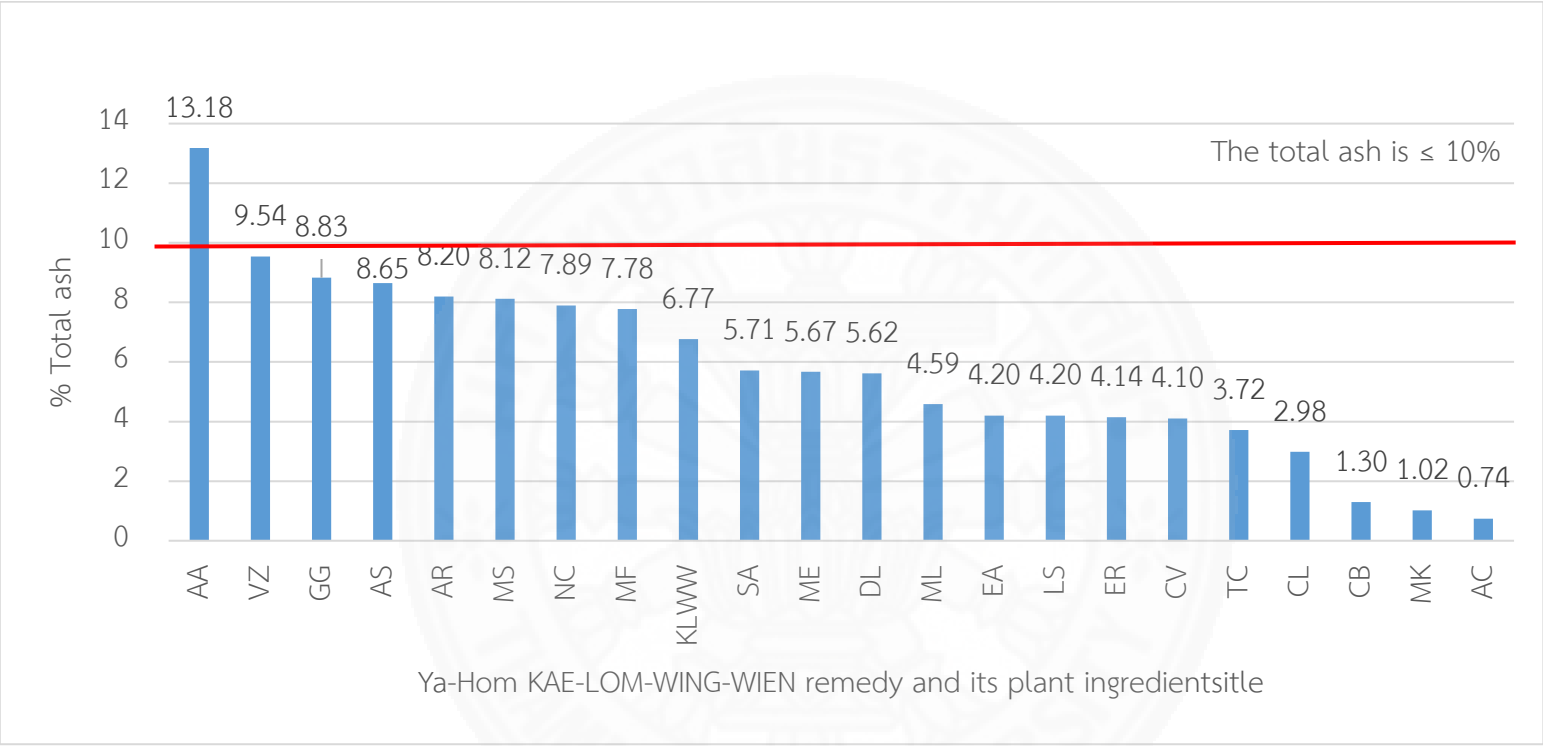

Figure 4-4 The percentage total ash of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients

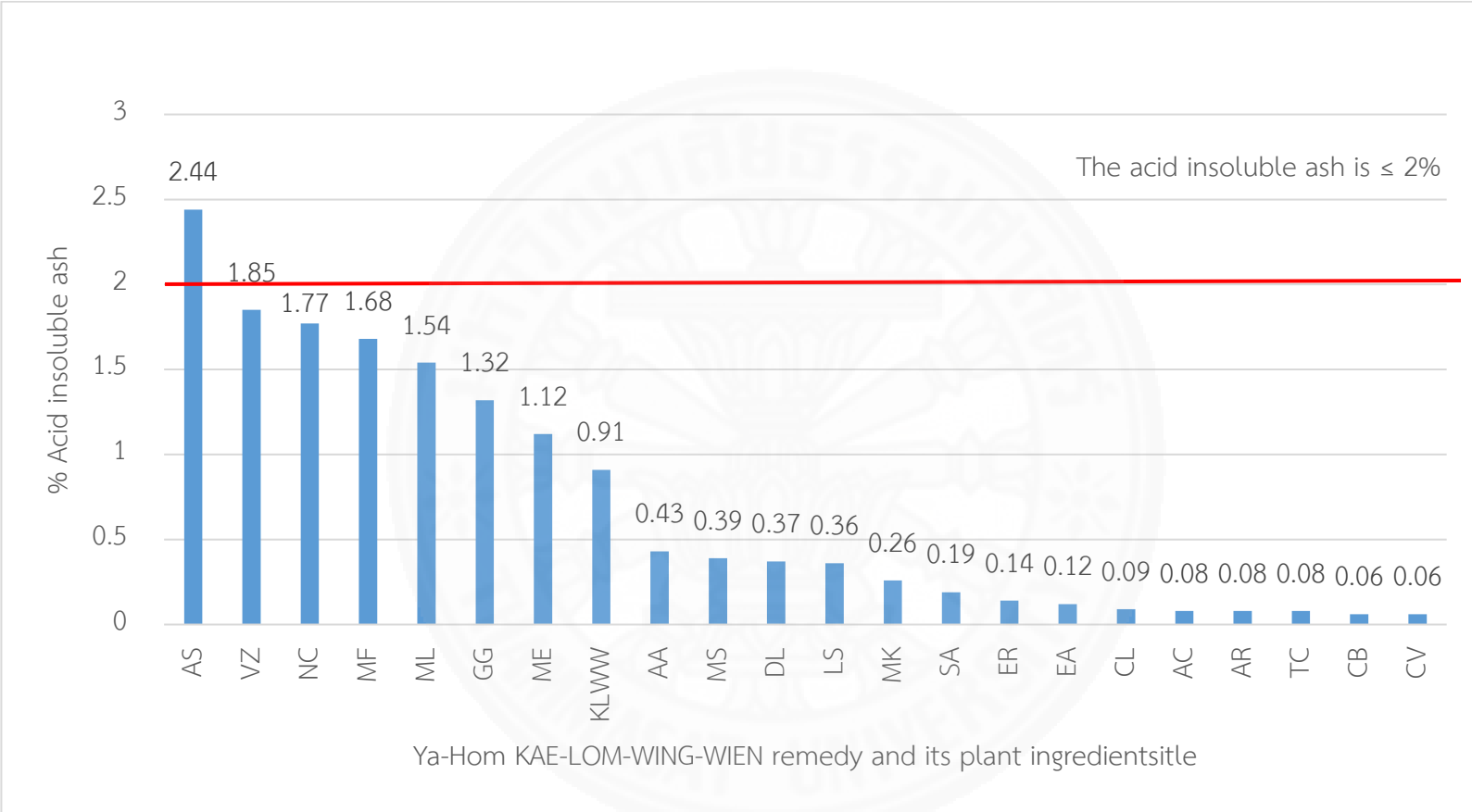

Figure 4-5 The percentage acid insoluble ash of Ya-Hom KAE-LOM-WING-WIEN remedy and its plant ingredients

4.3 Antioxidant activities

4.3.1 Nitroblue tetrazolium (NBT) dye reduction assay

The ethanolic, aqueous, and hydrolyzed extracts of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients were tested for antioxidant activity by scavenging PMA-stimulated superoxide production in HL-60 cells measured by NBT dye reduction assay. Cytotoxicity evaluation was performed using MTT assay as described in Chapter 3 **(section 3.2.3.1)**. The results are shown in **Table 4-3.**

Ya-Hom KAE-LOM-WING-WIEN remedy extracts consist of 70%, 95% ethanolic, aqueous, and hydrolyzed extracts which showed antioxidant activity with IC_{50} value more than 100 µg/ml, compared with propyl gallate (positive control) with IC_{50} value of 27.20±0.21 µg/ml.

The 95% ethanolic extracts of three plant ingredients showed antioxidant activity. The highest antioxidant activity was *M. siamensis* with IC_{50} value of 13.51±2.20 µg/ml. The second and third were *E. rosea* and *T. chebula* with IC_{50} values of 25.68±2.26 and 32.02±2.36 µg/ml, respectively. Finally, twenty-one extracts had no measurable antioxidant values (IC_{50} >100 µg/ml). There are seven plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy which had previously been reported. These reports found that the ethanolic extracts of *D. loureirin*, *M. ferrea*, *M. elengi*, *M. fragrans*, *M. siamensis, N. nucifera*, and *V. zizanioides* had also no measurable antioxidant values (Ouncharoen, Itharat, and Chaiyawatthananthn, 2017). However, in this study *M. siamensis* showed good antioxidant activity.

Table 4-3 Antioxidant activity by nitroblue tetrazolium (NBT) dye reduction assay and MTT assay of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (IC_{50} (µg/ml) ±SEM), (n=3)

Sample	Code	%Inhibition of superoxide production							IC_{50} (µg/ml)
		1 µg/ml	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	100 µg/ml	
A. annua	AAE95	-	-	-	-	-	23.07±7.36 (15.06±13.26)	43.17±11.86 (60.71±12.01)	>100
A. sinensis	ASE95	4.05±5.07 (54.62±4.22)	3.09±4.43 (-2.97±11.66)	-	-	-	18.57±0.28 (83.01±1.14)	74.82±6.90 (66.20±15.80)	>100
A. crassna	ACE95	3.23±10.65 (-3.38±3.75)	0.60±2.04 (0.29±0.84)	-	-	-	20.39±13.33 (70.31±15.23)	0.13±10.31 (78.50±6.47)	>100
A. reinwardtii	ARE95	-	-	-	-	-	-	12.83±3.73 (20.93±7.41)	>100
Borneo camphor	BSE95	0.97±0.82 (8.72±18.47)	11.82±15.88 (9.48±18.47)	-	-	-	29.68±0.13 (87.86±0.42)	-31.94±15.09 (87.45±0.72)	>100
C. bejolghota	CBE95	8.03±16.18 (40.62±2.87)	27.19±2.22 (18.95±7.23)	-	-	-	73.30±0.35 (35.82±3.34)	77.88±2.25 (11.36±2.18)	toxicity

Table 4-3 Antioxidant activity by nitroblue tetrazolium (NBT) dye reduction assay and MTT assay of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (IC_{50} (µg/ml) ±SEM), (n=3) (Continued)

Sample	Code	%Inhibition of superoxide production							IC_{50} (µg/ml)
		1 µg/ml	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	100 µg/ml	
C. loureirii	CLE95	-	-	-	-	-	-	-14.84±5.05 (9.68±10.30)	>100
C. verum	CVE95	-	-	-	-	-	-	-7.63±5.25 (-12.67±5.84)	>100
D. loureiri	DLE95	-	-	-	-	-	-	44.12±12.17 (13.52±7.53)	>100
E. antiquorum	EAE95	3.51±1.82 (-20.06±11.29)	13.00±14.08 (24.13±3.41)	-	46.04±0.58 (60.21±15.22)	52.46±2.92 (45.22±2.61)	79.80±15.22 (73.44±0.18)	93.39±3.76 (62.09±1.81)	>100
E. rosea	ERE95	-1.36±1.36 (3.21±1.97)	25.59±0.76 (-9.33±4.37)	-	-	-	54.06±0.42 (-17.26±1.93)	68.94±0.72 (-33.85±0.79)	25.68±2.26
G. glabra	GGE95	13.31±8.90 (79.45±1.06)	9.60±4.88 (8.55±0.32)	-	55.66±15.84 (49.54±10.53)	44.74±12.20 (62.04±12.19)	103.25±1.56 (5.92±6.65)	90.94±1.50 (64.3±2.52)	toxicity

Table 4-3 Antioxidant activity by nitroblue tetrazolium (NBT) dye reduction assay and MTT assay of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (IC_{50} (µg/ml) ±SEM), (n=3) (Continued)

Sample	Code	%Inhibition of superoxide production							IC_{50} (µg/ml)
		1 µg/ml	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	100 µg/ml	
L. sinense	LSE95	3.65±3.65 (73.23±0.71)	-17.02±12.70 (60.08±14.36)	-	-	-	-16.63±8.42 (77.26±3.83)	9.66±9.11 (74.95±11.13)	>100
M. ferrea	MLE95	-29.47±7.66 (69.86±12.40)	-5.01±12.79 (73.38±5.49)	-	-	-	37.87±1.37 (73.51±8.88)	85.27±15.08 (74.68±6.4)	>100
M. elengi	MEE95	-	-	-	-	-	-	-0.35±17.79 (22.88±2.34)	>100
*M. elengi**	MKE95	-	-	-	-	-	-	44.07±0.80 (2.49±7.19)	>100
M. fragrans	MFE95	0.90±0.62 (8.03±1.18)	-0.50±7.20 (16.88±6.15)	-	-	-	38.92±5.82 (-27.38±0.36)	70.52±7.79 (81.13±4.83)	>100
M. siamensis	MSE95	-8.22±10.33 (-44.04±12.13)	27.72±14.14 (-76.50±9.28)	81.56±7.71 (25.46±8.18)	94.04±2.69 (22.06±4.32)	-	-	-	13.51±2.20

Asterisk **(*)** indicates wood of *Mimusops elengi* Linn. known as Khon dok.

Table 4-3 Antioxidant activity by nitroblue tetrazolium (NBT) dye reduction assay and MTT assay of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (IC_{50} (µg/ml) ±SEM), (n=3) (Continued)

Sample	Code	%Inhibition of superoxide production							IC_{50} (µg/ml)
		1 µg/ml	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	100 µg/ml	
N. nucifera	NCE95	-5.86±7.40 (-8.55±11.22)	4.08±2.74 (-2.57±1.55)	-	-	-	17.24±13.34 (45.19±2.09)	89.43±1.39 (81.85±1.63)	>100
S. aromaticum	SAE95	-	-	-	-	-	-	42.58±8.82 (-7.67±12.24)	>100
Sodium borate	SBE95	-	-	-	-	-	-	17.31±0.48 (-31.72±6.17)	>100
Sodium borate(Satu)	SBEs 95	-	-	-	-	-	-	19.17±13.12 (5.58±5.43)	>100
T. chebula	TCE95	-4.78±13.99 (3.16±1.41)	36.27±0.58 (-27.12±14.58)	-	51.81±5.75 (4.49±6.18)	61.35±4.34 (46.76±4.18)	-		32.02±2.36
V. zizanioides	VZE95	-	-	-	-	-	-	29.58±5.87 (19.40±16.80)	>100

Table 4-3 Antioxidant activity by nitroblue tetrazolium (NBT) dye reduction assay and MTT assay of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (IC_{50} (µg/ml) ±SEM), (n=3) (Continued)

Sample	Code	%Inhibition of superoxide production							IC_{50} (µg/ml)
		1 µg/ml	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	100 µg/ml	
Ya-Hom KAE-LOM-WING-WIEN remedy	KLWW70	-	-	-	-	-	-	41.65±11.10 (-9.23±12.16)	>100
	KLWW95	-	-	-	-	-	28.86±1.23 (29.95±7.55)	82.71±9.08 (76.18±3.58)	>100
	KLWWA	-	-	-	-	-	-	40.06±16.04 (12.41±3.64)	>100
	KLWWH	-	-	-	-	-	-	47.93±4.37 (-1.3±2.22)	>100
Propyl gallate	PG	1.72±3.70 (-5.28±3.74)	25.06±0.41 (-6.38±4.49)	-	-	-	58.11±4.76 (1.79±5.25)	74.11±5.84 (-5.09±5.09)	27.20±0.21

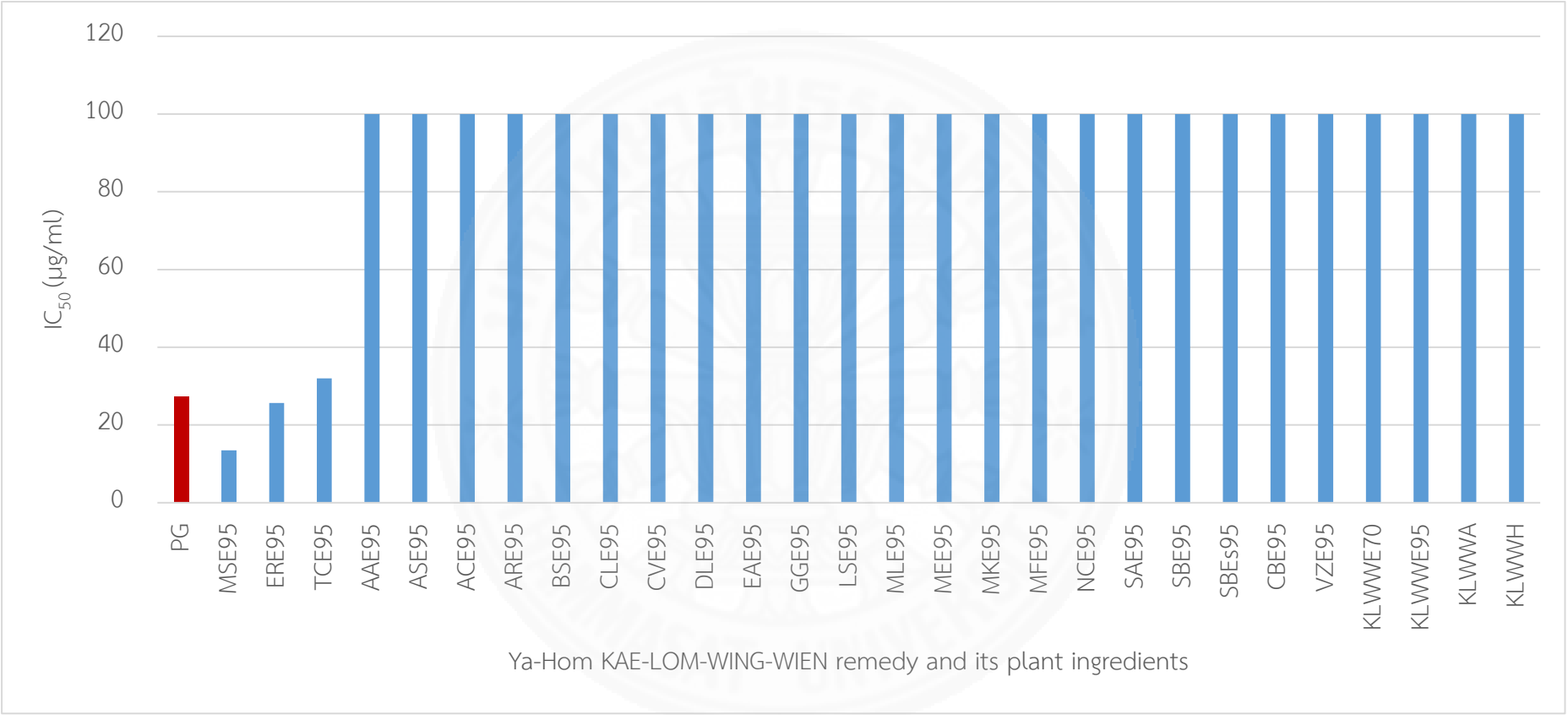

Figure 4-6 Antioxidant activity by nitroblue tetrazolium (NBT) dye reduction assay of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients extracts (IC_{50} (µg/ml) ±SEM), (n=3)

4.3.2 Thiobarbituric acid reactive substances (TBARS) assay

The MDA values were calculated from the standard graph of 1,1,3,3-tetramethoxypropane instead MDA. A line correlation between the absorbance at 532 nm and six concentrations of standard 1,1,3,3-tetramethoxypropane solution was observed in **Table 4-4** and **Figure 4-7**. The representative regression coefficient [R^2] was 0.999 and the linear regression equation was y=0.1025x+0.0459. Results showed that neurotoxic agents (Fe^{2+}) increase TBARS levels in porcine brain homogenate compared to the normal. The meaning of the MDA value was defined as the concentration of antioxidant preventing Fe^{2+} induced oxidative stress though the decreasing in TBARS levels.

Ya-Hom KAE-LOM-WING-WIEN remedy extracts consist of 70%, 95% ethanolic, aqueous, and hydrolyzed extracts which showed MDA values ranging between 7.72±0.91 and 16.10±1.73 nM. The hydrolyzed extract showed the highest antioxidant activity with MDA value of 7.72±0.91 nM, compared with propyl gallate (positive control) with MDA value of 10.42±0.66 nM.

The 95% ethanolic extracts of ten ingredients namely *A. sinensis*, *A. crassna*, borneo camphor, *M. elengi*, *M. elengi** (wood of *Mimusops elengi* Linn. known as Khon dok), *M. siamensis, S. aromaticum,* sodium borate, sodium borate (Satu), and *V. zizanioides* showed antioxidant activity. The highest antioxidant activity was *M. elengi* with MDA value of (-1.64)±3.22 nM. High antioxidant activity was *A. crassna* with MDA value of 4.41± 2.91 nM. Moderate antioxidant activity was *M. elengi**, *V. zizanioides*, *M. siamensis*, and sodium borate (Satu) with MDA values of 5.04±2.93, 5.06±1.96, 6.42±1.76, and 6.97±3.13 nM, respectively. Weak antioxidant activity was *S. aromaticum*, borneo camphor, sodium borate, and *A. sinensis* with MDA values of 7.09±2.44, 7.38±1.94, 8.72±0.55, and 9.06± 1.85 nM, respectively. However, these ingredients were higher antioxidant than propyl gallate. The previous study reported that there are two plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy namely *G. glabra* and *M. fragrans* which were tested by TBARS assay. These reports found that the methanolic/water (80:20, v/v) extract of *G. glabra* showed EC_{50} of 0.24±0.01 mg/ml (Martins *et a*l., 2015) and the concentration of 5% methanolic extract of *M. fragrans* was able to reduce range of TBARS value by 30-40% compared to untreated (Zakaria, Abas, and Rukayadi, 2015).

Table 4-4 A calibration curve of 1,1,3,3-tetramethoxypropane standards at 532 nm for determination of mean malondialdehyde (MDA) values (n=3)

Concentrations of 1,1,3,3-tetramethoxypropane(nM)	Absorbance at 532 nm			
	n1	n2	n3	Mean±SEM
4(4.42)	0.454	0.464	0.447	0.455±0.005
2(2.21)	0.253	0.261	0.244	0.253±0.005
1(1.10)	0.152	0.156	0.150	0.152±0.002
0.6(0.55)	0.099	0.099	0.099	0.099±0.0002
0.3(0.28)	0.074	0.074	0.077	0.075±0.001
0.1(0.14)	0.063	0.060	0.060	0.061±0.001

A linear correlation between a series of 1,1,3,3-tetramethoxypropane concentrations (nM) used as a standard and the absorbance at 532 nm (n=3)

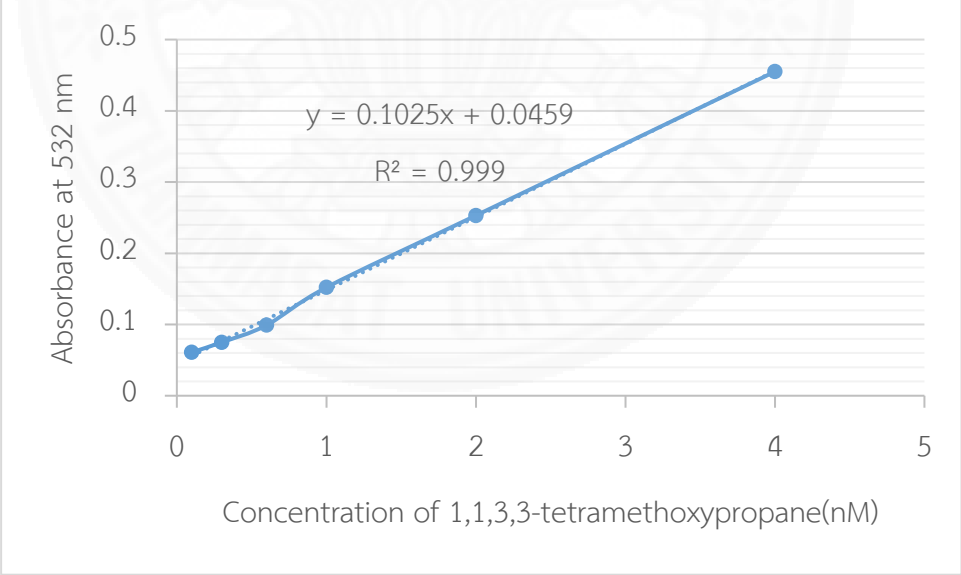

Figure 4-7 Calibration curve between the absorbance at 532 nm by TBARS assay and six concentrations of 1,1,3,3-tetramethoxypropane standard solution

Table 4-5 Antioxidant activity of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients expressed as the MDA values±SEM measuring by TBARS assay (n=3)

Sample	Code	MDA values(nM)±SEM				
		5 µg/ml	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml
A. annua	AAE95	12.13±3.37	13.36±3.62	11.68±3.13	12.47±2.98	14.77±1.77
A. sinensis	ASE95	10.84±2.57	8.32±1.13	9.25±1.36	11.76±4.21	9.06±1.85
A. crassna	ACE95	6.46±4.02	6.40±3.75	7.01±3.99	6.32±2.77	4.41±2.91
A. reinwardtii	ARE95	8.43±2.11	8.00±2.28	9.06±3.09	10.04±2.05	10.77±1.12
Borneo camphor	BSE95	12.36±2.27	13.44±2.56	12.11±2.30	10.29±2.26	7.38±1.94
C. bejolghota	CBE95	-	-	20.10±1.81	32.64±2.55	60.52±6.34
C. loureirii	CLE95	13.88±1.64	12.29±1.48	12.69±0.79	17.24±1.61	37.35±1.23
C. verum	CVE95	10.49±1.96	8.23±2.37	9.49±2.11	13.66±2.21	31.08±1.95
D. loureiri	DLE95	8.99±0.81	8.54±0.73	10.36±1.60	11.86±2.02	25.95±0.51
E. antiquorum	EAE95	-	-	14.29±4.82	8.11±3.39	20.72±1.61
E. rosea	ERE95	15.10±2.60	13.60±2.01	17.62±2.10	26.85±2.07	68.82±3.89
G. glabra	GGE95	6.43±2.18	5.72±2.00	5.75±1.34	8.76±0.83	26.49±1.01

Table 4-5 Antioxidant activity of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients expressed as the MDA values±SEM measuring by TBARS assay (n=3) (Continued)

Sample	Code	MDA values(nM)±SEM				
		5 µg/ml	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml
L. sinense	LSE95	14.43±3.07	11.17±1.87	10.15±1.22	10.96±3.22	12.15±1.84
M. ferrea	MLE95	16.19±2.08	16.19±0.47	11.77±1.04	11.27±1.01	14.49±0.13
M. elengi	MEE95	8.13±4.21	7.77±3.07	5.90±2.38	3.85±3.36	(-1.64)±3.22
*M. elengi**	MKE95	5.38±2.68	5.41±2.02	6.13±3.21	7.31±3.46	5.04±2.93
M. fragrans	MFE95	12.68±1.95	11.09±2.15	9.67±0.47	9.69±1.05	16.93±0.85
M. siamensis	MSE95	7.83±1.01	5.87±1.26	7.38±1.25	4.12±1.19	6.42±1.76
N. nucifera	NCE95	12.04±0.70	11.75±0.96	14.23±2.33	14.65±0.32	13.72±1.09
S. aromaticum	SAE95	7.28±0.81	8.85±1.05	4.53±2.74	2.55±1.95	7.09±2.44
Sodium borate	SBE95	18.54±1.13	19.63±3.03	18.13±1.82	16.55±2.39	8.72±0.55
Sodium borate(Satu)	SBEs95	9.76±3.18	10.08±3.44	9.59±3.06	8.67±3.18	6.97±3.13
T. chebula	TCE95	12.67±3.65	13.60±3.58	17.60±1.67	22.88±2.22	25.89±1.48
V. zizanioides	VZE95	10.95±1.26	10.36±0.70	7.99±1.31	8.02±1.41	5.06±1.96

Asterisk (*) indicates wood of Mimusops elengi Linn. known as Khon dok.

Table 4-5 Antioxidant activity of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients expressed as the MDA values±SEM measuring by TBARS assay (n=3) (Continued)

Sample	Code	MDA values(nM)±SEM					
		6.5 µM	5 µg/ml	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml
Ya-Hom KAE-LOM-WING-WIEN remedy	KLWW70	-	3.68±1.76	3.03±1.71	2.79±1.92	4.79±2.43	14.18±5.21
	KLWW95	-	4.96±2.78	3.12±2.87	3.82±3.15	5.86±2.84	15.97±5.29
	KLWWA	-	20.13±3.90	14.74±3.87	5.84±0.78	6.14±1.08	16.10±1.73
	KLWWH	-	9.43±2.30	6.53±1.40	5.78±1.53	5.01±1.69	7.72±0.91
Propyl gallate	PG	10.42±0.66	-	-	-	-	-

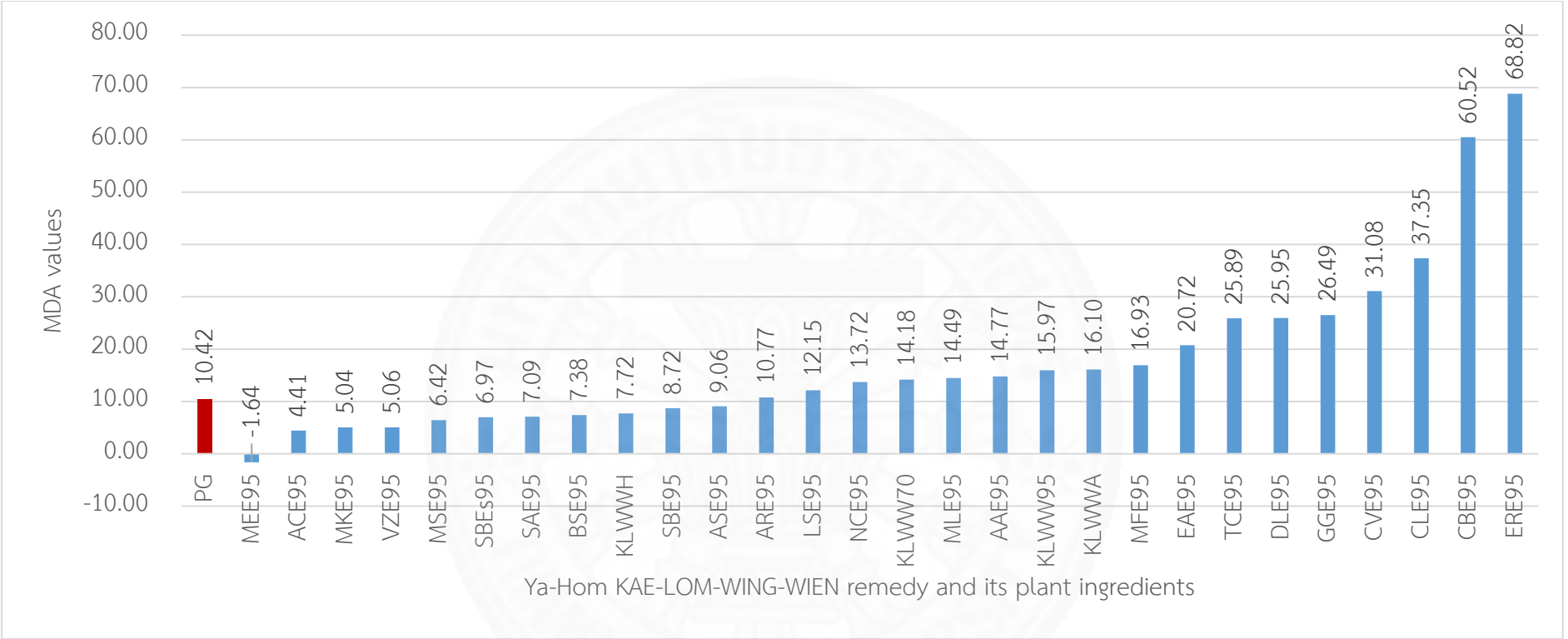

Figure 4-8 Antioxidant activity of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients extracts (at 500 µg/ml) using TBARS assay (n=3)

4.4 Anti-inflammatory activity

4.4.1 Nitric oxide (NO) inhibitory assay

The ethanolic, aqueous and hydrolyzed extracts of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients were tested for anti-inflammatory activity by inhibition of nitric oxide (NO) production in RAW 264.7 cell lines. Nitric oxide (NO) production was determined by measuring the Griess reagent and cytotoxicity was tested by MTT assay. The results are shown in **Table 4-6.**

Ya-Hom KAE-LOM-WING-WIEN remedy extracts consist of 70%, 95% ethanolic, aqueous, and hydrolyzed extracts which showed that the 95% ethanolic extract had the strongest anti-inflammatory activity with IC_{50} value of 19.53±1.95μg/ml, compared with prednisolone (positive control) with IC_{50} value of 0.16±0.01 μg/ml. The second was the hydrolyzed extract with IC_{50} value of 30.96±1.08μg/ml. Finally, the 70% ethanolic and aqueous extracts had IC_{50} values more than 100 μg/ml.

The 95% ethanolic extracts of ten plant ingredients namely *A. annua, A. sinensis, A. crassna, C. loureirii, D. loureirin, E. antiquorum, G. glabra, L. sinense, M. fragrans,* and *M. siamensis* showed anti-inflammatory activity. *A. sinensis* showed the highest anti-inflammatory activity with IC_{50} value of 14.70±1.64. High anti-inflammatory activity was *M. siamensis* with IC_{50} value of 17.13±0.84 μg/ml. Moderate anti-inflammatory activity was *A. crassna*, *E. antiquorum*, and *M. fragrans* with IC_{50} values of 21.08±1.20, 23.11±2.80, and 27.76±0.25 μg/ml, respectively. Weak anti-inflammatory activity was *D. loureirin*, *G. glabra*, *C. loureirii*, and *A. annua* with IC_{50} values of 31.32±2.68, 34.88±2.28, 39.68±1.55, and 43.38±1.14 μg/ml, respectively. Finally, thirteen extracts had no measurable anti-inflammatory activity (IC_{50} >100 μg/ml).

The 95% ethanolic extracts of ten plant ingredients in Ya-Hom KAE-LOM-WING-WIEN remedy have ever been reported in the previous study on the inhibition of nitric oxide production consisting of *A. sinensis*, *A. annua*, and *L. sinense* with the high anti-inflammatory activity. Moderate anti-inflammatory activity was *M. fragrans* and *M. ferrea* and weak anti-inflammatory activity was *D. loureirin*, *M. siamensis*, *M. elengi*, and *S. aromaticum*. *N. nucifera* was inactive (Makchuchit, Itharat, and Tewtrakul 2010). But in this study found that *A. annua* showed less anti-

inflammatory activity and *M. siamensis* showed higher than the previous study. Finally, *M. ferrea, M. elengi*, and *S. aromaticum* had no measurable anti-inflammatory activity. In addition, ethyl acetate extract of *A. crassna* significantly reduced the TNF-α level (Kumphune *et al*., 2011). The methanolic extract of *C. loureirii* had inhibition of iNOS and COX-2 (Hong, 2002). The sequential extraction of E-cinnamaldehyde and O-methoxycinnamaldehyde isolated from *C. verum* inhibited NO production (Gunawardena *et al*., 2015). Aqueous and alcoholic extracts of *E. antiquorum* produced significant inhibition of carrageenan induced rat paw edema (Kumar and Saikia, 2016). The ethanolic extract of *G. glabra* showed inhibition of NO production (Visavadiya, Soni, and Dalwad, 2009) and Ligustilides isolated from *L. sinense* showed anti-inflammatory activity by NO assay (Huang *et al*., 2013). *M. elengi* L. wood namely Khon dok showed significantly inhibitory the carrageenan- induced paw edema (Gupta, 2013). Essential oil from *V. zizanioides* showed reducing NO production (Chou *et al*., 2012).

Table 4-6 Anti-inflammatory activity by nitric oxide (NO) inhibitory assay and MTT assay of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (IC_{50} (µg/ml) ±SEM), (n=3)

Sample	Code	%Inhibition of nitric oxide production							IC_{50} (µg/ml)
		0.1 µg/ml	1 µg/ml	10 µg/ml	20 µg/ml	30 µg/ml	50 µg/ml	100 µg/ml	
A. annua	AAE95	-	-15.31±8.58 (-6.01±9.48)	-6.02±7.38 (5.38±6.48)	-	31.52±*1.58* (-0.81±3.45)	61.16±3.92 (6.25±1.96)	94.19±3.13 (4.88±3.43)	43.38±1.14
A. sinensis	ASE95	-	-12.91±4.00 (-6.94±6.36)	35.37±4.97 (16.26±7.47)	-	87.60±4.28 (7.49±6.25)	97.26±1.74 (-2.47±3.32)	100.11±0.58 (15.26±16.99)	14.70±1.64
A. crassna	ACE95	-	-2.92±2.20 (0.91±3.05)	27.14±3.05 (7.11±1.15)	-	59.43±0.36 (8.94±2.83)	73.75±0.52 (10.19±6.44)	89.61±1.26 (2.58±4.56)	21.08±1.20
A. reinwardtii	ARE95	-	-	-	-	-	-	48.93±9.05 (0.93±9.55)	>100
Borneo camphor	BSE95	-	-	-	-	-	-	27.98±8.17 (5.92±5.34)	>100
C. bejolghota	CBE95	-	-	-	-	-	-	10.50±-10.26 (0.12±10.38)	>100

Table 4-6 Anti-inflammatory activity by nitric oxide (NO) inhibitory assay and MTT assay of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (IC_{50} (µg/ml) ±SEM), (n=3) (Continued)

Sample	Code	%Inhibition of nitric oxide production							IC_{50} (µg/ml)
		0.1 µg/ml	1 µg/ml	10 µg/ml	20 µg/ml	30 µg/ml	50 µg/ml	100 µg/ml	
C. loureirii	CLE95	-	-15.89±1.67 (-12.94±5.42)	-4.56±0.71 (-10.79±8.14)	-	33.90±2.58 (-25.42±3.54)	64.51±1.89 (-22.43±8.34)	92.11±0.65 (-25.43±9.28)	39.68±1.55
C. verum	CVE95	-	-	-	-	-	12.96±3.62 (-15.56±7.60)	63.44±8.41 (-24.73±2.89)	>100
D. loureiri	DLE95	-	-9.53±0.29 (-0.20±13.90)	9.04±2.37 (6.94±8.19)	-	-	77.33±4.08 (16.71±13.90)	96.19±1.42 (31.37±11.43)	31.32±2.68
E. antiquorum	EAE95	-44.16±14.83 (-30.78±10.55)	-32.69±8.33 (-26.98±11.56)	-0.09±12.11 (-3.48±7.68)	46.90±10.02 (0.49±2.62)	68.44±8.63 (11.06±3.31)	-	-	23.11±2.80
E. rosea	ERE95	-	-	-	-	-	-	9.47±1.47 (0.34±14.54)	>100
G. glabra	GGE95	-	-1.62±8.10 (-19.80±9.01)	4.74±2.09 (-14.31±7.47)	-	41.97±3.65 (-36.24±8.28)	69.47±4.03 (-28.77±7.85)	-	34.88±2.28

Table 4-6 Anti-inflammatory activity by nitric oxide (NO) inhibitory assay and MTT assay of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (IC_{50} (µg/ml) ±SEM), (n=3) (Continued)

Sample	Code	%Inhibition of nitric oxide production							IC_{50} (µg/ml)
		0.1 µg/ml	1 µg/ml	10 µg/ml	20 µg/ml	30 µg/ml	50 µg/ml	100 µg/ml	
L. sinense	LSE95	-	-24.02±5.63 (-14.70±13.84)	9.81±3.96 (-11.22±12.01)	-	64.52±1.84 (4.43±3.31)	90.07±1.23 (-0.78±8.129)	98.69±0.39 (-16.67±4.25)	22.87±1.15
M. ferrea	MLE95	-	-	-	-	-	42.48±4.22 (50.68±5.20)	93.26±1.36 (83.94±1.15)	>100
M. elengi	MEE95	-	-	-	-	-	-	26.98±7.25 (4.35±7.43)	>100
*M. elengi**	MKE95	-	-	-	-	-	14.88±6.36 (23.26±12.94)	79.34±0.50 (52.6±3.23)	>100
M. fragrans	MFE95	-	-23.88±7.94 (7.79±0.37)	-7.69±7.24 (8.52±5.41)	26.72±4.37 (-1.61±10.42)	55.72±0.64 (-1.32±1.26)	-	-	27.76±0.25
M. siamensis	MSE95	-	7.757±2.63 (6.41±5.90)	35.22±2.12 (22.47±4.42)		64.46±1.81 (7.36±1.21)	81.30±6.29 (26.94±10.08)	-	17.13±0.84

Asterisk **(*)** indicates wood of *Mimusops elengi* Linn. known as Khon dok.

Table 4-6 Anti-inflammatory activity by nitric oxide (NO) inhibitory assay and MTT assay of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (IC_{50} (µg/ml) ±SEM), (n=3) (Continued)

Sample	Code	%Inhibition of nitric oxide production							IC_{50} (µg/ml)
		0.1 µg/ml	1 µg/ml	10 µg/ml	20 µg/ml	30 µg/ml	50 µg/ml	100 µg/ml	
N. nucifera	NCE95	-	-	-	-	-	-	48.34±2.19 (-36.41±3.41)	>100
S. aromaticum	SAE95	-	-	-	-	-	-	60.10±5.21 (3.96±10.08)	>100
Sodium borate	SBE95	-	-	-	-	-	-	44.14±1.92 (-37.58±3.59)	>100
Sodium borate(Satu)	SBEs95	-	-	-	-	-	-	45.18±2.23 (-17.62±1.06)	>100
T. chebula	TCE95	-	-	-	-	-	-	3.29±-21.82 (-9.26±12.56)	>100
V. zizanioides	VZE95	-	-	-	-	-	-	33.77±5.93 (18.01±7.59)	>100

Table 4-6 Anti-inflammatory activity by nitric oxide (NO) inhibitory assay and MTT assay of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (IC_{50} (µg/ml) ±SEM), (n=3) (Continued)

Sample	Code	%Inhibition of nitric oxide production								IC_{50} (µg/ml)
		0.01 µg/ml	0.1 µg/ml	1 µg/ml	10 µg/ml	20 µg/ml	30 µg/ml	50 µg/ml	100 µg/ml	
Ya-Hom KAE-LOM-WING-WIEN remedy	KLWW70	-	-	-	-	-	-	-	33.07±5.89 (-33.73±0.11)	>100
	KLWW95	-	-	-12.22±2.33 (-7.27±2.42)	25.62±4.31 (-21.37±9.82)	-	63.10±0.40 (0.52±8.07)	79.70±4.32 (-17.96±1.61)	97.20±1.16 (3.33±4.04)	19.53±1.95
	KLWWA	-	-	-	-	-	-	-	65.03±9.19 (26.46±6.17)	>100
	KLWWH	-	-	-26.61±5.41 **(-8.89±4.54)**	-3.00±3.18 **(-5.76±1.80)**	-	48.04±2.36 **(-6.57±4.29)**	79.65±0.81 **(4.02±2.52)**	97.37±0.03 **(-17.47±2.72)**	30.96±1.08
Prednisolone	Pred	-18.00±0.89 (-0.10±10.98)	26.08±2.31 (14.39±8.68)	50.23±0.59 (14.95±7.20)	88.34±1.69 (8.86±5.46)	-	-	95.19±0.88 (16.21±8.29)	-	0.16±0.01

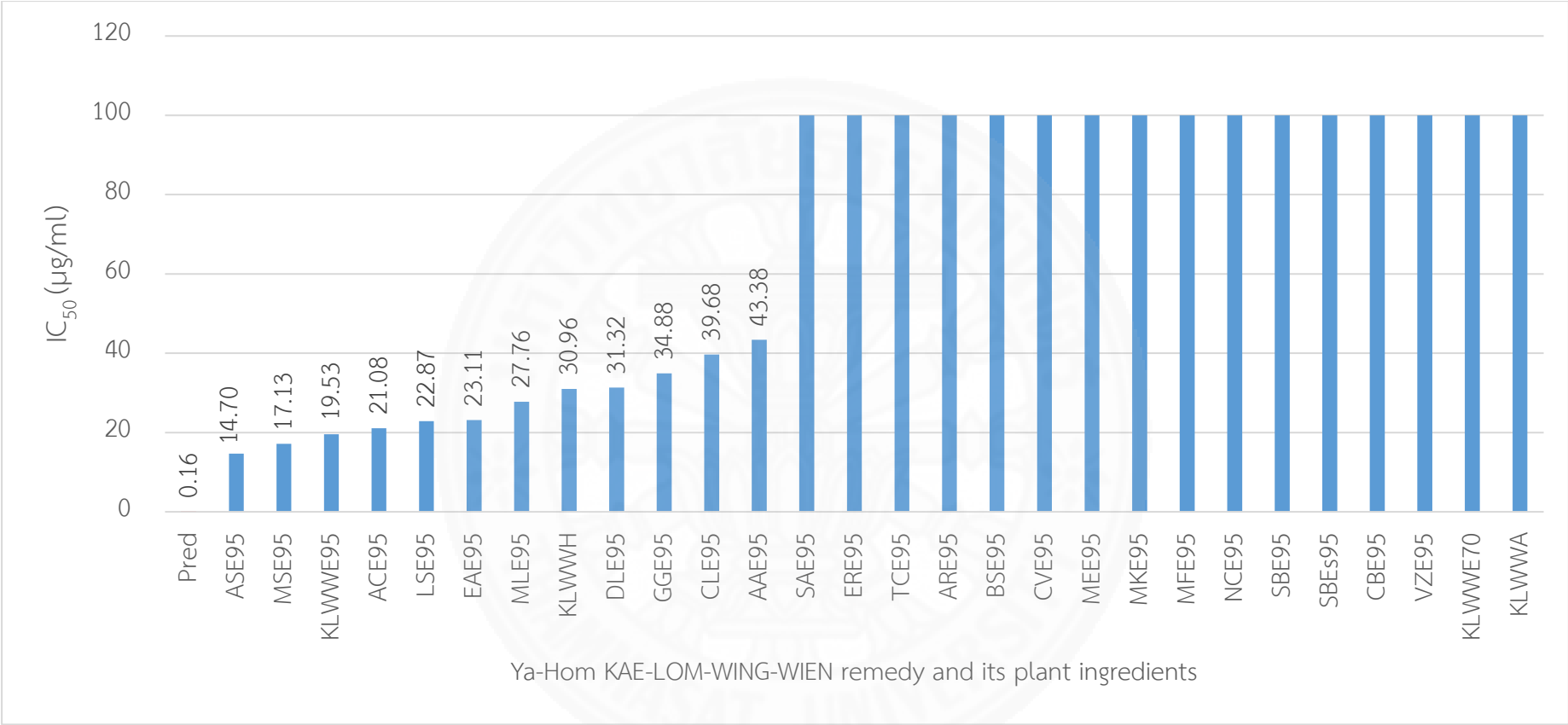

Figure 4-9 Anti-inflammatory activity by nitric oxide (NO) inhibitory assay and MTT assay of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients extracts (IC_{50} (µg/ml) ±SEM), (n=3)

4.5 The stability test of Ya-Hom KAE-LOM-WING-WIEN extract

4.5.1 The stability test of Ya-Hom KAE-LOM-WING-WIEN extract for anti-inflammatory activity by NO inhibitory assay

The stability test was the modified method from Grimm (1998), carried out in triplicate. The 95% ethanolic extract of Ya-Hom KAE-LOM-WING-WIEN remedy (KLWW) was stored over a six-month period, under 40 ± 2 ℃ with 75 ± 5% RH as accelerate conditions. Samples were taken at days 0 (control sample), 15, 30, 60, 90, 120, 150 and 180, which were evaluated the anti-inflammatory activity by NO inhibitory assay. The results showed that the 95% ethanolic extract of KLWW on days 0, 15, 30, 60, 90, 120, 150 and 180 inhibited nitric oxide (NO) production in RAW 264.7 cell lines with IC_{50} values of 19.38±1.13, 28.43±078, 29.06±1.71, 34.02±0.40, 33.82±0.44, 34.66±2.32, 34.13±1.68, and 32.04±2.32 µg/ml, respectively. All results of inhibition activity are shown in **Table 4-7**. The 95% ethanolic extract of KLWW showed statistically significant difference from control and crude extract on other days. Consequently, they are stable for at least two months.

Table 4-7 Stability of the 95% ethanolic extract of Ya-Hom KAE-LOM-WING-WIEN remedy under accelerated conditions at 40°C, 75% RH for 6 months by anti-inflammatory activity through inhibition of nitric oxide (NO) production in RAW 264.7 cell lines (IC_{50} µg/ml ± SEM), (n=3)

Storage time of extract	Stability of 95% ethanolic extract of Ya-Hom remedy by inhibition nitric oxide production IC_{50} ± SEM (µg/ml)
Day 0	19.38 ± 1.13
Day 15	28.43 ± 0.78
Day 30	29.06 ± 1.71
Day 60	34.02 ± 0.40
Day 90	33.82 ± 0.44
Day 120	34.66 ± 2.32
Day 150	34.13 ± 1.68
Day 180	32.04 ± 2.32

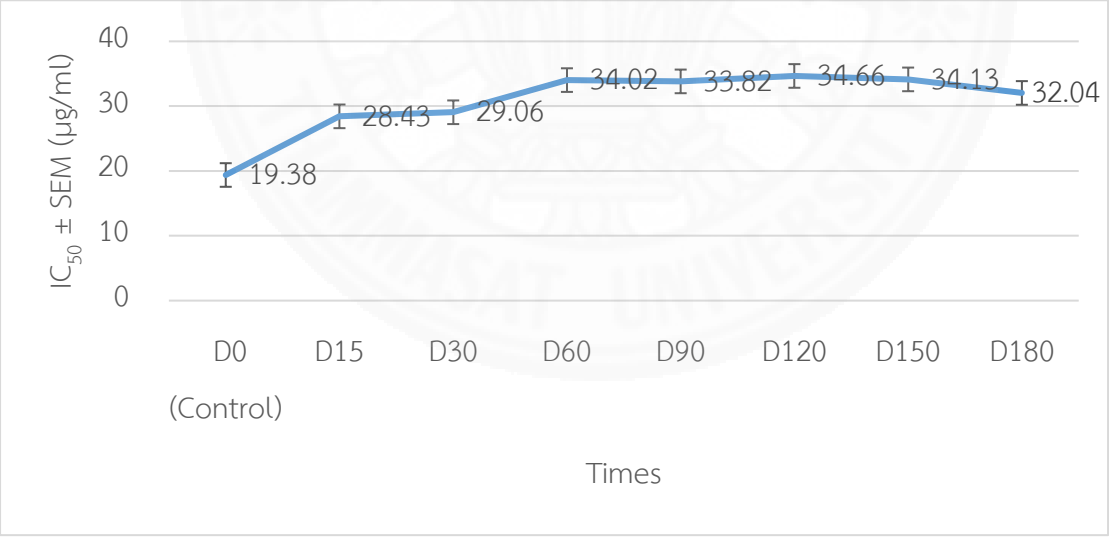

Figure 4-10 Anti-inflammatory activity through inhibition of nitric oxide (NO) production in RAW 264.7 cell lines of the 95% ethanolic extract of Ya-Hom KAE-LOM-WING-WIEN remedy in stability test (mean ± SEM), (n=3)

4.6 Phytochemical screening of the extracts of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS)

The 70%, 95% ethanolic, aqueous, and hydrolyzed extracts of Ya-Hom KAE-LOM-WING-WIEN remedy were analyzed by using gas chromatography-mass spectrometry (GC-MS). The 70% and 95% ethanolic extracts analysis results found that there are thirty-four components. The highest contents of the 70% and 95% ethanolic extract components were eugenol (34.89%) and borneol (33.31%), respectively. The aqueous extract analysis results found that there are twenty-eight components. The highest content of the aqueous extract components was eugenol (28.80%). The hydrolyzed extract analysis results found thirty-nine components. The highest content of the hydrolyzed extract components was eugenol (42.02%). All results are shown in **Table 4-8, 4-9, 4-10,** and **4-11.**

Table 4-8 Analysis results of the 70% ethanolic extract of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS)

RT	Text name	% Area
14.88	Endo-Borneol	13.03
15.19	Borneol	23.90
17.49	Benzaldehyde, ethyl	0.05
17.83	1-Methoxy-2-indanol	0.13
18.05	Cinnamic aldehyde	1.77
19.14	Phenol, 4-ethenyl-2-methoxy-	0.06
20.31	Eugenol	34.89
22.09	beta-caryophyllene	3.70
22.42	Coumarin	0.30
22.99	alpha-Caryophyllene	0.49
23.21	Cinnamic acid	0.05
23.46	Isoledene	0.13

Table 4-8 Analysis results of the 70% ethanolic extract of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS) (Continued)

RT	Text name	% Area
23.58	alpha-Curcumene	0.17
23.83	beta-Selinene	0.12
23.91	alpha-Amorphene	0.03
24.05	alpha-Muurolene	0.25
24.14	Unknown 1	0.14
24.39	Aromadendrene	0.12
24.51	delta-Cadinene	0.39
24.59	Calamenene	0.14
24.71	Unknown 2	0.18
28.01	BUTYLIDENE PHTHALIDE	0.38
28.14	(+) spathulenol	1.00
28.41	beta-Himachalene	1.17
28.99	Allyl phenoxyacetate	1.75
29.19	Curcumene	1.54
29.32	3 N BUTYL PHTHALIDE	3.08
30.01	Ledenoxid	0.40
33.30	Methyl palmitate	0.17
33.39	Unknown 3	0.46
33.80	Unknown 4	0.38
34.00	Palmitic acid	0.46
34.62	Stearic acid	1.15
39.08	2-Oxomanoyl oxide	8.05

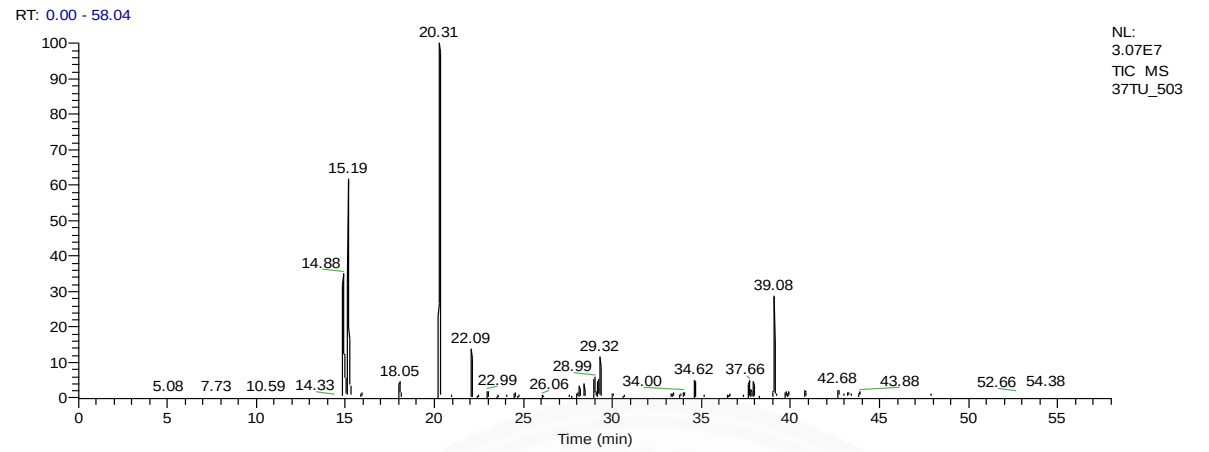

Figure 4-11 Chromatogram results of the 70% ethanolic extract of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS)

Table 4-9 Analysis results of the 95% ethanolic extract of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS)

RT	Text name	% Area
14.91	Endo-Borneol	18.79
15.24	Borneol	33.31
17.49	Benzaldehyde, ethyl	0.04
17.83	1-Methoxy-2-indanol	0.02
17.91	Linalool	0.02
18.05	Cinnamic aldehyde	0.34
19.12	Phenol, 4-ethenyl-2-methoxy-	0.08
20.30	Eugenol	24.26
22.09	beta-caryophyllene	2.25
22.43	Coumarin	0.19
23.00	alpha-Caryophyllene	0.27
23.21	Cinnamic acid	0.05
23.46	Isoledene	0.10
23.58	alpha-Curcumene	0.11
23.83	beta-Selinene	0.09
23.91	alpha-Amorphene	0.02
24.04	alpha-Muurolene	0.19
24.14	Unknown 1	0.07
24.38	Aceteugenol	5.54
24.52	delta-Cadinene	0.28
24.60	Calamenene	0.09
24.72	Unknown 2	0.10
28.01	BUTYLIDENE PHTHALIDE	0.27

Table 4-9 Analysis results of the 95% ethanolic extract of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS) (Continued)

RT	Text name	% Area
28.41	beta-Himachalene	0.62
28.99	Allyl phenoxyacetate	1.22
29.20	Curcumene	0.87
29.31	3 N BUTYL PHTHALIDE	1.92
30.01	Ledenoxid	0.21
33.30	Methyl palmitate	0.17
33.39	Unknown 3	0.46
33.80	Unknown 4	0.38
34.00	Palmitic acid	0.46
34.62	Stearic acid	1.15
39.08	2-Oxomanoyl oxide	8.05

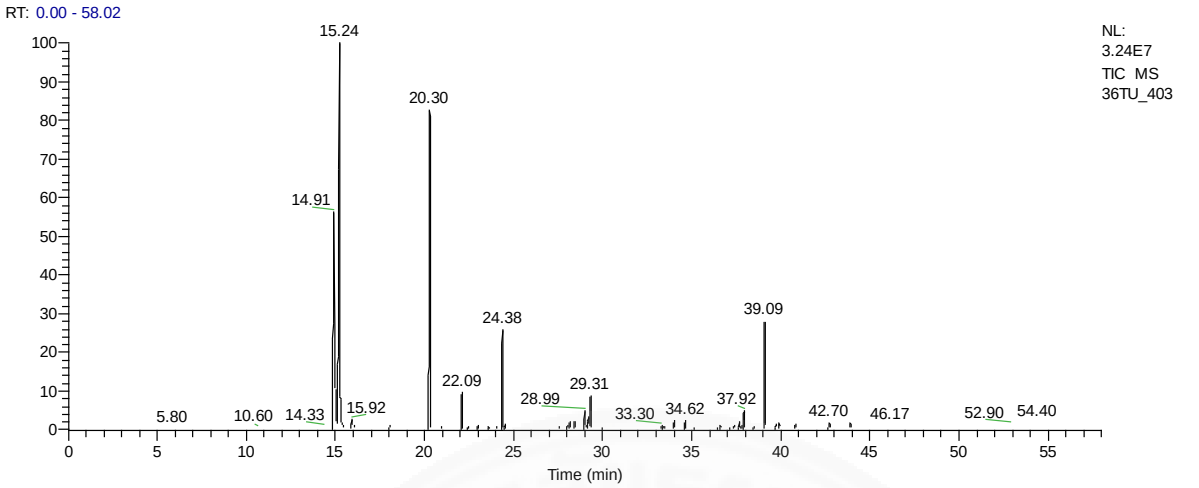

Figure 4-12 Chromatogram results of the 95% ethanolic extract of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS)

Table 4-10 Analysis results of the aqueous extract of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS)

RT	Text name	% Area
12.00	Serine methyl ester cyclic butaneboronate	1.64
14.18	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.13
14.84	Silver benzoate	1.01
15.12	Borneol	1.82
16.75	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	1.79
18.96	Unknown 1	4.53
19.13	Phenol, 4-ethenyl-2-methoxy-	3.06
19.99	1,4-Cyclohexadiene-1,2-dicarboxylic anhydride	0.91
20.24	Eugenol	28.80
22.42	Coumarin	5.87
23.29	Unknown 2	3.18
24.17	Phenol, 2,5-bis(1,1-dimethylethyl)-	1.60
24.35	Aceteugenol	2.31
24.59	Unknown 3	0.77
27.13	Cyclooctasiloxane, hexadecamethyl-	0.99
27.39	N2,O6-Dimethylguanine	0.84
28.85	Caryophyllene oxide	0.28
28.99	Allyl phenoxyacetate	1.58
29.15	Unknown 4	0.84
29.30	3 N BUTYL PHTHALIDE	1.56

Table 4-10 Analysis results of the aqueous extract of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS) (Continued)

RT	Text name	% Area
33.30	Palmitic acid	8.45
33.39	Unknown 5	2.35
36.46	Linoleic acid	2.54
36.58	Oleic acid	8.70
36.90	Tauromisin	1.51
39.07	2-Oxomanoyl oxide	1.97
41.60	Unknown 6	1.89
44.92	Unknown 7	3.07

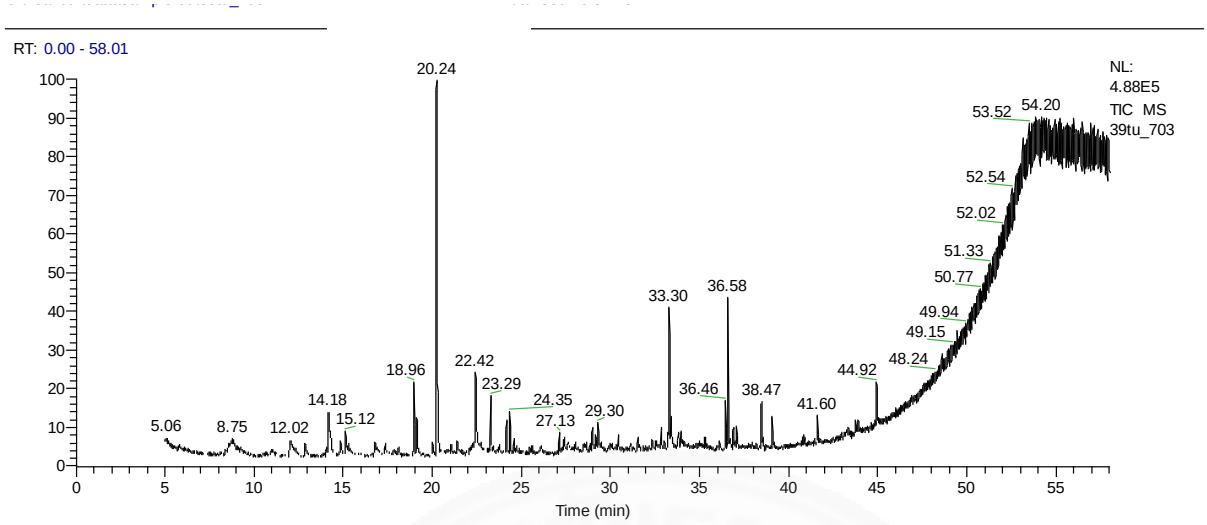

Figure 4-13 Chromatogram results of the aqueous extract of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS)

Table 4-11 Analysis results of the hydrolyzed extract of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS)

RT	Text name	% Area
12.31	4-Heptanol, 4-ethyl-2,6-dimethyl	0.04
13.48	4-Octadecenal	0.13
14.15	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.16
14.56	Dehydromevalonic lactone	0.38
14.85	Endo-Borneol	0.18
14.91	Silver benzoate	0.29
15.11	Borneol	0.92
17.48	Benzaldehyde, ethyl	0.26
17.94	Linalool	0.08
18.06	Cinnamic aldehyde	0.50
18.55	4-BROMO-2,3,3-TIMETHYLBUTEN-3-OIC ACID,METHYL ESTER	0.36
19.10	Phenol, 4-ethenyl-2-methoxy-	2.75
20.28	Eugenol	42.02
20.54	Unknown 1	3.34
22.46	Coumarin	11.96
23.42	6-OCTENOIC ACID, 3-ETHENYL-3,7-DIMETHYL-2-METHYLENE-, METHYL ESTER	0.30
23.58	Cuparene	0.27
24.35	Aceteugenol	3.79
24.70	Unknown 2	0.43
27.39	Unknown 3	0.47
27.58	Unknown 4	1.00
28.57	Deoxysericealactone	0.78
28.87	Caryophyllene oxide	0.95
29.00	Allyl phenoxyacetate	2.57

Table 4-11 Analysis results of the hydrolyzed extract of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS) (Continued)

RT	Text name	% Area
29.17	Unknown 5	0.82
29.30	3 N BUTYL PHTHALIDE	1.70
29.99	2(3H)-NAPHTHALENONE, 4,4A,5,6,7,8-HEXAHYDRO-1-METHOXY	1.55
31.55	longifolen	1.67
32.34	Diepi-alpha-Cedrenepoxide	1.74
33.24	Unknown 6	2.41
33.40	Unknown 7	3.81
33.81	Unknown 8	2.41
34.87	Linoleic acid	2.39
36.59	Oleic acid	0.50
36.91	Tauromisin	2.90
39.07	2-Oxomanoyl oxide	2.38
43.88	Unknown 9	1.81

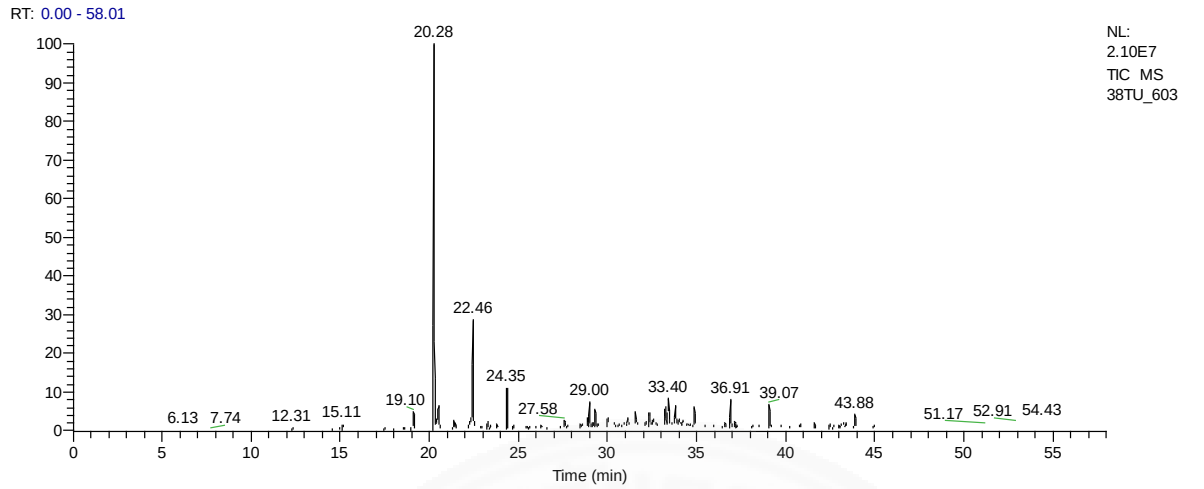

Figure 4-14 Chromatogram results of the hydrolyzed extract of Ya-Hom KAE-LOM-WING-WIEN remedy by using gas chromatography-mass spectrometry (GC-MS)

Table 4-12 Summary of biological activities of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients

Sample	Code	NBT dye reduction assay IC_{50} (µg/ml)	MDA values at 500 µg/ml (nM)±SEM	NO inhibitory assay IC_{50} (µg/ml)
A. annua	AAE95	>100	14.77±1.77	43.38±1.14
A. sinensis	ASE95	>100	9.06±1.85	14.70±1.64
A. crassna	ACE95	>100	4.41±2.91	21.08±1.20
A. reinwardtii	ARE95	>100	10.77±1.12	>100
Borneo camphor	BSE95	>100	7.38±1.94	>100
C. bejolghota	CBE95	toxicity	60.52±6.34	>100
C. loureirii	CLE95	>100	37.35±1.23	39.68±1.55
C. verum	CVE95	>100	31.08±1.95	>100
D. loureiri	DLE95	>100	25.95±0.51	31.32±2.68
E. antiquorum	EAE95	>100	20.72±1.61	23.11±2.80
E. rosea	ERE95	25.68±2.26	68.82±3.89	>100
G. glabra	GGE95	toxicity	26.49 ±1.01	34.88±2.28

Table 4-12 Summary of biological activities of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (Continued)

Sample	Code	NBT dye reduction assay IC_{50} (µg/ml)	MDA values at 500 µg/ml (nM)±SEM	NO inhibitory assay IC_{50} (µg/ml)
L. sinense	LSE95	>100	12.15±1.84	22.87±1.15
M. ferrea	MLE95	>100	14.49±0.13	>100
M. elengi	MEE95	>100	(-1.64)±3.22	>100
*M. elengi**	MKE95	>100	5.04±2.93	>100
M. fragrans	MFE95	>100	16.93±0.85	27.76±0.25
M. siamensis	MSE95	13.51±2.20	6.42±1.76	17.13±0.84
N. nucifera	NCE95	>100	13.72±1.09	>100
S. aromaticum	SAE95	>100	7.09±2.44	>100
Sodium borate	SBE95	>100	8.72±0.55	>100
Sodium borate(Satu)	SBEs95	>100	6.97±3.13	>100
T. chebula	TCE95	32.02±2.36	25.89±1.48	>100

Asterisk (*) indicates wood of *Mimusops elengi* Linn. known as Khon dok.

Table 4-12 Summary of biological activities of Ya-Hom KAE-LOM-WING-WIEN remedy and its ingredients (Continued)

Sample	Code	NBT dye reduction assay IC_{50} (µg/ml)	MDA values at 500 µg/ml (nM)±SEM	NO inhibitory assay IC_{50} (µg/ml)
V. zizanioides	VZE95	>100	5.06±1.96	>100
Ya-Hom KAE-LOM-WING-WIEN remedy	KLWW70	>100	14.18±5.21	>100
	KLWW95	>100	15.97±5.29	19.53±1.95
	KLWWA	>100	16.10±1.73	>100
	KLWWH	>100	7.72±0.91	30.96±1.08

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

Ya-Hom as a traditional herbal remedy had long been used as a cardiotonic agent and adjustment of the wind element for healthy circulation in Thai traditional medicine. Ya-Hom KAE-LOM-WING-WIEN remedy (KLWW) which is registered on List of Herbal Medicinal Products in Thai National List of Essential Medicines is used for treatment of dizziness, fatigue, and sleepiness. KLWW consists of twenty- three medicinal plants and has a cool, fragrant, and sweet taste. In the previous study there are antioxidant and anti-inflammatory activities of KLWW ingredients. Usually taking Ya-Hom may be able to prevent and slow down the progress of chronic disease. However, there are no research reports on KLWW to verify this. Therefore, the aims of this research were to study the antioxidant and anti- inflammatory activities of ethanolic, aqueous, and hydrolyzed extracts of KLWW and its ingredients.

Quality control of raw materials of KLWW was tested following Thai Herbal Pharmacopoeia (THP). All KLWW ingredients were within the standard value of THP, except *A. annua* and *A. sinensis*, which were total ash more than 10% and acid insoluble ash more than 2%. However, KLWW was within the standard value of THP consist of loss on drying, extractive value, total ash, and acid insoluble ash.

Extraction method of KLWW was extracted by maceration with 70% and 95% ethanol, decoction, and hydrolyzed extraction. KLWW ingredients were maceration with 95% ethanol. The percentage yields of KLWW were the 70% ethanolic extract (20.86%), aqueous extract (18.76%), 95% ethanolic extract (10.30%), and hydrolyzed extract (1.46%), respectively. The highest percentage yield of the 95% ethanolic extract of KLWW ingredients was *T. chebula* (35.44%).

Antioxidant activity of the ethanolic, aqueous, and hydrolyzed extracts of KLWW and the 95% ethanolic extracts of ingredients were tested by nitroblue tetrazolium (NBT) dye reduction assay. The results revealed that the ethanolic, aqueous, and hydrolyzed extracts of KLWW had no measurable antioxidant activity (IC_{50} > 100 µg/ml), compared with propyl gallate (positive control) with IC_{50} value of 27.20±0.21 µg/ml.

The highest antioxidant activity of KLWW ingredients was the 95% ethanolic extract of *M. siamensis*. However, *M. siamensis* extract was higher than propyl gallate. *E. rosea* and *T. Chebula* showed good antioxidant activity.

Antioxidant activity of the ethanolic, aqueous, and hydrolyzed extracts of KLWW and the 95% ethanolic extracts of ingredients were tested by thiobarbituric acid reactive substances (TBARS) assay. The results revealed that the hydrolyzed extract of KLWW showed the highest antioxidant activity, compared with propyl gallate (positive control) with MDA value of 10.42±0.66 nM. However, the hydrolyzed extract of KLWW was higher than propyl gallate, the ethanolic, and aqueous extracts of KLWW. the 95% ethanolic extracts of *A. sinensis*, *A. crassna*, borneo camphor, *M. elengi*, *M. elengi** (wood of *Mimusops elengi* Linn. known as Khon dok), *M. siamensis, S. aromaticum,* sodium borate, sodium borate (Satu), and *V. zizanioides* showed strong antioxidant activity. However, ten 95% ethanolic extracts of KLWW ingredients were higher than propyl gallate.

The previous study demonstrated that *G. glabra* and *M. fragrans* had antioxidant activity by inhibition of TBARS formation. The methanolic/water (80:20, v/v) extract of *G. glabra* showed EC_{50} of 0.24±0.01 mg/ml (Martins *et a*l., 2015). The concentration of 5% methanolic extract of *M. fragrans* was able to reduce range of by 30-40% (Zakaria, Abas, and Rukayadi, 2015). Although, the different methods may analyze the different results, *G. glabra* and *M. fragrans* had also antioxidant activity.

Anti-inflammatory activity of the ethanolic, aqueous, and hydrolyzed extracts of KLWW and the 95% ethanolic extracts of ingredients were tested by nitric oxide (NO) inhibitory assay. The results revealed that the 95% ethanolic extract of KLWW had the highest anti-inflammatory activity, compared with prednisolone (positive control) with IC_{50} value of 0.16±0.01 µg/ml. However, the 95% ethanolic extract of KLWW was higher than the hydrolyzed extract. The 70% ethanolic and aqueous extracts of KLWW had no measurable anti-inflammatory activity (IC_{50} > 100 µg/ml). High anti-inflammatory activity of KLWW ingredients was the 95% ethanolic extract of *A. sinensis and M. siamensis*. *A. crassna*, *L. sinense, E. antiquorum*, *M. fragrans*, *D. loureirin*, *G. glabra*, *C. loureirii*, and *A. annua* showed good anti-inflammatory activity.

Stability test of the 95% ethanolic extract of KLWW was stable in anti-inflammatory activity by NO inhibitory assay with IC_{50} value of 19.38±1.13 µg/ml for at least two months. Although, the results were reduction of activity, KLWW extract was still effective anti-inflammatory activity.

Phytochemical screening of the 70%, 95% ethanolic, aqueous, hydrolyzed extracts of KLWW were analyzed by using gas chromatography-mass spectrometry (GC-MS). The 70% and 95% ethanolic extracts were analyzed results found that there are thirty-four components. The highest contents of the 70% and 95% ethanolic extract components were eugenol and borneol, respectively. The aqueous extract was analyzed result found that there are twenty-eight components. The hydrolyzed extract was analyzed result found that thirty-nine components. The highest contents of both aqueous and hydrolyzed extract components were eugenol. The previous study demonstrated that eugenol is the main bioactive compound of *S. aromaticum*, which presented higher antioxidant activity than butylated hydroxyanisole, BHT, trolox, and α-tocopherol by DPPH, ABTS, N,N-dimethyl-p-phenylenediamine, CUPRAC and ferric reducing assay (Gulcin, 2011). Eugenol showed inhibited iron and ·OH (hydroxyl) radical initiated lipid peroxidation with IC_{50} values of 10 µM and 14 µM, respectively (Nagababu *et al.*, 2010) and showed anti-inflammatory activity *in vitro* nitric oxide scavenging activity with EC_{50} value of 14.17 µg/ml (Mahapatraa and Roy, 2014). Borneol found in several species of Artemisia and Dipterocarpaceae, which has shown various bioactivities such as improving energy metabolism and neuroprotection against cerebral ischemia/reperfusion injury (Gutierrez-Fernandez *et a*l., 2011; Ehrnhofer-Ressler *et al.*, 2013). In addition, borneol exhibited the most potent inhibition of lipid peroxidation (IC_{50} = 80.41 ± 0.24 µg/ml) and showed a strong inhibitory effect on LPS-induced NO production with %NO inhibition of 88 ± 0.05 at 45.0 µM (Abdossi and Kazemi, 2016).

In conclusion, the hydrolyzed extract of KLWW showed strong antioxidant activity by TBARS assay and showed good anti-inflammatory activity by NO inhibitory assay. The 95% ethanolic extract of KLWW showed also good anti-inflammatory activity by NO inhibitory assay. In addition, KLWW should study supercritical fluid extraction for comparison of other extraction methods. Because this technique is suitable for

constituents of volatile oils. And Ya-Hom has been used dissolving in warm water, which should compare with decoction extraction. Moreover, KLWW should study various hydrolyzed extraction in addition to the hydrolyzed extraction with chloroform. Because hydrolyzed extraction studied based on human gastrointestinal tract which system is complex. Thus, KLWW and its ingredients should be further studied for other antioxidant and anti-inflammatory activities. Eugenol and borneol may not be the main chemical compounds of KLWW because GC-MS is an analytical method for the identification of volatile and semi-volatile compounds. KLWW should be analyzed other methods to determine a major compound such as TLC, VLC, and HPLC. And the biological activities of eugenol and borneol of KLWW extract should be further studied both *in vitro* and *in vivo* for reducing the risk of chronic disease. In addition, product development of KLWW is in easily dosage form using or increasing the ratios of high activity of plant ingredients such as *A. sinensis and M. siamensis*.

These results support the use of Ya-Hom KAE-LOM-WING-WIEN remedy for antioxidant and inflammation-related disease because the hydrolyzed and 95% ethanolic extracts of KLWW exhibited inhibition of TBARS formation and nitric oxide production. In addition, these results related to use of KLWW for treatment of dizziness, fatigue, and sleepiness which are caused partly by free radicals and prolonged inflammatory response. Future study of the active compounds and herbal medicine development of KLWW with the aim of prevention and risk reduction of chronic diseases would bring significant benefit to the ageing Thai society.

REFERENCES

Books and Book Articles

Bondy, S. C., Campbell, A., editors. (2016*). Inflammation, aging, and oxidative stress*. Switzerland: Spring International Publishing.

Cotran, R. S., Kumar, V., Collins, T., editors. (1999). *Acute and chronic inflammation*. USA: Robbins Pathologic Basis of Disease, pp. 50–88.

Department for Development of Thai Traditional and Alternative Medicine. (2013). *List of Herbal Medicinal Products A.D. 2013 (2nd ed.)*. Bangkok: The war Veterans Organization of Thailand Under Royal of His Majesty The King.

Inprasit, J. (2014). *Biological activities of Sa-Tri-Lhung-Klod remedy for women's health*. (Master's thesis). Thammasat University, Faculty of Medicine.

Kim, L. S. (2006). *Chemical constituents of Mesua Ferrea L. and Piper Nigrum L. and their larvicidal activities*. (Master's thesis). University Putra Malaysia.

Makchucit, S. (2010). *Anti-inflammatory and anti-allergic activities of Thai traditional medicine preparation called Prasaprohyai*. (Master's thesis). Thammasat University, Faculty of Medicine.

Ministry of Public Health. (1987). *Thai Herbal Pharmacopoeia: Standardization of Thai Herbal Medicines (THP).* Bangkok: Department of Medicinal Science, Vol.1, Part 1, pp. 438-439.

Ouncharoen, K. (2016). *Antioxidant and wound healing activity of Kheaw-Hom oral preparation for recurrent aphthous stomatitis*. (Master's thesis). Thammasat University, Faculty of Medicine.

Sukkasem, K. (2015). *Biological activities of Thai traditional remedy called Kheaw-Hom and its plant ingredients*. (Master's thesis). Thammasat University, Faculty of Medicine.

Articles

Abdossi, V., and Kazemi, M. (2016). Bioactivities of *Achillea millefolium* essential oil and its main terpenes from Iran. *International Journal of Food Properties*, Vol. 19: 1798-1808.

Alinezhad, H., Zare, M., Nabavi, S. F., Naqinezhad. A., and Nabavi, S.M. (2012). Antioxidant, antihemolytic, and inhibitory activities of endemic *Primula heterochroma* against Fe^{2+} -induced lipid peroxidation and oxidative stress in rat brain *in vitro*. *Pharmaceutical Biology*, Vol. 50, Issue 11: 1391-1396.

Anuthakoengkun, A., and Itharat, A. (2014). Inhibitory effect on nitric oxide production and free radical scavenging activity of Thai medicinal plants in osteoarthritic knee treatment. *Journal of the Medical Association of Thailand*, Vol.97, Issue 8: 116-124.

Ayala, A., Munoz, M. F., and Arguelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity*, Vol. 2014: 360438.

Ayyappan, P., Palayyan, S. R., and Gopalan, K. (2016). Attenuation of oxidative damage by *Boerhaavia diffusa* L. against different neurotoxic agents in rat brain homogenate. *Journal of Dietary Supplements*, Vol. 13, Issue 3: 300-312.

Azziz, S., Arriffin, N. M., Alimon, H., and Naz, H. (2013). Chemical study of *Aquilaria crassna*. *Chemistry of Natural Compounds*, July; Vol. 49, Issue 3: 575-576.

Bagchi, K., and Puri, S. (1998). Free radicals and antioxidants in health and disease. *East Mediterranean Health Journal*, Vol. 4: 350–360.

Baliga, M. S., Pai, R. J., Bhat, H. P., Palatty, P. L., and Boloor, R. (2011). Chemistry and medicinal properties of the Bakul (*Mimusops elengi* Linn): A review. *Food Research International*, Aug; Vol. 44, Issue 7: 1823-1829.

Betteridge, D. J. (2000). What is oxidative stress? *Metabolism*, Feb; Vol. 49, Issue 2, Suppl. 1: 3-8.

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., and Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World Allergy Organ Journal*, Jan; Vol.5, Issue 1: 9-19.

Biswas, S. K. (2016). Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxidative Medicine and Cellular Longevity*, Jan; Vol.12: 1-9.

Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., and Gluud, C. (2007). Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *The Journal of the American Medical Association*, Vol. 297, no. 8: 842–857.

Cadenas, E. (2004). Mitochondrial free radical production and cell signaling. *Molecular Aspects of Medicine,* Feb-Apr; Vol. 25, Issue 1-2: 17-26.

Castellani, P., Balza, E., and Rubartelli, A. (2014). Inflammation, DAMPs, tumor development, and progression: a vicious circle orchestrated by redox signaling. *Antioxidants and Redox Signaling*, Mar; Vol. 20, Issue 7: 1086-1097.

Chao, W. W., and Lin, B. F. (2011). Bioactivities of major constituents isolated from *Angelica sinensis* (Danggui). *Chinese Medical Journal*, Aug; Vol. 6, Issue 29: 1-7.

Chou, S. T., Lai, C. P., Lin, C. C., and Shih, Y. (2012). Study of the chemical composition, antioxidant activity and anti-inflammatory activity of essential oil from *Vetiveria zizanioides*. *Food Chemistry*, Sep; Vol. 134, Issue 1: 262-268.

Cortes-Rojas, D. F., de Souza, C. R. F., and Oliveira, W. P. (2014). Clove (*Syzygium aromaticum*): a precious spice. *Asian Pacific Journal of Tropical Biomedicine*, Feb; Vol. 4, Issue 2: 90-96.

Dahham, S. S., Tabana, Y. M., Iqbal, M. A., Ahamed, M.B., Ezzat, M. O., *et al*. (2015). The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna*. *Molecules*, Jun; Vol. 20, Issue 7: 11808-11829.

Fialkow, L., Wang, Y., and Downey, G. P. (2007). Reactive oxygen and nitrogen species Jan as signaling molecules regulating neutrophil function. *Free Radical Biology and Medicine*, Jan; Vol. 42, Issue 2: 153-164.

Gami, B., Pathak, S., and Parabia, M. (2012). Ethnobotanical, phytochemical and pharmacological review of *Mimusops elengi* Linn. *Asian Pacific Journal of Tropical Biomedicine*, Sep; Vol. 2, Issue 9: 743–748.

Girotti, A. W. (1998). Lipid hydroperoxide generation, turnover, and effector action in biological systems. *Journal of Lipid Research*, Vol. 39, no. 8: 1529–1542.

Gunawardena, D., Karunaweera, N., Lee, S., Kooy F, V. D., Harman, D. G., Raju, R., *et al*. (2015). Anti-inflammatory activity of cinnamon (*C. zeylanicum* and *C. cassia*) extracts - identification of E-cinnamaldehyde and o-methoxy cinnamaldehyde as the most potent bioactive compounds. *Food and Function*, Mar; Vol. 6, Issue 3: 910-919.

Gupta, A. D., Bansal, V. K., Babu, V., and Maithil, N. (2013). Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* Houtt). *Journal of Genetic Engineering and Biotechnology*, Jun; Vol. 11, Issue 1: 25-31.

Guzik, T. J., Korbut, R., and Adamek-Guzik, T. (2003). Nitric oxide and superoxide in inflammation and immune regulation. *Journal of physiology and pharmacology,* Vol. 54: 469-487.

Halliwell, B. (1989). Tell me about free radicals, doctor: a review. *Journal of the Royal Society of Medicine*, Vol. 82, Issue 12: 747–752.

Halliwell, B., and Whiteman, M. (2004). Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *British Journal of Pharmacology*, Vol. 142, Issue 2: 231–255.

Hong, C. H., Hur, S. K., Oh, O. J., Kim, S. S., Nam, K. A., and Lee, S. K. (2002). Evaluation of natural products on inhibition of inducible cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) in cultured mouse macrophage cells. *Journal of Ethnopharmacology*, Nov; Vol. 83, Issue 1-2: 153-159.

Huang, J., Lu, X. Q., Zhang, C., Lu, J., Li, G. Y., Lin, R. C., *et al.* (2013). Anti-inflammatory ligustilides from *Ligusticum chuanxiong* Hort. *Fitoterapia Journal*, Dec; Vol. 91: 21-27.

Ishikawa, T., Kondo, Y., Goda, K., and Fujisawa, M. (2005). Overexpression of endothelial nitric oxide synthase in transgenic mice accelerates testicular germ cell apoptosis induced by experimental cryptorchidism. *Journal of Andrology*, Mar-Apr; Vol. 26, Issue 2: 281-288.

Itharat, A., Makchuchit, S., and Tewtrakul, S. (2009). Anti-inflammatory activity of Thai traditional medicine preparation called Prasaprohyai. *Journal of Medicinal Plant and Natural Product Research*, Vol. 75, Issue 9: 1043-1044.

Jensen, A., and Meilby, H. (2012). Assessing the population status of a tree species using distance sampling: *Aquilaria crassna* (Thymelaeaceae) in Northern Laos. *International Journal of Forestry Research*, Vol. 2012, 265831.

Jiang, Y., and Li, B. T. (1977). Flora of China. *Science Press and Missouri Botanical Garden*, Vol. 63: 234.

Jirovetz, L., Buchbauer, G., Stoilova, I., Stoyanova, A., Krastanov, A., and Schmidt, E. (2006). Chemical composition and antioxidant properties of clove leaf essential oil. *Journal of Agricultural and Food Chemistry*, Aug; Vol. 54, Issue 17: 6303-6307.

Kadam, P. V., Yadav, K. N., Deoda, R. S., Shivatare, R. S., and Patil, M. J. (2012). *Mimusops elengi*: a review on ethnobotany, phytochemical and pharmacological profile. *Journal of Pharmacognosy and Phytochemistry*, Vol. 1, No. 3: 64-74.

Kang, J. S., Yoon, Y. D., Cho, I. J., Han, M. H., Lee, C. W., Park, S. K., and Kim, H. M. (2005). Glabridin, an isoflavan from licorice root, inhibits inducible nitric-oxide synthase expression and improves survival of mice in experimental model of septic shock. *Journal of Pharmacology and Experimental Therapeutics*, Vol. 312, Issue 3: 1187-1194.

Kanner, J., German, J. B., and Kinsella, J. E. (1987). Initiation of lipid peroxidation in biological systems. *Critical Reviews in Food Science and Nutrition*, Vol. 25, no. 4: 317–364.

Kar, B., Kumar, R. B. S., Karmakar, I., Dola, N., Bala, A., K, U., *et al*. (2012). Antioxidant and i*n vitro* anti-inflammatory activities of *Mimusops elengi* leaves. *Asian Pacific Journal of Tropical Biomedicine*, Vol. 2, Issue 2: S976-S980.

Khansari, N., Shakiba, Y., and Mahmoudi, M. (2009). Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Patents on Inflammation and Allergy Drug Discovery*, Jan; Vol. 3, Issue 1: 73-80.

Kim, W. S., Choi, W. J., Lee, S., Kim, W. J., Lee, D. C., Sohn, U. D., *et al*. (2015). Anti-inflammatory, antioxidant and antimicrobial effects of Artemisinin extracts from *Artemisia annua* L. *The Korean Journal of Physiology and Pharmacology*, Jan; Vol. 19, Issue 1: 21-27.

Kim, H. J., Chen, F. Wang, X., Chung, H. Y., and Jin, Z. (2005). Evaluation of antioxidant activity of vetiver (*Vetiveria zizanioides* L.) oil and identification of its antioxidant

constituents. *Journal of Agricultural and Food Chemistry*, Oct; Vol. 53, Issue 20: 7691-7695.

Klinthong, S., Khammanit, R., Phornchirasilp, S., Temsiririrkkul, R., and Siriwatanametanon, N. (2015). *In vivo* anti-inflammatory and *in vitro* antioxidant activities of a Thai traditional formula, Rid-si-duang-ma-ha-kan, for hemorrhoid treatment. *Mahidol University Journal of Pharmaceutical Sciences*, Vol. 42, Issue 3: 144-152.

Koseywattana, I. (2002). Quality control of food supplement products and cosmetics from herbs. *The Fourth National Seminar on Pharmaceutical Biotechnology*, Sep 10-12; Chiang Mai, Thailand.

Kumar, A., and Saika, D. (2016). *Euphorbia Antiquorum* Linn: a comprehensive review of ethnobotany, phytochemistry and pharmacology. *Journal of Analytical and Pharmaceutical Research*, Vol. 2, Issue 4: 00024.

Latha, P. G., Sindhu, P. G., Suja, S. R., Geetha, B. S., Pushpangadan, P., and Rajasekharan, S. (2005). Pharmacology and chemistry of *Myristica fragrans* Houtt. *Journal of Spices and Aromatic Crops,* Vol. 14, Issue 2: 94–101.

Li, D., Wang, Z., Chen, H., Wang, J., Zheng, Q., Shang, J., and Li, J. (2009). Isoliquiritigenin induces monocytic differentiation of HL-60 cells. *Free Radical Biology and Medicine*, Mar; Vol. 46, Issue 6: 731-736.

Li, H., Horke, S., and Forstermann, U. (2013). Oxidative stress in vascular disease and its pharmacological prevention. *Trends in Pharmacological Sciences*, Jun; Vol. 34, Issue 6: 313-319.

Lobo, V., Patil, A., Phatak, A., and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, Jul; Vol.4, Issue 8: 118-126.

Mahapatraa, S. K., and Roy, S. (2014). Phytopharmacological approach of free radical scavenging and anti-oxidative potential of eugenol and *Ocimum gratissimum* Linn. *Asian Pacific Journal of Tropical Medicine*, Sep; Vol. 7, Suppl. 1: S391-397.

Mahidol, C., Kaweetripob, W., Prawat, H., and Ruchirawat, S. (2002). Mammea coumarins from the flowers of *Mammea siamensis*. *Journal of natural products*, Vol. 65, Issue 5: 757-760.

Makchuchit, S., Itharat, A., and Tewtrakul, S. (2010). Antioxidant and nitric oxide inhibition activities of Thai medical plants. *Journal of the Medical Association of Thailand*, Vol. 93: 227-233.

Makisshima M, Okabe-Kado J, and Honma Y. (1966). Growth inhibition and differentiation induction in human monoblastic leukaemia cells by 1 α-hydroxyvitamin D derivatives and their enhancement by combination with hydroxyurea. *British Journal of Cancer*, Jun; Vol. 77, Issue 1: 33-39.

Martins, N., Barros, L., Duenas, M., Santos-Buelgac, C., and Ferreira, I. C. F. R. (2015). Characterization of phenolic compounds and antioxidant properties of *Glycyrrhiza glabra* L. rhizomes and roots. *Royal Society of Chemistry Journals*, Mar; Vol. 5: 26991–26997.

Mitsuru, U., and Midori, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, May; Vol. 86, Issue 1: 271-278.

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., and Malik, A. B. (2014). Reactive oxygen species in inflammation and tissue injury. *Antioxidants and Redox Signaling*, Mar; Vol. 20, Issue 7: 1126-1167.

Morikawa, T., Sueyoshi, M., Chaipech, S., Matsuda, H., Nomura, Y., Yabe, M., *et al*. (2012). Suppressive effects of coumarins from *Mammea siamensis* on inducible nitric oxide synthase expression in RAW264.7 cells. *Bioorganic and Medicinal Chemistry*, Vol. 20, Issue 16: 4968-4977.

Nagababu, E., Rifkind, J. M., Sesikeran, B., and Lakshmaiah, N. (2010). Assessment of antioxidant activities of eugenol by *in vitro* and *in vivo* methods. *Methods in Molecular Biology*, Vol. 610: 165-180.

Nanavati, B. (2014). *Glycyrrhiza glabra* (Liquorice) a potent medicinal herb. *International Journal of Herbal Medicine*, Vol. 2, Issue 2: 132-136.

Neveu, V., Perez-Jiménez, J., Vos, F., Crespy, V., du Chaffaut, L., Mennen, L., *et al*. (2010). Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *The Journal of Biological Databases and Curation*, Jan; Vol. 2010: bap024.

Pacher, P., Beckman, S., and Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. *Physiological Reviews*, Jan; Vol. 87, Issue 1: 315-424.

Paudel, K. R., and Panth, N. (2015). Phytochemical profile and biological activity of *Nelumbo nucifera*. *Evidence-Based Complementary and Alternative Medicine*, Dec; Vol. 2015: 789124.

Pham-Huy, L. A., He, H., and Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science*, Jun; Vol. 4, no. 2: 89-96.

Rattanapan, J., Sichaem, J., and Tip-pyang, S. (2012). Chemical constituents and antioxidant activity from the stems of *Alyxia reinwardtii*. *Academy of Chemistry of Globe Publications*, Vol. 6, Issue 3: 288-291.

Reanmongkol, W., Subhadhirasakul, S., and Bouking, P. (2003). Antinociceptive and antipyretic activities of extracts and fractions from *Dracaena loureiri* in experimental animals. *Songklanakarin Thai Journal of Science and Technology*, Vol. 25, Issue 4: 467-476.

Sesso, H. D., Buring, J. E., Christen, W. G., Kurth, T., Belanger, C., Macfadyen, J. *et al.*, (2008). Vitamins E and C in the prevention of cardiovascular disease in men: the physicians' health study II randomized controlled trial. *The Journal of the American Medical Association*, Vol. 300, no. 18: 2123–2133.

Sharma, J. N., Al-Omran, A., and Parvathy, S. S. (2007). Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*, Vol. 6: 252-259.

Shan, B., Cai, Y. Z., Sun, M., and Corke, H. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry*, Oct; Vol. 53, Issue 20: 7749-7759.

Sharma, V., Katiyar, A., and Agrawal, R. (2016). *Glycyrrhiza glabra*: chemistry and pharmacological activity. *Springer International Publishing*, Jan: 1-14.

Singh, S. P., Dharma, S. K., Singh, T., and Singh, L. (2013). Review on *Vetiveria zizanioides*: a medicinal herb. *Journal of Drug Discovery and Therapeutics*, Vol. 1, Issue 7: 80-83.

Song, C. W., Lunga, P. K., Qin, X. J., Cheng, G. G., Gu, J. L., Liu, Y. P., and Luo, X. D. (2014). New antimicrobial pregnane glycosides from the stem of *Ecdysanthera rosea*. *Fitoterapia*, Dec; Vol. 99: 267-275.

Srisawat, U., Panunto, W., Kaendee, N., Tanuchit, S., Itharat, A., Lerdvuthisopon, N., *et al*. (2010). Determination of phenolic compounds, flavonoids, and antioxidant

activities in water extracts of Thai red and white rice cultivars. *Journal of Medical Association Thailand*, Dec; Vol. 93, Suppl. 7: S83-S91.

Steinrut, L., Itharat, A., and Ruangnoo, S. (2011). Free radical scavenging and lipid peroxidation of Thai medicinal plants used for diabetic treatment. *Journal of the Medical Association of Thailand*, 2011 Dec; Vol. 94, Issue 7: 178-182.

Suantawee, T., Wesarachanon, K., Anantsuphasak, K., Daenphetploy, T., Thien-Ngern, S., Thilavech, T., *et al.* (2015). Protein glycation inhibitory activity and antioxidant capacity of clove extract. *Journal of Food Science and Technology*, Jun; Vol. 52, Issue 6: 3843-3850.

Subhadhirasakul, S., and Pechpong, P. (2005). A terpenoid and two steroids from the flowers of *Mammea siamensis*. *Journal of science and technology Mahasarakham University*, Vol. 27, Issue 2: 555-561.

Tanko, Y., Mohammed, A., Okasha, M. A., Umar, A. H., and Magaji, R. A. (2008). Anti-nociceptive and anti-inflammatory activities of ethanol extract of *Syzygium aromaticum* flower bud in wistar rats and mice. *The African Journal of Traditional, Complementary and Alternative medicines*, Vol. 5, Issue 2: 209–212.

The, S. S., Ee, G. C. L., Mah, S. H., Yong, Y. K., Lim, Y. M., Rahmani, M., and Ahmad, Z. (2013). *In vitro* cytotoxic, antioxidant, and antimicrobial activities of *Mesua beccariana* (Baill.) Kosterm., *Mesua ferrea* Linn., and *Mesua congestiflora* extracts. *BioMed Research International*, Vol. 2013: 517072.

Tewtrakul, S., and Itharat, A. (2007). Nitric oxide inhibitory substances from the rhizomes of *Dioscorea membranacea*. *Journal of Ethnopharmacology*, Feb; Vol. 109, Issue 3: 412-416.

Tewtrakul, S., and Subhadhirasakul, S. (2008). Effects of compounds from *Kaempferia parviflora* on nitric oxide, prostaglandin E_2 and tumor necrosis factor-alpha productions in RAW264.7 macrophage cells. *Journal of Ethnopharmacology*, Vol. 120: 81-84.

Tiwari, P., and Nandy, S. (2012). Screen of anti-inflammatory activity of *Mesua ferrea* Linn flower. *International Journal of Biomedical Research*, Vol. 3, Issue 5: 245-252.

Uttara, B., Singh, A. V., Zamboni, P., and Mahajan, R. T. (2009). Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology*, Mar; Vol.7, Issue 1: 65–74.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and cell biology*, Vol.39, Issue 1: 44-84.

Verdian-rizi, M. R. (2009). Chemical composition and antimicrobial activity of the essential oil of *Artemisia annua* L. from Iran. *Pharmacognosy Research*, Vol. 1, Issue 1: 21-24.

Victor, V. M., Rocha, M., and De la Fuente, M. (2004). Immune cells: free radicals and antioxidants in sepsis. *International Immunopharmacology*, Mar; Vol. 4, Issue 3: 327-347.

Yin, H., Xu, L., and Porter, N. A. (2011). Free radical lipid peroxidation: mechanisms and analysis. *Chemical Reviews*, Vol. 111, no. 10: 5944–5972.

Zahid, H., Rizwani, G. H., Khan, M., and Shaukat, K. (2016). Anti-inflammatory and antioxidant activities of *Mimusops elengi* L. *British Journal of Pharmaceutical Research*, Vol. 9, Issue 3, 1-10.

Zakaria, M. P. M., Abas, F., and Rukayadi, Y. (2015). Effects of *Myristica fragrans* Houtt. (Nutmeg) extract on chemical characteristic of raw beef during frozen storage. *International Food Research Journal*, Nov; Vol. 22, Issue 3: 902-909.

Zhang, C., Liu, R., He, J., Ma, Z., and Zhang, X. (2016). Chemical compositions of *Ligusticum chuanxiong* Oil and lemongrass oil and their joint action against *Aphis citricola* Van Der Goot (hemiptera: aphididae). *Molecules*, Oct; Vol. 21, Issue 10: 1359.

Zhang, S. M., Cook, N. R., Albert, C. M., Gaziano, J. M., Buring, J. E., and Manson, J. E. (2008). Effect of combined folic acid, vitamin B6, and vitamin B12 on cancer risk in women: a randomized trial. *Journal of the American Medical Association*, Vol. 300, no. 17: 2012–2021.

Electronic Media

Herbal Information Office, Faculty of Pharmacy, Mahidol University. (2017). *Cinnamomum bejolghota* (Buch.-Ham.) Sweet, Available from: http://medplant.mahidol.ac.th/pharm/botanic.asp ?bc=0774&kw=%CA%C1%D8%C5%E1%C7%E9%A7.

International Programme on Chemical Safety. (2017). *Myristica fragrans* Houtt., Available from: http://www.inchem.org/documents/pims/plant/pim355.htm.

Medicinal plants of Bangladesh. (2017). *Euphorbia antiquorum* L., Available from: http://www.mpbd.info/plants/euphorbia-antiquorum.php.

Thaicrudedrug, Faculty of Phamaceutical Science, Ubon Ratchathani University (2017). *Mimusops elengi* L. (Khon dok), Available from: http://www.thaicrudedrug.com/main.php?action=viewpage&pid=36.

Thaicrudedrug, Faculty of Phamaceutical Science, Ubon Ratchathani University (2017). *Terminalia chebula* Retz. var chebula, Available from: http://www.thaicrudedrug.com/main.php?action=viewpage&pid=31.

Thaicrudedrug, Faculty of Phamaceutical Science, Ubon Ratchathani University (2017). *Cinnamomum bejolghota* (Buch.-Ham.) Sweet, Available from: http://www.thaicrudedrug.com/main.php?action=viewpage&pid=163.

The Royal Botanic Gardens, Kew science Plants of the world online. (2017). *Artemisia annua* L., Available from: http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:304416-2.

The Royal Botanic Gardens, Kew science Plants of the world online. (2017). *Glycyrrhiza glabra* L., Available from: http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:496941-1.

The Royal Botanic Gardens, Kew science Plants of the world online. (2017). *Syzygium aromaticum* Merr. & L.M. Perry, Available from: http://powo.science.kew.org/taxon/urn: lsid: ipni.org: names:601421-1.

University of Maryland Medical Center. (2017). *Angelica sinensis* (Oliv.) Diels, Available from: https://www.umm.edu/health/medical/altmed/herb/dong-quai.

APPENDICES

APPENDIX A

Chemical Reagents for Laboratory Experiments

1. Reagent for Nitroblue tetrazolium (NBT) dye reduction assay

1.1 RPMI 1640 medium

10.4 g of RPMI 1640 medium powder is dissolved in 500 ml sterile water. Add 2.0 g of sodium bicarbonate and dilute to 10,000 ml with sterile water. Adjust pH to 7.2-7.4 with 10% sodium hydroxide or 10% hydrochloric acid and filter though 0.2 micron membrance filter and keep in sterile bottle.

The complete medium is mixture of 200 ml of RPMI, 20 ml fetal bovine serum and 2 ml penicillin/streptomycin. The medium is stored at 4°C.

1.2 10% Hydrochloric acid (HCl)

Conc. HCl (37%)	27	ml
Distilled water to	100	ml

1.3 10% Sodium hydroxide (NaOH)

NaOH	10	g
Distilled water to	100	ml

1.4 Fetal bovine serum (FBS)

Slowly thaw the FBS (inactive), heat 56°C, 60 minuites

(Aliquot, kept at -20°C)

1.5 Penicillin-Streptomycin (P/S)

Slowly thaw the frozen P/S in water bath at 37°C till completely thawed (Aliquot, kept at -20°C)

1.6 Nitroblue tetrazolium (NBT) solution

NBT	1.25	mg
Hank's buffer to	1	ml

The reagent was prepared freshly and protected from light with aluminum foil.

1.7 Phorbol 12 myristate 13-actate (PMA)

Conc. PMA	2	mg/ml
Hank's Balanced Salt solution to	250	ng/ml

1.8 MTT

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H -tetrazolium bromide or Thiazolyl blue tetrazolium bromide	200	mg
Phosphase buffer saline (PBS)	40	ml

The reagent was protected from light with aluminum foil and stored at 4°C.

2. Reagent for Thiobarbituric acid reactive substances (TBARS) assay

2.1 10mM Tris-HCl buffer

Tris base	1,211	g
Distilled water to	1,000	ml
Adjust pH to 7.5 by HCl		

2.2 2.8% Trichloroacetic acid (TCA)

TCA	2.8	ml
Distilled water to	100	ml

2.3 8.1% Sodium dodecyl sulphate (SDS)

SDS	8.1	g
Distilled water to	100	ml

2.4 0.6% Thiobarbituric acid (TBA) in 0.1 M NaOH

TBA	0.6	g
0.1 M NaOH to	100	ml

2.5 0.1 M NaOH

NaOH	2	g
Distilled water to	500	ml

2.6 Fe^{2+}/EDTA

Fe^{2+} ($FeSO_4$)	0.139	g

EDTA	0.1862	g
Distilled water to	500	ml

3. Reagent for Nitric oxide (NO) inhibitory assay

3.1 RPMI 1640 medium

10.4 g of RPMI 1640 medium powder is dissolved in 500 ml sterile water. Add 2.0 g of sodium bicarbonate and dilute to 10,000 ml with sterile water. Adjust pH to 7.2-7.4 with 10% sodium hydroxide or 10% hydrochloric acid and filter though 0.2 micron membrance filter and keep in sterile bottle.

The complete medium is mixture of 200 ml of RPMI, 20 ml fetal bovine serum and 2 ml penicillin/streptomycin. The medium is stored at 4°C.

3.2 10% Hydrochloric acid (HCl)

Conc. HCl (37%)	27	ml
Distilled water to	100	ml

3.3 10% Sodium hydroxide (NaOH)

NaOH	10	g
Distilled water to	100	ml

3.4 Fetal bovine serum (FBS)

Slowly thaw the FBS (inactive), heat 56°C, 60 minuites

(Aliquot, kept at -20°C)

3.5 Phosphase buffer saline (PBS)

PBS	1	Tablet
Distilled water to	100	ml

Sterilize by autoclave before use

3.6 Penicillin-Streptomycin (P/S)

Slowly thaw the frozen P/S in water bath at 37°C till completely thawed (Aliquot, kept at -20°C)

3.7 Trypsin-EDTA

Slowly thaw the frozen 0.5% trypsin-EDTA in water bath at 37°C, 60 minutes till completely thawed (Aliquot, kept at -20°C)

3.8 Griess reagent

Sulfanilamide	1.0	g
N-(1-Naphthyl) ethylenediamine dihydrochloride	0.1	g
Phosphoric acid	2.5	g
Distilled water to	1,000	ml

The reagent was protected from light with aluminum foil and stored at 4°C.

3.9 MTT

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H -tetrazolium bromide or Thiazolyl blue tetrazolium bromide	200	mg
Phosphase buffer saline (PBS)	40	ml

The reagent was protected from light with aluminum foil and stored at 4°C.

3.10 0.04 HCl in Isopropanol

Conc. HCl	0.83	ml
Isopropanol to	250	ml

APPENDIX B
ยาหอมแก้ลมวิงเวียน

ยาผง (รพ.) ยาเม็ด (รพ.)

สูตรตำรับ

ในผงยา 225 กรัมประกอบด้วย รากชะเอมเทศ หนัก 32 กรัม แก่นจันทน์เทศ หนัก 24 กรัม ดอกกานพลู โกฐเชียง โกฐหัวบัว รากแฝกหอม เกสรบัวหลวง หนักสิ่งละ 12 กรัม เปลือกสมุลแว้ง หนัก 10 กรัม เปลือกอบเชยญวน เปลือกอบเชยเทศ กฤษณา กระลำพัก โกฐจุฬาลัมพา โกฐพุงปลา เปลือกชะลูด หนักสิ่งละ 8 กรัม พิมเสน ขอนดอก ดอกบุนนาค ดอกพิกุล ดอกสารภี หนักสิ่งละ 6 กรัม เถามวกแดง หนัก 5 กรัม น้ำประสานทองสะตุ แก่นจันทน์แดง หนักสิ่งละ 4 กรัม

คำแนะนำ

แก้ลมวิงเวียน อ่อนเพลีย นอนไม่หลับ

ขนาดและวิธีใช้

รับประทานครั้งละ 600 มิลลิกรัม – 1 กรัม ละลายน้ำสุก เมื่อมีอาการ ทุก 3 - 4 ชั่วโมง ไม่ควรเกินวันละ 3 ครั้ง

คำเตือน

- ควรระวังการรับประทานร่วมกับยาในกลุ่มสารกันเลือดเป็นลิ่ม (anticoagulant) และยาต้านการจับตัวของเกล็ดเลือด (antiplatelets)

- ควรระวังการใช้กับผู้ป่วยที่มีประวัติแพ้เกสรดอกไม้

BIOGRAPHY

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Publications

Jantarach, N., Chaiyawatthanananthn, P., and Itharat, A. Quality standard values and stability study of ethanolic Ya-Hom KAE-LOM-WING-WIEN remedy extract on nitric oxide inhibition in LPS-stimulated RAW 264.7 macrophage cells. *Thammasat Medical Journal*. In press 2018.

Conference and Presentation

Jantarach, N., and Itharat, A. (2017). Anti-inflammatory Activity of Thai Traditional "Ya-Hom" Remedy. *Diversity in Multidisciplinary Approach to Patient Self Care*, Faculty of Medicine, Thammasat University, Thailand. (Poster presentation)

Jantarach, N., and Itharat, A. (2017). Anti-inflammatory and antioxidant activities of three KOT using in all Ya-Hom remedies. *The 2nd One Health International Conference 2017*, Centara Grand at Central Plaza Ladprao, Bangkok, Thailand. (Poster presentation)