BIOLOGICAL ACTIVITIES OF THAI TRADITIONAL REMEDY CALLED KHEAW-HOM AND ITS PLANT INGREDIENTS

BY

MISS KANMANEE SUKKASEM

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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was approved as partial fulfillment of the requirements for
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ABSTRACT

Kheaw-Hom is a Thai traditional remedy which folk doctors have long used to treat fever in the exanthematous fever group such as chickenpox, measles, herpes zoster and hand, foot and mouth disease. It consists of eighteen Thai medicinal plants as follows: Phim sen ton (*Pogostemon cablin* (Blanco) Benth.), Phak krachom (*Limnophila rugosa* Merr.), Mak phu (*Cordyline fruticosa* (L.) Goeppert.), Mak mia (*Cordyline fruticosa* (L.) Goeppert.), San phra hom (*Eupatorium stoechadosmum* Hance), Faek hom (*Vetiveria zizanioides* (L.) Nash ex Small), Proh hom (*Kaempferia galanga* Linn.), Chan thet (*Myristica fragrans* Houtt.), Chan dang (*Dracaena loureiri* Gagnep.), Wan kep rat (*Angiopteris evecta* (G.Forst) Hoffm.), Wan ron thong (*Globba malaccensis* Ridl.), Nae ra phu sri (*Tacca chantrieri* Andre), Phit sa nat (*Sophora exigua* Craib), Ma has sa dam (*Cyathea gigantea* Holtt.), Phi kul (*Mimusops elengi* Linn.), Bun nak (*Mesua ferrea* Linn.), Sa ra phi (*Mammea siamensis* Kosterm.) and Bua luang (*Nelumbo nucifera* Gaertn.). Some plant ingredients in this remedy have been investigated for antimicrobial and anti-inflammatory activities. However, for Kheaw-Hom remedy, there is no report to verify this. Consequently, the objectives of this research were to study the antiviral, antimicrobial and anti-inflammatory activities that are related to exanthematous fever and skin infection complications of ethanolic and aqueous extracts of Kheaw-Hom

remedy and its plant ingredients. All extracts were tested for antiviral activity by antiviral activity based CPE assay, antimicrobial activities by using disc diffusion method and microtitre plate-based antimicrobial assay and anti-inflammatory activities by determination of inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines. The extract which showed the strongest activity was selected to study for stability testing.

Kheaw-Hom remedy and each of its plant ingredients were extracted by maceration in 95% ethanol and decoction in water to obtain ethanolic and aqueous extracts, respectively. The percentage yields of the ethanolic and aqueous extracts of Kheaw-Hom remedy were 8.75% and 13.36%, respectively. The highest percentage yield of the ethanolic extract was *D. loureiri* (19.49%) and the highest percentage yield of the aqueous extract was *M. siamensis* (30.56%).

All extracts were tested for biological activities. First, antiviral activity was tested by using the antiviral activity based cytopathic effect (CPE) assay of enterovirus 71 (EV71) on Vero cell lines. The aqueous extracts of Kheaw-Hom remedy and its plant ingredients which cytotoxicity was less than 20%, were tested for antiviral activities against 100, 50 and 25 $TCID_{50}$ (50% tissue culture infective dose) EV71 in duplicate experiments. Morphological changes of Vero cells which were infected with EV71 at 100, 50 and 25 $TCID_{50}$ were observed after incubated 5 days. The results showed that the aqueous extract of Kheaw-Hom remedy (KHA) had antiviral activity against 25 $TCID_{50}$ EV71 with cytopathic effect less than 50% at a concentration 400 μg/ml. In contrast, the ethanolic extract of Kheaw-Hom remedy (KHE) exhibited toxic to Vero cells. The aqueous extract of *T. chantrieri* (TCA) showed the best antiviral activity against EV71 at 100, 50 and 25 $TCID_{50}$ with cytopathic effect less than 50% at a concentration 50, 50 and 100 μg/ml, respectively.

Second, all extracts were tested for antimicrobial activity by disc diffusion method to determine the inhibition zone and by using microtitre plate-based antimicrobial assay to determine minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) against four gram positive bacteria (*Staphylococcus aureus* ATCC 25923, Methicillin-resistant *Staphylococcus aureus* DMST 20651, *Staphylococcus epidermidis* ATCC 12228 and *Streptococcus pyogenes* ATCC 19615), one gram negative bacterium (*Klebsiella pneumoniae* ATCC 700603)

and one fungus (*Candida albicans* ATCC 90028). KHE showed inhibition zone against four gram positive bacteria *S. aureus,* methicillin-resistant *S. aureus, S. epidermidis* and *S. pyogenes* with the inhibition zone of 7.33±0.58, 7.00±0.00, 8.00±0.00 and 12.67±0.58 mm, respectively. KHA was not able to inhibit all microbes. The ethanolic extract of *S. exigua* (SEE) showed the highest antimicrobial activity against these five gram positive bacteria with an inhibition zone of 12.67±0.58, 13±0.00, 14.33±0.58 and 16.00±1.00 mm, respectively and showed a low inhibition zone of 8.67±0.58 mm against *C. albicans*. Neither ethanolic nor aqueous extracts of Kheaw-Hom remedy and its plant ingredients were able to inhibit gram negative *K. pneumoniae.*

In addition, all extracts were tested for antimicrobial activity by microtitre plate-based antimicrobial assay to determine minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC). The results showed that KHE had antimicrobial activity against *S. aureus,* methicillin-resistant *S. aureus, S. epidermidis* and *S. pyogenes* with MIC values of 0.625, 0.625, 1.25 and 0.625 mg/ml and MMC values of 1.25, 0.625, 2.5 and 0.625 mg/ml, respectively but had no activity against *K. pneumonia* and *C. albicans*. The ethanolic extract of *S. exigua* (SEE) showed the highest antimicrobial activity against these four gram positive bacteria with MIC values of 0.005, 0.005, 0.039 and 0.019 mg/ml and MMC values of 0.005, 0.005, 0.039 and 0.019 mg/ml, respectively. Most of the aqueous extracts of Kheaw-Hom remedy and its plant ingredients had no activity against all bacteria and fungi.

Third, the anti-inflammatory activities by determination of inhibitory effects on lipopolysaccharide (LPS) induced nitric oxide (NO) release from murine macrophages cell lines (RAW 264.7) were investigated. The results found that the KHA showed anti-inflammatory activity with IC_{50} value of 46.86±0.82 μg/ml which was higher than the KHE showed IC_{50} value of 59.77±3.76 μg/ml. The ethanolic extract of *M. siamensis* (MSE) showed the highest anti-inflammatory activity with IC_{50} value of 11.55±2.70 μg/ml. The aqueous extract of *S. exigua* (SEA) showed the highest anti-inflammatory activity with IC_{50} value of 3.17±0.68 μg/ml.

The stability test of Kheaw-Hom extract found that the ethanolic extract of Kheaw-Hom remedy was stable in antimicrobial activity against MRSA with MIC

value of 0.625 mg/ml for at least 8 months and the aqueous extract of Kheaw-Hom remedy was stable in antiviral activity against 25 $TCID_{50}$ of EV71 for at least 1 year and 8 months.

Kheaw-Hom remedy was analyzed by using gas chromatography-mass spectrometry (GC-MS). There are forty-seven components found in the ethanolic extract of Kheaw-Hom remedy. The highest content was ethyl p-methoxycinnamate (18.64%) and the second highest was patchouli alcohol (16.38%).

In conclusion, the aqueous extract of Kheaw-Hom remedy (KHA) showed antiviral and anti-inflammatory activities while the ethanolic extract of Kheaw-Hom remedy (KHE) showed good antimicrobial activities. The aqueous extract of *T. chantrieri* (TCA) showed the best antiviral activity against EV71 and the aqueous extract of *S. exigua* (SEA) showed the best anti-inflammatory activities. Furthermore, the ethanolic extract of *M. siamensis* (MSE) showed the best antimicrobial activities and had good antiviral and anti-inflammatory activities. Thus, these results support the use of Kheaw-Hom remedy to treat exanthematous fevers and skin infection complications such herpes zoster, chickenpox and hand, foot and mouth disease (HFMD) because this remedy showed antiviral properties against EV71, antimicrobial properties against microbes related to skin infection complications and anti-inflammatory activities. Moreover these results are related to Thai traditional medical practice.

Keywords: Kheaw-Hom, Thai Traditional Remedy, Antiviral, Antimicrobial, Anti-inflammatory

หัวข้อวิทยานิพนธ์ ฤทธิ์ทางชีวภาพของตำรับยาไทยชื่อยาเขียวหอม และสมุนไพรในตำรับ

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บทคัดย่อ

ตำรับยาเขียวหอมเป็นตำรับยาสมุนไพรไทยซึ่งแพทย์แผนไทยและหมอพื้นบ้านนิยมใช้รักษาไข้ตัวร้อนหรือไข้ออกผื่น เช่น อีสุกอีใส หัด โรคมือเท้าปากในเด็ก และยังใช้บรรเทาอาการร้อนในกระหายน้ำกันมานานหลายทศวรรษ ประกอบด้วยด้วยสมุนไพรทั้งหมด 18 ชนิด ได้แก่ ใบพิมเสนต้น (*Pogostemon cablin* (Blanco) Benth.), ใบผักกระโฉม (*Limnophila rugosa* Merr.), ใบหมากผู้ (*Cordyline fruticosa* (L.) Goeppert.), ใบหมากเมีย (*Cordyline fruticosa* (L.) Goeppert.), ใบสันพร้าหอม (*Eupatorium stoechadosmum* Hance), รากแฝกหอม (*Vetiveria zizanioides* (L.) Nash ex Small), หัวเปราะหอม (*Kaempferia galanga* Linn.), แก่นจันทน์เทศ (*Myristica fragrans* Houtt.), แก่นจันทน์แดง (*Dracaena loureiri* Gagnep.), ว่านกีบแรด (*Angiopteris evecta* (G.Forst) Hoffm.), ว่านร่อนทอง (*Globba malaccensis* Ridl.), เนระพูสี (*Tacca chantrieri Andre*), พิษนาศน์ (*Sophora exigua* Craib), มหาสดำ (*Cyathea gigantea* Holtt.), ดอกพิกุล (*Mimusops elengi* Linn.), ดอกบุนนาค (*Mesua ferrea* Linn.), ดอกสารภี (*Mammea siamensis* Kosterm.) และเกสรบัวหลวง (*Nelumbo nucifera* Gaertn.). สมุนไพรบางชนิดมีงานวิจัยถึงฤทธิ์ต้านจุลชีพและฤทธิ์ต้านการอักเสบ แต่อย่างไรก็ตามตำรับยาเขียวหอมยังไม่มีรายการวิจัยถึงฤทธิ์ดังกล่าว ดังนั้นวัตถุประสงค์ของการวิจัยนี้เพื่อศึกษาฤทธิ์ต้านไวรัส ต้านจุลชีพ และต้านการอักเสบที่เกี่ยวข้องกับไข้ออกผื่นและภาวะแทรกซ้อนจากการติดเชื้อผิวหนังของสารสกัดเอทานอลและสารสกัดน้ำของตำรับยาเขียวหอมและสมุนไพรในตำรับ สารสกัดทั้งหมดนำมาทดสอบฤทธิ์ต้านไวรัสด้วยวิธี antiviral activity based CPE assay ส่วนฤทธิ์ต้านจุลชีพทดสอบด้วยวิธี disc diffusion method และวิธี microtitre plate-based antimicrobial assay และทดสอบฤทธิ์ต้าน

การอักเสบโดยดูการยับยั้งการหลั่งไนตริกออกไซด์จากเซลล์ RAW 264.7 เมื่อถูกกระตุ้นด้วย LPS สารสกัดตำรับยาเขียวหอมที่มีฤทธิ์ดีจะถูกนำมาทดสอบความคงตัวของสารสกัด

ตำรับยาเขียวหอมและสมุนไพรแต่ละชนิดถูกนำมาสกัดด้วยวิธีการหมักด้วยเอทานอลความเข้มข้น 95% และสกัดด้วยวิธีการต้มน้ำจะได้สารสกัดเอทานอลและสารสกัดน้ำตามลำดับ สารสกัดเอทานอลและสารสกัดน้ำของตำรับยาเขียวหอมได้ผลผลิตร้อยละ (Percentage yield) เท่ากับ 8.75% และ 13.36% ตามลำดับ สารสกัดเอทานอลของแก่นจันทน์แดงมีร้อยละของผลผลิตสูงที่สุดเท่ากับ 19.49% และสารสกัดน้ำของดอกสารภีมีร้อยละของผลผลิตสูงที่สุดเท่ากับ 30.56%

สารสกัดทั้งหมดถูกนำมาทดสอบฤทธิ์ต้านไวรัส enterovirus 71 (EV71) ด้วยวิธี antiviral activity based cytopathic effect (CPE) assay ในเซลล์ Vero สารสกัดน้ำของตำรับยาเขียวหอมและสมุนไพรเดี่ยวที่มีค่าความเป็นพิษต่อเซลล์ Vero น้อยกว่า 20% จะถูกนำมาทดสอบฤทธิ์ต้านไวรัส EV71 ที่ปริมาณความเข้มข้นของไวรัส 100, 50 และ 25$TCID_{50}$ (50% tissue culture infective dose หรือปริมาณไวรัสที่ทำให้เซลล์เพาะเลี้ยงครึ่งหนึ่งเกิดการติดเชื้อ) แล้วนำมาสังเกตการเปลี่ยนแปลงทางพยาธิสภาพของเซลล์หลังการติดเชื้อไวรัสภายใต้กล้องจุลทรรศน์ พบว่าสารสกัดน้ำของตำรับยาเขียวหอมที่ความเข้มข้น 400 ไมโครกรัมต่อมิลลิลิตรสามารถยับยั้งไวรัส EV71 ที่มีปริมาณความเข้มข้น 25$TCID_{50}$ ได้โดยไม่มีความเป็นพิษต่อเซลล์ Vero ในทางกลับกันนั้นสารสกัดเอทานอลของตำรับยาเขียวหอมไม่มีฤทธิ์ในการยับยั้งไวรัส EV71 สารสกัดเอทานอลของตำรับยาเขียวหอมและสมุนไพรเดี่ยวส่วนใหญ่มีความเป็นพิษต่อเซลล์ Vero ส่วนสารสกัดน้ำของเนระพูสีมีฤทธิ์ดีที่สุดในการยับยั้งไวรัส EV71 ที่มีปริมาณความเข้มข้น 100, 50 และ 25$TCID_{50}$ ด้วยความเข้มข้น 50, 50 และ 100 ไมโครกรัมต่อมิลลิลิตรตามลำดับ

การศึกษาฤทธิ์ต้านเชื้อจุลชีพด้วยวิธี Disc diffusion เพื่อหาเส้นผ่าศูนย์กลางของ inhibition zone และวิธี microtitre plate-based antimicrobial assay เพื่อหาค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญเติบโตของเชื้อจุลชีพ (minimum inhibitory concentration หรือ MIC) และค่าความเข้มข้นต่ำสุดที่สามารถฆ่าเชื้อจุลชีพ (minimum microbicidal concentration หรือ MMC) โดยทดสอบกับเชื้อแบคทีเรียแกรมบวก 4 สายพันธุ์ คือ *Staphylococcus aureus* ATCC 25923, Methicillin-resistant *Staphylococcus aureus* DMST 20651, *Staphylococcus epidermidis* ATCC 12228 และ *Streptococcus pyogenes* ATCC 19615 เชื้อแบคทีเรียแกรมลบ 1 สายพันธุ์ คือ *Klebsiella pneumoniae* ATCC 700603 และเชื้อรา

Candida albicans ATCC 90028 พบว่าสารสกัดเอทานอลของตำรับยาเขียวหอมมีฤทธิ์ยับยั้งเชื้อแบคทีเรียแกรมบวก *S. aureus*, Methicillin-resistant *S. aureus*, *S. epidermidis* และ *S. pyogenes* โดยมีค่าเส้นผ่าศูนย์กลางของ inhibition zone เท่ากับ 7.33±0.58, 7.00±0.00, 8.00±0.00 และ 12.67±0.58 มิลลิเมตรตามลำดับ ส่วนสารสกัดน้ำของตำรับยาเขียวหอมไม่มีฤทธิ์ยับยั้งเชื้อแบคทีเรียแกรมบวก สารสกัดเอทานอลของพิษนาศน์มีฤทธิ์ยับยั้งเชื้อแบคทีเรียแกรมบวกเหล่านี้ได้ดีที่สุดโดยมีค่าเส้นผ่าศูนย์กลางของ inhibition zone เท่ากับ 12.67±0.58, 13±0.00, 14.33±0.58 และ 16.00±1.00 มิลลิเมตรตามลำดับและยังมีฤทธิ์ยับยั้งเชื้อรา *C. albicans* โดยมีค่าเส้นผ่าศูนย์กลางของ inhibition zone เท่ากับ 8.67±0.58 มิลลิเมตร ทั้งสารสกัดเอทานอลและสารสกัดน้ำของตำรับยาเขียวหอมและสมุนไพรเดี่ยวไม่มีฤทธิ์ยับยั้งเชื้อ *K. pneumoniae*

การศึกษาฤทธิ์ต้านจุลชีพด้วยวิธี microtitre plate-based antimicrobial assay เพื่อหาค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญเติบโตของเชื้อจุลชีพ (minimum inhibitory concentration หรือ MIC) และค่าความเข้มข้นต่ำสุดที่สามารถฆ่าเชื้อจุลชีพ (minimum microbicidal concentration หรือ MMC) พบว่าสารสกัดเอทานอลของตำรับยาเขียวหอมมีฤทธิ์ยับยั้งเชื้อแบคทีเรียแกรมบวก *S. aureus*, Methicillin-resistant *S. aureus*, *S. epidermidis*, *S. pneumoniae* และ *S. pyogenes* โดยมีค่า MIC เท่ากับ 0.625, 0.625, 1.25 และ 0.625 มิลลิกรัมต่อมิลลิลิตรและมีค่า MMC เท่ากับ 1.25, 0.625, 2.5 และ 0.625 มิลลิกรัมต่อมิลลิลิตรตามลำดับ แต่ไม่มีฤทธิ์ยับยั้งเชื้อ *K. pneumonia* และ *C. albicans* สารสกัดเอทานอลของพิษนาศน์มีฤทธิ์ยับยั้งเชื้อแบคทีเรียแกรมบวกเหล่านี้ได้ดีที่สุดโดยมีค่า MIC เท่ากับ 0.625, 0.625, 1.25 และ 0.625 มิลลิกรัมต่อมิลลิลิตรและค่า MMC เท่ากับ 1.25, 0.625, 2.5 และ 0.625 มิลลิกรัมต่อมิลลิลิตร สารสกัดน้ำของตำรับยาเขียวหอมและสมุนไพรเดี่ยวส่วนใหญ่ไม่มีฤทธิ์ยับยั้งเชื้อทั้งหมด

การทดสอบฤทธิ์ต้านการอักเสบโดยดูการยับยั้งการสร้างไนตริกออกไซด์จากเซลล์ RAW 264.7 เมื่อถูกกระตุ้นด้วย LPS พบว่าสารสกัดน้ำของตำรับยาเขียวหอมมีฤทธิ์ต้านการอักเสบโดยมีค่า IC_{50} เท่ากับ 46.86±0.82 ไมโครกรัมต่อมิลลิลิตรซึ่งมีค่าสูงกว่าสารสกัดเอทานอลของตำรับยาเขียวหอมโดยมีค่า IC_{50} เท่ากับ 59.77±3.76 ไมโครกรัมต่อมิลลิลิตร สารสกัดเอทานอลของสมุนไพรเดี่ยวในตำรับที่มีฤทธิ์ต้านการอักเสบดีที่สุดคือสารภีโดยมีค่า IC_{50} เท่ากับ 11.55±2.70 ไมโครกรัมต่อมิลลิลิตร ส่วนสารสกัดน้ำของสมุนไพรเดี่ยวในตำรับที่มีฤทธิ์ต้านการอักเสบดีที่สุดคือพิษนาศน์โดยมีค่า IC_{50} เท่ากับ 3.17±0.68 ไมโครกรัมต่อมิลลิลิตร

การศึกษาความคงตัวของสารสกัดตำรับยาเขียวหอมภายใต้สภาวะเร่ง เมื่อนำสารสกัดเอทานอลของตำรับยาเขียวหอมมาทดสอบฤทธิ์ต้านเชื้อแบคทีเรีย Methicillin-resistant *S.aureus* พบว่ามีความคงตัวประมาณ 8 เดือนโดยมีค่า MIC เท่ากับ 0.625 มิลลิกรัม/มิลลิลิตร และเมื่อนำสารสกัดน้ำของตำรับยาเขียวหอมมาทดสอบฤทธิ์ต้านเชื้อไวรัส enterovirus 71 พบว่ามีความคงตัวประมาณ 1ปี 8 เดือน

การศึกษาองค์ประกอบทางเคมีของตำรับยาเขียวหอม โดยวิธีแก๊สโครมาโตกราฟี พบสาระสำคัญหลักได้แก่ ethyl p-methoxycinnamate (18.64%) และ patchouli alcohol (16.38%) ตามลำดับ

จากผลการทดลองพบว่าสารสกัดน้ำของตำรับยาเขียวหอมมีฤทธิ์ต้านไวรัสและฤทธิ์ต้านการอักเสบในขณะที่สารสกัดเอทานอลมีฤทธิ์ต้านแบคทีเรียและเชื้อราได้ดี ส่วนสมุนไพรเดี่ยวในตำรับที่มีฤทธิ์ต้านไวรัส EV71 ที่ดีที่สุดคือสารสกัดน้ำของเนระพูสีและสารสกัดน้ำของพิษนาศน์มีฤทธิ์ต้านการอักเสบที่ดีที่สุด นอกจากนี้สารสกัดเอทานอลของสารภีมีฤทธิ์ต้านเชื้อจุลชีพได้ดีที่สุดและยังมีฤทธิ์ต้านไวรัสและต้านการอักเสบได้ดีเช่นกัน จากผลการทดลองที่กล่าวมาข้างต้นนั้นสามารถนำมาสนับสนุนการใช้ตำรับยาเขียวหอมในการรักษาไข้ออกผื่น เช่น งูสวัด อีสุกอีใส และโรคมือเท้าปาก รวมทั้งภาวะแทรกซ้อนจากการติดเชื้อผิวหนังในโรคเหล่านี้ เนื่องจากตำรับยาเขียวหอมมีฤทธิ์ต้านไวรัส EV71 ซึ่งก่อให้เกิดโรคมือเท้าปาก มีฤทธิ์ต้านเชื้อแบคทีเรียเชื้อราที่เกี่ยวข้องในภาวะแทรกซ้อนจากการติดเชื้อผิวหนัง และยังมีฤทธิ์ต้านอักเสบได้ นอกจากนี้ผลที่ศึกษาได้ยังสอดคล้องกับวิธีการใช้ทางการแพทย์แผนไทย

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Kanmanee Sukkasem

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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
%	Percent
>	More than
≥	More than or equal
<	Less than
≤	Less than or equal
=	Equal
µg	Microgram
µg/ml	Microgram per milliliter
µm	Micrometer
µl	Microliter
^{o}C	Degree celsius
^{o}F	Degree Fahrenheit
Amp	Amphotericin B
ATCC	American type culture colletion
CFU	Colony forming unit
CFU/ml	Colony forming unit per milliliter
$CHCl_3$	Chloroform
cm	Centimeter
CO_2	Carbon dioxide
CPE	Cytopathic effect
DMSO	Dimethylsulfoxide
EMEM	Earle's minimal essential medium
et al.	Et alii, and others
EV71	Enterovirus 71
FBS	Fetal bovine serum
g	Gram
g/kg	Gram per kilogram
Gen	Gentamicin

LIST OF ABBREVIATIONS (CONTINUED)

Symbols/Abbreviations	Terms
HCl	Hydrochlioric acid
HFMD	Hand, foot, and mouth disease
H_3PO_4	Phosphoric acid
HT-29	Human colorectal adenocarcinoma cell line
IC_{50}	Concentration causing 50% inhibition effect
IMR-90	Human foetal lung cell
iNOS	Inducible nitric oxide synthase
IL-1β, 6	Interleukin-1β, 6
LPS	Lipopolysaccharide
m	Meter
MBC	Minimal bactericidal concentration
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
MIC	Minimumi inhibition concentration
MMC	Minimum microbicidal concentration
ml	Milliliter
mm	Millimeter
mg/kg	Millimeter per kilogram
mg/ml	Milligram per milliliter
MRSA	Methicillin-resistant *Staphylococcus aureus*
MTT	Thiazolyl blue tetrazolium bromide or 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide
NA	Nutrient agar
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NI	No inhibition zone
nm	Nanometer
NO	Nitric oxide

LIST OF ABBREVIATIONS (CONTINUED)

Symbols/Abbreviations	Terms
NT	Not tested
PBS	Phosphate buffer saline
PGE2	Prostaglandin E2
PGs	Prostaglandins
P/S	Penicillin-Streptomycin
RAW 264.7	Murine macrophage leukemia
RH	Relative humidity
RNA	Ribonucleic acid
RPMI 1640	Roswell Park Memorial Institute 1640
SEM	Standard error of mean
$TCID_{50}$	50% tissue culture infectious dose
THP	Thai Herbal Pharmacopoeia
TNF	Tumor necrosis factor

CHAPTER 1
INTRODUCTION

1.1 Introduction

Hand, foot, and mouth disease (HFMD) is a common infectious disease that belongs to the exanthematous fever group and mainly affects infants and young children. However, it can occasionally occur in adults. HFMD is most commonly caused by infection with coxsackie virus A16 and enterovirus 71. Enterovirus 71 infection is a particular concern as it can have more severe complications and sometimes death in children. (Ventarola *et al*., 2015)

The epidemic situation of HFMD in Thailand has been reported that the morbidity rate of HFMD increased every year and was more severe in 2012-2014. The morbidity rate of HFMD in 2014 is eighty times higher than that in 2004. The highest morbidity rate occurs in children 0-6 years old and outbreaks frequently occur in the end of summer to the beginning of rainy season. Additionally, epidemiological studies indicate that HFMD caused by enterovirus71 infection is a global public health issue, especially in Asia-Pacific region. (Bureau of Epidemiology Annual, 2014)

The clinical symptoms of HFMD include fever, headache, sore throat, loss of appetite, ulcers in the throat, mouth, and tongue, and skin rashes or blisters that are usually on the palms of the hands and soles of the feet or buttocks. (Wen *et al*., 2015) The rash is rarely itchy for children, but can be extremely itchy for adults. HFMD is spread from one person to another through direct contact with nasal discharge, saliva, feces, and fluids from the blisters of an infected person. The symptoms are generally self-limiting and can heal in about a week. Nevertheless, enterovirus 71 infection can have more severe complications including secondary infections, pulmonary edema, aseptic meningitis or death in infants and young children. (Ji *et al*., 2015)

The bacteria that cause secondary infections are belongs to the *Staphylococcus* group such as *Staphylococcus aureus*, Methicillin resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Most blisters will not leave scars unless it is infected with bacteria which may probably be introduced by scratching. In addition, the

Streptococcus group such as *Streptococcus pyogenes* also causes secondary infection. These bacteria that cause secondary infection can found in another exanthematous fever such as chickenpox. Skin infection can be treated by antibiotic drugs but they are high in price due to being imported from abroad and some bacteria can become resistant to antibiotics.

However, there are no effective antiviral drugs and vaccines to prevent and treat HFMD. (Chong *et al*., 2015) In clinical medicine, the common therapies are treatment of the viral infections with broad-spectum antiviral drugs, including Ribavirin®, Ganciclovir®, and Acyclovir® which only partially alleviate the symptoms. In addition, symptomatic treatment is the primary care in HFMD such as taking analgesic medications to relief pain from the sores. Therefore, many trials have been conducted to find antiviral components from plants for the treatment of HFMD. (Solomon *et al.*, 2010)

Kheaw-Hom remedy is a Thai traditional medicine which folk doctors have long been using to treat fever in the exanthematous fever group such as chickenpox, measles, Herpes zoster, and HFMD. The principle in Thai traditional medicine for exanthematous fever is to stimulate the toxin so that the patient will have more rashes. This method will not cause an internal rash and leads to shorter time for recovery. Kheaw-Hom remedy has a cooling and bitter characteristic which decreases the toxin in the blood. Regarding Thai traditional medicine treatment methods, it is recommended to both ingest and apply medicine on the skin. Taking medicine will stimulate the toxin to appear on the skin while applying Kheaw-Hom will decrease the heat on the skin. Comparing to modern medicine, it is possible that Kheaw-Hom might have anti-viral, anti-microbial and anti-inflammation activities. However, there is no research report to verify this.

Kheaw-Hom remedy has been published in National List of Essential Medicines 2011 (Department for Development of Thai Traditional and Alternative Medicine, 2011). This remedy consists of eighteen Thai medicinal plants as follows: Phim sen ton (*Pogostemon cablin* (Blanco) Benth.), Phak krachom (*Limnophila rugosa* Merr.), Mak phu (*Areca catechu* Linn.), Mak mia (*Cordyline fruticosa* (L.) Goeppert.), San phra hom (*Eupatorium stoechadosmum* Hance), Faek hom (*Vetiveria zizanioides* (L.) Nash ex Small), Proh hom (*Kaempferia galanga* Linn.), Chan thet

(*Myristica fragrans* Houtt.), Chan dang (*Dracaena loureiri* Gagnep.), Wan kep rat (*Angiopteris evecta* (G.Forst) Hoffm.), Wan ron thong (*Globba malaccensis* Ridl.), Nae ra phu sri (*Tacca chantrieri* Andre), Phit sa nat (*Sophora exigua* Craib), Ma has sa dam (*Cyathea gigantea* Holtt.), Phi kul (*Mimusops elengi* Linn.), Bun nak (*Mesua ferrea* Linn.), Sa ra phi (*Mammea siamensis* Kosterm.) and Bua luang (*Nelumbo nucifera* Gaertn.).

There has been no report on the biological activities of this remedy but previous studies report that some herbs show antimicrobial activities, namely *Vetiveria zizanioides, Kaempferia galanga, Angiopteris evecta, Dracaena loureiri, Mesua ferrea, Mimusops elengi* and *Mammea siamensis*. Some herbs show anti-inflammatory activities, namely *Pogostemon cablin, Kaempferia galangal, Dracaena loureiri, Mesua ferrea, Mimusops elengi, Mammea siamensis* and *Nelumbo nucifera.* Consequently, the objective of this investigation is to study the antiviral, antimicrobial and anti-inflammatory activities that are related to exanthematous fever such as hand, foot, and mouth disease of Kheaw-Hom extract and its plant ingredients. The results are likely to support the use of Kheaw-Hom remedy for treating exanthematous fever and secondary infection.

1.2 Objectives of this study

1.2.1 Overall objectives

1.2.1.1 The overall objectives of this research are to study the antiviral, antimicrobial and anti-inflammatory activities that are related to exanthematous fever such as hand, foot, and mouth disease of ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients.

1.2.2 Specific objectives

1.2.2.1 To investigate antiviral activities of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients.

1.2.2.2 To investigate antimicrobial activities of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients.

1.2.2.3 To investigate anti-inflammatory activities on nitric oxide inhibition induced by lipopolysaccharide in RAW 264.7 cell line of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients.

1.2.2.4 To study the quality control and stability on accelerated condition of Kheaw-Hom remedy extract.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Fever

Fever (pyrexia and febrile response) is defined as having a body temperature rises above normal range because of an increase in the body's temperature set-point. There is not a single agreed-upon upper limit for normal temperature with sources using values between 37.5 and 38.3 °C. The increase in set-point triggers increased muscle contraction and causes a feeling of cold. This results in greater heat production and efforts to conserve heat. When the set-point temperature returns to normal a person feels hot, becomes flushed, and may begin to sweat. Rarely a fever may trigger a febrile seizure. This is more common in young children. Fevers do not typically go higher than 41 to 42 °C. (Lowth, 2014)

Fever can be caused by many medical conditions ranging from the not serious to potentially serious. This includes viral, bacterial and parasitic infections such as the common cold, urinary tract infections, meningitis, malaria and appendicitis among others. Non-infectious causes include vasculitis, deep vein thrombosis, side effects of medication, and cancer among others. It differs from hyperthermia, in that hyperthermia is an increase in body temperature over the temperature set-point, due to either too much heat production or not enough heat loss. (Garmel and Mahadevan, 2009)

Exanthematous fever is a one type of diseases that present with fever and rash. (Mckinnon *et al*., 2000) Exanthematous fever is defined as widespread generalized rash of different types associated with pyrexia particularly occurring in children but can occur in adults. It can cause by toxins, drugs or microorganisms such as bacteria and virus. (Munjal *et al*., 2015)

2.2 Enterovirus 71

2.2.1 Taxonomy of enterovirus 71

Enterovirus 71 (EV71) belongs to human enterovirus species A of the genus *Enterovirus*, is a members of the *Picornaviridae* family. (Hsiung and Wang, 2000) EV71 is a single stranded RNA and nonenveloped viruses about 20-30 nanometer in diameter. EV71 contains a positive-strand RNA genome of approximately 7,400 nucleotides. (Shih *et al.,* 2011) The receptors for EV71 have been identified as P-selectin glycoprotein ligand-1 and scavenger receptor class B, member 2 (SCARB2); both are transmembrane proteins. The basic reproductive number (R0) for enterovirus 71 (EV71) was estimated to a median of 5.48 with an interquartile range of 4.20 to 6.51. (Wang and Liu, 2014)

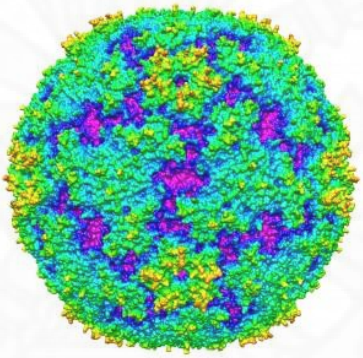

Figure 2-1 Structure of enterovirus 71 (Plevka *et al*., 2012)

2.2.2 Epidemiology of enterovirus 71

EV71 first appeared in California, USA in 1969 with caused sporadic cases or small outbreaks of hand, foot, and mouth disease (HFMD) and neurological disease. The first isolation of EV71 was in fecal matter of a baby with encephalitis. It spread to Europe with the first outbreak in Bulgaria in 1975 and then in Hungary in 1978. It has since spread to various countries in Asia where it has been responsible for several outbreaks. In 1973, EV71 was first appeared as causing epidemics of HFMD in Japan. The virus caused an unexpectedly large and severe outbreak in Sarawak, Malaysia, in 1997 with high mortality. Regular epidemics have since been seen in countries across the Asia-Pacific region, including an epidemic in Taiwan in 1998 that was thought to involve millions of people, and an outbreak of HFMD in China, during which

approximately 490,000 cases of EV71 infection and 128 deaths were reported. (Ooi *et al.*, 2010)

The epidemic situation of HFMD in Thailand has been reported that the morbidity rate of HFMD increased every year and was more severe in 2012-2014. The morbidity rate of HFMD in 2014 is eighty times higher than that in 2004. Six deaths were caused by HFMD in 2011 and two deaths last year. The highest morbidity rate occurs in children 0-6 years old and outbreaks frequently occur in the end of summer to the beginning of rainy season. Additionally, epidemiological studies indicate that HFMD caused by enterovirus71 infection is a global public health issue, especially in Asia-Pacific region. (Bureau of Epidemiology Annual, 2014)

2.2.3 Clinical symptoms of hand, foot, and mouth disease

Hand, foot, and mouth disease is an illness that is usually caused by coxsackievirus A16. Other types of coxsackievirus have also been associated with this disease, such as coxsackievirus A4, A5, A9, A10, B2, B5, and enterovirus 71 (EV71). (Grossman, 2012) EV71 infection is a particular concern as it can have more severe complications and sometimes death in children. (Ventarola *et al.*, 2015) Symptoms include sores in the mouth followed by a blistery rash on the hands and/or the feet. (Wen *et al.*, 2015) In addition, the buttocks are sometimes involved but the rash there tends not to be vesicular. Symptoms may also include a mild fever, sore throat, stomachache and diarrhea. Resolution is usually complete within a week.

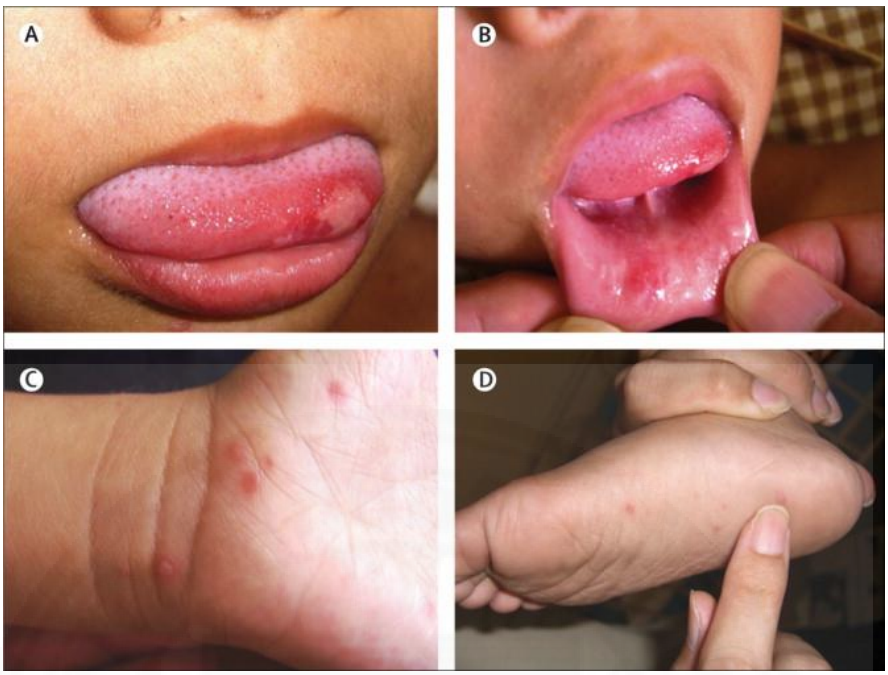

Figure 2-2 Ulcers on the tongue (A) and inside the lip (B), and vesicular and macular lesions on the wrists (C) and the soles (D) of children with hand, foot, and mouth disease caused by enterovirus 71. (Ooi *et al.*, 2010)

Nevertheless, EV71 infection can have more severe complications including secondary infections, pulmonary edema, aseptic meningitis or death in infants and young children. (Ji *et al*., 2015)

2.2.4 Treatment

Nowadays, There is no vaccine and specific therapy to be effective in prevention and treatment the hand, foot, and mouth disease that caused by EV71. (Chong *et al*., 2015) Symptoms typically clear up within seven to 10 days from the day of infection. The common therapies are treatment of the viral infections with broad-spectum antiviral drugs, including Ribavirin®, Ganciclovir®, and Acyclovir® which only partially alleviate the symptoms. In addition, symptomatic treatment is the primary care in HFMD such as taking analgesic medications to relief pain from the sores. (Solomon *et al.*, 2010)

2.3 Pathogenic microorganism

2.3.1 *Staphylococcus aureus*

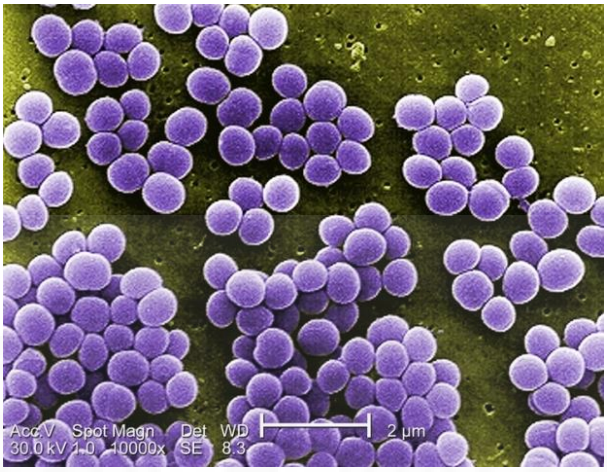

Figure 2-3 Scanning electron micrograph of *S.aureus*
(Available from http://www.bacteriainphotos.com/
Staphylococcus%20aureus%20electron%20microscopy.html, 2015)

Staphylococcus aureus is a facultative anaerobic gram-positive coccal bacterium which appears as grape-like cluster and belongs to the Staphylococcaceae family. (Murray *et al*., 2003) It is approximately 0.5-1.5 μm in diameter, non-motile, non-spore forming and catalase positive. *S. aureus* is part of human flora. It is found in the human respiratory tract and on the skin. (Kluytmans *et al*., 1997)

The pathogenic strains produce staphylococcal enterotoxins, toxic shock syndrome toxins-1 (TSST-1), exfoliative toxins, and cytolytic toxins. (Balaban and Rasooly, 2000) This bacterium is a leading cause of food poisoning from the consumption of food contaminated with enterotoxins. Certain strains of *S. aureus* produce the superantigen TSST-1. The clinical presentation is severe and acute symptoms include high fever, rash, nausea, vomiting, watery diarrhea and dehydration. (Parsonnet *et al*., 2008) Mortality is very high and death can occur within 2 hours. Scaled skin syndrome is caused by exfoliative toxins secreted on the epidermis and mostly affects neonates and young children. Other skin and wound infection are caused by Staphylococcal exfoliative toxins include blisters, pimples, furuncles, impetigo, folliculitis, abscesses, and the staphylococcal scalded-skin syndrome (SSSS) (Ladhani *et al*., 1999)

2.3.2 Methicillin-resistant *Staphylococcus aureus* (MRSA)

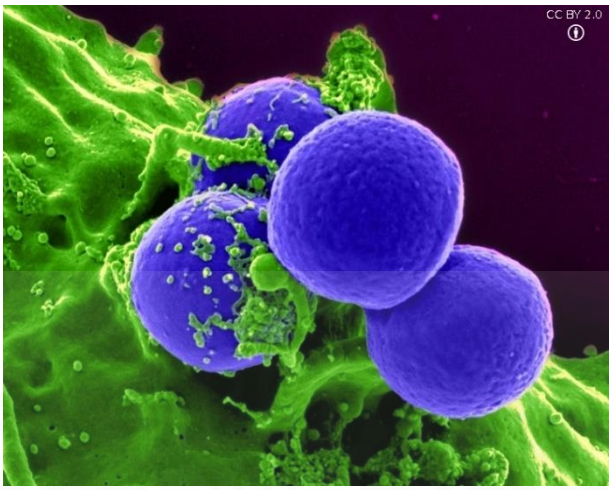

Figure 2-4 Scanning electron micrograph of MRSA
(Available from http://www.bacteriainphotos.com/
Staphylococcus_aureus_MRSA.html, 2015)

Staphylococcus aureus has developed to Methicillin-resistant *S. aureus* (MRSA) through the process of natural selection, resistance to beta-lactam antibiotics which include the penicillins, tetracyclines, sulfonamides, methicillin. (Becker and Sunderkotter, 2012) Therefore, MRSA infection is difficult to treat with standard antibiotics. Patients with compromised immune systems are at a significantly greater risk of symptomatic secondary infection. It is a worldwide problem in clinical medicine. MRSA began as a hospital-acquired infection, but has developed limited endemic status and is now sometimes community-acquired. (Peltroche-Llacsahuanga *et al*., 1998)

2.3.3 *Staphylococcus epidermidis*

Figure 2-5 Scanning electron micrograph of *Staphylococcus epidermidis* (Available from http://www.sciencephoto.com/media/390714/view, 2015)

S. epidermidis is a gram-positive coccal bacterium which arranged in grape-like cluster. It is approximately 0.5-1.5 μm in diameter, non-motile, catalase-positive and coagulase-negative. (Murray *et al*., 2003) S. epidermidis is a facultative anaerobe that can grow in aerobic conditions.

S. epidermidis is part of the normal human flora that found on the skin. S. epidermidis has become a leading cause of nosocomial infections. It produces a thick, multilayered biofilm allowing *S.epidermidis* to colonise polymer surfaces and consequently a wide range of implanted medical devices. *S. epidermidis* remains a clinically important pathogen. (Fey and Olson, 2010)

2.3.4 *Streptococcus pyogenes*

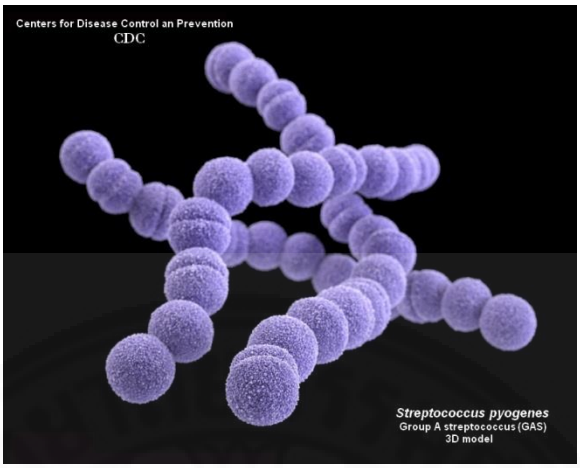

Figure 2-6 Scanning electron micrograph of *Streptococcus pyogenes* (Available from http://www.bacteriainphotos.com/ streptococcus_pyogenes_3D.html, 2015)

S. pyogenes is group A β-hemolytic streptococcus (GAS), aerobic gram-positive extracellular bacterium. It is non-motile, non-sporing cocci that are less than 2 μm in length and that form chains and large colonies greater than 0.5 mm in size. This bacterium belongs to the Streptococcaceae family. (Murray *et al*., 2003)

S. pyogenes is responsible for a wide array of infections. It can cause streptococcal sore throat which is characterized by fever, enlarged tonsils, tonsillar exudate, sensitive cervical lymph nodes and malaise. Scarlet fever (pink-red rash and fever) as well as impetigo (infection of the superficial layers of skin) and pneumonia are also caused by this bacterium. (Cohen *et al*., 2005) Septicaemia, otitis media, mastitis, sepsis, cellulitis, erysipelas, myositis, osteomyelitis, septic arthritis, meningitis, endocarditis, pericarditis, and neonatal infections are all less common infections due to *S. pyogenes*. (Madigan *et al*., 2006)

2.3.5 *Klebsiella pneumoniae*

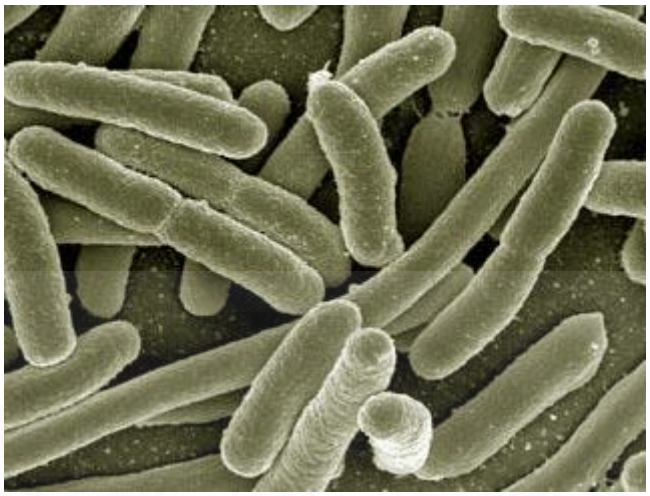

Figure 2-7 Scanning electron micrograph of *Klebsiella pneumoniae* (Available from http://klebsiella-pneumoniae.org/klebsiella_pneumoniae_urinary_tract_infection.html, 2015)

K. pneumoniae is a gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. This bacterium belongs to the Enterobacteriaceae family. (Murray *et al*., 2003)

K. pneumonia is an important cause of human infections. Although found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human lungs if aspirated, specifically to the alveoli resulting in bloody sputum. *K. pneumoniae* has been identified as an important common pathogen for nosocomial pneumonia, septicaemia, urinary tract infection, wound infections, intensive care unit (ICU) infections, and neonatal septicaemias. (Podschun and Ullmann, 1998)

2.3.6 *Candida albicans*

Figure 2-8 Scanning electron micrograph of *Candida albicans*
(Available from http://www.sciencephoto.com/media/677666/view, 2015)

C. albicans is a diploid fungus that grows both as yeast and filamentous cells. The yeast form is 10-12 µm in diameter. It belongs to the Saccharomycetaceae family. *C. albicans* appears as large, round, white or cream colonies with a yeasty odor on agar plates at room temperature. (Murray *et al.*, 2003)

C. albicans is a part of the normal human flora that is found in the mouth, gut, and vagina. Although *C. albicans* most frequently infects the skin and mucosal surfaces, it can cause systemic infections manifesting as pneumonia, septicaemia or endocarditis in severely immunocompromised patients. In addition, hospital-acquired infections by *C. albicans* have become a cause of major health concerns. (Hietala *et al*., 1982)

2.4 Nitric oxide and Fever

Fever is a phenomenon characterized by a raised thermoregulatory set point that leads to an elevation in body temperature. It is well known that fever can be initiated by a number of agents including lipopolysaccharide (LPS), viruses, yeast and Gram-positive bacteria. Considerable efforts have been made to identify the mechanisms of fever and it is generally believed that fever results from the induction of cytokines, such as interleukin (IL)-1β, IL-6, interferons, and tumor necrosis factor (TNF), and subsequent generation of prostaglandins (PGs) in the CNS, particularly prostaglandin E2 (PGE2), thought to act as a proximal mediator of fever (Blatteis *et al.*, 1998; Kluger, 1991).

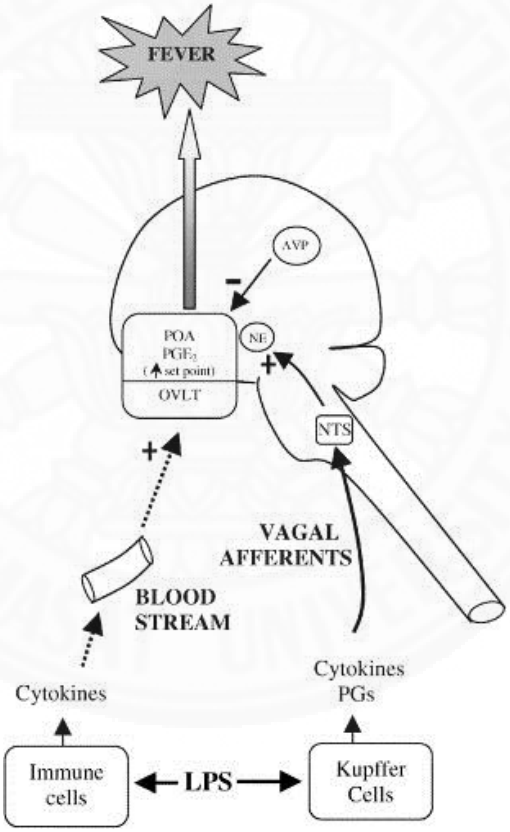

Figure 2-9 The mechanisms involved in fever. (Steiner and Branco *et. al.*, 2001)

NO participates in several systems that are involved in body temperature regulation under euthermic conditions. However, when a pyrogen is administered to an animal, a number of pathways in which NO might participate are thought to be activated. Actually, NO has been shown to participate in the febrile response by acting at both peripheral and central sites. (Steiner and Branco *et. al.*, 2001)

2.5 Kheaw-Hom remedy and its plant ingredients

2.5.1 *Angiopteris evecta* (G.Forst) Hoffm. (MARATTIACEAE)

Figure 2-10 *Angiopteris evecta* (G.Forst) Hoffm.

Common names: Wan gieb rad (ว่านกีบแรด)**,** Giant fern, King fern

Family: MARATTIACEAE

Botanical characteristics

A.evecta is tree growing to 120 cm high and 100 cm in diameter. On either side of the petiole insertion the rhizome bears two flat, rounded, dark brown, leathery, stipule-like outgrowths, ca. 10-15 cm long that bear proliferous buds and can grow into new plants when broken off. The petioles are thick and fleshy and can reach ca. 2 m long with a swollen base and bipinnate lamina which are glabrous, very large and spreading, usually to ca. 6 m long and to ca. 2.5-3 m broad. The pinnae are ca. 30 cm wide; ultimate segments (pinnules) are numerous, alternate, shortly stalked, commonly 10-13 cm long, 1.5-2.5 cm wide, linear, the base unequally wedge-shaped to more or less rounded, the margins serrate towards the apical part, the apices acuminate. Sporangia are clustered in short double-rows of three to seven with no indusium. (GISD, 2010)

Part used: Rhizomes

Traditional used

The plant is used to treat oral wound healing, fever, diarrhea, and as a diuretic. Rhizome is used to stop bleeding. Leaves are used to treat coughs. (Wutthithammawet, 2002)

2.5.2 *Cordyline fruticosa* (L.) A.Chev (Green leaves) (AGAVACEAE)

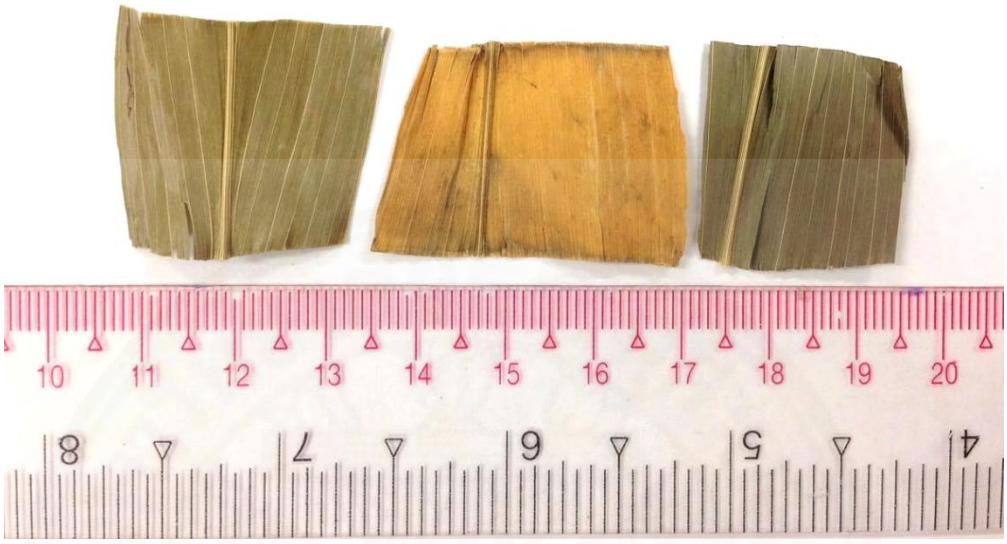

Figure 2-11 *Cordyline fruticosa* (L.) A.Chev (Green leaves)

Common names: Maak mia (Thai)

Family: AGAVACEAE

Botanical characteristics

C. fruticosa is an erect, smooth shrub which grows from 1 to 3 m high from tuberous roots. Stems are simple or somewhat branched, and marked with leaf-scars. Leaves are mostly near the apex of the stem, lanceolate to oblanceolate, and usually tinged with green, 30 to 50 cm long. Panicles are terminal, purplish, laxly branched; the branches up to 30 cm in length, and slender. Flowers are pink, and about 1 cm long, slender, tubular, with the perianth split to the middle into 6 equal lobes. Stamens are 6, ovary 3-celled, with 4 to 16 ovules. Fruits are globose berries, and about 5 mm in diameter, few or one-seeded. (Stuartxchange, 2014)

Part used: Leaves

Traditional used

Thai traditional medicine has used juice from leaves of *C. fruticosa* to treat colds, cough, and whooping cough. (Wutthithammawet, 2002)

2.5.3 *Cordyline fruticosa* (L.) A.Chev (Red leaves) (AGAVACEAE)

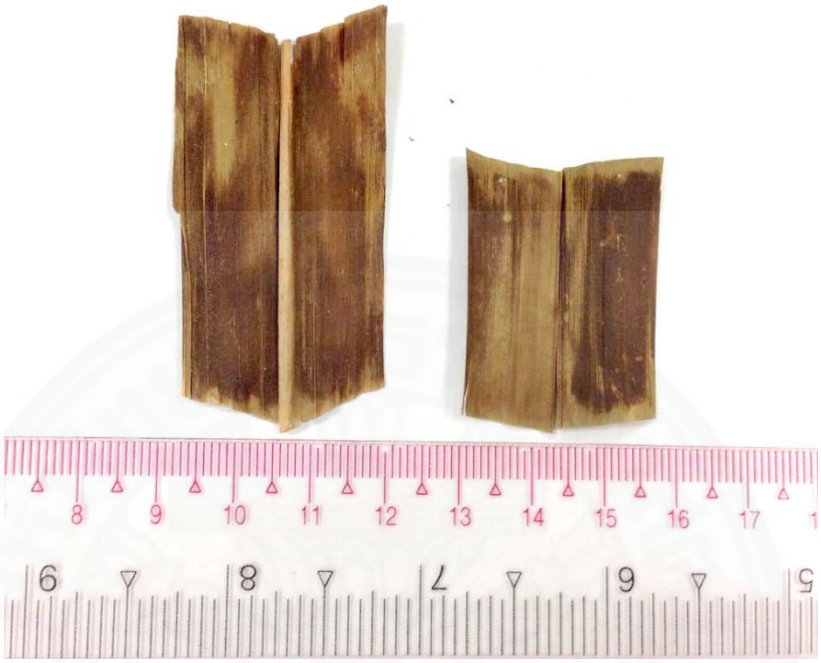

Figure 2-12 *Cordyline fruticosa* (L.) A.Chev (Red leaves)

Common names: Maak phu (Thai)

Family: AGAVACEAE

Botanical characteristics

C. fruticosa is an erect, smooth shrub which grows from 1 to 3 m high from tuberous roots. Stems are simple or somewhat branched, and marked with leaf-scars. Leaves are mostly near the apex of the stem, lanceolate to oblanceolate, and usually tinged with red or purple, 30 to 50 cm long. Panicles are terminal, purplish, laxly branched; the branches up to 30 cm in length, and slender. Flowers are pink, and about 1 cm long, slender, tubular, with the perianth split to the middle into 6 equal lobes. Stamens are 6, ovary 3-celled, with 4 to 16 ovules. Fruits are globose berries, and about 5 mm in diameter, few or one-seeded. (Stuartxchange, 2014)

Part used: Leaves

Traditional used

Thai traditional medicine has used juice from leaves of *C. fruticosa* to treat colds, cough, and whooping cough. (Wutthithammawet, 2002)

2.5.4 *Cyathea gigantea* Holtt. (CYATHEACEAE)

Figure 2-13 *Cyathea gigantea* Holtt.

Common names: Ma has sa dam (Thai)

Family: CYATHEACEAE

Botanical characteristics

C. gigantea is a fern and growing up to 2 m high. Petioles are 50 cm long, nearly black or deep castaneous, polished, densely covered with spreading scales. Scales up to 1.5 cm long, 2 mm broad, dark brown to nearly black, shining, stiff, edges pale, thinner, ragged and eroding with age, rather broad, pale. Pneumathodes are small, in a single row, distinct; main rachis castaneous to nearly black, minutely scaly, smooth. Pinnules about 2.5 cm apart, patent or ascending, straight or slightly falcate lanceolate, caudate-acuminate at apex, cordate at base, very shortly stalked, up to 12 cm long, 2 cm wide, lobed to more than 1/3 way towards costae. Lobes round subdeltoid, round at apex, oblique, falcate, serrate at margin, up to 4 mm broad, with narrow sinus. Texture thin, papyraceous, green, veins pinnate, veinlets simple, all free. Sori close to costule or medial, exindusiate. (Royal Botanic Garden Edinburgh, 2015)

Part used: Stem

Traditional used

Rhizome is used to treat fever, cough and to reduce pain. Wood is used for fever and diarrhea treatment. (Wutthithammawet, 2002)

2.5.5 *Dracaena loureiri* Gagnep. (DRACAENACEAE)

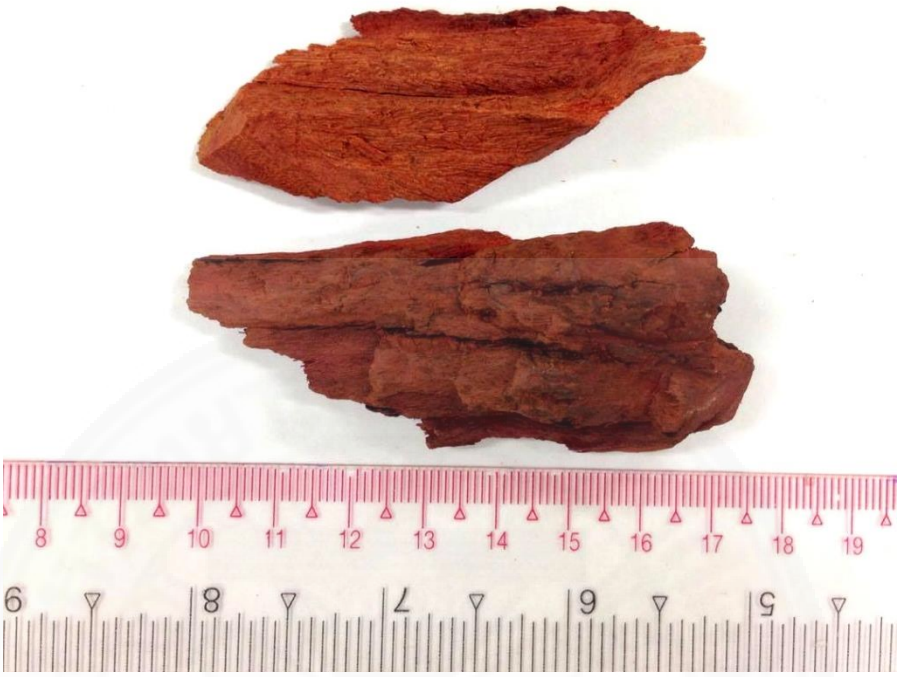

Figure 2-14 *Dracaena loureiri* Gagnep.

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

Family: DRACAENACEAE

Botanical characteristics

D. loureiri is a plant with grayish-white or brown, branched, woody stems. Leaves crowded at the apex of the branches, sessile, sword-shaped, and leathery. Inflorescence branches with flowers in clusters of 2-5. Flowers are bisexual, 3-merous with shortly pedicellate. Perainth is milky-white with a short tube and 3 similar lobes. 6 stamens are near the throat of the perianth. Ovary is superior, 3-loculed with ovules 1 or 2 per lobed stigma. Fruits are an orange sub-globose berry with 1-3 seeds.

Part used: Stem

Chemical constituents

The chemical constituents of *D. loureiri* are stillbenoids including 4,3',5'-trihydroxystilbene, 4,3'-dihydroxy-5'-methoxystilbene and 4-hydroxy-3', 5'-dimethoxystilbene. (Likhitwitayawuid *et al*., 2002)

Traditional used

It has been used as a folk medicine such as antipyretic, anti-inflammatory and for pain relief. Its root has been used for antidiarrhea. (Wutthithammawet, 2002)

2.5.6 *Eupatorium stoechadosmum* Hance. (ASTERACEAE)

Figure 2-15 *Eupatorium stoechadosmum* Hance.

Common names: San phra hom (Thai)

Family: COMPOSITAE (ASTERACEAE)

Botanical characteristics

E. stoechadosmum is an erect, leafy branched, smooth herb, 60 to 90 m high. Leaves are fragrant, up to 19 cm long, divided quite to the base into three segments, the upper leaves subtending the branches of the inflorescence being deeply divided. Segments are elliptic-lanceolate or elliptic-ovate, up to 13 cm long, pointed at both ends, and toothed at the margins. Inflorescence is terminal, measuring up to 14 cm across. Flowering heads are 3 to 4 mm across. Flowers are white and fragrant.

Part used: Leaves

Chemical constituents

The major constituents were found in the essential oil of *E. stoechadosmum* including thymohydroquinone dimethyl ether (73.6%), β-caryophyllene (8.9%) and selina-4,11-diene (11%). (Dung *et al.*, 1991)

2-Hydroxy-4-methylacetophenone, 8, 10-Epoxy-9-acetoxythymol angelate, 9-Isobutyryloxy-8, 10-dihydroxythymol and 9-Angeloyloxy-8, 10-dihydroxythymol was isolated from aerial parts of *E. stoechadosmum*. (Trang *et al*., 1993)

The coumarin contents in dichloromethane extract of *E. stoechadosmum* were found to be 0.44±0.02 and 0.45±0.04 g/100g by TLC-densitometry performed using winCATS software and image analysis using imageJ software (Pengnaummuang, 2014)

Traditional used

Folk doctors used the leaves of *E. stoechadosmum* to treat ulcer, fever, headaches and fractures. (Wutthithammawet, 2002)

2.5.7 *Globba malaccensis* Ridl. (ZINGIBERACEAE)

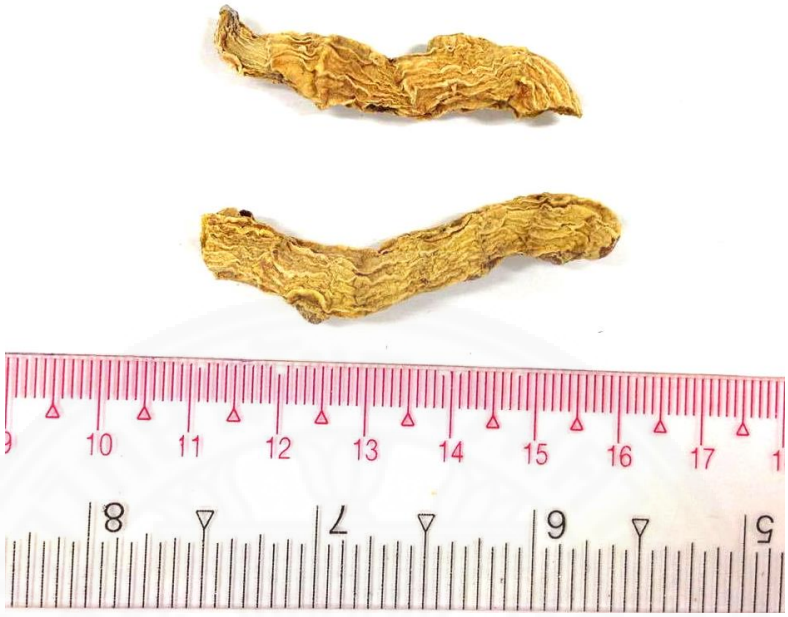

Figure 2-16 *Globba malaccensis* Ridl.

Common names: Wan ron thong (Thai)

Family: ZINGIBERACEAE

Botanical characteristics

G. malaccensis is a perennial plant growing to 1 m high. The leaves are alternate and arranged in rows. Leaf stalk is green and short about 5-7 cm. The leaf sheath of sapling plant is red. Leaves are lanceolate, long, petioled, tapering at each end, smooth, 10-15 cm wide, 30-35 cm long. Flowers are yellow. The roots have an aromatic and spicy fragrance.

Part used: Rhizomes

Traditional used

Folk doctors used the rhizomes of *G. malaccensis* to treat diarrhea and centipede or scorpion bites. (Wutthithammawet, 2002)

2.5.8 *Kaempferia galanga* Linn. (ZINGIBERACEAE)

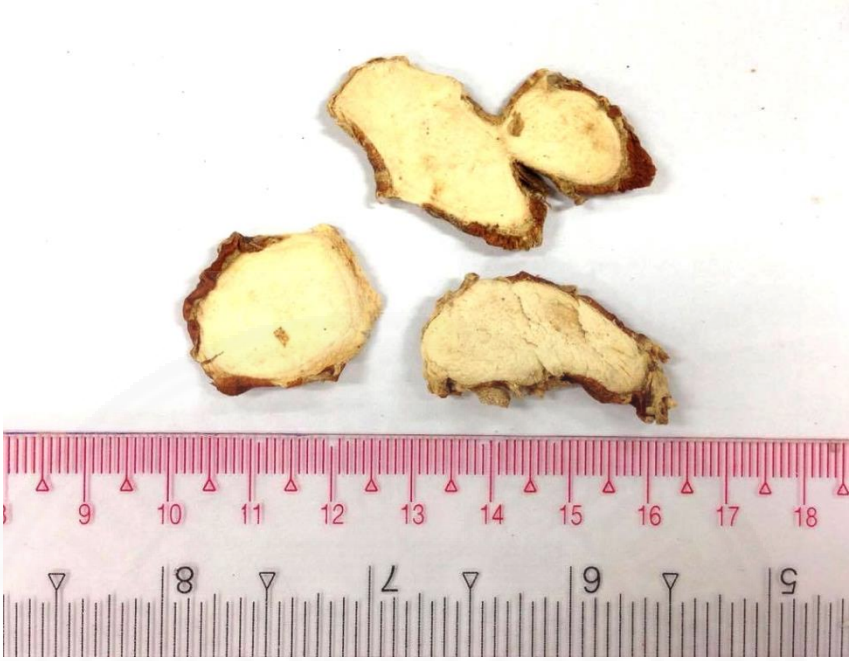

Figure 2-17 *Kaempferia galanga* Linn.

Common names: Proh hom, Wan hom (Thai), Cekur (Malaysia), Kencur (Indonesia), Shan jiang (Chinese)

Family: ZINGIBERACEAE

Botanical characteristics

K. galanga is a smooth, stemless herb arising from tuberous aromatic rootstocks with fibrous cylindric roots. Leaves are horizontally spreading, orbicular to broadly ovate, 7 to 15 cm long, with rounded base. Flowers are few, about 4 to 6 or more, with lanceolate bracts which are about 3.5 cm long. Corolla tube is slender, 2.5 to 3 cm long; with a lip cleft to the middle, about 2.5 cm wide, white or pale pink spotted with violet. Staminodes are obovate, about 1 to 2 cm long. Staminal crest is quadrate, and 2-lobed.

Part used: Rhizomes

Chemical constituents

Thirty-eight aroma compounds were found in volatile oil of the rhizome of *K. galanga* by using Gas chromatography-mass spectrometry (GC-MS). The major compounds were trans-ethyl-p-methoxycinnamate and trans-ethyl cinnamate. Other chemical compounds were δ-3-carene, 1,8-cineole, borneol, pentadecane (Raina and Abraham, 2016), methyl cinnamate, carvone, eucalyptol, pentadecane borneol, camphene, benzene and α-pinene (Tewtrakul *et al.*, 2005)

Traditional used

The rhizomes are used for inflammatory diseases, diabetes and obesity. Leaves are used for sore throat. (Wutthithammawet, 2002) Thai traditional medicine use rhizomes for treatment of scariasis, bacterial infections, tumor and it is also applied externally for abdominal pain in women and used topically for treatment of rheumatism. (Tewtrakul *et al.*, 2005)

2.5.9 *Limnophila rugosa* (Roth) Merr. (SCROPHULARIACEAE)

Figure 2-18 *Limnophila rugosa* (Roth) Merr.

Common names: Phak kra chom (Thai)

Family: SCROPHULARIACEAE

Botanical characteristics

L. rugosa is an erect herb reaching a height of about 50 cm Leaves are opposite, oblong-ovate, 3 to 10 cm long, 1.5 to 4 cm wide, pointed at both ends, and toothed at the margins. Upper surface of the leaves is rough. Flowers are about 1 cm long, purplish, and clustered on stems which are found in the axils of the leaves or which terminate the leafy branches.

Part used: Leaves

Chemical constituents

A flavones, 5-hydroxy-7,8,2′,4′-tetramethoxyflavone was isolated from petrol extract of aerial parts and roots. (Mukherjee *et al*., 2003)

The major constituents were found in the essential oil of the aerial parts of *L.rugosa* from foothills include methyl chavicol (76.6%) and (*E*)-anethole (19.1%).

Other constituents were pogostol (0.4%), linalool (0.1%), and (*Z*)-anethole (0.1%), *p*-anisal dehyde (0.1%) and (*E*) caryophyllene (0.1%). However, *L.rugosa* from mid-hills was rich in (*E*)-anethole (88.5%). Other constituents were (*Z*)-anethole (2.2%), anisyl acetone (1.5%), anisyl methyl ketone (1.4%), methyl chavicol (0.7%) and linalool (0.3%). (Verma *et al.*, 2014)

Traditional used

The leaves are antipyretic. The plant is used in the treatment of cough and cold. Decoction of leaves is used as an expectorant. (Wutthithammawet, 2002)

2.5.10 *Mammea siamensis* Kosterm. (GUTTIFERAE)

Figure 2-19 *Mammea siamensis* Kosterm.

Common names: Sarapi (Thai)

Family: GUTTIFERAE

Botanical characteristics

M. siamensis is a small evergreen, which grows up to 15 cm tall. Leaves simple, opposite, 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, 1-seeded.

Part used: Flowers

Chemical constituents

Four 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 *M. siamensis* flowers. (Mahidol *et al.*, 2002)

Beta-sitosterol and stigmasterol (steroids) and friedelin (terpenoid) were isolated from the chloroform extract of *M. siamensis* flowers by means of chromatography technique. (Subhadhirasakul and Pechpongs, 2005)

The chemical constituents of the hexane extract of *M. siamensis* flowers are six new compounds, deacetylinammea E/AC cyclo D, deacetylmammea E/AA cyclo D, deacetylmammea E/AB cyclo D, deacetylmammea E/BC cyclo D, deacetylmammea E/1313 cyclo D, and deacetylmammea E/BA cyclo D. (Mahidol *et al.*, 2007)

Traditional used

The flowers have traditionally been used as a heart tonic, reducing of fever**,** and enhancement of appetite. (Wutthithammawet, 2002)

2.5.11 *Mesua ferrea* Linn. (GUTTIFERAE)

Figure 2-20 *Mesua ferrea* Linn.

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

Family: GUTTIFERAE

Botanical characteristics

M.ferrea is a tree reaching up to 15 m tall, young shoots red or white. Leaves are sample, opposite, lanceolate or oblong-leanceolate, 2-4 cm wide, 7-12 cm long, coriaceous. Flower solitary, terminal or leaf-axil, white, fragrant, stamens numerous, yellow. Fruit is ellipsoid drupe.

Part used: Flowers

Traditional used

Folk doctors used the flowers of *M.ferrea* use to treat astringent, carminative, blood tonic and cardiac tonic. (Wutthithammawet, 2002)

2.5.12 *Mimusops elengi* Linn. (SOPOTACEAE)

Figure 2-21 *Mimusops elengi* Linn.

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

Family: SOPOTACEAE

Botanical characteristics

M. elengi is a medium-size evergreen tree reach up to 25 m high, lactiferous. Leaf is simple, alternate, ovate or elliptic, 3-6 cm wide, 5-12 cm long. Flowers solitary or 2-6 flowered fascicle, axillary, fragrant, white turning brown. Fruit is berry, ovoid, yellow or orange. Seeds are dark brown.

Part used: Flowers

Chemical constituents

There are 74 compounds of *M. elengi* flowers were identified by using GC-MS. The major compounds were 2-phenylethanol (37.80%), methyl benzoate

(13.40%), p-methylanisole (9.94%) and 2-phenylethyl acetate (7.16%). (Wong and Teng, 1994)

M. elengi exhibited the presence of flavonoids, alkaloids, glycosides and resins. Ursolic acid, β-sitosterol, lupeol, gallic acid, quercetin and kaempferol were major compounds that found in *M. elengi* flowers by using GC-MS. (Shailajan and Gurjar, 2015)

Traditional used

This flower is an ingredient in Ya-Hom used for cardiac tonic, treatment of sore and muscular pain. (Wutthithammawet, 2002)

2.5.13 *Myristica fragrans* Houtt. (MYRISTICACEAE)

Figure 2-22 *Myristica fragrans* Houtt.

Common names: Chan thet (Thai)

Family: MYRISTICACEAE

Botanical characteristics

M. fragrans is an aromatic evergreen tree growing 9-12 m high with spreading branches and a yellow fleshy fruit similar in appearance to an apricot or peach. Flowers are dioecious, small in axillary racemes. Peduncles and pedicles are glabrous. Fruit is a pendulous, globose drupe, consisting of a succulent pericarp - the mace arillus covering the hard endocarp, and a wrinkled kernel with ruminated endosperm. The seed or nutmeg is firm, fleshy, whitish, transversed by red-brown veins, abounding in oil. The fruit is gathered by means of a barb attached to a long stick. The mace is separated from the nut and both are dried separately.

Part used: Stem

Chemical constituents

Sabinene, terpineol and myristicin were major compounds that isolated from the essential oil of nutmeg seeds of *M. fragrans.* (Muchtaridi *et al.,* 2010)

Eugenol, methylisoeugenol, methyleugenol, sabinene and terpinen-4-ol were components of essential oil extracted from nutmeg seeds of *M. fragrans.* (Du *et al.*, 2014)

Traditional used

The plant is used as a stomachic stimulant, carminative and to treat intestinal catarrh and colic to stimulate appetite, and aromatic. (Wutthithammawet, 2002)

2.5.14 *Nelumbo nucifera* Gaertn. (NELUMBONACEAE)

Figure 2-23 *Nelumbo nucifera* Gaertn.

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

Family: NELUMBONACEAE

Botanical characteristics

N. nucifera is a perennial, aquatic herb with creeping rootstocks. Leaves are raised above the water, large, rounded, peltate, and 50 to 90 cm wide. Flowers are attractive, pink, red, or white, and 15 to 25 cm in diameter, standing out of the water. Flower has about 20 pink petals, 7 to 15 cm length. Center of the flower is a large structure shaped like an inverted cone, on top of which are located ovules. Around the inverted cone are numerous yellow stamens. Mature fruit is formed by the enlargement of the spongy, cone-shaped structure in the center of the flower. Rich carpel (fruit and seed in one) is about 13 mm long, with a black, bony and smooth pericarp.

Part used: Pollen

Traditional used

Folk doctors used the stemen of *N. nucifera* to treat cardiac tonic, vertigo and faintness (Wutthithammawet, 2002)

2.5.15 *Pogostemon cablin* (Blanco) Benth. (LAMIACEAE)

Figure 2-24 *Pogostemon cablin* (Blanco) Benth.

Common names: Phim sen ton (Thai), Pachouli (English)

Family: LABIATAE (LAMIACEAE)

Botanical characteristics

P. cablin is an aromatic, erect, branched and hairy herb, growing to a height of 0.5 to 1 m Leaves are oval in shape measuring about 10 cm long and wide, serrated with doted glands beneath sitting on a petiole that is about 8 cm long. Flowers are white and light purple, very small, crowded and borne in hairy, terminal, axillary spikes 2 to 8 cm long, 1 to 1.5 cm in diameter. Calyx is about 6 mm long. Corolla is 8 mm long, with obtuse lobes.

Part used: Leaves

Chemical constituents

The chemical constituents of *P. cablin* are patchouli alcohol, pogostone, friedelin, epifriedelinol, pachypodol, retusine, oleanolic acid, beta-sitosterol and

daucosterol. (Guan *et al.*, 1994) The chemical constituents from involatile moiety of *P.* cablin are epifriedelinol, 5-hydroxymethol-2-furfural, succinic acid, beta-sitosterol, daucosterol, crenatoside, 3'"-O-methylcrenatoside, isocrenatoside, and apigenin-7-O-beta-D-(6"-p-coumaryl)-glucoside. (Huang *et al.,* 2009) The nonvolatile chemical constituents of *P.* cablin are tilianin, diosmetin-7-O-beta-D-glucopyranoside, 3"-O-methylcrenatoside, uracil , soya-cerebroside I and II, agastachoside , apigenin-7-O-(3", 6"-di-(E) -p-coumaroyl) -beta-D-galactopyranoside, 5-hydroxy-3, 3', 4', 7-tetramethoxy flavone, 4', 5-dihydroxy-3, 3', 7-trimethoxyflavone, acacetin, crenatoside and isocrenatoside. (Wang *et al*., 2010)

Traditional used

P. cablin is cool flavor. Thai traditional medicine has used the leaves of *P. cablin* to treat fever, diuretic and carminative. (Ramya *et al*., 2013)

2.5.16 *Sophora exigua* Craib. (FABACEAE)

Figure 2-25 *Sophora exigua* Craib.

Common names: Phit sa naat (Thai)

Family: FABACEAE

Botanical characteristics

S. exigua is a shrub which grows to 15-30 cm high. Stem is very short. Leaves are pinnately compound, alternate and basal. Leaf shape is elliptic or ovate or ovate-oblong. At the end of leaf is obovate, 1.5-3 cm wide, 2-5 cm long. Leaf surface is white pubescent. Inflorescence is a raceme with many florets. Flowers are purple papalionaceous polypetalous corolla with long peduncle. Fruits are parallel-margin pod with white pubescent single seed.

Part used: Trunk

Chemical constituents

The chemical constituents of the roots of *S. exigua* are 5,7,2′-trihydroxy-8-lavandulylflavanone and maackiain, (2*S*)-5,7,2′,4′,6′- pentahydroxy-6-

lavandulylflavanone (exiguaflavanone C), (2*S*)-6-γ,γ-dimethylallyl-5,7,2′, 6′-tetrahydroxy-8-lavandulylflavanone (exiguaflavanone D), (2*S*)-5,2′,4′-trihydroxy-8-lavandulyl-7,5′-dimethoxyflavanone (exiguaflavanone E), 5,2′,5′-trihydroxy-8-lavandulyl-7- methoxyflavanone (exiguaflavanone F) and 5,7-dihydroxy-8-lavandulylbenzochromone (exiguachromone A). (Linuma *et al.*, 1993)

Traditional used

Folk doctors used *S. exigua* to treat fever, cough and increase breast milk. (Wutthithammawet, 2002)

2.5.17 *Tacca chantrieri* Andre. (TACCACEAE)

Figure 2-26 *Tacca chantrieri* Andre.

Common names: Na era phu sri (Thai)

Family: TACCACEAE

Botanical characteristics

T. chantrieri is a popular species and most commonly known for uniquely strange inflorescence shape and colour. This monocotyledon has long stalked, broad leaves of an olive- (dark) green colour growing up to 70 cm wide. The most eye-catching feature of the plant is its dramatic inflorescence. Its dark purple, maroon or black inflorescence can grow up to 50 cm wide, sometimes made up of 25 flowers. The inflorescence has two pairs of large spread, wing-like bracts with thread-like whiskers growing beneath them, known as bracteoles. The inflorescence also has smaller black flowers with 5 petals which hang like berries, giving it a bat shaped appearance.

Part used: Rhizomes

Chemical constituents

The chemical constituents of the rhizomes of *T. chantrieri* are diarylheptanoids, diarylheptanoid glucosides and steroidal saponins. (Yokosuka *et al*., 2002)

Traditional used

The plant is used to treat burns, gastric ulcers, stomach-aches and incised wounds. In addition, it is used to treat hypertension. (Wutthithammawet, 2002)

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

Figure 2-27 *Vetiveria zizanioides* (L.) Nash ex Small

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

Family: GRAMINEAE

Botanical characteristics

V. zizanioides is a coarse, erect, tufted perennial, growing 1 to 2 m high. Roots are fibrous and fragrant. Leaves are arranged in two rows, about 1 m long, 1 cm or less in width, and folded. Panicles are terminal, erect, purple or greenish, about 20 cm long; the branches are slender, whorled, spreading or ascending, 5 to 12 cm long. Sessile spikelets are about 4 mm long and muricate.

Part used: Roots

Chemical constituents

The volatiles from *V. zizanioides* in the roots was valencene, while in the shoots and leaves were 9-octadecenamide, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene and 1,2-benzendicarboxylic acid, diisooctyl ester. (Huang *et al*., 2004)

Traditional used

Folk doctors used the decoction of roots to dissolve or break kidney stones, and treat fever. (Wutthithammawet, 2002)

2.6 Biological activities of Kheaw-Hom remedy and its plant ingredients

Table 2-1 Biological activities of Ya-Kheaw and Kheaw-Hom remedies

Name	Activities	Biological activities	References
Ya-Kheaw remedy	Antiviral	The 20% ethanolic extract of the three most popular brand names of Ya-Kheaw remedy at 250 μg/ml significantly reduced varicella zoster virus infection in pre-treatment and no toxic to the IMR-90 cell line.	Sanguansermsri *et al*., 2005
Kheaw-Hom remedy	Antimicrobial	The 95% ethanolic extract of Kheaw-Hom remedy exhibited antimicrobial activity against *S.aureus,* MSRA and *S.epidermic* with MIC values of 31, 125 and 31 μg/ml.	Chusri *et al.,* 2014
	Cytoxicity	The 95% ethanolic extract of Kheaw-Hom remedy had no cytotoxic effects on Vero cells.	Chusri *et al.,* 2014
	Phytochemmical screening test	Kheaw-Hom remedy exhibited the presence of alkaloids, condensed tannins and hydrolysable tannins by using Dragendorff reagent and ferric chlorid reagent, respectively.	Chusri *et al.,* 2014

Table 2-2 Biological activities of *Angiopteris evecta* (G.Forst) Hoffm.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Angiopteris evecta (G.Forst) Hoffm. MARATTIACEAE	Antimicrobial	Tubers	The petrolic, dichloromethane, ethyl acetate, butanol and methanolic fraction of tubers of *A. evecta* inhibited *S. aureus* with inhibition zone of 8, 20, 20, 20 and 16 mm, inhibited *S.epidermidis* with inhibition zone of 10, 22, 18, 18 and 18 mm, inhibited *S.pneumoniae* with inhibition zone of 8, 22, 18, 18 and 18 mm, inhibited *K.pneumoniae* with inhibition zone of 10, 20, 18, 18 and 18 mm, respectively and did not inhibit *C.albicans* by disc diffusion method.	Khan and Omoloso, 2008
		Roots	The petrolic, dichloromethane, ethyl acetate, butanol and methanolic fraction of tubers of *A. evecta* inhibited *S. aureus* with inhibition zone of 14, 10, 8, 8 and 8 mm, inhibited *S.epidermidis* with inhibition zone of 16, 12, 10, 8 and 10 mm, inhibited *S.pneumoniae* with inhibition zone of 14, 12, 8, 8 and 8 mm, inhibited	Khan and Omoloso, 2008

Table 2-2 Biological activities of *Angiopteris evecta* (G.Forst) Hoffm.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
			K.pneumoniae with inhibition zone of 16, 10, 10, 10 and 10 mm, respectively and did not inhibit *C.albicans*	
	Phytochemical screening test	Tubers	The methanolic fraction of tubers of *A. evecta* exhibited positive results on alkaloids, flavonoids, saponins and tannins tests.	Khan and Omoloso, 2008
		Roots	The methanolic fraction of roots of *A. evecta* exhibited positive results on alkaloids, flavonoids, saponins, sterol, tannins and triterpenoids tests.	Khan and Omoloso, 2008
		Rhizomes	The methanolic extract of rhizome of *A. evecta* exhibited the presence of steroids, reducing sugars, sugars, alkaloids, phenolic compounds, flavonoids, saponins and anthraquinones on phytochemical screening.	Gracelin *et al.*, 2011

Table 2-3 Biological activities of *Cordyline fruticosa* (L.) A.Chev

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Cordyline fruticosa (L.) A.Chev AGAVACEAE	Antimicrobial	Leaves	Three new steroidal saponins, spirosta-5,25(27)-diene-1β,3β-diol-1-O-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside (fruticoside H) **1**, 5α-spirost-25(27)-ene-1β,3β-diol-1-O-α-L-rhamnopyranosyl-(1→2)-(4-O-sulfo)-β-D-fucopyranoside (fruticoside I) **2**, and (22S)-cholest-5-ene-1β,3β,16β,22-tetrol 1-O-β-galactopyranosyl-16-O-α-L-rhamnopyranoside (fruticoside J) **3**, were isolated from the leaves of *C. fruticosa*. All the isolated compounds were not able to inhibit *S. aureus* (MIC>256 mg/L) and *C.albicans* (MIC>256mg/L).	Fouedjou *et al.*, 2014
			The *Phyllosticta* fungus that isolated from *C. fruticosa* was not able to inhibit *S. aureus* by using disc diffusion method.	Chukeatirote *et al.*, 2014

Table 2-4 Biological activities of *Dracaena loureiri* Gagnep.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Dracaena loureiri Gagnep. DRACAENACEAE	Anti-HIV-1 integrase	Heart wood	The ethanol and water extracts of heart wood of *D. loureiri* exhibited anti-HIV-1 integrase activity with IC_{50} values of 28.0 and 22.1 μg/ml.	Bunluepuech and Tewtrakul, 2009
	Anti-inflammatory	Stems	The 95% ethanolic extract of *D. loureiri* showed anti-inflammatory effect by inhibition NO production (IC_{50} = 38.37±1.66 μg/ml).	Makchuchit, 2010
		Stillbenoids	The stillbenoids including 4,3',5'-trihydroxystilbene, 4,3'-dihydroxy-5'-methoxystilbene and 4-hydroxy-3', 5'-dimethoxystilbene were isolated form stem wood. The result found these compounds exhibited the enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) with IC_{50} value of 1.29 - 4.92 microM.	Likhitwitayawuid *et al*., 2002

Table 2-4 Biological activities of *Dracaena loureiri* Gagnep.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
	Antimicrobial	Stems	The 95% ethanolic extract of *D. loureiri* inhibited *S. aureus and S.aureus* MRSA with inhibition zone value of 9 and 16 mm. respectively by disk diffusion method., MIC value of 2.5 and 0.625 mg/ml and MBC value of 2.5 and 1.25 mg/ml.	Sattaponpan and Kondo, 2011
	Antipyretic	Stems	The methanolic fraction of *D. loureiri* exhibited antipyretic effect on brewer's yeast induced fever in rats.	Reanmongkol *et al.*, 2003

Table 2-5 Biological activities of *Globba malaccensis* Ridl.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Globba malaccensis Ridl. ZINGIBERACEAE	Anti-inflammatory	Rhizomes	The 95% ethanolic extract of *G. malaccensis* exhibited inhibitory effect on NO production with IC_{50} value of 8.15±0.01 μg/ml which more potent then indomethacin (IC_{50} = 20.32 μg/ml).	Anuthakoengkun and Itharat, 2014

Table 2-6 Biological activities of *Kaempferia galanga* Linn.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Kaempferia galanga Linn. ZINGIBERACEAE	Anti-inflammatory	Rhizomes	The 95% ethanolic extract of *K. galanga* showed anti-inflammatory effect by inhibition NO production (IC_{50} =30.30±1.23 μg/ml)	Makchuchit, 2010
			The chloroform extract of *K. galanga* (2 g/kg) showed the highest inhibition (42.9%) compared to control in carrageenan-induced paw edema model. In addition, the chloroform fraction (1g/kg) showed the highest inhibitory effect (51.9%) on carrageenan-induced edema and the hexane-chloroform subfraction was the most effective in inhibiting edema (53.7%).	Umar *et al.*, 2012
			The alcoholic extract of *K. galanga* at 600 mg/kg and 1200 mg/kg reduced edema in carrageenan induced paw edema model and cotton pellet granuloma model.	Vittalrao *et al*., 2011

Table 2-6 Biological activities of *Kaempferia galanga* Linn.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
			The essential oil of *K. galanga* inhibited *S. aureus* and *C. albicans* with inhibition zone of 12 and 31 mm which was stronger than standard antifungal Clotrimazole with inhibition zone of 25 mm by using disc diffusion method.	Tewtrakul *et al.*,2005
		Ethyl-*p*-methoxycinnamate	The 25, 50, 100, and 200 µg/ml of ethyl-*p*-methoxycinnamate (EMPC) showed inhibitory effect on IL-1 production with percent inhibition of 18.09, 29.23, 39.84 and 55.04%, respectively and showed IC_{50} values of 166.4 µg/ml. The concentrations of 50, 100, and 200 ug/ml of EPMC showed inhibitory effect on TNF-α production with percent inhibition of 53.25, 48.76 and 35.71 %, respectively and showed IC_{50} value of 96.84 µg/ml.Only 25 ug/ml of EPMC	Umar *et al.*, 2014

Table 2-6 Biological activities of *Kaempferia galanga* Linn.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
			showed inhibitory effect on NO production with percent inhibition of 12.4% in human macrophages cells. The concentrations of 200, 400, and 800 mg/kg of EPMC inhibited IL-1 in blood samples of rats by 11.35, 20.9 and 37.67%, respectively and inhibited TNF-α by 24.43, 37.95 and 57.40%, respectively by using the cotton pellet assay.	
		Ethyl-*p*-methoxycinnamate	Ethyl-*p*-methoxycinnamate (EPMC) inhibited COX-1 and COX-2 with % inhibition of 42.9 and 57.82%, respectively.	Umar *et al.*, 2012
			100, 200, 400 and 800 mg/kg of ethyl-*p*-methoxycinnamate (EPMC) inhibited the rat paw edema 800 mg/kg was not different from indomethacin.	Umar *et al.*, 2012

Table 2-6 Biological activities of *Kaempferia galanga* Linn.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
	Antimicrobial	Rhizomes	The 95% ethanolic extract of *K. galanga* inhibited *S. aureus* with inhibition zone value of 8 mm. by disk diffusion method, MIC value of 5 mg/ml and MBC value of 10 mg/ml.	Sattaponpan and Kondo, 2011
			Ethyl p-methoxycinnamate (EPMC) inhibited *S.aureus* and *C.albicans* with MIC values of 0.333 and 0.111 mg/ml.	Omar *et al.*, 2014
	Antipyretic	Rhizomes	The methanolic extract of *K.galanga* did not exhibit antipyretic activity in rat induced pyrexia by brewer's yeast injection.	Saewong, 2007

Table 2-7 Biological activities of *Limnophila rugosa* (Roth) Merr.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Limnophila rugosa (Roth) Merr. ZINGIBERACEAE	Antimicrobial	Aerial parts	The essential oils of the aerial parts of *L. rugosa* showed antimicrobial activity against *S.aureus* and *C.albicans* with inhibition zones of 6-27 mm and 6-37.5 mm by using disc diffusion method.	Linh and Thach, 2010
		Leaves	The methanolic extract of *L. rugosa* leaves at 25, 50, 100, and 250 μg/ml showed antimicrobial activity against *S.aureus* and *S.pyrogenes* with inhibition zone of 13, 16, 17, and 19 mm and 12, 13, 16, and 18 mm, respectively by using disc diffusion method.	Acharya *et al.*, 2014

Table 2-8 Biological activities of *Mammea siamensis* Kosterm.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Mammea siamensis Kosterm. GUTTIFERAE	Anti-inflammatory	Flowers	The 95% ethanolic extract of *M. siamensis* showed anti-inflammatory effect by inhibition NO production (IC_{50} = 74.62±8.77μg/ml).	Makchuchit, 2010
			A methanol extract of the flowers of *M. siamensis* was found to inhibit nitric oxide (NO) production in lipopolysaccharide-activated RAW264.7 cells. From the extract, mammeasins A (IC_{50}=1.8 muM), kayeassamins G (IC_{50}=0.8 muM) and mammea A/AD (IC_{50}=1.3 muM) were found to inhibit induction of inducible nitric oxide synthase (iNOS).	Morikawa *et al.*, 2012
	Antimicrobial	Flowers	The chloroform extract of *M. siamensis* flowers showed antimicrobial activity against *S. aureus* with inhibition zone of 7.8 mm and no inhibition zone against *C. albicans*.	Subhadhirasakul and Pechpongs, 2005

Table 2-8 Biological activities of *Mammea siamensis* Kosterm.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
			The 95% ethanolic extract of *M. siamensis* inhibited *S. aureus and S. aureus* MRSA with inhibition zone value of 10 and 10 mm. respectively by disc diffusion method., MIC value of 1.25 and 0.62 mg/ml and MBC value of 1.25 and 0.62 mg/ml.	Sattaponpan and Kondo, 2011

Table 2-9 Biological activities of *Mesua ferrea* Linn.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Mesua ferrea Linn. GUTTIFERAE	Anti-inflammatory	Flowers	The 95% ethanolic extract of *M. ferrea* showed anti-inflammatory effect by inhibition NO production (IC_{50} = 26.23±3.42μg/ml).	Makchuchit, 2010
	Antimicrobial	Flowers	The 95% ethanolic extract of *M. ferrea* inhibited *S. aureus and S. aureus* MRSA with inhibition zone value of 9 and 8 mm. respectively by disk diffusion method., MIC value of 0.62 and 0.31 mg/ml and MBC value of 0.62 and 0.62 mg/ml.	Sattaponpan and Kondo, 2011
	Immunomodulatory	Seeds	Mesuol isolated from *M. ferrea* seed oil showed immunomodulatory activity increase in antibody titer values in cyclophamide (50 mg/kg, i.p. 9^{th} and 16^{th} day) which was sensitized with sheep red blood cells on the 7^{th} and 14^{th} day of the experiment. Mesuol potentiated percentage of	Chahar *et al.*, 2012

Table 2-9 Biological activities of *Mesua ferrea* Linn.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
			neutrophil adhesion in neutrophil adhesion test in rats and phagocytosis in carbon clearance assay.	
	Immunomodulatory	Flower buds	ACII contain *M. ferrea* flower buds showed immunomodulatory activity on radiation induced immunosuppression. The lowered total white blood cell count was significantly increased. There was no significant change in the hemoglobin content of irradiated animals when compared with drug treated or normal animals.	Tharaka *et al.*, 2006

Table 2-10 Biological activities of *Mimusops elengi* Linn.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Mimusops elengi Linn. SOPOTACEAE	Anti-inflammatory	Flowers	The 95% ethanolic extract of *M. elengi* showed anti-inflammatory effect by inhibition NO production (IC_{50} = 69.24±5.30 μg/ml).	Makchuchit, 2010
	Antimicrobial	Flowers	The 95% ethanolic extract of M. elengi inhibited S. aureus and S. aureus MRSA with inhibition zone value of 8 and 9 mm. respectively by disk diffusion method., MIC value of 5 and 5 mg/ml and MBC value of 10 and 10 mg/ml.	Sattaponpan and Kondo, 2011
	Antipyretic	Flowers	The 70% ethanol extract of *M.elegi* at dose of 200 mg/kg deceased body temperature on brewer's yeast induced pyxeria in rats.	Purnima *et al.*, 2010

Table 2-11 Biological activities of *Nelumbo nucifera* Gaertn.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Nelumbo nucifera Gaertn. NELUMBONACEAE	Antimicrobial	Pollen	The essential oil of *N. nucifera* pollen was not able to inhibit *S. aureus, S. epidermidis,* and *K. pneumonia* by disc diffusion method.	Chaiyasit, 2009
			The 95% ethanolic extract of *N. nucifera* inhibited *S. aureus* MRSA with inhibition zone value of 9 mm. by disk diffusion method, MIC value of 10 mg/ml and MBC value of 10 mg/ml.	Sattaponpan and Kondo, 2011
	Antipyretic		The methanolic extract of *N. nucifera* showed significant decrease body temperature.	Mukherjee et al., 1996
	Immunomodulatory		% neutrophil adhesion was increased.	Mukherjee *et al.*, 2010

Table 2-12 Biological activities of *Pogostemon cablin* (Blanco) Benth.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Pogostemon cablin (Blanco) Benth. LABIATAE	Anti-influenza	Patchouli alcohol	Patchouli alcohol showed activity against H1N1, reduced the number of plaque by 75% at 2 µg/ml and 89% at 10 µg/m by plaque forming assay	Kiyohara *et al.*, 2012
			Patchouli alcohol inhibited the expression of cytokines IL-4 and IFN-γ after 16HBE (human respiratory epithelial cell) was infected by H1N1	Wu *et al.*, 2013
	Anti-inflammatory	Patchouli alcohol	Patchouli alcohol which is extracted from *P. cablin* inhibited the over-expression of iNOS and IL-6 in protein and mRNA levels in LPS-stimulated RAW264.7 and TNF-alpha stimulated HT-29 cells. In addition Patchouli alcohol inhibited IkappaB-alpha degradation and p65 nuclear translocation. These studies suggest that Patchouli alcohol shows anti-inflammatory	Jeong *et al.*, 2013

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
			activities through suppressing ERK-mediated NF-jB activation and subsequent down-regulation of inflammation cytokines in mouse macrophage and human colorectal cancer cells.	
	Anti-inflammatory	Patchouli alcohol	10-40 mg/kg of PA inhibited the ear edema induced by xylene in mice and the paw edema induced by carrageenin in rats and decreased the production of TNF-α, IL-1, PGE_2 and NO.	Yu *et al.*, 2011
		Leaves	The methanol extract of *P. cablin* was able to reduce the edema in Carrageenan-induced mouse paw edema within 3-4 hours after the Carrageenan injection.	Lu *et al*., 2011
	Antimicrobial	Patchouli alcohol	Patchouli alcohol inhibited both gram negative and gram positive bacteria, against MRSA (*in vitro*) and MRSA *(in vivo)*	Wan *et al*., 2016

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
	Immunomodulatory	Patchouli alcohol	Patchouli alcohol (40 or 80 mg/kg) increased the phagocytic index. PA (80 mg/kg) boosted the production of circulating serum IgM and IgG.	Jin *et al.*, 2013

Table 2-13 Biological activities of *Sophora exigua* Craib.

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Sophora exigua Craib**.** GRAMINEAE	Antimicrobial	5,7,2′,4′-Tetrahydroxy-8-lavandulyl-flavanone	5,7,2′,4′-Tetrahydroxy-8-lavandulyl-flavanone, isolated from *Sophora exigua*, showed antimicrobial activity against 21 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) at concentrations of 3.13–6.25 μg/ml.	Sato *et al.*, 1995

Table 2-14 Biological activities of *Vetiveria zizanioides* (L.) Nash ex Small

Scientific Name	Activities	Part of used/Bioactive compounds	Biological activities	References
Vetiveria zizanioides (L.) Nash ex Small GRAMINEAE	Antimicrobial	Roots	The methanolic extract of *V. zizanioides* collected in winter showed antibacterial activity by agar dilution method against *Staphylococcus aureus* at 250 µg/ml. In addition, *Vetiveria zizanioides* collected in spring showed antifungal activity against *Candida albicans* at 250 µg/ml.	Dos *et al*., 2014

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Materials

3.1.1 Chemicals and reagents

3.1.1.1 Extraction

Table 3-1 List of chemicals and reagents of extraction

Name	Source
Ethanol 95%, commercial grade	C.M.J Anchor company, Thailand
Deionized water	Milford, USA

3.1.1.2 Quality control

(1) Acid insoluble ash

Table 3-2 List of chemicals and reagents of quality control

Name	Source
Hydrochloric acid (HCl)	Merck, Germany
Deionized water	Milford, USA

(2) Extractive value

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

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

3.1.1.3 Antiviral activities

(1) Antiviral activity based CPE assay

Table 3-5 List of chemicals and reagents of antiviral activity based CPE assay

Name	Source
Amphotericin B	Pacific Health Care, Thailand
Deionized water	Milford, USA
Dimethyl sulfoxide (DMSO)	RCL Labscan, Thailand
Earle's minimal essential medium (EMEM)	Gibco, USA
Fetal bovine serum (FBS)	Gibco, USA
Gentamicin	Vesco Pharmaceutical, Thailand
L-glutamin	Hyclone, USA
Penicillin	General Drugs House, Thailand
Phosphase-buffered saline (PBS)	Gibco, USA
Ribavirin	Sigma-Aldrich, USA
Sodium bicarbonate ($NaHCO_3$)	Amresco, India
Trypsin-EDTA	Gibco, USA

3.1.1.4 Antimicrobial activities

(1) Disc diffusion method

Table 3-6 List of chemicals and reagents of disc diffusion method

Name	Source
Brain Heart Infusion Agar	Difco, USA
Brain Heart Infusion Broth	Difco, USA
Dimethyl sulfoxide (DMSO)	RCL Labscan, Thailand
Distilled water	Milford, USA
Mueller Hinton Agar	Difco, USA
Mueller Hinton Broth	Difco, USA
Nutrient Agar	Difco, USA
Sabouraud Dextrose Agar	Difco, USA

(2) Microtitre plate-based antimicrobial assay

Table 3-7 List of chemicals and reagents of microtitre plate-based antimicrobial assay

Name	Source
Brain Heart Infusion Agar	Difco, USA
Brain Heart Infusion Broth	Difco, USA
Dimethyl sulfoxide (DMSO)	RCL Labscan, Thailand
Distilled water	Milford, USA
Mueller Hinton Agar	Difco, USA
Mueller Hinton Broth	Difco, USA
Nutrient Agar	Difco, USA
Resazurin sodium salt	Sigma-Aldrich, USA
Sabouraud Dextrose Agar	Difco, USA

3.1.1.5 Anti-inflammatory activities

(1) Assay for NO inhibitory effects

Table 3-8 List of chemicals and reagents of assay for NO inhibitory effect

Name	Source
Dimethyl sulfoxide (DMSO)	RCL Labscan, Thailand
Distilled water	Milford, USA
Fetal bovine serum (FBS)	Biochem, Germany
Hydrochloric acid (HCl)	Univar, Australia
Isopropanol	RCI labscan, Thailand
Lipopolysaccharide from *E.coli*O55:B5 (LPS)	Sigma, USA
N-(1-Naphthyl)ethylenediamine dihydrochloride	Sigma, USA
Pennisilin-Streptomycin (P/S)	Sigma, USA
Phosphase buffered saline (PBS)	Amresco, USA
Phosphoric acid 85% (H_3PO_4)	Sigma, USA
Prednisolone ≥ 90 %	Sigma, USA
RPMI medium 1640	Gibco, USA
Sodium bicarbonate ($NaHCO_3$)	BHD, England
Sodium hydroxide (NaOH)	Univar, Australia
Sulfanilamide ($H_2NC_6H_4SO_2NH_2$)	Sigma, USA
Thiazolyl blue tetrazolium bromide (MTT)	Sigma, USA
Trypan blue 0.4%	Gibco, USA
Trypsin-EDTA	Gibco, USA

3.1.2 Instruments

Table 3-8 List of instruments, plastic, and glass wares

Name	Source
75 cm^2 plastic tissue culture flasks	Costar Corning, USA
96-well microplates flat, bottom with lid	Costar Corning, USA
96-well microplates flat, bottom without lid	Costar Corning, USA
96-well microplates U, bottom with lid	Costar Corning, USA
Autoclave	Hirayama, Japan
Balance 0.01 mg-41 g	Mettler-Toledo, Swizerland
Balance 0.01 g-220 g	Precica, Switzerland
Balance 0.5 g-3100 g	Mettler-Toledo, Swizerland
Buchner Funnel	Schott Duran, Germany
Cell culture flask, canted neck 75 cm^3	Costar Corning, USA
Centrifugulation	Beckman Coulter, USA
Centrifuge tube 15, 50 ml	Costar Corning, USA
CO_2 humidified incubator	Shel lab, USA
Crusibles	Coorstex, USA
Disposable pipatte 2, 5, 10, 25 mL	Costar Corning, USA
Erlenmeyer flasks	Schott Duran, Germany
Eppendrofs	Costar Corning, USA
Examination glove	Sritrang gloves, Thailand
Filter paper no.1 (125 mmØ)	Whatman, USA
Filter paper no.40 (125 mmØ)	Whatman, USA
Freezer	Sanyo, Japan
Glass bottle 50, 250, 500, 1000 mL	Schott Duran, Germany
Glasswares 10, 25, 50, 100, 250, 600, 1000 mL	Schott Duran, Germany Pyrex, USA
Hematocytometer	Boeco, Germany
Hot air oven	Memmert, germany
Hot plate	Thermolyne, USA
Incubated tabletop orbital shaker	Thermo Scientific, USA

Table 3-8 List of intruments, plastic, and glass wares (continued)

Name	Source
Inverted microscope	Nikon, Japan
Laminar air flow	Boss tech, Thailand
Liquid nitrogen tank	Taylor-Wharton, USA
Lipophilizer	Telster, Spain
Litmus paper pH-fix 4.5-10.0	Macherey-Nagel, Germany
McFarland densitometer	Grant-Bio, England
Membran filter with pore-size rating of 0.22 micron	Millipore, Germany
Micropipettes 20 µl, 200 µl, 1000 µl	Gibson, USA
Microplate reader	Bio Tek, USA
Moisture analyzer	Scaltec instrument, Germany
Muffle furnace	Nabertherm, Germanny
Multi-channels pipette	Costar Corning, USA
Paper discs (0.6 cm diameter)	Whatman, USA
Petri dish	Hycon, USA
pH buffer	Thermo Scientific, USA
pH meter	WTW inolab, Germany
Pipette tips	Costar Corning, USA
Pipetteboy	Integra biosciences, Switzerland
Reagent reservoir (Sterile)	Costar Corning, USA
Refrigerrator (4^{o}C)	Sharp, Japan
Refrigerrator (-20^{o}C)	Sanyo, Japan
Rotary evaporator	Buchi, Switzerland
Shaking incubator	Vision Scientific, Korea
Sonicator	Elma, Germany
Stability incubator	Termarks, Norway
Syringes	Nipro, Thailand
Vacuum desiccator	Simax, USA
Vacuum pump	Rocker, Taiwan

Table 3-8 List of intruments, plastic, and glass wares (continued)

Name	Source
Vortex mixer	Scientific industries, USA
Water bath	Memmert, Germany
Water purification machine	Elga, UK

3.1.3 Plant materials

Kheaw-Hom remedy consists of 18 herbs. Each herb was purchased from Charoensuk Osot Pharmacy. The voucher specimens were referenced from the herbarium of Thai Medicinal Plants at Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla Province, Thailand. The herbal plants detail are shown in Table 3-9

Table 3-9 List of plant materials in Kheaw-Hom remedy

Scientific Name	Family Name	Voucher specimen number	Thai name	Part used	Flavor	% in remedy
Angiopteris evecta (G.Forst) Hoffm.	MARATTIACEAE	SKP 110-1 01 05 01	ว่านกีบแรด	Rhizome	Flavorless	5.56
Cordyline fruticosa (L.) A.Chev (Green leaf)	AGAVACEAE	SKP 005 03 06 01	หมากเมีย	Leaf	Flavorless	5.56
Cordyline fruticosa (L.) A.Chev (Red leaf)	AGAVACEAE	SKP 005 03 06 01	หมากผู้	Leaf	Flavorless	5.56
Cyathea gigantea Holtt.	CYATHEACEAE	SKP 059 03 07 01	มหาสดำ	Stem	Cool	5.56
Dracaena loureiri Gagnep.	DRACAENACEAE	SKP 065 04 12 01	จันทน์แดง	Stem	Bitter	5.56
Eupatorium stoechadosmum Hance	COMPOSITAE	SKP 051 05 19 01	สันพร้าหอม	Leaf	Cool& Flavorless	5.56
Globba malaccensis Ridl.	ZINGIBERACEAE	SKP 206 07 13 01	ว่านร่อนทอง	Rhizome	Hot& Fragrant	5.56
Kaempferia galanga Linn.	ZINGIBERACEAE	SKP 206 11 07 01	เปราะหอม	Rhizome	Hot& Fragrant	5.56
Limnophila rugosa Merr.	SCROPHULARIACEAE	SKP 177 12 18 01	ผักกระโฉม	Leaf	Cool& Fragrant	5.56
Mammea siamensis Kosterm.	GUTTIFERAE	SKP 083 13 19 01	สารภี	Flower	Cool& Fragrant	5.56
Mesua ferrea Linn.	GUTTIFERAE	SKP 083 13 06 01	บุนนาค	Flower	Cool& Fragrant	5.56
Mimusops elengi Linn.	SAPOTACEAE	SKP 171 13 05 01	พิกุล	Flower	Cool& Fragrant	5.56
Myristica fragrans Houtt.	MYRISTICACEAE	SKP 121 13 06 01	จันทน์เทศ	Stem	Hot& Fragrant	5.56

Table 3-9 List of plant materials in Kheaw-Hom remedy (Continued)

Scientific Name	Family Name	Voucher specimen number	Thai name	Part used	Flavor	% in remedy
Nelumbo nucifera Gaertn.	NELUMBONACEAE	SKP 125 14 14 01	บัวหลวง	Pollen	Astringent& Fragrant	5.56
Pogostemon cablin (Blanco) Benth.	LABIATAE	SKP 095 16 03 01	พิมเสนต้น	Leaf	Cool& Fragrant	5.56
Sophora exigua Craib	FABACEAE	SKP 072 19 05 01	พิษนาศน์	Trunk	Bitter	5.56
Tacca chantrieri Andre	TACCACEAE	SKP 189 20 03 01	เนระพูสี	Rhizome	Astringent	5.56
Vetiveria zizanioides (L.) Nash ex Small	GRAMINEAE	SKP 081 22 26 01	แฝกหอม	Root	Cool& Fragrant	5.56
Kheaw-Hom	-	-	เขียวหอม	-	**Bitter&Cool**	**100**

3.2 Methods

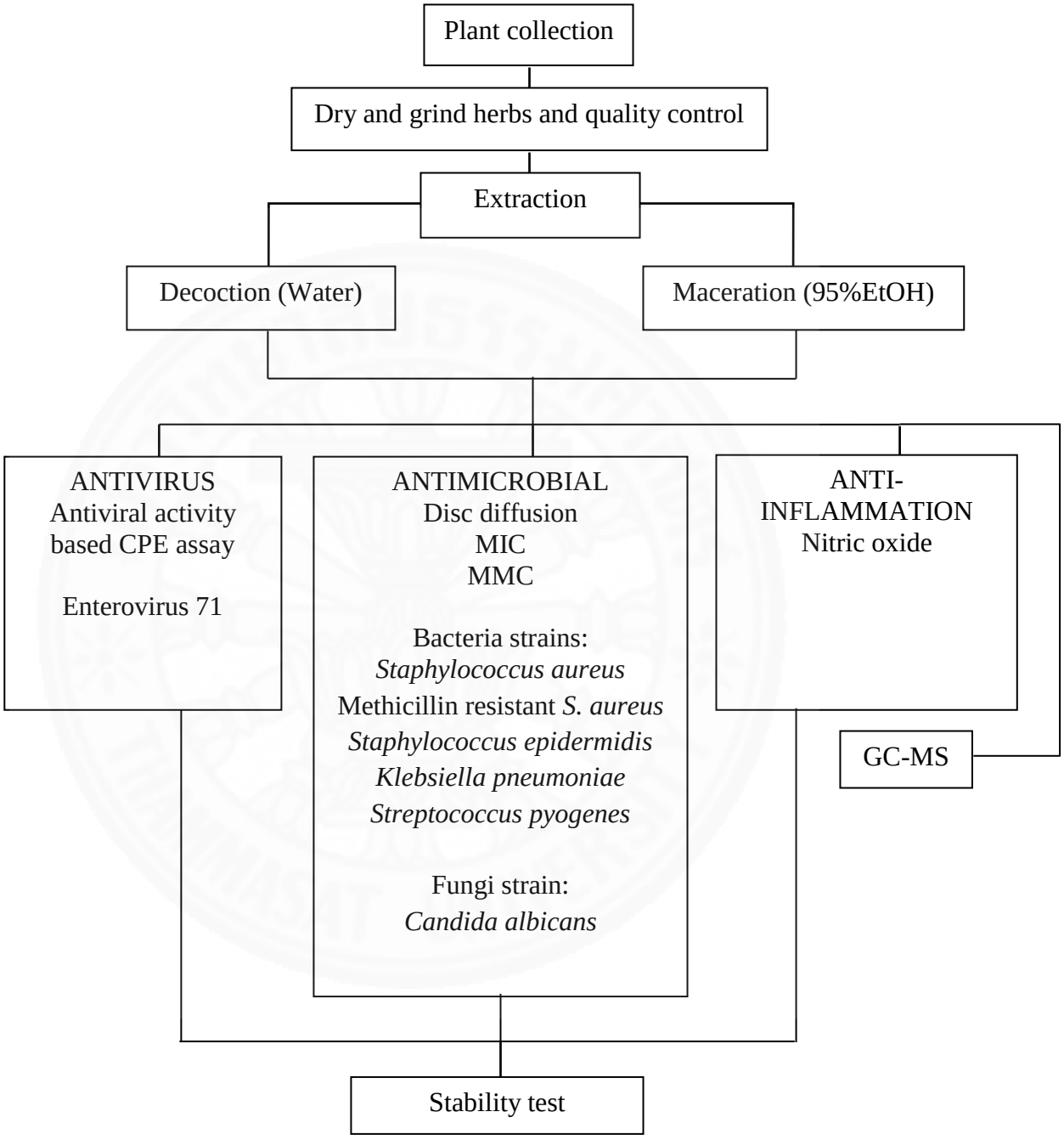

Figure 3-1 Conceptual framework of thesis

3.2.1 Preparation of crude extracts

Plant materials were cleaned with water, sliced into small pieces and dried in a hot air oven at 50^{o}C. These were ground to crude powder. These plant ingredients were weighed and mixed to the Kheaw-Hom formula. The preparation and each plant were macerated in 95% ethanol and decocted in distilled water.

3.2.1.1 Maceration

The crude powder of the remedy and its ingredients were macerated in 95% ethanol for 3 days and filtered through a Whatman No.1 filter paper. Filtrate was dried by rotary evaporator. The maceration was repeated twice with residue and dried again by vacuum drying. Percentage yields of all the ethanolic extracts were calculated.

3.2.1.2 Decoction

The crude powder of remedy and its ingredients were boiled in distilled water for 15 minutes and filtered through a Whatman No.1 filter paper. This boiling was repeated twice with the residue and dried by lyophilizer. Percentage yields of all the aqueous extracts were calculated using the following equation:

$$\%\text{Yield} = \frac{\text{Weight of the extract (g)}}{\text{Weight of dried powder (g)}} \times 100$$

The crude extracts were kept in a freezer (-20^{o}C) until use.

3.2.2 Quality control

The quality control methods were performed following Thai Herbal Pharmacopeia. Moisture content, total ash, acid insoluble ash and extractive value were determined. These methods were carried out in triplicate.

3.2.2.1 Loss on drying

Moisture content is one of the most important for standardization of herbal materials because it affects the quality of raw material and storage. The method was to analyze loss on drying by an electric moisture analyzer. Two grams of the sample is put on moisture analyzer at 105^{o}C. The weight of dried sample is displayed and loss on drying is calculated using the following equation:

$$\% \text{ Moisture content} = \frac{\text{Weight of beginning sample (g)- Weight of drying sample (g)}}{\text{Weight of beginning sample (g)}} \times 100$$

3.2.2.2 Total ash

This method investigated the physiological ash and non-physiological ash or inorganic compounds that contaminate the raw material. The crucible was dried until the weight of crucible was stable. Two grams of sample was weighed in crucible and burned in a muffle furnace at 450^{o}C until the ash was changed to grey or white and then the crucible was put in a desiccator until cool and weighed. This process was repeated until the weight was constant. Total ash was calculated using the following equation:

$$\% \text{ Total ash} = \frac{\text{Weight of beginning sample (g)- Weight after burning sample (g)}}{\text{Weight of beginning sample (g)}} \times 100$$

3.2.2.3 Acid insoluble ash

This method was continued from total ash method. Twenty five ml of 10% Hydrochloric acid (HCl) was boiled with the ash for 5 minutes and filtered through Whatman ashless filter paper No. 42. Residue was washed to pH 7 with distilled water. The ashless filter paper was dried and burned at 500^{o}C in a muffle furnace for 9 hours, repeating until the weight is constant. Acid insoluble ash was calculated using following equation:

$$\% \text{ Acid insoluble ash} = \frac{\text{Weight of beginning sample (g)- Weight after burning sample (g)}}{\text{Weight of beginning sample (g)}} \times 100$$

3.2.2.4 Extractive value

The ethanol soluble (ethanolic extract) and water soluble (aqueous extract) were evaluated for extractive value. Five grams of the dried powder was macerated in Erlenmeyer flask. 100 ml of 95% ethanol was added for ethanolic extract and 100 ml of chloroform water added for aqueous extract. The flask was shaken for 6 hours and allowed to stand at room temperature for 18 hours, then filtered 20 ml of filtrate from each extract was evaporated and dried at 105^{o}C. This process was repeated until the

weight was constant. Percentage yields of all extracts were calculated using the following equation:

$$\%\text{Yield} = \frac{\text{Weight of the extract (g)}}{\text{Weight of dried powder (g)}} \times 100$$

3.2.3 Antiviral activities

3.2.3.1 Animal cell lines

African green monkey kidney epithelial cell line (Vero cell) was obtained from American Type Culture Collection (ATCC CCL-81). This cell line was cultured in Earle's minimal essential medium (EMEM) containing L-glutamine, penicillin, gentamycin, fungizone and supplemented with 10% heat-inactivated fetal bovine serum. The cells was incubated at 37^{o}C in a 5% CO2 incubator and subpassaged twice a week.

3.2.3.2 Virus propagation

The enterovirus 71 was propagated in Vero cells maintained in EMEM plus 2% FBS at 37^{o}C in5% CO_2 incubator. The infected culture was observed daily for the appearance of cytopathic effect (CPE). The culture supernatant was harvested when the infected cell showed 3+ to 4+ degree of CPE by spinning at 2,000 rpm for 10 minutes at 4^{o}C. The supernatant was collected and aliquoted into vials, then kept at -80^{o}C as virus stocks until used.

3.2.3.3 Preparation of sample solution

The 95% ethanolic extracts were dissolved in sterile dimethyl sulfoxide (DMSO) to a final concentration of 50 mg/ml but the aqueous extracts were dissolved in sterile distilled water to a final concentration of 10 mg/ml and filtered with Millipore filter 0.22 μm. Ribavirin (positive control) was dissolved in sterile distilled water to a final concentration of 1 mg/ml and filtered with Millipore filter 0.22 μm. Each stock solution was diluted with 1X EMEM to obtain final concentrations of 800, 400, 200, 100 and 50μg/ml.

3.2.3.4 Antiviral activity based CPE assay

(1) Virus titration

The virus stock was titrated in quadruplicate in 96-well culture plate to determine 50% tissue culture infectious dose ($TCID_{50}$) by Reed and Muench method. Vero cells were cultured in the 96-well flat-bottom sterile plate (4×10^4 cells/well) with 200 µl each well and incubated for 24 hours at 37°C in 5% CO_2 incubator. The virus stock was diluted to 1:10 with 1X EMEM in a microcentrifuge tube (virus 70 µl + 1X EMEM 630 µl). 1X EMEM was added into the 96-well U-bottom sterile plate with 100 µl each well, except the wells in column 1 and control wells. The diluted virus stock was added in column 1 with 146 µl each well, serially transferred 46 µl from column 1 to 2, 3, …, to 11, respectively and 46 µl discarded from each well in the last column. 100 µl of 1X EMEM was added into each well and incubated for 2 hours at 37°C in 5% CO_2 incubator. 150 µl of media from Vero cultured plate which cultured in the 96-well plate for 24 hours was discarded from each well, then 50 µl of 1X EMEM was added into each well except control wells, which received 150 µl. 100 µl of each virus dilution was transferred into the Vero cultured plate and incubated for 5 days at 37°C in 5% CO_2 incubator. The infected cells were observed for CPE appearance under inverted microscope.

(2) Antiviral activity based CPE assay

Vero cells were cultured in the 96-well flat-bottom sterile plate (4×10^6cells/plate) and incubated for 24 hours at 37°C in 5% CO_2 incubator. The virus stock was diluted in 1X EMEM to obtain the concentrations of 200, 100 and 50 $TCID_{50}$/100 µl. The extracts were diluted with 1X EMEM in a serial 2-fold dilution beginning from the highest concentration that showed no toxicity to the Vero cells. 60 µl of the diluted virus was mixed with 60 µl of each extract concentration in the 96-well U-bottom sterile plate in duplicate wells. The diluted virus was replaced with 1X EMEM for the extract control wells. 60 µl of 1X EMEM and 60 µl of the diluted virus were used for virus control wells. The plate was incubated for 1 hour at 37°C in 5% CO_2 incubator. The Vero cells monolayer in 96-well flat-bottom sterile plate were washed with 1X EMEM. 100 µl of the virus-extract mixture in 96-well U-bottom sterile plate was transferred to the 96-well Vero cells monolayer. At this step, the final

concentrations of the test virus are 100, 50, and 25 $TCID_{50}$/well. The plate was incubated for 1 hour at 37°C in 5% CO_2 incubator. The virus-extract mixture was removed and washed with 1X EMEM. 100 µl of 4% EMEM and 100 µl of extract were added into the plate. The plate was incubated for 5 days at 37°C in 5% CO_2 incubator. CPE development was observed. The antiviral activity of the extracts was defined as the concentration that reduced at least 50% of CPE production (degree of CPE≤ 2+).

3.2.4 Antimicrobial activities

3.2.4.1 Microbial strains

The gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* MRSA, *Staphylococcus epidermidis* ATCC 12228 and *Streptococcus pyogenes* ATCC 19615), gram negative bacterium (*Klebsiella pneumoniae* ATCC 700603) and fungus (*Candida albicans* ATCC 90028) were used for this study. The cultures of bacteria were maintained at 4°C throughout the study and used as stock cultures. The cultivation medium is Mueller Hinton Agar (MHA) as base medium for the screening of anti-microbial activity. Mueller-Hinton Broth (MHB) will be used for preparation of inoculums. The antimicrobial test was performed in triplicate. Gentamicin was used as positive for bacteria and amphotericin B for the fungus. Dimethylsulphoxide (DMSO) was used as negative control.

3.2.4.2 Preparation of inoculums

Each type of microorganism was streaked on a non-inhibitory NA plate to obtain isolated colonies and incubated at 37°C for 18-24 hours for bacteria and 36-48 hours for fungi. One single colony was selected with an inoculating loop, and transferred into 3ml of Mueller-Hinton Broth (MHB) and incubated in a shaking incubator at 37°C for 2 hours. The turbidity of bacteria was adjusted to 0.5 McFarland standards (1.5×10^8CFU/ml) by Mueller-Hinton Broth (MHB)

3.2.4.3 Preparation of test disc

The 95% ethanol extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration 500 mg/ml and the aqueous extracts were dissolved in distilled

water to a final concentration of 100 mg/ml and filtered with Millipore filter 0.22 μm. Then 10 μl of extracts were applied to sterilized paper discs (6 mm in diameter)

3.2.4.4 Disc diffusion method (Lorian, 1996)

This method was used to screen antimicrobial activity of the extracts, as described by Lorian, 1996 Sterilized paper discs (6 mm in diameter) were impregnated with 10 μl of the extracts. The standardized suspensions were swabbed on Mueller Hinton Agar (MHA) surface evenly in three directions with sterile cotton. The plates were left for 3-5 minutes. Then, the extract paper discs and the positive control discs (Gentamicin for bacteria and amphotericin B for fungus) were placed on the Mueller Hinton Agar (MHA) with sterile forceps. The plates with bacteria and test samples were incubated at 37^{o}C for 24 hours and fungus for 48 hours. The zone of inhibition was determined by measuring the diameter.

3.2.4.5 Microtitre plate-based antimicrobial assay (Sarker *et al.*, 2007)

The microtitre plate-based antimicrobial assay is a technique used to determine the minimum inhibitory concentration (MIC) of an extract that will inhibit the microorganism, described by Sarker *et al*., 2007. The inoculum was adjusted to 0.5 McFarland standards (1.5×10^{8}CFU/ml) and diluted with sterile Mueller-Hinton Broth (MHB) at 1:200 to give a final concentration of microorganism of 5×10^{5}CFU/ml. The ethanolic extracts were diluted with DMSO to 500 mg/ml and diluted with MHB to 10 mg/ml. The aqueous extracts were diluted with sterile distilled water to 10 mg/ml and filtered through 0.22 μm nylon-66 membrane filter. Serial two-fold dilutions of each extracts were prepared. The 50 μl of each concentration of extract solution and 50 μl of the inoculum were added into a sterile 96-well plate. Positive control was diluted to 100 μg/ml. The plates were covered with a sealer to ensure that bacteria did not become dehydrated in a shaking incubator at 37^{o}C for 18-24 hours and fungus for 36-48 hours. MIC of tested sample was determined after adding 10 μl of resazurin and incubated in a shaking incubator at 37^{o}C for 2 hours. The result was interpreted by the change of color of resazurin. The MIC value was the lowest dilution of crude extract solution that was able to inhibit

microorganism by changing blue color of resazurin to pink. Resazurin is a redox dye commonly used as an indicator of chemical cytotoxicity in cultured cells. Resorufin produced as a result of resazurin bioreduction is measured colorimetrically. The assay was repeated in triplicate. The Minimum microbicidal concentration (MMC) values were determined immediately after taking the MIC values. The MMC was taken from a row of all the wells with no visible growth in them and transferred to agar plates. The plates were incubated at 37^{o}C for 24 hours. The lowest concentration of the extract showing no growth was evaluated as the MMC value. The assay was repeated in triplicate.

3.2.5 Anti-inflammatory activities

3.2.5.1 Animal cell lines

Murine leukemia macrophage cell line (RAW 264.7) was obtained from American Type Culture Collection (ATCC TIB-71). This cell line was cultured in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, penicillin and streptomycin. The cells were incubated at 37^{o}C in a 5% CO_2 incubator and subpassaged every 4-5 days.

3.2.5.2 Preparation of sample solution

The ethanolic extracts were dissolved in sterile dimethyl sulfoxide (DMSO) to a final concentration of 50 mg/ml but the aqueous extracts were dissolved in sterile distilled water to a final concentration of 10 mg/ml and filtered with Millipore filter 0.22 μm. Each extract was diluted with RPMI to obtain final concentration of 1-100 μg/ml.

3.2.5.3 Assay for NO inhibitory effects in RAW 264.7 cells (Tewtrakul and Itharat, 2007)

This modified method evaluated the inhibition of nitric oxide (NO) production produced by mouse macrophage leukemia-like (RAW 264.7) in inflammatory conditions. The cells (RAW 264.7) were cultured in a flask with RPMI 1640 medium containing 10% FBS, penicillin (100 unites/ml) and streptomycin (100 unites/ml), RAW 264.7 cells were washed by phosphate buffer saline (PBS) and suspended by

0.25% trypsin-EDTA. The cells were cultured in a sterile 96-well plate ($1x10^5$cells/well) with 100 µl complete RPMI and incubated in 5% CO_2, 37^oC overnight. Complete RPMI (100 µl/well) containing 10ng/ml of lipopolysaccharide (LPS) was replaced in control and only complete RPMI was replaced in normal. Next, 100 µl/well of each sample concentration were added but 100 µl /well of complete RPMI was added in control medium, 100 µl/well of 0.2% DMSO in control solvent, then incubated overnight. Supernatant 100 µl was transferred to another sterile 96-well plate, followed by 100 µl of Griess reagent. The NO production was determined by measuring the accumulation of nitrite which interacted with Griess reagent. The absorbance were measured by spectrophotometer at wavelength 570 nm. This method were carried out in triplicate. The inhibition (%) was calculated using the following equation and IC_{50} value was calculated using Prism program.

$$\% \text{ inhibition} = \frac{C - S}{C} \times 100$$

Control(C) : [LPS (+), sample (-)] - [LPS (-), sample (-)]
Sample (S) : [LPS (+), sample (+)] - [LPS (-), sample (+)]

3.2.5.4 MTT assay (Tewtrakul and Itharat, 2007)

MTT assay was determined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromine(MTT) colorimetric method. This method continued from NO assay above. The plates were incubated at 37^oC in 5% CO_2 incubator for 24 hours. MTT solution (10 µl, 5mg/ml in PBS) was added in each well and incubated 2 hours. Supernatant was removed and 100 µl of isopropanol contained 0.04 M HCl was added to dissolve the formazan production in cells. The density of formazan solution was measured by micro spectrophotometer at wavelength 570 nm. If the density of cell viability is less than 70% that sample was considered to toxic.

$$\%\text{Toxicity} = \frac{C - S}{C} \times 100$$

Control (C) : LPS (-), sample (-)
Sample (S) : LPS (-), sample (+)

3.2.6 The stability test of Kheaw-Hom extract

Stability testing was done using transparent vials. Kheaw-Hom extracts were put in these vials and exposed to $40^{o}C \pm 2$ with $75\% \pm 5$ RH as accelerated conditions testing for 6 months period. The 95% ethanol extracts were tested for antimicrobial activity and the aqueous extracts were tested for antiviral activity on days 0, 15, 30, 60, 90, 120, 150, 180, and results compared with those of day 0.

3.2.7 Phytochemical of the ethanolic extract of Kheaw-Hom remedy by using gas chromatography-mass spectrometry (GC-MS)

The ethanolic extract of Kheaw-Hom remedy (50 mg) was transferred into 10 ml volumetric flask and diluted to volume with methanol. The solution was analyzed by using a gas chromatography-mass spectrometry (GC-MS) with column Thermo TG-5 slims. Helium gas (He) was carrier with flow rate 1.0 ml/min. The initial temperature of column oven was programed $60^{o}C$ and then heated to $300^{o}C$ with a rate of $5^{o}C$/min and kept constant at $300^{o}C$ for 10 minutes. The mass spectrum of each peak was recorded. Chemical components were analysed by Herb and Thai Traditional Medicine Devision, BIOTEC Pilot Plant, Thailand Science Park.

3.2.8 Statistical analysis

All data are the mean of three replications. The values of IC_{50} were evaluated by using Prism program. Values of different parameters was expressed as the mean ± standard error of mean (SEM). Statistical analysis was performed using SPSS statistical software.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Preparation of crude extracts

4.1.1 Percentage of yield

The ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients were prepared by using maceration and decoction methods as described in Chapter 3 **(section 3.2.1)**. The extraction methods are related with Thai folk doctor's uses. Most of the crude ethanolic extracts were obtained in the forms of sticky liquid or sticky solid. Most of the crude aqueous extracts were obtained in the forms of powder or stick mass. The percentages of yield of Kheaw-Hom remedy and its plant ingredients are shown as percentage by weight in **Table 4-1**.

The percentage yields of all ethanolic extracts were in range from 1.05% to 19.49%. The maximum percentage yield of the ethanolic extracts was *Dracaena loureiri* (19.49%) and the minimum percentage yield of the ethanolic extracts was *Myristica fragrans* (1.05%). As for the aqueous extracts, the percentage yields were in range from 1.70% to 41.13%. The maximum percentage yield of the aqueous extracts was *Vetiveria zizanioides* (41.13%) and the minimum percentage yield of the aqueous extracts was *Myristica fragrans* (1.70%). The percentage yields of the ethanolic and aqueous extracts of Kheaw-Hom remedy were 8.75% and 13.36%, respectively.

Most of the aqueous extracts were higher in percentage yield than the ethanolic extracts. The different chemical constituents in the plants behave respond differently to the method of extraction and the solvents used.

Table 4-1 The percentage yields of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients.

Sample	Thai name	Ethanolic extract		Aqueous extract	
		Code	%Yield	Code	%Yield
Angiopteris evecta (G.Forst) Hoffm.	ว่านกีบแรด	AEE	1.27	AEA	10.77
Cordyline fruticosa (L.) A.Chev (Green leaf)	หมากเมีย	CFE	10.15	CFA	16.82
Cordyline fruticosa (L.) A.Chev (Red leaf)	หมากผู้	COE	9.09	COA	18.01
Cyathea gigantea Holtt.	มหาสดำ	CGE	1.53	CGA	4.41
Dracaena loureiri Gagnep.	จันทน์แดง	DLE	19.49	DLA	2.25
Eupatorium stoechadosmum Hance	สันพร้าหอม	ESE	7.70	ESA	20.29
Globba malaccensis Ridl.	ว่านร่อนทอง	GME	7.38	GMA	7.29
Kaempferia galanga Linn.	เปราะหอม	KGE	3.52	KGA	13.41
Limnophila rugosa Merr.	ผักกระโฉม	LRE	8.45	LRA	20.88
Mammea siamensis Kosterm.	สารภี	MSE	17.21	MSA	30.56
Mesua ferrea Linn.	บุนนาค	MLE	17.50	MLA	13.36
Mimusops elengi Linn.	พิกุล	MEE	8.40	MEA	13.61
Myristica fragrans Houtt.	จันทน์เทศ	MFE	1.05	MFA	1.70
Nelumbo nucifera Gaertn.	บัวหลวง	NNE	8.40	NNA	19.59
Pogostemon cablin (Blanco) Benth.	พิมเสนต้น	PCE	5.67	PCA	16.25
Sophora exigua Craib	พิษนาศน์	SEE	11.60	SEA	9.11
Tacca chantrieri Andre	เนระพูสี	TCE	3.27	TCA	14.25
Vetiveria zizanioides (L.) Nash ex Small	แฝกหอม	VZE	2.73	VZA	41.13
Kheaw-Hom	**เขียวหอม**	**KHE**	**8.75**	**KHA**	**13.36**

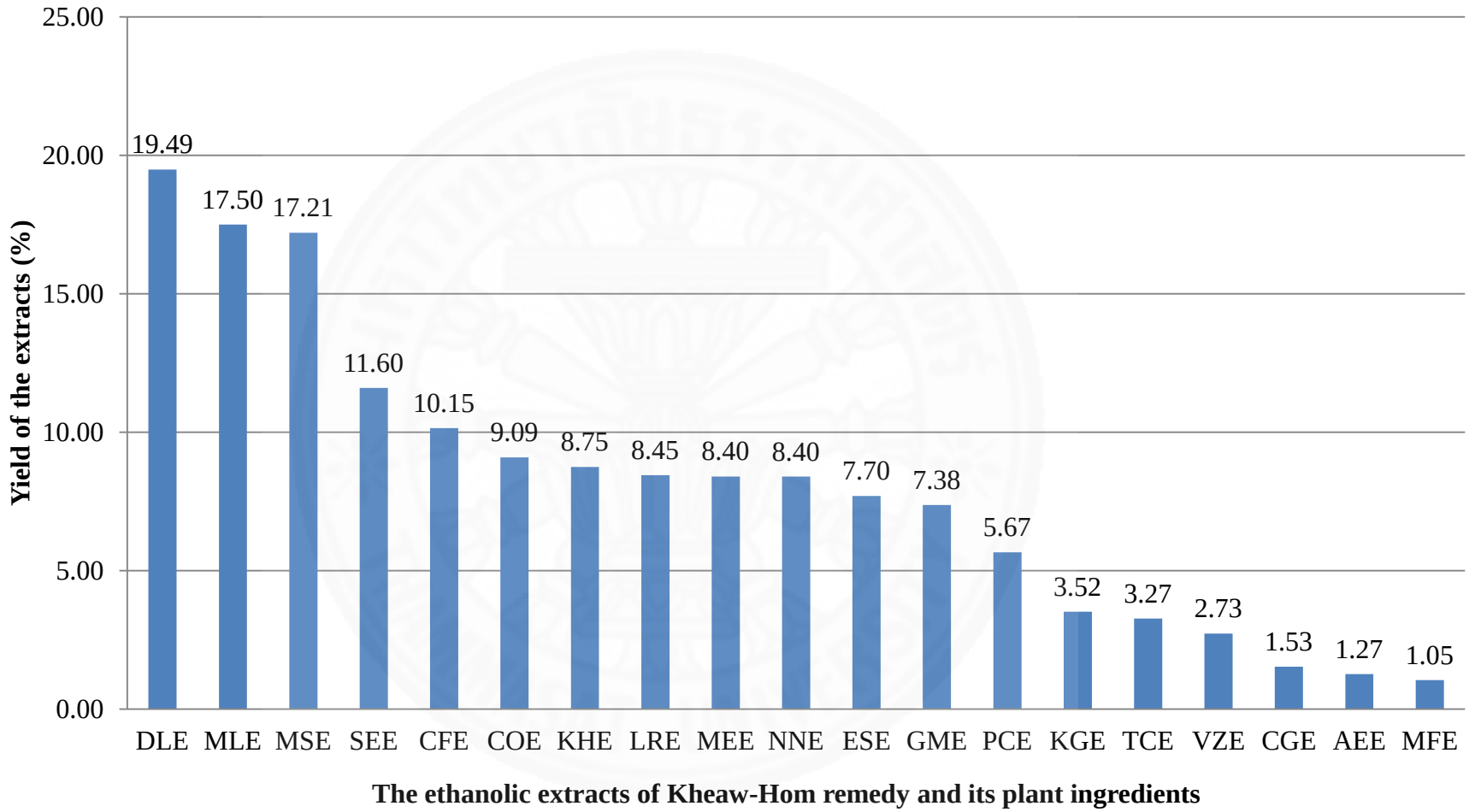

Figure 4-1 The percentage yield of the ethanolic extracts of Kheaw-Hom remedy and its plant ingredients.

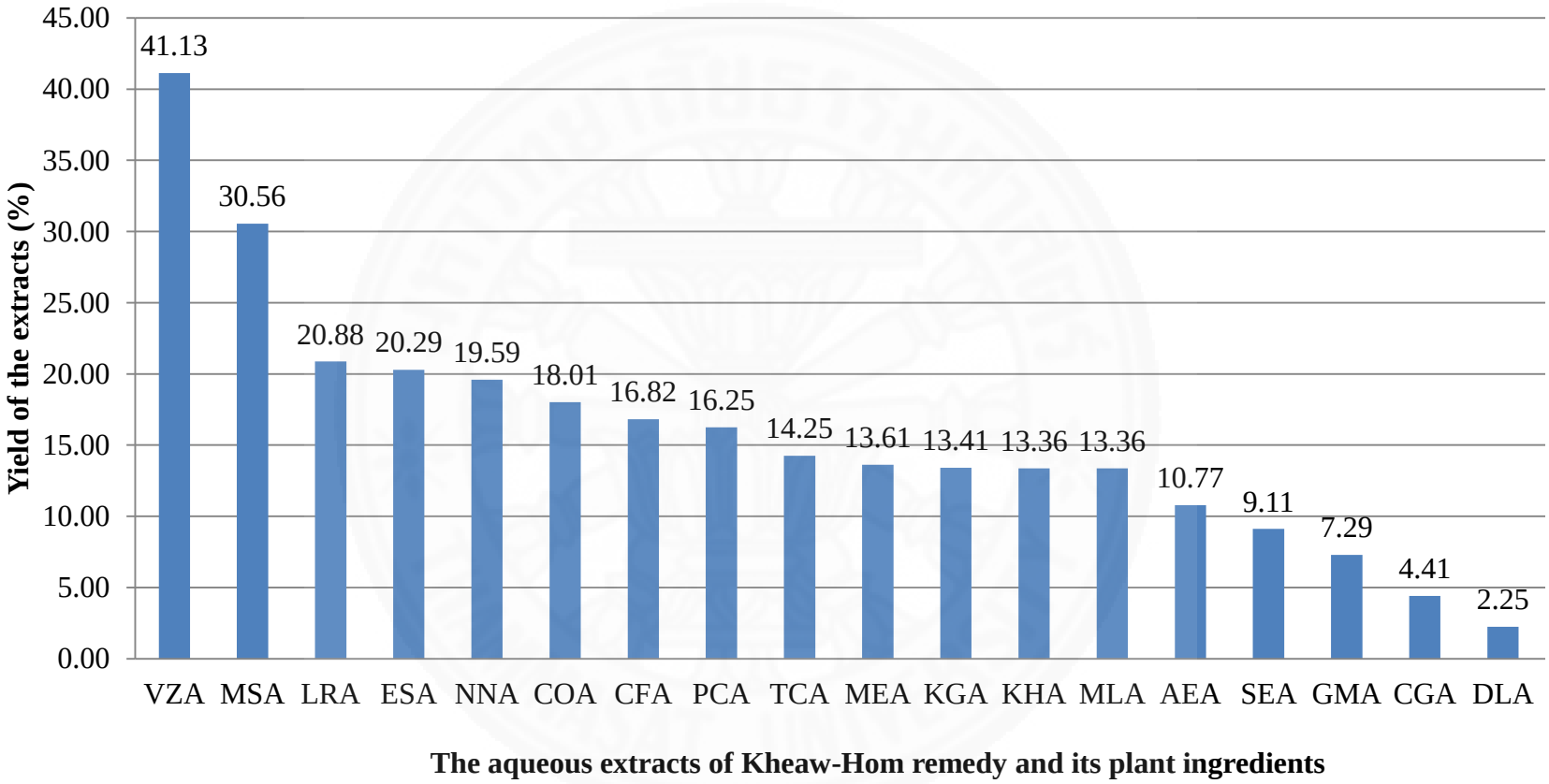

Figure 4-2 The percentage yield of the aqueous extracts of Kheaw-Hom remedy and its plant ingredients.

4.2 Quality control

Quality controls of Kheaw-Hom remedy and its plant ingredients included loss on drying, total ash, acid insoluble ash and extractive values according to the standard values set by the Thai Herbal Pharmacopoeia (Thai herbal pharmacopeia, 2009)

4.2.1 Loss on drying

The standard value of Thai Herbal Pharmacopoeia indicated that loss on drying is ≤ 10% after drying at 105°C to constant weight. The results of loss on drying of Kheaw-Hom remedy and its plant ingredients are shown in **Table 4-2**. The percentage loss on drying of all plant ingredients ranged from 5.98% to 9.93%. The maximum percentage loss on drying was *Angiopteris evecta* (9.93±0.27%) and the minimum percentage loss on drying was *Dracaena loureiri* (5.98±0.33%). As for Kheaw-Hom remedy, the percentage loss on drying was 8.75±0.24%.

All plant ingredients and Kheaw-Hom remedy were within standard in Thai Herbal Pharmacopoia criteria.

4.2.2 Total ash

The standard value of Thai Herbal Pharmacopoeia indicated that total ash content is ≤ 10% after burning at 450°C to constant weight. The results of total ash content of Kheaw-Hom remedy and its plant ingredients are shown in **Table 4-2**. The percentage total ash of all plant ingredients ranged from 3.15 % to 10.94 %. The maximum percentage total ash was *Vetiveria zizanioides* (10.94±3.57%) and the minimum percentage total ash was *Angiopteris evecta* (3.15±0.11%). As for Kheaw-Hom remedy, the percentage total ash was 6.01±0.05%.

Kheaw-Hom remedy was within standard criteria in being not more than 10 % after burning at 450°C to constant weight but some plant ingredients were more than 10% such as *Pogostemon cablin* (10.52±0.11%) and *Vetiveria zizanioides* (10.94±3.57%).

4.2.3 Acid insoluble ash

The standard value of Thai Herbal Pharmacopoeia indicated that acid insoluble ash is ≤ 2% after burning at 500°C to constant weight. The results of acid

insoluble ash content of Kheaw-Hom remedy and its plant ingredients are shown in **Table 4-2**. The percentage acid insoluble ash of all plant ingredients ranged from 0.32% to 2.23%. The maximum percentage acid insoluble ash was *Vetiveria zizanioides* (2.23±0.05%) and the minimum percentage acid insoluble ash was *Dracaena loureiri* (0.32±0.03%). As for Kheaw-Hom remedy, the percentage acid insoluble ash was 1.29±0.00%.

Kheaw-Hom remedy was within standard criteria in being not more than 2% after burning at 500^{o}C to constant weight but some plant ingredients were more than 2% such as *Pogostemon cablin* (2.18±0.14%) and *Vetiveria zizanioides* (2.23±0.05%).

4.2.4 Extractive value

The percentage of ethanol-soluble extractive ranged from 0.45% to 25.25%. The maximum percentage of ethanol-soluble extractive was 25.25±1.04% (*Dracaena loureiri*) and the minimum percentage of ethanol-soluble extractive was 0.45±1.21% (*Kaempferia galanga*). The percentage of water-soluble extractive ranged from 1.69% to 56.49%. The maximum percentage of water-soluble extractive was 56.49±0.13% (*Vetiveria zizanioides*) and the minimum percentage of water-soluble extractive was 1.69±0.06% (*Myristica fragrans*). The percentages of ethanol-soluble and water-soluble extractive of Kheaw-Hom remedy were 3.77±0.25 and 6.12±0.31%, respectively.

Most water-soluble extractive values were higher than the ethanolic-soluble extractive values. The extractive values related with the percentage yield.

Table 4-2 Results of quality controls of Kheaw-Hom remedy and its plant ingredients (mean±SD), (n=3)

Sample	Thai name	Code	% Loss on drying	% Ash contents		% Extractive values	
				Total ash	Acid insoluble ash	Ethanol-soluble	Water-soluble
Angiopteris evecta (G.Forst) Hoffm.	ว่านกีบแรด	AE	9.93±0.27	3.15±0.11	0.46±0.01	2.08±0.08	14.43±0.33
Cordyline fruticosa (L.) A.Chev (Green leaf)	หมากเมีย	CF	7.11±0.70	7.68±0.22	0.59±0.04	5.24±0.28	17.96±0.76
Cordyline fruticosa (L.) A.Chev (Red leaf)	หมากผู้	CO	7.17±0.66	7.99±0.46	0.53±0.12	5.71±1.12	17.42±0.58
Cyathea gigantea Holtt.	มหาสดำ	CG	7.63±0.12	3.37±0.33	0.57±0.23	2.89±0.31	6.72±0.35
Dracaena loureiri Gagnep.	จันทน์แดง	DL	5.98±0.33	4.45±0.32	0.32±0.03	25.25±1.04	3.04±0.23
Eupatorium stoechadosmum Hance	สันพร้าหอม	ES	6.39±0.18	5.63±0.52	0.67±0.08	3.36±0.17	18.48±0.85
Globba malaccensis Ridl.	ว่านร่อนทอง	GM	8.85±0.64	7.42±0.16	1.10±0.02	4.84±1.08	11.34±0.94
Kaempferia galanga Linn.	เปราะหอม	KG	6.67±0.23	5.52±0.14	1.32±0.04	0.45±1.21	9.61±0.55
Limnophila rugosa Merr.	ผักกระโฉม	LR	6.39±0.18	9.82±0.71	0.83±0.21	7.62±0.70	15.55±1.12
Mammea siamensis Kosterm.	สารภี	MS	7.74±0.17	7.98±0.32	0.43±0.02	2.84±0.17	25.39±1.27
Mesua ferrea Linn.	บุนนาค	ML	8.57±0.59	5.01±0.21	1.49±0.34	1.66±0.10	8.24±0.49
Mimusops elengi Linn.	พิกุล	ME	8.59±0.36	5.97±0.19	1.29±0.30	5.23±1.31	10.30±0.27
Myristica fragrans Houtt.	จันทน์เทศ	MF	6.10±0.22	8.58±0.26	1.78±0.02	1.39±0.09	1.69±0.06
Nelumbo nucifera Gaertn.	บัวหลวง	NN	8.69±0.34	8.01±0.63	1.69±0.31	1.22±0.02	8.62±0.49

Table 4-2 Results of quality controls of Kheaw-Hom remedy and its plant ingredients (mean±SD), (n=3)

Sample	Thai name	Code	% Loss on drying	% Ash contents		% Extractive values	
				Total ash	Acid insoluble ash	Ethanol-soluble	Water-soluble
Pogostemon cablin (Blanco) Benth.	พิมเสนต้น	PC	9.55±0.39	10.52±0.11	2.18±0.14	3.37±0.29	13.13±0.37
Sophora exigua Craib	พิษนาศน์	SE	6.35±0.32	4.60±0.06	1.07±0.08	13.52±0.58	15.78±0.65
Tacca chantrieri Andre	เนระพูสี	TC	7.31±0.98	4.89±0.07	0.82±0.08	5.61±0.84	17.55±0.23
Vetiveria zizanioides (L.) Nash ex Small	แฝกหอม	VZ	8.84±0.74	10.94±0.57	2.23±0.05	2.36±0.21	56.49±0.13
Kheaw-Hom	**เขียวหอม**	**KH**	**8.75±0.24**	**6.01±0.05**	**1.29±0.00**	**3.77±0.25**	**6.12±0.31**

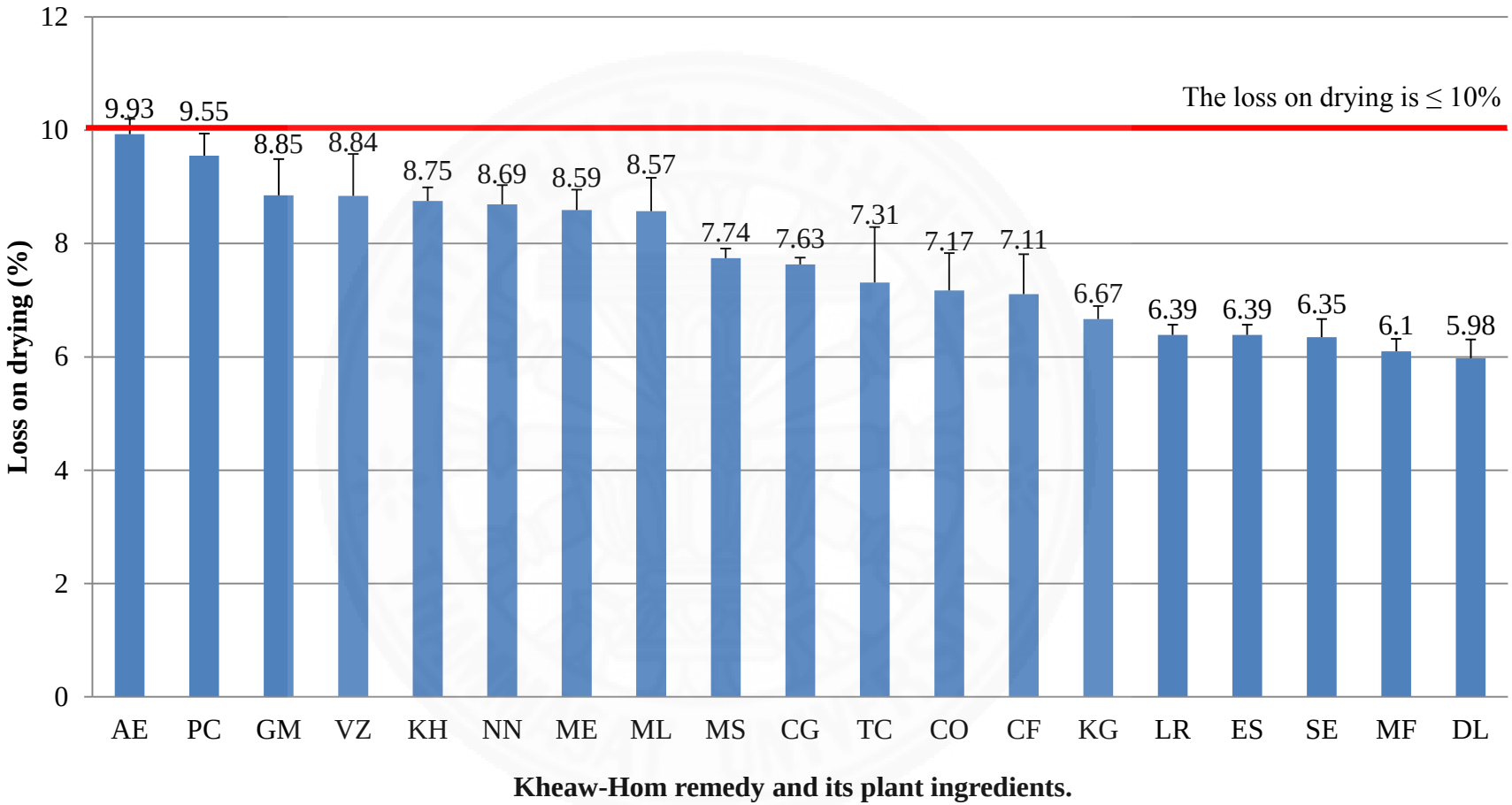

Figure 4-3 The loss on drying (%) of Kheaw-Hom remedy and its plant ingredients.

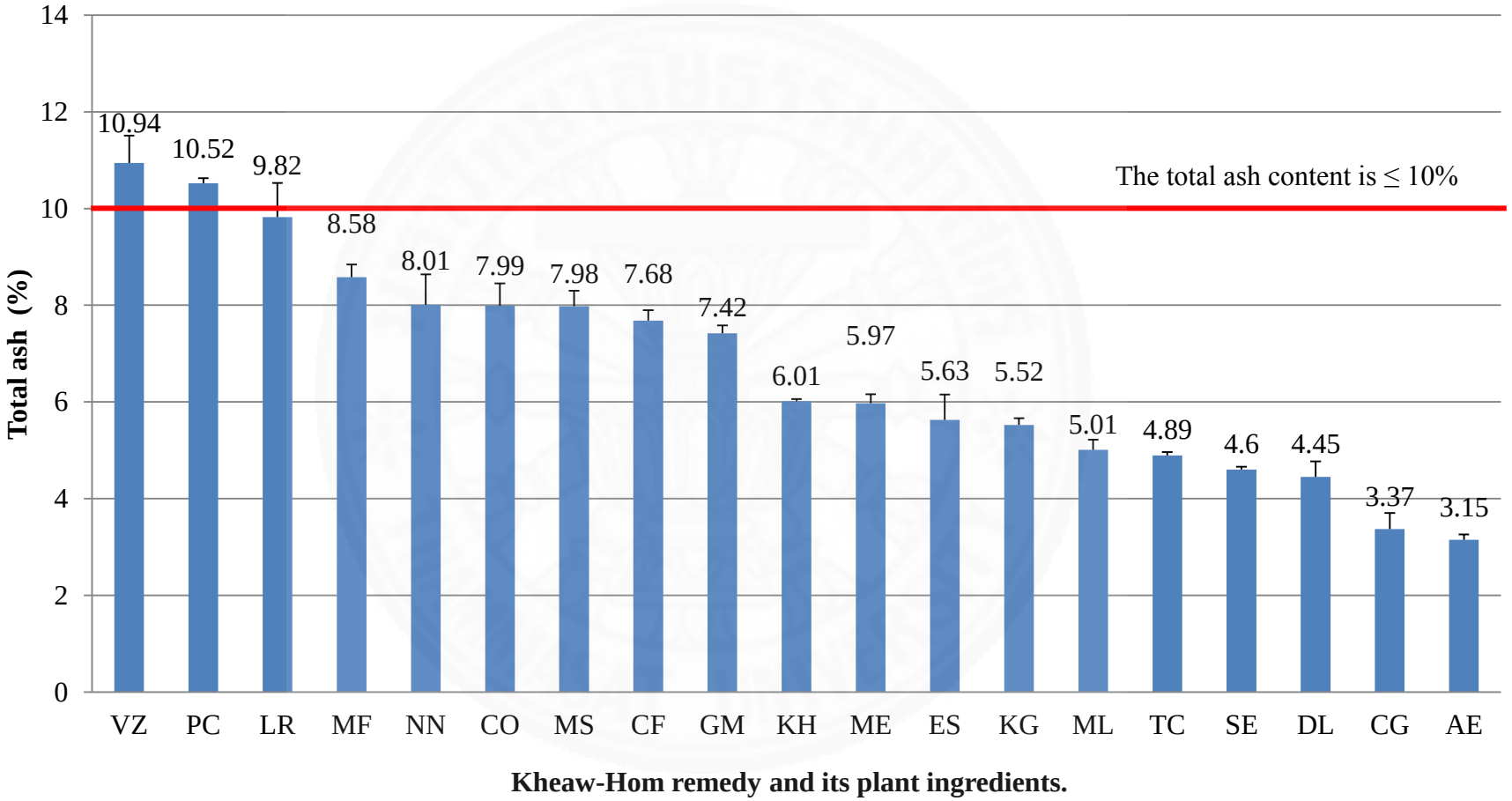

Figure 4-4 The total ash contents of Kheaw-Hom remedy and its plant ingredients.

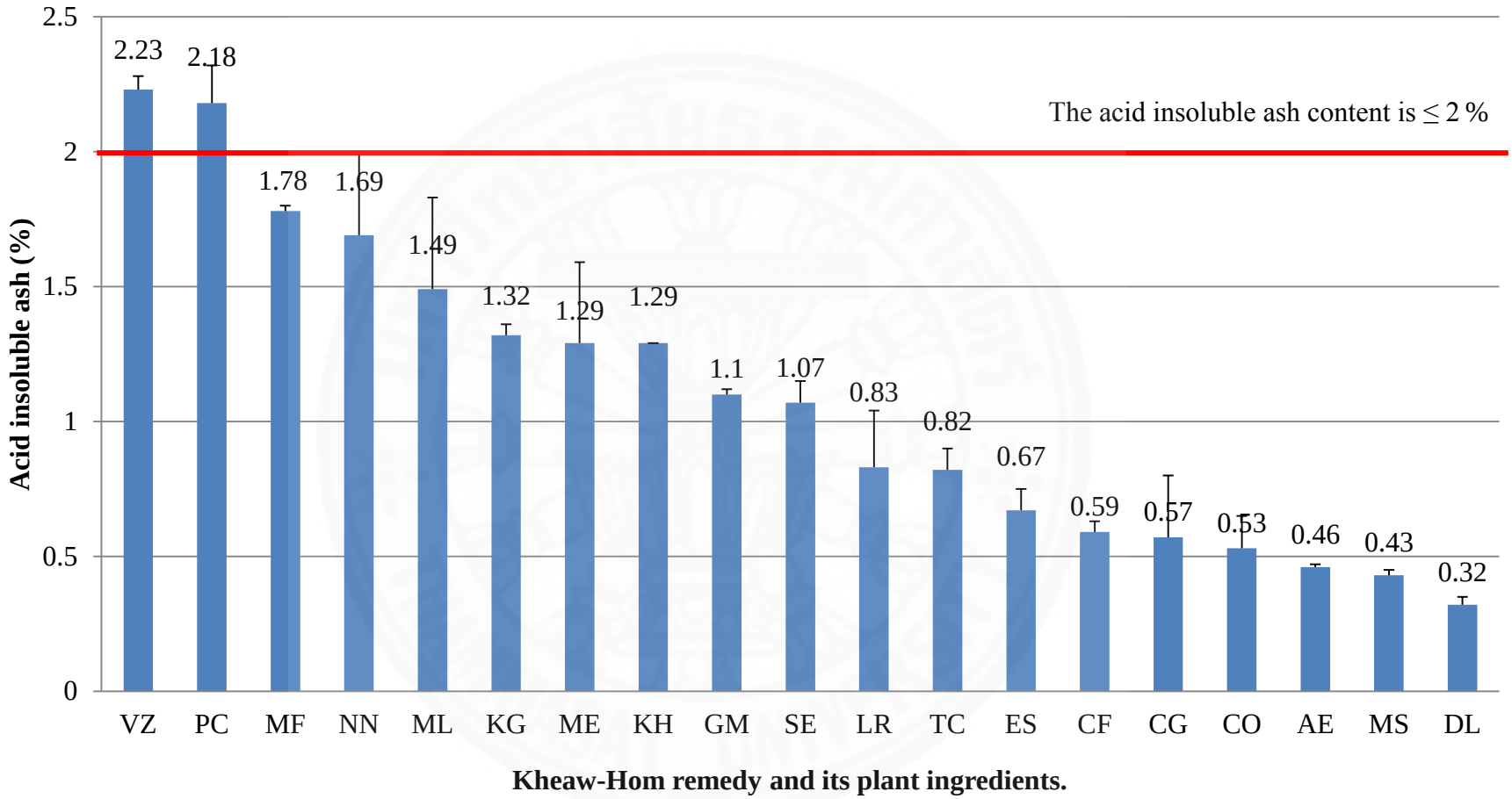

Figure 4-5 The acid insoluble ash contents of Kheaw-Hom remedy and its plant ingredients.

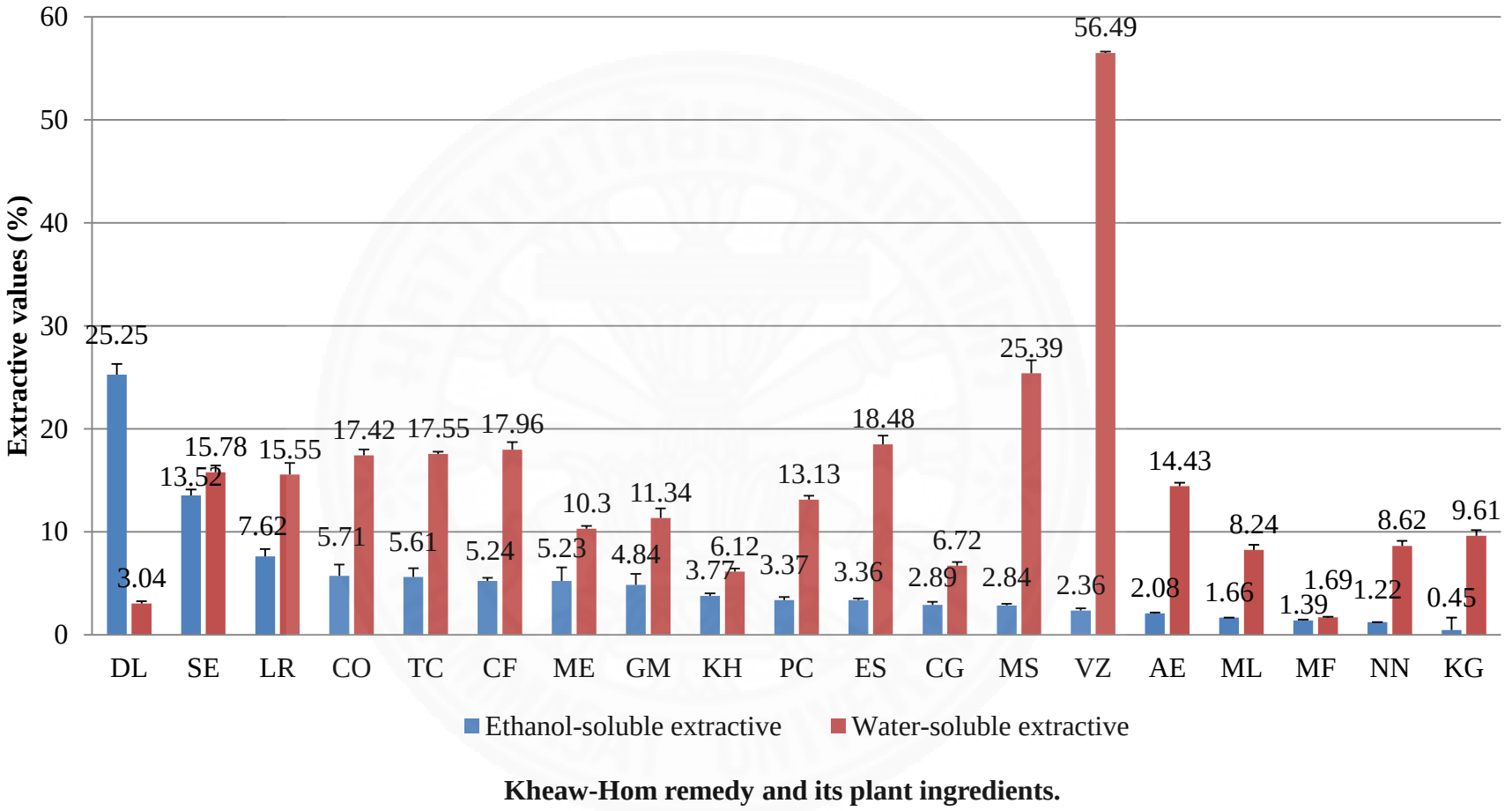

Figure 4-6 The extractive values of Kheaw-Hom remedy and its plant ingredients.

4.3 Antiviral activities

4.3.1 MTT assay

The cytotoxicity of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients were measured in Vero cell lines by using MTT assay to determine % toxicity at varied concentration from 50, 100, 200, 400 and 800 μg/ml. If the density of cell death is more than 20%, it was considered to be toxic. All results of the ethanolic extracts are shown in **Table 4-3** and the aqueous extracts in **Table 4-4**.

The ethanolic extract of Kheaw-Hom remedy was toxic to Vero cells (cell toxicity ≥ 20%). There are three ethanolic extracts of plant ingredients namely *C.fruticosa* green leaves (CFE), *C.fruticosa* red leaves (COE) and *N.nucifera* (NNE) that were not toxic to Vero cells at concentrations 50, 100 and 200 μg/ml and the ethanolic extract of *M.elengi* (MEE) was not toxic to Vero cells at concentrations 50 and 100 μg/ml. *E.stoechadosmum* (ESE), *G.malaccensis* (GME) and *T.chantrieri* (TCE) showed toxicity at all concentrations except 50 μg/ml. There are eleven ethanolic extracts which showed toxicity to Vero cells at all concentrations. Most ethanolic extracts of Kheaw-Hom remedy and its plant ingredients were toxic to Vero cells thus the ethanolic extracts were not able to be evaluated for antiviral activity.

For the aqueous extracts of Kheaw-Hom ingredients, *L.rugosa* (LRA) showed toxicity to Vero cells at all concentrations. In addition, the aqueous extracts of *C.gigantea* (CGA) and *K.galanga* (KGA) were toxic to Vero cells at 100, 200, 400 and 800μg/ml. There are seven extracts namely *C.fruticosa* green leaves (CFA), *C.fruticosa* red leaves (COA), *E.stoechadosmum* (ESA), *G.malaccensis* (GMA), *P.cablin* (PCA), *S.exigua* (SEA) and *V.zizanioides* (VZA) which showed no toxicity to Vero cells at all concentrations. The aqueous extract of Kheaw-Hom remedy was not toxic to Vero cells at all concentrations except at concentration 800 μg/ml. Thus, the aqueous extracts at maximum non-toxic concentration (that exhibited toxicity less than 20 %) were able to be evaluated for antiviral activity.

Although most the ethanolic extracts of Kheaw-Hom remedy and its plant ingredients were toxic to Vero cells by MTT assay, previous work reported that the ethanolic extract of Kheaw-Hom remedy had no cytotoxic effects on Vero cell by green fluorescent protein based assay. These contrasting results could be due to the use of different experimental methods.

Table 4-3 Cytotoxicity of the ethanolic extracts of Kheaw-Hom remedy and its plant ingredients on Vero cell line by using MTT assay

Plant name	Code	Concentration				
		50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml
A.evecta	AEE	+	+	+	+	+
C.fruticosa (green leaves)	CFE	-	-	-	+	+
C.fruticosa (red leaves)	COE	-	-	-	+	+
C. gigantea	CGE	+	+	+	+	+
D.loureiri	DLE	+	+	+	+	+
E.stoechadosmum	ESE	-	+	+	+	+
G.malaccensis	GME	-	+	+	+	+
K.galanga	KGE	+	+	+	+	+
L.rugosa	LRE	+	+	+	+	+
M.siamensis	MSE	+	+	+	+	+
M.ferrea	MLE	+	+	+	+	+
M.elengi	MEE	-	-	+	+	+
M.fragrans	MFE	+	+	+	+	+

"+" = % toxicity more than 20

"-" = % toxicity less than 20

Table 4-3 Cytotoxicity of the ethanolic extracts of Kheaw-Hom remedy and its plant ingredients on Vero cell line by using MTT assay (Continued)

Plant name	Code	Concentration				
		50 µg/ml	**100 µg/ml**	**200 µg/ml**	**400 µg/ml**	**800 µg/ml**
N.nucifera	NNE	-	-	-	+	+
P.cablin	PCE	+	+	+	+	+
S.exigua	SEE	+	+	+	+	+
T.chantrieri	TCE	-	+	+	+	+
V.zizanioides	VZE	+	+	+	+	+
Kheaw-Hom	**KHE**	**+**	**+**	**+**	**+**	**+**

"+" = % toxicity more than 20

"-" = % toxicity less than 20

Table 4-4 Cytotoxicity of the aqueous extracts of Kheaw-Hom remedy and its plant ingredients on Vero cell line by using MTT assay

Plant name	Code	Concentration				
		50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml
A.evecta	AEA	-	-	-	-	+
C.fruticosa (green leaves)	CFA	-	-	-	-	-
C.fruticosa (red leaves)	COA	-	-	-	-	-
C. gigantea	CGA	-	+	+	+	+
D.loureiri	DLA	-	-	+	+	+
E.stoechadosmum	ESA	-	-	-	-	-
G.malaccensis	GMA	-	-	-	-	-
K.galanga	KGA	-	+	+	+	+
L.rugosa	LRA	+	+	+	+	+
M.siamensis	MSA	-	-	-	+	+
M.ferrea	MLA	-	-	-	+	+
M.elengi	MEA	-	-	-	-	+
M.fragrans	MFA	-	-	-	-	+

"+" = % toxicity more than 20

"-" = % toxicity less than 20

Table 4-4 Cytotoxicity of the aqueous extracts of Kheaw-Hom remedy and its plant ingredients on Vero cell line by using MTT assay (Continued)

Plant name	Code	Concentration				
		50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml
N.nucifera	NNA	-	-	+	+	+
P.cablin	PCA	-	-	-	-	-
S.exigua	SEA	-	-	-	-	-
T.chantrieri	TCA	-	-	+	+	+
V.zizanioides	VZA	-	-	-	-	-
Kheaw-Hom	**KHA**	**-**	**-**	**-**	**-**	**+**

"+" = % toxicity more than 20

"-" = % toxicity less than 20

4.3.3 Antiviral activity based CPE assay

All the aqueous extracts of Kheaw-Hom remedy and its ingredients that were non-toxic to Vero cells were tested for antiviral activities against 100 $TCID_{50}$, 50 $TCID_{50}$ and 25 $TCID_{50}$ of enterovirus 71 (EV71) in duplicate experiments. Ribavirin, a commonly used antiviral drug was used as positive control. The results are shown in **Table 4-5, Table 4-6** and **Table 4-7**.

At 100 $TCID_{50}$ and 50 $TCID_{50}$ of EV71, four aqueous extracts exhibited antiviral activity against EV71 namely *A.evecta* (AEA), *G.malaccensis* (GMA), *M.siamensis* (MSA) and *T.chantrieri* (TCA) with cytopathic effect less than 50% at concentrations 400, 400, 200 and 50 μg/ml, respectively. The aqueous extract of Kheaw-Hom remedy showed no activity. All results are shown in **Table 4-5** and **Table 4-6**.

At 25 $TCID_{50}$ of EV71, the aqueous extract of Kheaw-Hom remedy exhibited antiviral activity against EV71 with cytopathic effect less than 50% at concentration 400 μg/ml. There are five plant ingredients that exhibited antiviral activity namely *A.evecta* (AEA), *G.malaccensis* (GMA), *M.siamensis* (MSA), *T.chantrieri* (TCA) and *N.nucifera* (NNA) with cytopathic effect less than 50% at concentrations 200, 400, 100, 100 and 50 μg/ml, respectively. All results are shown in **Table 4-7.**

In summary, there are four plant ingredients in Kheaw-Hom remedy that exhibited antiviral activity against all doses of 100 $TCID_{50}$, 50 $TCID_{50}$, and 25 $TCID_{50}$ of EV71 namely *A.evecta* (AEA), *G.malaccensis* (GMA), *M.siamensis* (MSA) and *T.chantrieri* (TCA). *N.nucifera* (NNA) and Kheaw-Hom remedy (KHA) exhibited antiviral effects only at the low dose of 25 $TCID_{50}$ of EV71. All results are shown in **Table 4-8**.

These results of Kheaw-Hom remedy and its plant ingredients have never been reported. This study is the first report on antiviral activity against EV71. Previous work demonstrated the antiviral activity against varicella zoster virus (VZV) of Ya-Kheaw which is a Thai traditional remedy to treat fever, measles, and chickenpox and has some ingredients identical to Kheaw-Hom remedy. The results showed that pre-treatment of the virus with the 20% ethanolic extract of Ya-Kheaw at 250 μg/ml significantly reduced virus infection in the cell line because the interaction of the Ya-Kheaw and VZV particle may have an effect on virus's ability to infect the cells. (Sanguansermsri

et al., 2005) However, post-treatment of the extract after infection has not significantly reduced the number of plaque by using plaque reduction assay. For this reasons, Thai traditional medical practices recommend that Kheaw-Hom should be used immediately in the early stage of disease. In addition a study of *P. cablin* which is a plant ingredient in Kheaw-Hom remedy showed that patchouli alcohol isolated from *P. cablin* had anti-H1N1 activity (Kiyohara *et al.,* 2012) and inhibited the expression of IL-4 and IFN-γ after H1N1 infection (Wu *et al.,* 2013). The ethanolic and aqueous extracts of *D. loureiri* exhibited anti-HIV-1 integrase activity (Bunluepuech and Tewtrakul, 2009).

Table 4-5 Antiviral activities of the aqueous extracts of Kheaw-Hom remedy and its ingredients against 100 $TCID_{50}$ of enterovirus 71 in duplicate experiments

Plant name	Code	Concentration of the aqueous extracts of Kheaw-Hom remedy and its ingredients (µg/ml)							
		6.25	12.5	25	50	100	200	400	Conclusion
A.evecta	AEA	NT	NT	NT	-	-	-	+	400
C.fruticosa (green leaves)	CFA	NT	NT	NT	-	-	-	-	>400
C.fruticosa (red leaves)	COA	NT	NT	NT	-	-	-	-	>400
D.loureiri	DLA	NT	-	-	-	-	Toxic	Toxic	>100
E.stoechadosmum	ESA	NT	NT	NT	-	-	-	-	>400
G.malaccensis	GMA	NT	NT	NT	-	-	-	+	400
K.galanga	KGA	-	-	-	-	Toxic	Toxic	Toxic	>50
M.siamensis	MSA	NT	NT	-	-	-	+	Toxic	200
M.ferrea	MLA	NT	NT	-	-	-	-	Toxic	>200
N.nucifera	NNA	NT	-	-	-	-	Toxic	Toxic	>100
P.cablin	PCA	NT	NT	NT	-	-	-	-	>400
S.exigua	SEA	NT	NT	NT	-	-	-	-	>400

"+" = less than 50% cytopathic effect

"-" = more than 50% cytopathic effect

NT = not tested

Table 4-5 Antiviral activities of the aqueous extracts of Kheaw-Hom remedy and its ingredients against 100 $TCID_{50}$ of enterovirus 71 in duplicate experiments (Continued)

Plant name	Code	Concentration of the aqueous extracts of Kheaw-Hom remedy and its ingredients (µg/ml)							
		6.25	12.5	25	50	100	200	400	Conclusion
T.chantrieri	TCA	NT	-	-	+	+	Toxic	Toxic	50
V.zizanioides	VZA	NT	NT	NT	-	-	-	-	>400
Kheaw-Hom	**KHA**	**NT**	**NT**	**NT**	**-**	**-**	**-**	**-**	**>400**
Ribavirin	**RBV**	**NT**	**NT**	**NT**	**-**	**-**	**-**	**-**	**>400**

"+" = less than 50% cytopathic effect

"-" = more than 50% cytopathic effect

NT = not tested

Table 4-6 Antiviral activities of the aqueous extracts of Kheaw-Hom remedy and its ingredients against 50 $TCID_{50}$ of enterovirus 71 in duplicate experiments

Plant name	Code	Concentration of the aqueous extracts of Kheaw-Hom remedy and its ingredients (µg/ml)							
		6.25	12.5	25	50	100	200	400	Conclusion
A.evecta	AEA	NT	NT	NT	-	-	-	+	400
C.fruticosa (green leaves)	CFA	NT	NT	NT	-	-	-	-	>400
C.fruticosa (red leaves)	COA	NT	NT	NT	-	-	-	-	>400
D.loureiri	DLA	NT	-	-	-	-	Toxic	Toxic	>100
E.stoechadosmum	ESA	NT	NT	NT	-	-	-	-	>400
G.malaccensis	GMA	NT	NT	NT	-	-	-	+	400
K.galanga	KGA	-	-	-	-	Toxic	Toxic	Toxic	>50
M.siamensis	MSA	NT	NT	-	-	-	+	Toxic	200
M.ferrea	MLA	NT	NT	-	-	-	-	Toxic	>200
N.nucifera	NNA	NT	-	-	-	-	Toxic	Toxic	>100
P.cablin	PCA	NT	NT	NT	-	-	-	-	>400
S.exigua	SEA	NT	NT	NT	-	-	-	-	>400

"+" = less than 50% cytopathic effect, "-" = more than 50% cytopathic effect

NT = not tested

Table 4-6 Antiviral activities of the aqueous extracts of Kheaw-Hom remedy and its ingredients against 50 $TCID_{50}$ of enterovirus 71 in duplicate experiments (Continued)

Plants name	Code	Concentration of the aqueous extracts of Kheaw-Hom remedy and its ingredients (µg/ml)							
		6.25	12.5	25	50	100	200	400	Conclusion
T.chantrieri	TCA	NT	-	-	+	+	Toxic	Toxic	50
V.zizanioides	VZA	NT	NT	NT	-	-	-	-	>400
Kheaw-Hom	**KHA**	**NT**	**NT**	**NT**	**-**	**-**	**-**	**-**	**>400**
Ribavirin	**RBV**	**NT**	**NT**	**NT**	**-**	**-**	**-**	**-**	**>400**

"+" = less than 50% cytopathic effect

"-" = more than 50% cytopathic effect

NT = not tested

Table 4-7 Antiviral activities of the aqueous extracts of Kheaw-Hom remedy and its ingredients against 25 $TCID_{50}$ of enterovirus 71 in duplicate experiments

Plants name	Code	Concentrations of the aqueous extracts of Kheaw-Hom remedy and its ingredients (µg/ml)							
		6.25	12.5	25	50	100	200	400	Conclusion
A.evecta	AEA	NT	NT	NT	-	-	+	+	200
C.fruticosa (green leaves)	CFA	NT	NT	NT	-	-	-	-	>400
C.fruticosa (red leaves)	COA	NT	NT	NT	-	-	-	-	>400
D.loureiri	DLA	NT	-	-	-	-	Toxic	Toxic	>100
E.stoechadosmum	ESA	NT	NT	NT	-	-	-	-	>400
G.malaccensis	GMA	NT	NT	NT	-	-	-	+	400
K.galanga	KGA	-	-	-	-	Toxic	Toxic	Toxic	>50
M.siamensis	MSA	NT	NT	-	-	+	+	Toxic	100
M.ferrea	MLA	NT	NT	-	-	-	-	Toxic	>200
N.nucifera	NNA	NT	-	-	-	+	Toxic	Toxic	100
P.cablin	PCA	NT	NT	NT	-	-	-	-	>400
S.exigua	SEA	NT	NT	NT	-	-	-	-	>400

"+" = less than 50% cytopathic effect

"-" = more than 50% cytopathic effect

NT = not tested

Table 4-7 Antiviral activities of the aqueous extracts of Kheaw-Hom remedy and its ingredients against 25 $TCID_{50}$ of enterovirus 71 in duplicate experiments (Continued)

Plants name	Code	Concentrations of the aqueous extracts of Kheaw-Hom remedy and its ingredients (µg/ml)							
		6.25	12.5	25	50	100	200	400	Conclusion
T.chantrieri	TCA	NT	-	-	+	+	Toxic	Toxic	50
V.zizanioides	VZA	NT	NT	NT	-	-	-	-	>400
Kheaw-Hom	**KHA**	**NT**	**NT**	**NT**	**-**	**-**	**-**	**+**	**400**
Ribavirin	**RBV**	**NT**	**NT**	**NT**	**-**	**+**	**+**	Toxic	**100**

"+" = less than 50% cytopathic effect

"-" = more than 50% cytopathic effect

NT = not tested

Table 4-8 Antiviral activities of the aqueous extracts of Kheaw-Hom remedy and some of its ingredients against different concentrations of enterovirus 71

Plants name	**Code**	**Concentrations of enterovirus 71 ($TCID_{50}$)**		
		100	**50**	**25**
A.evecta	AEA	+ (400 μg/ml)	+ (400 μg/ml)	+ (200 μg/ml)
G.malaccensis	GMA	+ (400 μg/ml)	+ (400 μg/ml)	+ (400 μg/ml)
M.siamensis	MSA	+ (200 μg/ml)	+ (200 μg/ml)	+ (100 μg/ml)
T.chantrieri	TCA	+ (50 μg/ml)	+ (50 μg/ml)	+ (50 μg/ml)
N.nucifera	NNA	-	-	+ (100 μg/ml)
Kheaw-Hom	**KHA**	**-**	**-**	**+** (400 μg/ml)

"+" = less than 50% cytopathic effect

"-" = more than 50% cytopathic effect

4.4 Antimicrobial activities

4.4.1 Disc diffusion method

All extracts were tested for their antimicrobial activity by disc diffusion method to determine the inhibition zone against four gram positive bacteria (*Staphylococcus aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus* DMST 20651, *Staphylococcus epidermidis* ATCC 12228 and *Streptococcus pyogenes* ATCC 19615), one gram negative bacterium (*Klebsiella pneumoniae* ATCC 700603) and one fungus (*Candida albicans* ATCC 90028) that relate to skin infection complications in exanthematous fever such as hand, foot, and mouth disease and chickenpox. The results of disc diffusion method are summarized in **Table 4-9.**

Staphylococcus aureus, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, and *Streptococcus pyogenes* are facultive anaerobic gram-positive coccal bacteria. They are found in the human respiratory tract and on the skin (Kluytmans *et al.,* 1997). The results found that most ethanolic extracts of Kheaw-Hom remedy and its plant ingredients were able to inhibit the four gram positive bacteria. The range of the inhibition zone was 7 to 16 mm. The ethanolic extracts of Kheaw-Hom remedy showed antimicrobial activity against four gram positive bacteria with inhibition zone of 7.33±0.58, 7.00±0.00, 8.00±0.00, and 12.67±0.58 mm. The result of Kheaw-Hom remedy has never been reported. This study is the first report on antimicrobial activity by using disc diffusion method.

There are nine ethanolic extracts of its plant ingredients namely *P. cablin* (PCE), *E. stoechadosmum* (ESE), *D. loureiri* (DLE), *G. malaccensis* (GME), *T. chantrieri* (TCE), *S. exigua* (SEE), *C. gigantea* (CGE), *M. ferrea* (MLE), and *M. siamensis* (MSE) which exhibited antimicrobial activity against *S. aureus*, MRSA, and *S. epidermidis* with range of inhibition zone 7 to 12.67 mm, 7 to 13 mm and 7 to 14.33 mm, respectively. Specifically, the ethanolic extract of *S.exigua* (SEE) showed the highest antimicrobial activity against these three positive bacteria with inhibition zone of 12.67±0.58, 13.00±0.00 and 14.33±0.58 mm, respectively. The aqueous extract of *N.nucifera* (NNA) was the only one of all the aqueous extracts which showed antimicrobial activity against these three gram positive bacteria with low inhibition zone of 7.33±0.58, 8±0.00 and 7±0.00 mm.

Kheaw-Hom remedy and its all plant ingredients showed antimicrobial activity against *S. pyogenes* with range of inhibition zone 8 to16 mm. The ethanolic extract of Kheaw-Hom remedy showed antimicrobial activity against *S. pyogenes* with inhibition zone of 12.67±0.58 mm. The ethanolic extract of *S. exigua* showed the highest activity with inhibition zone 16.00±1.00 mm. The result of *S.exigua* is the first report on antimicrobial activity by using disc diffusion method. The ethanolic extract of *K. galanga* showed the lowest activity with inhibition zone of 8.00±1.00 mm. However, all extracts showed antimicrobial activity lower than gentamicin (positive control) except methicillin-resistant *S. aureus* that is resistant to gentamicin.

K. pneumoniae is a facultive anaerobic gram negative bacterium (Murray *et al.,* 2003). It is an important cause of human infections (Podschun and Ullmann, 1998). Neither ethanolic nor aqueous extracts of Kheaw-Hom remedy and its plant ingredients were able to inhibit gram negative *K. pneumoniae* while gentamicin (positive control) showed antimicrobial activity with inhibition zone of 13.33±0.58 mm.

C. albicans is a diploid fungus (Murray *et al.,* 2003). It is a part of the normal human flora found in the mouth, gut, and vagina (Hietala *et al.,* 1982) The ethanolic extract of Kheaw-Hom remedy had no activity against *C. albicans*. However, there are three ethanolic extracts of plant ingredients namely *E. stoechadosmum* (ESE), *K. galangal* (KGE) and *S. exigua* (SEE) which showed antimicrobial activity against *C. albicans* with low inhibition zone of 8.67±1.15, 9.33±0.58 and 8.67±0.58, respectively that are lower than amphotericin B (positive control) with inhibition zone of 21.00±0.00 mm. All the aqueous extracts of Kheaw-Hom remedy and its plant ingredients were not able to inhibit *C.albicans*.

There are five plant ingredients that were reported in the previous study on antimicrobial activity by using disc diffusion method. Firstly, the methanolic fraction of *A. evecta* inhibited *S. aureus, S. epidermidis,* and *K. pneumoniae* (Khan and Omoloso, 2008) and the essential oils of *L. rugosa* inhibited *S. aureus, S. pyrogenes,* and *C. albicans* (Linh and Thach, 2010; Acharya *et al.,* 2014) but both the ethanolic and aqueous extracts of *A. evecta* and *L. rugosa* in this study were not able to inhibit. It may be due to differences among of the active compounds in the plants because of different extraction methods. The steroidal saponins isolated from the leaves of *C. fruticosa* were not able to inhibit *S.aureus* and *C. albicans* (Fouedjou *et al.,* 2014) as in this study. Subhadhirasakul

and Pechpongs reported that the chloroform extract of *M. siamensis* inhibited *S. aureus* (Subhadhirasakul and Pechpongs, 2005) and finally, the ethanolic extract of *M. ferrea* inhibited *S. aureus* and MRSA (Sattaponpan and Kondo, 2011) which agrees with this study.

Table 4-9 Antimicrobial activity of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients by disc diffusion method (mean±SD) (n=3)

Sample	Code	Inhibition zone (mm)					
		S.aureus	**MRSA**	***S.epidermidis***	***K.pneumoniae***	***S.pyogenes***	***C.albicans***
A.evecta	AEE	NI	NI	NI	NI	9.33±1.53	NI
	AEA	NI	NI	NI	NI	NI	NI
C.fruticosa (green leaves)	CFE	NI	NI	NI	NI	9.00±1.73	NI
	CFA	NI	NI	NI	NI	NI	NI
C.fruticosa (red leaves)	COE	NI	NI	NI	NI	8.33±1.15	NI
	COA	NI	NI	NI	NI	NI	NI
C.gigantea	CGE	7.00±0.00	7.00±0.00	7.00±0.00	NI	9.00±0.00	NI
	CGA	NI	NI	NI	NI	NI	NI
D.loureiri	DLE	10.33±0.58	10.33±0.58	11.33±0.58	NI	14.67±0.58	NI
	DLA	NI	NI	NI	NI	NI	NI
E.stoechadosmum	ESE	8.00±1.73	7.67±1.15	9.33±1.15	NI	13.00±0.00	8.67±1.15
	ESA	NI	NI	NI	NI	NI	NI
G.malaccensis	GME	7.00±0.00	7.00±0.00	7.67±0.58	NI	11.67±0.58	NI
	GMA	NI	NI	NI	NI	NI	NI
K.galanga	KGE	NI	NI	NI	NI	8.00±1.00	9.33±0.58
	KGA	NI	NI	NI	NI	NI	NI

NI = No inhibition zone

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Table 4-9 Antimicrobial activity of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients by disc diffusion method (mean±SD) (n=3)

Sample	Code	Inhibition zone (mm)					
		S.aureus	**MRSA**	***S.epidermidis***	***K.pneumoniae***	***S.pyogenes***	***C.albicans***
L.rugosa	LRE	NI	NI	NI	NI	11.33±1.53	NI
	LRA	NI	NI	NI	NI	NI	NI
M.siamensis	MSE	8.33±0.58	9.33±0.58	9.67±0.58	NI	13.67±2.08	NI
	MSA	NI	NI	NI	NI	NI	NI
M.ferrea	MLE	8.00±0.00	8.00±0.00	8.00±1.00	NI	10.33±0.58	NI
	MLA	NI	NI	NI	NI	NI	NI
M.elengi	MEE	NI	NI	NI	NI	8.67±0.58	NI
	MEA	NI	NI	NI	NI	NI	NI
M.fragrans	MFE	NI	NI	NI	NI	10.00±0.00	NI
	MFA	NI	NI	NI	NI	NI	NI
N.nucifera	NNE	NI	NI	NI	NI	8.67±1.53	NI
	NNA	NI	NI	NI	NI	NI	NI
P.cablin	PCE	7.00±0.00	7.67±0.58	7.00±0.00	NI	9.67±0.58	NI
	PCA	NI	NI	NI	NI	NI	NI
S.exigua	SEE	12.67±0.58	13.00±0.00	14.33±0.58	NI	16.00±1.00	8.67±0.58
	SEA	NI	NI	NI	NI	NI	NI

NI = No inhibition zone

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Table 4-9 Antimicrobial activity of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients by disc diffusion method (mean±SD) (n=3)

Sample	Code	Inhibition zone (mm)					
		S.aureus	**MRSA**	***S.epidermidis***	***K.pneumoniae***	***S.pyogenes***	***C.albicans***
T.chantrieri	TCE	8.00±0.00	8.00±0.00	9.33±0.58	NI	11.33±0.58	NI
	TCA	NI	NI	NI	NI	NI	NI
V.zizanioides	VZE	NI	NI	NI	NI	11.67±0.58	NI
	VZA	NI	NI	NI	NI	NI	NI
Kheaw-Hom	KHE	7.33±0.58	7.00±0.00	8.00±0.00	NI	12.67±0.58	NI
	KHA	NI	NI	NI	NI	NI	NI
Gentamicin	Gen	22.00±0.00	NI	25.00±1.00	13.33±0.58	20.33±0.58	NT
Amphotericin B	Amp	NT	NT	NT	NT	NT	21.00±0.00

NI = No inhibition zone, NT = Not tested

4.4.2 Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) by microtitre plate-based antimicrobial assay

All extracts were tested for antimicrobial activity by microtitre plate-based antimicrobial assay to determine minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) against four gram positive bacteria (*Staphylococcus aureus* ATCC 25923, Methicillin-resistant *Staphylococcus aureus* DMST 20651, *Staphylococcus epidermidis* ATCC 12228 and *Streptococcus pyogenes* ATCC 19615), one gram negative bacterium (*Klebsiella pneumoniae* ATCC 700603) and one fungus (*Candida albicans* ATCC 90028). MIC and MMC values of all extracts are shown in **Table 4-10** and **Table 4-11.**

The results show that most ethanolic extracts of Kheaw-Hom remedy and its plant ingredients were able to inhibit five gram positive bacteria. The ethanolic extract of Kheaw-Hom remedy (KHE) showed antimicrobial activity against *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *S. epidermidis* and *S. pyogenes* with MIC values of 0.625, 0.625, 1.25 and 0.625 mg/ml and MMC values of 1.25, 0.625, 2.5 and 0.625 mg/ml, respectively. The ethanolic extract of Kheaw-Hom remedy had no activity against *K. pneumonia* and *C.albicans*. Furthermore, the aqueous extract of Kheaw-Hom remedy had no activity against all bacteria and the fungus.

There are eleven ethanolic extracts of plant ingredients namely *P. cablin* (PCE), *L. rugosa* (LRE), *E. stoechadosmum* (ESE), *M. fragrans* (MFE), *D. loureiri* (DLE), *G. malaccensis* (GME), *T. chantrieri* (TCE), *S. exigua* (SEE), *C. gigantea* (CGE), *M. ferrea* (MLE) and *M. siamensis* (MSE) which exhibited antimicrobial activity against *S. aureus* with range of MIC values of 0.005 to 5 mg/ml and MMC values showed the same range. The ethanolic extract of *M. siamensis* (MSE) showed strong antimicrobial activity against *S. aureus* with MIC value of 0.005 mg/ml and MMC value of 0.005 mg/ml while the ethanolic extract of *S. exigua* (SEE) and *M. ferrea* (MLE) showed moderate antimicrobial activity with MIC values of 0.156 and 0.156 mg/ml and MMC values of 0.313 and 0.625 mg/ml, respectively. There are eight aqueous extracts of plant ingredients namely *P. cablin* (PCE), *D. loureiri* (DLE), *T. chantrieri* (TCE), *S. exigua* (SEE), *M. elengi* (MEE), *M. ferrea* (MLE), *M. siamensis* (MSE) and *N. nucifera* (NNE) which showed antimicrobial activity against *S. aureus* with range of low MIC and MMC

values of 1.25 to 5 mg/ml. All extracts showed antimicrobial activity lower than gentamicin (positive control) with MIC value of 0.195 µg/ml.

There are nine ethanolic extracts of plant ingredients namely *P. cablin* (PCE), *E. stoechadosmum* (ESE), *D. loureiri* (DLE), *G. malaccensis* (GME), *T. chantrieri* (TCE), *S. exigua* (SEE), *C. gigantea* (CGE), *M. ferrea* (MLE) and *M. siamensis* (MSE) which exhibited antimicrobial activity against MRSA with range of MIC values of 0.005 to 2.5 mg/ml and MMC values of 0.005 to 5 mg/ml. The ethanolic extract of *M. siamensis* (MSE) showed strong antimicrobial activity against MRSA with MIC value of 0.005 mg/ml and MMC value of 0.005 mg/ml and the ethanolic extract of *S.exigua* (SEE) showed the second highest antimicrobial activity with MIC value of 0.039 mg/ml and MMC value of 0.313 mg/ml. The ethanolic extract of *M. ferrea* (MLE) showed moderate antimicrobial activity with MIC value of 0.156 mg/ml and MMC value of 0.625 mg/ml, respectively. There are nine aqueous extracts of of plant ingredients namely *P. cablin* (PCE), *M. fragrans* (MFE), *D. loureiri* (DLE), *T. chantrieri* (TCE), *S. exigua* (SEE), *M. elengi* (MEE), *M. ferrea* (MLE), *M. siamensis* (MSE) and *N. nucifera* (NNE) which showed antimicrobial activity against *S. aureus* with range of low MIC and MMC values of 1.25 to 5 mg/ml.

Results of antimicrobial activity against *S. epidermidis* showed that nine ethanolic extracts of plant ingredients were able to inhibit it namely *P. cablin* (PCE), *E. stoechadosmum* (ESE), *D. loureiri* (DLE), *G. malaccensis* (GME), *T. chantrieri* (TCE), *S. exigua* (SEE), *C. gigantea* (CGE), *M. ferrea* (MLE) and *M. siamensis* (MSE) with range of MIC values of 0.039 to 5 mg/ml and MMC values of 0.039 to 5 mg/ml. The highest activity against *S. epidermidis* was *M. siamensis* (MSE) with MIC value of 0.039 mg/ml and MMC value of 0.039 mg/ml. The second highest activity was *S. exigua* (SEE) that showed MIC value of 0.039 mg/ml and MMC value of 0.313 mg/ml. There are six aqueous extracts of plant ingredients namely *P. cablin* (PCE), *D. loureiri* (DLE), *S. exigua* (SEE), *M. elengi* (MEE), *M. ferrea* (MLE) and *N. nucifera* (NNE) which showed antimicrobial activity against *S. aureus* with range of low MIC and MMC values of 1.25 to 5 mg/ml. However, all extracts showed antimicrobial activity lower than gentamicin (positive control) with MIC value of 0.098 µg/ml and MMC value of 0.098 µg/ml.

Results of antimicrobial activity against *S.pyogenes* showed that eleven ethanolic extracts of plant ingredients were able to inhibit it namely *P. cablin* (PCE),

E. stoechadosmum (ESE), *V. zizanioides* (VZE), *K. galangal* (KGE), *M. fragrans* (MFE), *D. loureiri* (DLE), *G. malaccensis* (GME), *S. exigua* (SEE), *M. ferrea* (MLE) and *M. siamensis* (MSE) and *N. nucifera* (NNE) with range of MIC values of 0.019 to 1.25 mg/ml and MMC values of 0.019 to 2.5 mg/ml. The highest activity against *S. pyogenes* was *M. siamensis* (MSE) with MIC value of 0.019 mg/ml and MMC value of 0.019 mg/ml. The secondary highest activity were *M. fragrans* (MFE) and *S. exigua* (SEE) that showed the same MIC values of 0.039 mg/ml and MMC values of 0.313 mg/ml. All the aqueous extracts of Kheaw-Hom remedy and its plant ingredients were able to inhibit *S. pyogenes*.

Neither ethanolic nor aqueous extracts of Kheaw-Hom remedy and its plant ingredients were able to inhibit gram negative *K. pneumoniae* while gentamicin (positive control) showed the antimicrobial activity with MIC value of 6.25 µg/ml and MMC value of 25 µg/ml.

The ethanolic extract of Kheaw-Hom remedy had no activity against *C. albicans*. However, the ethanolic extract of *S. exigua* that is an ingredient in this remedy showed the highest activity against *C. albicans* with MIC value of 0.625 mg/ml and MMC value of 0.625 mg/ml whereas the aqueous extract of Kheaw-Hom remedy and its plant ingredients had no activity against *C. albicans*.

The ethanolic extracts of *D. loureiri, K. galangal, M. elengi* and *N. nucifera* had weak activity against *S. aureus* and MRSA (Sattaponpan and Kondo, 2011) which agrees with this study. Previous work found that ethy-*p*-methoxycinnamate (EPMC), which was isolated from *K. galanga,* was able to inhibit *S. aureus* and *C. albicans* (Omar *et al.*, 2014). The ethanolic extract of *M. ferrea* had moderate activity against *S. aureus* (Sattaponpan and Kondo, 2011) with MIC value of 0.62 mg/ml, which is 4 times less powerful than that in this study, and against MRSA with MIC value of 0.31 mg/ml which is twice more powerful than that shown in this study. The same study reported that the ethanolic extract of *M. siamensis* had moderate efficacy against *S.aureus* and MRSA (Sattaponpan and Kondo, 2011). This present study showed higher antimicrobial activity against both strains than Sattaponpan and Kondo found with MIC values 250 and 125 times, respectively. Different sources, stage of growth, and mouth of collection of plant materials may have influenced results. Finally, the ethanolic extract of *S.exigua* had good

activity against MRSA while flavanone isolated from *S.exigua* also inhibited this microbe in previous study (Sato *et al.*, 1995).

Table 4-10 Minimum inhibitory concentration (MIC) of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients by using microtitre plate-based antimicrobial assay (n=3)

Sample	Code	Minimum inhibitory concentration (mg/ml)					
		S.aureus	**MRSA**	***S.epidermidis***	***K.pneumoniae***	***S.pyogenes***	***C.albicans***
A.evecta	AEE	NI	NI	NI	NI	NI	NI
	AEA	NI	NI	NI	NI	NI	NI
C.fruticosa (green leaves)	CFE	NI	NI	NI	NI	NI	NI
	CFA	NI	NI	NI	NI	NI	NI
C.fruticosa (red leaves)	COE	NI	NI	NI	NI	NI	NI
	COA	NI	NI	NI	NI	NI	NI
C.gigantea	CGE	5	2.5	5	NI	NI	NI
	CGA	NI	NI	NI	NI	NI	NI
D.loureiri	DLE	2.5	2.5	1.25	NI	1.25	2.5
	DLA	2.5	2.5	1.25	NI	NI	NI
E.stoechadosmum	ESE	1.25	2.5	1.25	NI	0.625	1.25
	ESA	NI	5	NI	NI	NI	NI
G.malaccensis	GME	2.5	2.5	2.5	NI	1.25	2.5
	GMA	NI	NI	NI	NI	NI	NI
K.galanga	KGE	NI	NI	NI	NI	0.625	0.625
	KGA	NI	NI	NI	NI	NI	NI

NI = No inhibition, NT = Not tested

Table 4-10 Minimum inhibitory concentration (MIC) of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients by using microtitre plate-based antimicrobial assay (n=3)

Sample	Code	Minimum inhibitory concentration (mg/ml)					
		S.aureus	**MRSA**	***S.epidermidis***	***K.pneumoniae***	***S.pyogenes***	***C.albicans***
L.rugosa	LRE	5	NI	NI	NI	NI	NI
	LRA	NI	NI	NI	NI	NI	NI
V.zizanioides	VZE	NI	NI	NI	NI	1.25	NI
	VZA	NI	NI	NI	NI	NI	NI
M.siamensis	MSE	0.005	0.005	0.039	NI	0.019	NI
	MSA	2.5	2.5	NI	NI	NI	NI
M.ferrea	MLE	0.156	0.625	0.625	NI	1.25	NI
	MLA	2.5	2.5	2.5	NI	NI	NI
M.elengi	MEE	NI	NI	NI	NI	NI	NI
	MEA	5	5	5	NI	NI	NI
M.fragrans	MFE	5	NI	NI	NI	0.156	NI
	MFA	NI	5	NI	NI	NI	NI
N.nucifera	NNE	NI	NI	NI	NI	0.625	NI
	NNA	1.25	1.25	2.5	NI	NI	NI
P.cablin	PCE	0.625	1.25	0.625	NI	0.156	2.5
	PCA	2.5	NI	2.5	NI	NI	NI

NI = No inhibition, NT = Not tested

Table 4-10 Minimum inhibitory concentration (MIC) of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients by using microtitre plate-based antimicrobial assay (n=3)

Sample	Code	Minimum inhibitory concentration (mg/ml)					
		S.aureus	**MRSA**	***S.epidermidis***	***K.pneumoniae***	***S.pyogenes***	***C.albicans***
T.chantrieri	TCE	2.5	2.5	5	NI	NI	NI
	TCA	5	5	NI	NI	NI	NI
S.exigua	SEE	0.156	0.156	0.156	NI	0.156	0.625
	SEA	5	5	5	NI	NI	NI
Kheaw-Hom	KHE	0.625	0.625	1.25	NI	0.625	NI
	KHA	NI	NI	NI	NI	NI	NI
Gentamicin	Gen	0.195 (µg/ml)	>100(µg/ml)	0.195 (µg/ml)	6.25(µg/ml)	0.391(µg/ml)	NT
Amphotericin B	Amp	NT	NT	NT	NT	NT	1 (µg/ml)

NI = No inhibition, NT = Not tested

Table 4-11 Minimum microbicidal concentration (MMC) of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients by subculturing method (n=3)

Sample	Code	Minimum microbicidal concentration (mg/ml)					
		S.aureus	**MRSA**	***S.epidermidis***	***K.pneumoniae***	***S.pyogenes***	***C.albicans***
A.evecta	AEE	NI	NI	NI	NI	NI	NI
	AEA	NI	NI	NI	NI	NI	NI
C.fruticosa (green leaves)	CFE	NI	NI	NI	NI	NI	NI
	CFA	NI	NI	NI	NI	NI	NI
C.fruticosa (red leaves)	COE	NI	NI	NI	NI	NI	NI
	COA	NI	NI	NI	NI	NI	NI
C.gigantea	CGE	5	2.5	5	NI	NI	NI
	CGA	NI	NI	NI	NI	NI	NI
D.loureiri	DLE	2.5	2.5	5	NI	2.5	5
	DLA	2.5	2.5	1.25	NI	NI	NI
E.stoechadosmum	ESE	1.25	2.5	1.25	NI	0.625	1.25
	ESA	NI	5	NI	NI	NI	NI
G.malaccensis	GME	2.5	5	5	NI	1.25	5
	GMA	NI	NI	NI	NI	NI	NI
K.galanga	KGE	NI	NI	NI	NI	0.625	2.5
	KGA	NI	NI	NI	NI	NI	NI

NI = No inhibition, NT = Not tested

Table 4-11 Minimum microbicidal concentration (MMC) of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients by subculturing method (n=3)

Sample	Code	Minimum microbicidal concentration (mg/ml)					
		S.aureus	**MRSA**	***S.epidermidis***	***K.pneumoniae***	***S.pyogenes***	***C.albicans***
L.rugosa	LRE	5	NI	NI	NI	NI	NI
	LRA	NI	NI	NI	NI	NI	NI
M.siamensis	MSE	0.005	0.005	0.039	NI	0.195	NI
	MSA	5	5	NI	NI	NI	NI
M.ferrea	MLE	0.625	0.625	0.625	NI	1.25	NI
	MLA	2.5	2.5	2.5	NI	NI	NI
M.elengi	MEE	NI	NI	NI	NI	NI	NI
	MEA	5	5	5	NI	NI	NI
M.fragrans	MFE	5	NI	NI	NI	0.156	NI
	MFA	NI	5	NI	NI	NI	NI
N.nucifera	NNE	NI	NI	NI	NI	0.625	NI
	NNA	1.25	1.25	2.5	NI	NI	NI
P.cablin	PCE	0.625	2.5	1.25	NI	0.156	5
	PCA	2.5	NI	2.5	NI	NI	NI
S.exigua	SEE	0.313	0.313	0.313	NI	0.156	0.625
	SEA	5	5	5	NI	NI	NI

NI = No inhibition, NT = Not tested

Table 4-11 Minimum microbicidal concentration (MMC) of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients by subculturing method (n=3)

Sample	Code	Minimum microbicidal concentration (mg/ml)					
		S.aureus	**MRSA**	***S.epidermidis***	***K.pneumoniae***	***S.pyogenes***	***C.albicans***
T.chantrieri	TCE	2.5	2.5	5	NI	NI	NI
	TCA	5	5	NI	NI	NI	NI
V.zizanioides	VZE	NI	NI	NI	NI	1.25	NI
	VZA	NI	NI	NI	NI	NI	NI
Kheaw-Hom	KHE	1.25	0.625	2.5	NI	0.625	NI
	KHA	NI	NI	NI	NI	NI	NI
Gentamicin		0.195 (μg/ml)	>100 (μg/ml)	0.098 (μg/ml)	25 (μg/ml)	0.391 (μg/ml)	NT
Amphotericin B		NT	NT	NT	NT	NT	1 (μg/ml)

NI = No inhibition, NT = Not tested

4.5 Anti-inflammatory activites

4.5.1 Assay for NO inhibitory effects in RAW 264.7 cells

Anti-inflammatory activities of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients were tested by measuring their inhibitory effects on LPS-induced nitric oxide (NO) release from murine macrophages cell lines (RAW 264.7). Measurement of nitric oxide (NO) production was performed by using Griess reaction and cytotoxicity was performed by MTT assay. All results were shown in **table 4-12.**

For the Kheaw-Hom remedy extracts, the aqueous extract (KHA) showed anti-inflammatory activity with IC_{50} value of 46.86±0.82 μg/ml which is higher than the ethanolic extract (KHE) with IC_{50} value of 59.77±3.76 μg/ml. However, both of the Kheaw-Hom remedy extracts were less than prednisolone (positive control) with IC_{50} value of 1.31±0.05μg/ml.

There were ten extracts of plant ingredients namely *P. cablin* (PCE), *E. stoechadosmum* (ESE), *K. galanga* (KGE), *M. fragrans* (MFE), *D. loureiri* (DLE), *A. evecta* (AEE), *G. malaccensis* (GME), *S. exigua* (SEE), *M. ferrea* (MLE) and *M. siamensis* (MSE) which exhibited anti-inflammatory activity. The ethanolic extract of *M. siamensis* (MSE) showed the highest anti-inflammatory activity with IC_{50} value of 11.55±2.70 μg/ml. The second highest anti-inflammatory activity was *A. evecta* (AEE) with IC_{50} value of 14.26±1.25 μg/ml. The third was *S. exigua* (SEE) which showed IC_{50} value of 22.84±3.95 μg/ml. Moderate anti-inflammatory activity was *M. fragrans* (MFE) with IC_{50} value of 65.71±1.09 μg/ml. There are two extracts of plant ingredients namely *E.stoechadosmum* (ESE) and *M. ferrea* (MLE) which showed weak anti-inflammatory activity with IC_{50} values of 78.12±7.86 and 88.67±5.21 μg/ml, respectively. Finally, eight extracts namely *L. rugosa* (LRE), *C. fruticosa* red leaf (COE), *C. fruticosa* green leaf (CFE), *V. zizanioides* (VZE), *T. chantrieri* (TCE), *C. gigantea* (CGE) *M. elengi* (MEE) and *N. nucifera* (NNE) had no measurable activity (IC_{50} >100 μg/ml).

For the aqueous extract of plant ingredients, there were five extracts namely *K. galanga* (KGA), *A. evecta* (AEA), *S. exigua* (SEA), *M. elengi* (MEA) and *N. nucifera* (NNA) which exhibited anti-inflammatory activity. In particular, *S. exigua* (SEA) and *K. galanga* (KGA) showed high anti-inflammatory activity with IC_{50}

values of 3.17±0.68 and 10.30±0.99 μg/ml, respectively. The two aqueous extracts of *M. elengi* (MEA) and *N. nucifera* (NNA) showed moderate anti-inflammatory activity with IC_{50} values of 48.25±5.02 and 43.91±2.60 μg/ml. The aqueous extract of *A. evecta* (AEA) showed weak anti-inflammatory activity with IC_{50} value of 82.98±3.08 μg/ml. Finally, thirteen other extracts had no measurable activity (IC_{50} >100 μg/ml). All the extracts were lower than prednisolone (IC_{50} = 1.31±0.05 μg/ml) which is a positive anti-inflammatory drug.

There are six plant ingredients in Kheaw-Hom remedy that were reported in the previous study on anti-inflammatory activity by inhibition of nitric oxide (NO) production including the ethanolic extract of *M. ferrea,* which showed high anti-inflammatory activity, *D. loureiri* and *K. galanga,* which showed moderate anti-inflammatory activity. *M. siamensis* and *M. elengi* showed weak anti-inflammatory activity (Makchuchit, 2010) but the ethanolic of *M. siamensis* in this study showed highest anti-inflammatory activity. Finally, the ethanolic extract of *G. malaccensis* showed high anti-inflammatory activity in previous studies (Anuthakoengkun and Itharat, 2014). In addition, the stillbenoid isolated from stem wood of *D. loureiri* showed anti-inflammatory activity by inhibition COX-1 and COX-2 production (Likhitwitayawuid *et al.*, 2002). Ethyl-*p*-methoxycinnamate (EMPC) isolated from *K. galanga* showed anti-inflammatory activity by inhibition IL-1, TNF-α production (Umar *et al.*, 2014), COX-1 and COX-2 (Umar *et al.*, 2012) and patchouli alcohol isolated from *P. cablin* inhibited the over-expression of iNOS, IL-1, IL-6, TNF-α, and PGE_2 (Jeong *et al.*, 2013; Yu *et al.*, 2011).

Table 4-12 Anti-inflammatory activity by Griess reagent and MTT assay on RAW 264.7 cells at various concentrations

Plant name	Code	% Inhibition of NO production / (% Toxicity)								IC_{50} (µg/ml)
		0.1 µg/ml	1 µg/ml	5 µg/ml	10 µg/ml	30 µg/ml	50 µg/ml	70 µg/ml	100 µg/ml	
A.evecta	AEE	-	-3.26±2.33 (-10.65±0.76)	-	34.36±3.76 (-10.03±0.76)	92.681±3.318 (-5.98±1.44)	99.40±0.66 (16.46±5.71)	-	-	14.26±1.25
	AEA	-	-2.96±6.77 (0.20±5.75)	-	0.92±5.63 (3.60±0.34)	-	24.68±2.69 (-2.76±4.74)	-	64.29±3.34 (-2.18±8.40)	82.98±3.08
C.fruticosa (red leaf)	CFE	-	-	-	-	-	-	-	27.59±0.71 (-25.70±9.35)	>100
	CFA	-	-	-	-	-	-	-	19.26±1.36 (12.76±14.65)	>100
C.fruticosa (green leaf)	COE	-	-	-	-	-	-	-	36.32±1.56 (6.46±0.55)	>100
	COA	-	-	-	-	-	-	-	18.54±4.31 (5.13±11.82)	>100
C.gigantea	CGE	-	-	-	-	-	-	-	19.34±3.18 (-29.41±18.05)	>100
	CGA	-	-	-	-	-	-	-	13.83±5.52 (-9.96±6.88)	>100

Note: All data represents the mean ± SEM in triplicate experiments.

Table 4-12 Anti-inflammatory activity by Griess reagent and MTT assay on RAW 264.7 cells at various concentrations

Plant name	Code	% Inhibition of NO production / (% Toxicity)								IC_{50} (µg/ml)
		0.1 µg/ml	1 µg/ml	5 µg/ml	10 µg/ml	30 µg/ml	50 µg/ml	70 µg/ml	100 µg/ml	
D.loureiri	DLE	-	2.06±3.01 (-13.34±12.67)	-	14.08±4.70 (-11.87±4.43)	-	58.12±4.39 (-12.35±7.40)	-	87.94±5.07 (20.57±5.48)	40.73±4.99
	DLA	-	-	-	-	-	-	-	29.98±4.12 (4.28±4.57)	>100
E.stoechadosmum	ESE	-	-3.44±0.74 (-8.89±8.97)	-	8.19±5.97 (-1.38±4.71)	-	27.56±5.73 (-8.62±6.20)	-	72.66±6.45 (-7.38±9.18)	78.12±7.86
	ESA	-	-	-	-	-	-	-	38.08±5.81 (-19.05±3.06)	>100
G.malaccensis	GME	-	-1.78±1.91 (-18.80±7.00)	-	25.62±5.55 (-14.83±3.02)	58.63±6.43 (-0.89±11.16)	81.21±4.25 (-1.89±6.34)	-	-	24.11±4.82
	GMA	-	-	-	-	-	-	-	19.40±3.67 (-5.09±7.56)	>100
K.galanga	KGE	-	-5.90±2.56 (12.42±4.52)	-	3.26±3.04 (4.64±1.79)	-	54.05±5.85 (-7.15±3.07)	-	86.58±3.50 (1.54±10.93)	46.15±5.39
	KGA	-	-16.30±5.05 (-7.37±1.81)	-	50.40±5.66 (-0.03±3.42)	-	76.78±4.48 (13.60±2.02)	84.75±4.23 (6.26±4.90)	-	10.30±0.99

Note: All data represents the mean ± SEM in triplicate experiments.

Table 4-12 Anti-inflammatory activity by Griess reaction and MTT assay on RAW 264.7 cells at various concentrations

Plant name	Code	% Inhibition of NO production / (% Toxicity)								IC_{50} (µg/ml)
		0.1 µg/ml	1 µg/ml	5 µg/ml	10 µg/ml	30 µg/ml	50 µg/ml	70 µg/ml	100 µg/ml	
L.rugosa	LRE	-	-	-	-	-	-	-	26.56±5.31 (6.43±17.17)	>100
	LRA	-	-	-	-	-	-	-	47.00±0.41 (30.94±8.80)	>100
M.siamensis	MSE	-	3.50±3.46 (-5.23±5.96)	-	48.46±7.45 (16.62±2.27)	63.21±4.06 (4.20±6.26)	78.13±2.66 (16.64±12.74)	-	-	11.55±2.70
	MSA	-	-	-	-	-	-	-	0.67±2.65 (-12.36±5.77)	>100
M.ferrea	MLE	-	-15.05±2.24 (-12.02±4.32)	-	-1.33±1.61 (-15.80±6.94)	-	33.82±3.25 (0.21±12.54)	54.60±1.04 (18.77±5.11)	-	65.71±1.09
	MLA	-	-	-	-	-	-	-	31.53±4.76 (-1.85±2.54)	>100
M.elengi	MEE	-	-	-	-	-	-	-	13.08±0.37 (-26.29±8.79)	>100
	MEA	-	-7.47±0.96 (3.96±4.40)	-	-2.10±2.65 (-3.59±3.69)	-	53.00±6.02 (12.10±2.95)	-	70.59±1.57 (6.84±5.91)	48.25±5.02

Note: All data represents the mean ± SEM in triplicate experiments.

Table 4-12 Anti-inflammatory activity by Griess reaction and MTT assay on RAW 264.7 cells at various concentrations

Plant name	Code	% Inhibition of NO production / (% Toxicity)								IC_{50} (μg/ml)
		0.1 μg/ml	1 μg/ml	5 μg/ml	10 μg/ml	30 μg/ml	50 μg/ml	70 μg/ml	100 μg/ml	
M.fragrans	MFE	-	-0.79±2.09 (-2.47±3.28)	-	0.59±2.19 (-3.38±9.52)	-	25.13±2.69 (-20.42±9.18)	-	56.62±2.97 (-15.54±11.12)	88.67±5.21
	MFA	-	-	-	-	-	-	-	32.06±0.29 (-5.94±5.19)	>100
N.nucifera	NNE	-	-	-	-	-	-	-	28.31±3.09 (-33.93±0.70)	>100
	NNA	-	-2.44±3.62 (-0.32±7.24)	-	3.71±1.35 (-6.98±4.86)	-	58.01±3.95 (14.86±6.65)	-	73.10±3.34 (15.84±10.95)	43.91±2.60
P.cablin	PCE	-	1.68±3.52 (2.65±5.61)	-	12.81±4.21 (-0.31±2.47)	39.16±3.87 (9.14±5.24)	68.33±0.77 (8.37±4.89)	-	-	37.16±2.12
	PCA	-	-	-	-	-	-	-	11.65±0.93 (19.69±2.78)	>100
S.exigua	SEE	-6.47±2.81 (-23.87±8.17)	-3.22±2.75 (-21.42±4.61)	23.78±5.83 (-58.70±5.47)	62.43±7.26 (-5.57±24.65)	-	-	-	-	22.84±3.95
	SEA	-11.71±8.03 (-3.99±6.30)	12.05±4.86 (1.37±4.57)	62.99±3.35 (16.54±3.83)	75.37±3.58 (21.30±3.06)	-	-	-	-	3.17±0.68

Note: All data represents the mean ± SEM in triplicate experiments.

Table 4-12 Anti-inflammatory activity by Griess reaction and MTT assay on RAW 264.7 cells at various concentrations

Plant name	Code	% Inhibition of NO production (% Toxicity)								IC_{50} (μg/ml)
		0.1 μg/ml	1 μg/ml	5 μg/ml	10 μg/ml	30 μg/ml	50 μg/ml	70 μg/ml	100 μg/ml	
T.chantrieri	TCE	-	-	-	-	-	-	-	16.89±1.14 (-35.51±3.86)	>100
	TCA	-	-	-	-	-	-	-	10.43±2.65 (-19.05±3.06)	>100
V.zizanioides	VZE	-	-	-	-	-	-	-	32.36±2.46 (36.24±5.38)	Toxic
	VZA	-	-	-	-	-	-	-	5.20±3.27 (8.65±12.97)	>100
Kheaw-Hom	KHE	-	-7.26±1.55 (-4.95±3.42)	-	2.41±3.33 (13.39±5.37)	-	42.02±3.29 (0.74±5.97)	-	77.07±1.80 (10.00±6.56)	59.77±3.76
	KHA	-	1.36±2.35 (2.70±6.33)	-	2.38±2.29 (3.71±5.08)	-	54.31±1.40 (15.05±8.50)	-	76.01±1.73 (20.51±8.47)	46.86±0.82
Prednisolone	Pred	32.35±1.87 (3.97±1.29)	46.11±0.48 (7.44±3.18)	-	58.45±1.17 (8.71±1.8)	64.86±2.03 (11.79±1.07)	74.03±1.17 (6.57±6.65)	-	56.68±1.65 (14.73±2.02)	1.31±0.05

Note: All data represents the mean ± SEM in triplicate experiments.

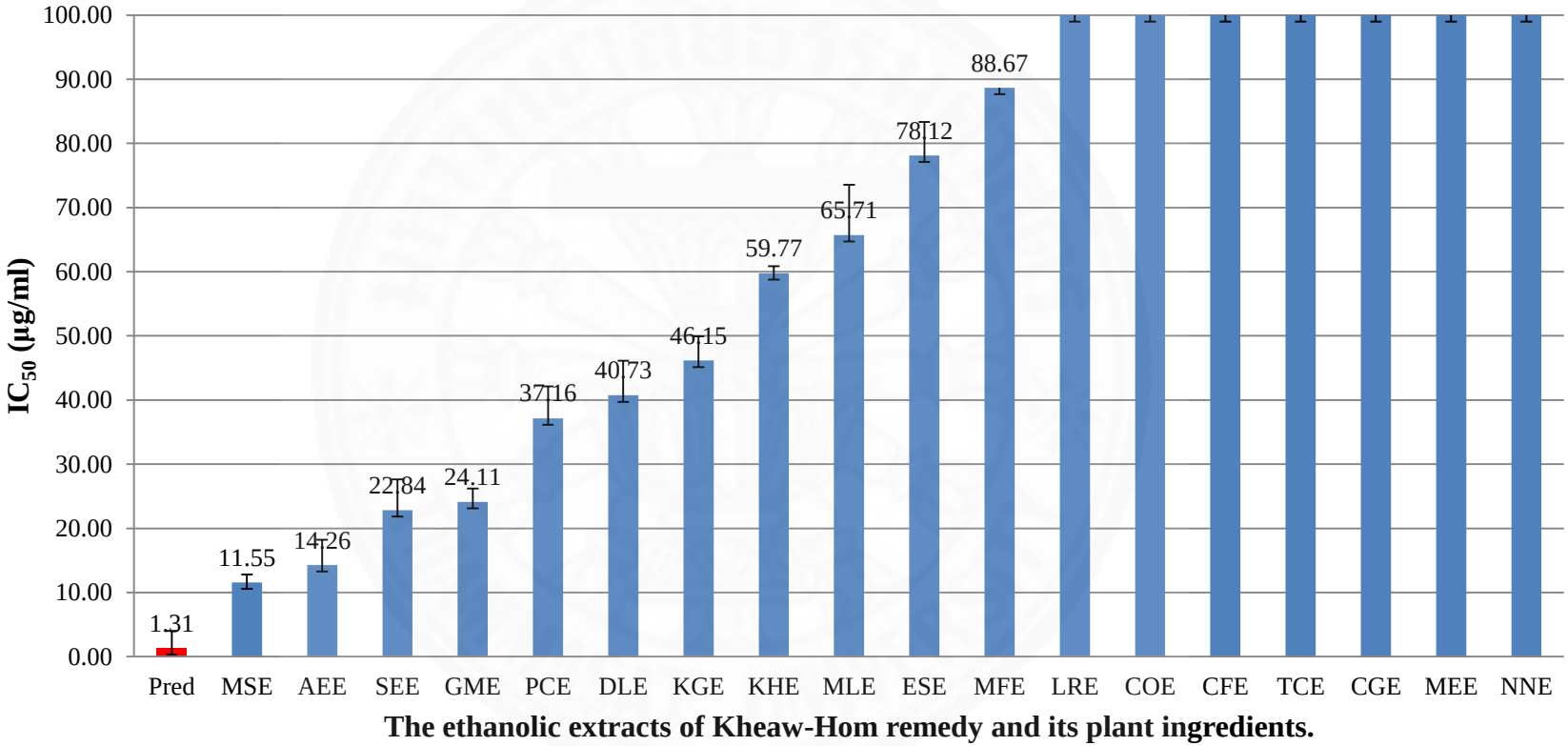

Figure 4-7 Anti-inflammatory activity IC_{50} (µg/ml) ± SEM by Griess reagent on RAW 264.7 cells (N=3) of ethanolic extracts

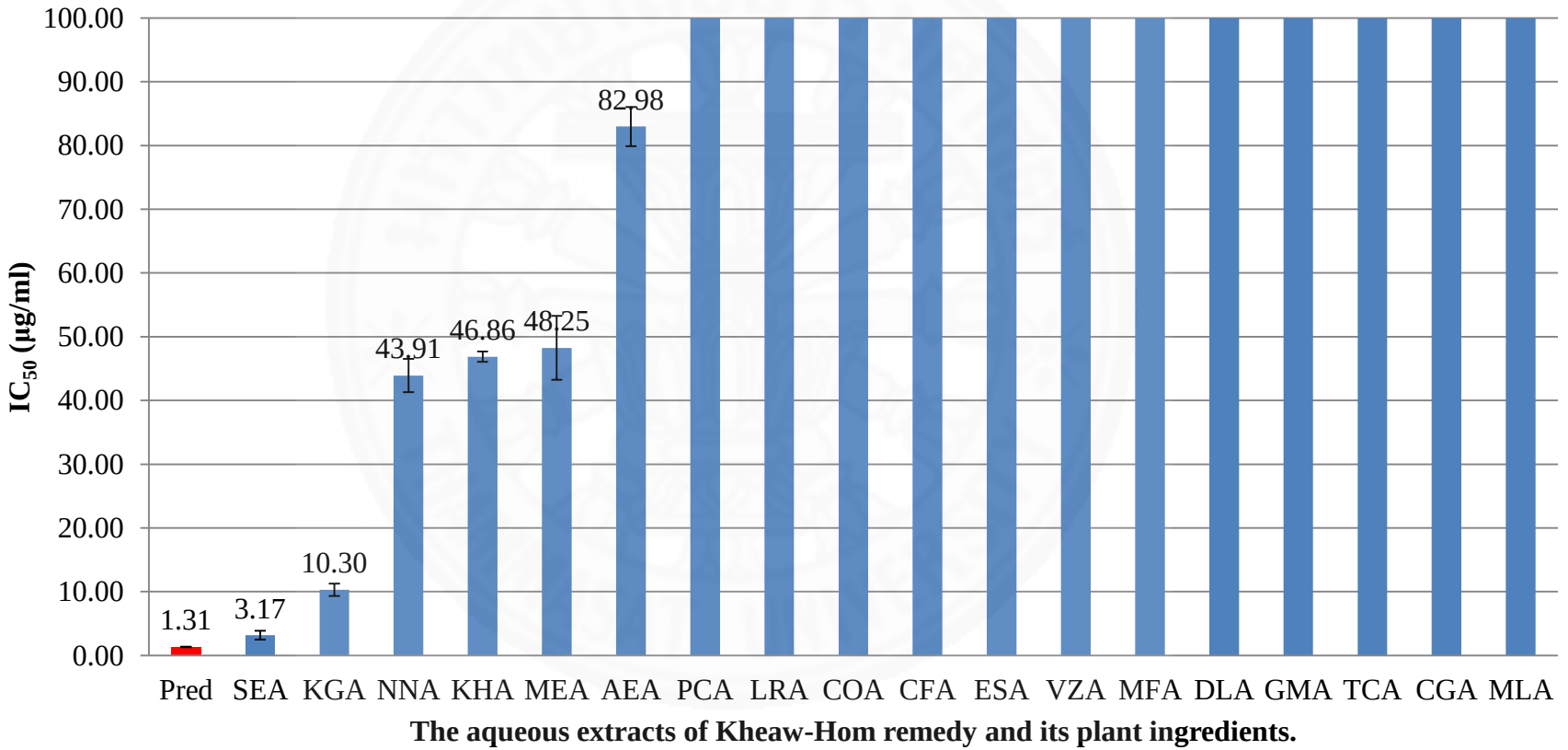

Figure 4-8 Anti-inflammatory activity IC_{50} (µg/ml) ± SEM by Griess reagent on RAW 264.7 cells (N=3) of aqueous extracts

4.5 The stability test of Kheaw-Hom extracts

The ethanolic and aqueous extracts of Kheaw-Hom remedy were studied for stability according to Thai FDA guideline. The samples on the beginning day 0 (control sample), day 15, day 30, day 60, day 90, day 120, day 150 and day 180 were exposed under 40 ± 2^{o}C with 75 ± 5% RH as accelerated testing for 6 months period. The purpose of this study was to investigate whether the ethanolic and aqueous extracts of Kheaw-Hom remedy are stable when kept in a closed container protected from light and stored at the room temperature for at least 2 years. All aqueous extracts were tested for antiviral property by using antiviral activity based CPE assay and all ethanolic extracts were tested for antimicrobial property by using microtitre plate-based antimicrobial assay.

4.5.1 The stability test of Kheaw-Hom extract for antimicrobial activity by microtitre plate-based assay

The ethanolic extracts of Kheaw-Hom remedy on the beginning day 0 (control sample), day 15, 30, 60, 90, 120, 150 and 180 were kept under accelerated condition at 40 ± 2^{o}C with 75 ± 5% RH and were tested for antimicrobial activity to determine minimum inhibitory concentration (MIC) by using microtitre plate-based antimicrobial assay. The results showed that the ethanolic extract of Kheaw-Hom remedy (KHE) on days 0, 15, 30 and 60 exhibited antimicrobial activity against methicillin-resistant S. aureus (MRSA) with MIC values of 0.625, 0.625, 0.625 and 0.625 mg/ml while days 90, 120, 150 and 180 exhibited MIC values of 1.25, 1.25, 1.25 and 1.25mg/ml. MIC values of all extracts are shown in **Table 4-13**. Therefore, the ethanolic extracts of Kheaw-Hom remedy are stable (MIC=0.625 mg/ml) for at least 8 months.

4.5.2 The stability test of Kheaw-Hom extract for antiviral activity based CPE assay

The aqueous extracts of Kheaw-Hom remedy on the beginning day 0 (control sample) and days 15, 30, 60, 90, 120, 150 and 180 were kept under accelerated condition at 40 ± 2^{o}C with 75 ± 5% RH and tested for antiviral activity based CPE assay. The results showed that the aqueous extract of Kheaw-Hom remedy (KHA) on

days 0, 15, 30, 60, 90, 120 and 150 exhibited antiviral activity against 25 $TCID_{50}$ of enterovirus 71 with cytopathic effect less than 50% at concentration 400 μg/ml while day 180 did not exhibit antiviral activity against 25 $TCID_{50}$ of enterovirus 71. All results are shown in **Table 4-14.** Therefore, the aqueous extracts of Kheaw-Hom remedy are stable for at least 1 year and 8 months.

Table 4-13 Minimum inhibitory concentration (MIC) of the stability test of Kheaw-Hom extract by using microtitre plate-based antimicrobial assay (n=3)

Sample	MIC (mg/ml)
	MRSA
Day 0	0.625
Day 15	0.625
Day 30	0.625
Day 60	0.625
Day 90	1.25
Day 120	1.25
Day 150	1.25
Day 180	1.25

Figure 4-9 Minimum inhibitory concentration (MIC) of the stability test of Kheaw-Hom extract by using microtitre plate-based antimicrobial assay (n=3)

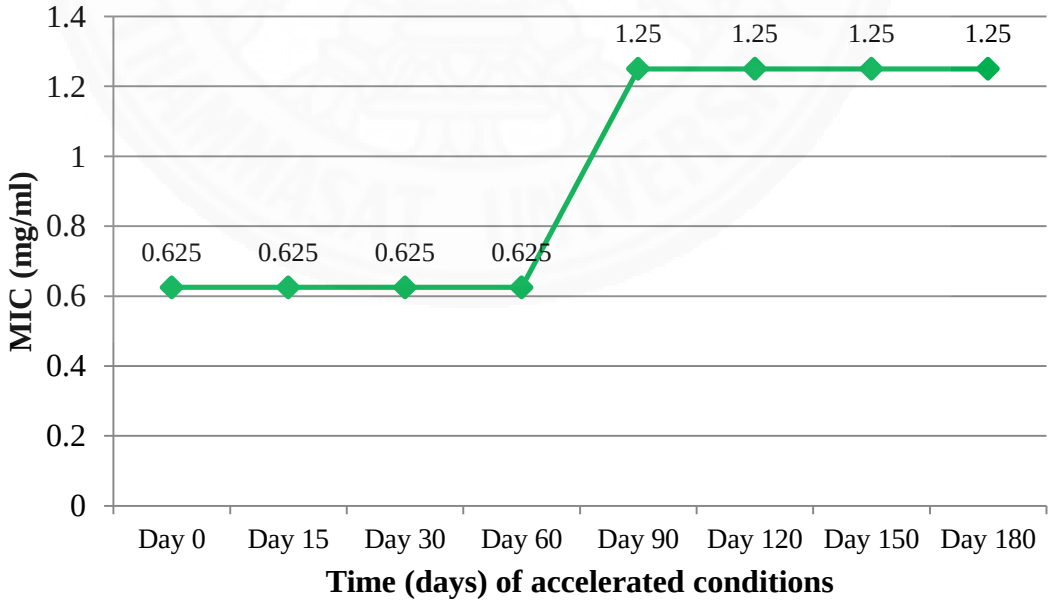

Table 4-14 Stability test of antiviral activity against 25 $TCID_{50}$ of enterovirus 71 by using antiviral activity based CPE assay

KHA (400 µg/ml)	**Result 25$TCID_{50}$**
Day 0	+
Day 15	+
Day 30	+
Day 60	+
Day 90	+
Day 120	+
Day 150	+
Day 180	-

"+" = less than 50% cytopathic effect
"-" = more than 50% cytopathic effect

4.6 Phytochemical analysis of the ethanolic extract of Kheaw-Hom remedy by using gas chromatography-mass spectrometry (GC-MS)

The ethanolic extract of Kheaw-Hom remedy was analyzed by using gas chromatography-mass spectrometry (GC-MS). There are forty-seven components that found in the ethanolic extract of Kheaw-Hom remedy. The component that exhibited the highest content was ethyl p-methoxycinnamate (18.64%) and the second highest was patchouli alcohol (16.38%). All results are shown in **Table 4-14**.

Table 4-15 Analysis results of Kheaw-Hom remedy by using gas chromatography-mass spectrometry (GC-MS).

No.	RT	CAS No.	Text name	% Area
1	16.56	63-75-2	Arecaline	0.55
2	18.05	585-34-2	Phenol	0.19
3	18.44	499-75-2	Antioxine	0.27
4	20.09	97-54-1	Isoeugenol	0.18
5	20.25	13524-76-0	3,3-Dimethyl-1-benzofuran-2(3H)-one	0.16
6	21.03	514-51-2	beta-Patchoulene	1.80
7	21.13	3691-12-1	ALPHA-GUAIEN	0.22
8	21.51	489-40-7	alpha-Gurjunene	0.10
9	21.62	2010-15-3	Pth-glycine	0.17
10	21.71	14753-08-3	Thymohydroquinone dimethyl ether	0.07
11	21.93	N/A	Caryophyllene	0.24
12	22.32	3691-12-1	Azulene	1.07
13	22.69	20085-93-2	Seychellene	1.82
14	23.00	3691-11-0	alpha-Bulnesene	0.85
15	23.06	4192-77-2	2-Propenoic acid	4.68
16	23.29	527-35-5	Durenol	0.40
17	23.68	25246-27-9	Alloaromadendrene	0.41
18	23.85	88-84-6	Beta-Guaiene	0.30
19	23.93	544-76-3	Hexadecane	2.78
20	24.26	95910-36-4	(-)-ISOLEDENE	0.17
21	24.37	489-39-4	AROMADENDRENE VI	0.17

Table 4-15 Analysis results of Kheaw-Hom remedy by using gas chromatography-mass spectrometry (GC-MS).

No.	RT	CAS No.	Text name	% Area
22	24.67	79718-83-5	4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane	0.31
23	25.07	86803-90-9	Scentenal	0.19
24	25.49	34545-87-4	4,4a,5,6-Tetrahydro-2(3H)-naphthalenone	0.29
25	25.65	3155-71-3	(2E)-2-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal	0.18
26	26.02	62924-17-8	(2E)-2-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-buten-1-ol	0.23
27	26.27	94609-18-4	TRICYCLO[3.3.0.0E4,6]OCTAN-3,8-DION, 2,7,7-TRIMETHYL-2-(2-METHYL-2-PROPEN-1-YL)-	1.17
28	26.78	24063-71-6	Isocurcumenol	0.74
29	26.95	77171-55-2	ent-Spathulenol	0.38
30	27.03	N/A	Propanoic acid	0.29
31	27.56	1929-30-2	2-Propenoic acid	0.54
32	27.64	22567-17-5	Delta-Gurjunene	1.23
33	27.98	5986-55-0	Patchouli alcohol	16.38
34	28.44	6902-91-6	Germacrone	4.16
35	29.06	24063-71-6	Isocurcumenol	7.10
36	29.41	93175-74-7	2-Butanone	3.99
37	29.55	N/A	2R,6s-2,6,8,8-Tetramethyltricyclo[5.2.2.0(4,6)]undecan-3-one	4.36
38	29.72	24393-56-4	Ethyl p-methoxycinnamate	18.64
39	30.32	N/A	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol	0.64
40	32.47	N/A	cassiffix	1.72
41	33.91	57-10-3	Palmitic acid	0.46

Table 4-15 Analysis results of Kheaw-Hom remedy by using gas chromatography-mass spectrometry (GC-MS).

No.	RT	CAS No.	Text name	% Area
42	34.05	N/A	5R,8R,9S,10R-2-Formyl-3-hydroxy-5-isopropenyl-8-8-methyl-(3a10)-octahydronaphthO	1.75
43	34.56	1731-92-6	Heptadecanoic acid	8.49
44	34.76	1159-25-7	Pregna-5,17(20)-dien-3-ol, (3beta,17E)	0.39
45	37.62	544-35-4	Linolein	4.51
46	37.72	55268-58-1	Nonanoic acid	4.20
47	38.23	111-61-5	Octadecanoic acid	1.03

Chromatogram of Kheaw-Hom remedy by using gas chromatography-mass spectrometry (GC-MS)

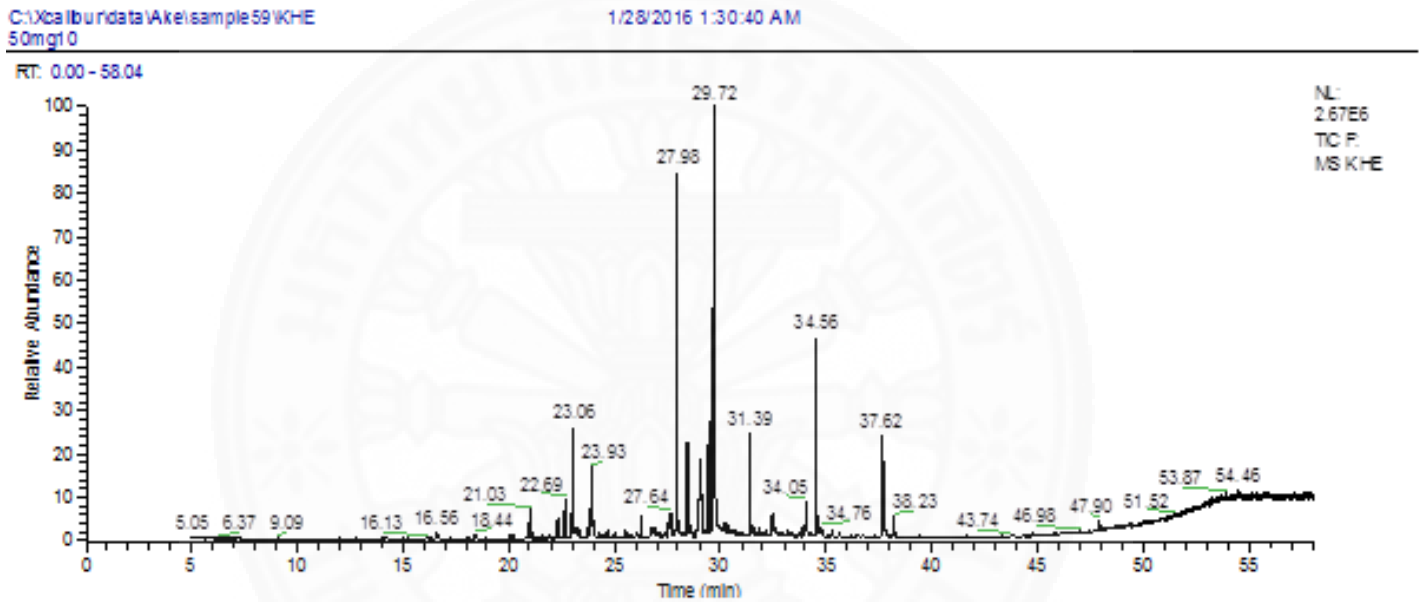

Figure 4-10 Analysis results of Kheaw-Hom remedy by using gas chromatography-mass spectrometry (GC-MS)

4.16 Summary of the biological activities of Kheaw-Hom remedy extracts and its ingredients

Table 4-15 Summary of the biological activities of Kheaw-Hom remedy extracts and its ingredients

Plants name	Code	Antiviral (µg/ml)			Antimicrobial Inhibition zone (mm)/ MIC (mg/ml)/ MMC (mg/ml)						Anti-inflammatory
		Enterovirus 71			*S.aureus*	MRSA	*S.epidermidis*	*K.pneumoniae*	*S.pyogenes*	*C.albicans*	NO IC50 (µg/ml)
		100 TCID50	50 TCID50	25 TCID50							
A.evecta	AEE	-	-	-	NI	NI	NI	NI	9.3/NI/NI	NI	14.26±1.25
	AEA	400	400	200	NI	NI	NI	NI	NI	NI	82.98±3.08
C.fruticosa (green)	CFE	-	-	-	NI	NI	NI	NI	9.0/NI/NI	NI	>100
	CFA	>400	>400	>400	NI	NI	NI	NI	NI	NI	>100
C.fruticosa (red)	COE	-	-	-	NI	NI	NI	NI	8.3/NI/NI	NI	>100
	COA	>400	>400	>400	NI	NI	NI	NI	NI	NI	>100
C. gigantea	CGE	-	-	-	7/5/5	7/2.5/2.5	7/5/5	NI	9/NI	NI	>100
	CGA	-	-	-	NI	NI	NI	NI	NI	NI	>100
D.loureiri	DLE	-	-	-	10.3/2.5/2.5	10.3/2.5/2.5	11.3/1.25/5	NI	14.6/1.25/2.5	NI/2.5/5	40.73±4.99
	DLA	>100	>100	>100	NI/2.5/2.5	NI/2.5/2.5	NI/1.25/1.25	NI	NI	NI	>100
E.stoechadosmum	ESE	-	-	-	8/1.25/1.25	7.6/2.5/2.5	9.3/1.25/1.25	NI	13/0.625/0.625	8.67/1.25/1.25	78.12±7.86
	ESA	>400	>400	>400	NI	NI/	NI	NI	NI	NI	>100

Table 4-15 Summary of the biological activities of Kheaw-Hom remedy extracts and its ingredients

Plants name	Code	Antiviral (µg/ml)			Antimicrobial Inhibition zone (mm)/ MIC (mg/ml)/ MMC (mg/ml)						Anti-inflammatory
		Enterovirus 71			*S.aureus*	MRSA	*S.epidermidis*	*K.pneumoniae*	*S.pyogenes*	*C.albicans*	NO IC50 (µg/ml)
		100 TCID50	50 TCID50	25 TCID50							
G.malaccensis	GME	-	-	-	7/2.5/2.5	7/2.5/5	7.67/2.5/5	NI	11.6/1.25/1.25	NI/1.25/5	24.11±4.82
	GMA	400	400	400	NI	NI	NI	NI	NI	NI	>100
K.galanga	KGE	-	-	-	NI	NI	NI	NI	8/0.625/0.156	9.3/0.625/2.5	46.15±5.39
	KGA	>50	>50	>50	NI	NI	NI	NI	NI	NI	10.30±0.99
L.rugosa	LRE	-	-	-	NI/5/5	NI	NI	NI	11.3/NI/NI	NI	>100
	LRA	-	-	-	NI	NI	NI	NI	NI	NI	>100
M.siamensis	MSE	-	-	-	8.3/0.005/0.005	9.3/0.005/0.005	9.6/0.039/0.039	NI	13.6/0.019/0.195	NI	11.55±2.70
	MSA	200	200	100	NI/2.5/5	NI/2.5/5	NI	NI	NI	NI	>100
M.ferrea	MLE	-	-	-	8/0.156/0.625	8/0.156/0.625	8/0.156/0.625	NI	10.33/0.156/1.25	NI/0.625/NI	65.71±1.09
	MLA	>200	>200	>200	NI/2.5/2.5	NI/2.5/2.5	NI/2.5/2.5	NI	NI	NI	>100
M.elengi	MEE	-	-	-	NI	NI	NI	NI	8.67/NI/NI	NI	>100
	MEA	-	-	-	NI/5/5	NI/5/5	NI/5/5	NI	NI	NI	48.25±5.02
M.fragrans	MFE	-	-	-	NI/5/5	NI	NI	NI	10/0.156/0.156	NI	88.67±5.21
	MFA	-	-	-	NI	NI/5/5	NI	NI	NI	NI	>100

Table 4-15 Summary of the biological activities of Kheaw-Hom remedy extracts and its ingredients

Plants name	Code	Antiviral (µg/ml)			Antimicrobial Inhibition zone (mm)/ MIC (mg/ml)/ MMC (mg/ml)						Anti-inflammatory
		Enterovirus 71			*S.aureus*	MRSA	*S.epidermidis*	*K.pneumoniae*	*S.pyogenes*	*C.albicans*	NO IC50 (µg/ml)
		100 TCID50	50 TCID50	25 TCID50							
N.nucifera	NNE	-	-	-	NI	NI	NI	NI	8.6/0.625/0.625	NI	>100
	NNA	>100	>100	100	NI/1.25/1.25	NI/1.25/1.25	NI/2.5/2.5	NI	NI	NI	43.91±2.60
P.cablin	PCE	-	-	-	7/0.625/0.625	7.67/1.25/2.5	7/0.625/1.25	NI	9.6/0.156/0.156	NI/2.5/5	37.16±2.12
	PCA	>400	>400	>400	NI/2.5/2.5	NI	NI/2.5/2.5	NI	NI	NI	>100
S.exigua	SEE	-	-	-	12.6/0.156/0.313	13/0.156/0.313	14.3/0.156/0.313	NI	16/0.156/0.156	8.6/0.625/0.625	22.84±3.95
	SEA	>400	>400	>400	NI/5/5	NI/5/5	NI/5/5	NI	NI	NI	3.17±0.68
T.chantrieri	TCE	-	-	-	8.00/2.5/2.5	8.00/2.5/2.5	9.33/5/5	NI	11.33/NI/NI	NI	>100
	TCA	50	50	50	NI/5/5	NI/5/5	NI	NI	NI	NI	>100
V.zizanioides	VZE	-	-	-	NI	NI	NI	NI	11.6/1.25/1.25	NI	Toxic
	VZA	>400	>400	>400	NI	NI	NI	NI	NI	NI	>100
Kheaw-Hom	KHE	-	-	-	7.3/0.625/1.25	7/0.625/0.625	8/1.25/2.5	NI	12.6/0.625/0.625	NI	59.77±3.76
	KHA	>400	>400	400	NI	NI	NI	NI	NI	NI	46.86±0.82

NI = No inhibition

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

Kheaw-Hom remedy which consists of eighteen Thai medicinal plants has long been used to treat exanthematous fever and skin infection complications such as chickenpox, measles, Herpes zoster, and hand, foot and mouth disease (HFMD). Kheaw-Hom remedy has a cooling and bitter characteristic**.** Taking medicine will stimulate the toxin to appear on the skin while applying Kheaw-Hom will decrease the heat on the skin. Comparing to modern medicine, it is possible that Kheaw-Hom might have anti-viral, anti-microbial and anti-inflammation activities. However, there is no research report to verify this. Thus, the objectives of this research were to study the antiviral, antimicrobial and anti-inflammatory activities that are related to exanthematous fever of ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients.

The raw materials of Kheaw-Hom ingredients were tested for standardization following the Thai Herbal Pharmacopoeia (THP). All plant ingredients and Kheaw-Hom remedy were within standard criteria except *Pogostemon cablin* and *Vetiveria zizanioides* which showed total ash more than 10% and acid insoluble more than 2%. However, Kheaw-Hom remedy was within standard criteria with loss on drying 8.75±0.24%, total ash 6.01±0.05% and acid insoluble ash was 1.29±0.00%.

Kheaw-Hom remedy and each of its plant ingredients were extracted by maceration in 95% ethanol and decoction in water to obtain ethanolic and aqueous extracts, respectively. The percentage yields of the ethanolic and aqueous extract of Kheaw-Hom remedy were 8.75% and 13.36%, respectively. The highest percentage yield of the ethanolic and aqueous extract was *D. loureiri* (19.49%) and *M. siamensis* (30.56%), respectively.

Both ethanolic and aqueous extracts of Kheaw-Hom remedy and its ingredients were subjected to cytotoxicity tests in Vero cell lines by using MTT assay to determine % toxicity. The results found that most of the ethanolic extracts were toxic to Vero cells. All the aqueous extracts of Kheaw-Hom remedy and its ingredients that were non-toxic to Vero cells were tested for antiviral activities against

100 $TCID_{50}$, 50 $TCID_{50}$ and 25 $TCID_{50}$ of enterovirus 71 (EV71) in duplicate experiments. At 100 $TCID_{50}$, 50 $TCID_{50}$ and 25 $TCID_{50}$ of EV71, the aqueous extracts of *A.evecta* (AEA), *G.malaccensis* (GMA), *M.siamensis* (MSA), *T.chantrieri* (TCA) and *N.nucifera* (NNA) exhibited antiviral activity against EV71with cytopathic effect less than 50% at concentration 400, 400, 200, and 50 μg/ml, respectively except that *N.nucifera* (NNA) exhibited antiviral activity against only 25 $TCID_{50}$ of EV71. In addition, the aqueous extract of Kheaw-Hom remedy at concentration 400 μg/ml exhibited antiviral activity in only the low dose of 25 $TCID_{50}$ of EV71. For this reason Thai traditional medical practices recommend that Kheaw-Hom remedy should be used immediately in the early stages of disease. This is the first report on antiviral activity against EV71.

Antimicrobial activity was investigated against five gram positive bacteria *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Streptococcus pneumonia*, and *Streptococcus pyogenes*, one gram negative bacterium *Klebsiella pneumonia* and one fungus *Candida albicans* which are the most common secondary bacterial infection skin in exanthematous fevers such as varicella and hand foot and mouth disease.

All extracts were tested for their antimicrobial activity by disc diffusion method to determine the inhibition zone. The results found that most ethanolic extracts of Kheaw-Hom remedy and its plant ingredients were able to inhibit five gram positive bacteria. The ethanolic extract of Kheaw-Hom remedy (KHE) showed antimicrobial activity against *S. aureus*, methicillin-resistant *S. aureus*, *S. epidermidis* and *S. pyogenes* with inhibition zone of 7.33±0.58, 7.00±0.00, 8.00±0.00 and 12.67±0.58 mm, respectively. Nevertheless, all extracts showed antimicrobial activity lower than gentamicin (positive control) Neither ethanolic nor aqueous extracts of Kheaw-Hom remedy and its plant ingredients were able to inhibit gram negative *K. pneumonia* The ethanolic extract of Kheaw-Hom remedy and all the aqueous extracts of Kheaw-Hom remedy and its plant ingredients were not able to inhibit *C.albicans*.

Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) were determined by using microtitre plate-based antimicrobial assay. The results found that most ethanolic extracts of Kheaw-Hom remedy and its plant ingredients were able to inhibit five gram positive bacteria. The ethanolic extract

of Kheaw-Hom remedy (KHE) showed antimicrobial activity against *S. aureus*, methicillin-resistant *S. aureus*, *S. epidermidis* and *S. pyogenes* with MIC values of 0.625, 0.625, 1.25 and 0.625 mg/ml and MMC values of 1.25, 0.625, 2.5 and 0.625 mg/ml, respectively. The ethanolic extract of Kheaw-Hom remedy had no activity against *K. pneumonia* and *C.albicans*. Furthermore, the aqueous extract of Kheaw-Hom remedy had no activity against all bacteria and fungi. Particularly, the ethanolic extracts *M.siamensis* (MSE) exhibited strong antimicrobial activity against *S. aureus*, methicillin-resistant *S. aureus*, *S. epidermidis* and *S. pyogenes* with MIC values of 0005, 0.005, 0.039 and 0.019 mg/ml and MMC values of 0.005, 0.005, 0.039 and 0.019 mg/ml, respectively. Neither ethanolic nor aqueous extracts of its plant ingredients were able to inhibit gram negative *K. pneumonia*.

The previous study demonstrated the antimicrobial activity against *S. aureus* and Methicillin-resistant *S. aureus* by the ethanolic extract of *D. loureiri, K. galangal, M. elengi*. *N. nucifera* had weak activity (Sattaponpan and Kondo, 2011). The present study agrees with this data. Secondly, the ethanolic extracts of *M. ferrea* had moderate activity against *S.aureus* (Sattaponpan and Kondo, 2011) with better MIC value than the previous study estimate 4 times and against Methicillin-resistant *S. aureus* with lower MIC values estimate 2 times. The previous study reported that the ethanolic extract of *M. siamensis* had moderate efficacy against *S.aureus* and methicillin-resistant *S. aureus* (Sattaponpan and Kondo, 2011). This present study showed higher antimicrobial activity against both strains than the previous study with MIC values 250 and 125 times, respectively. The different source of plant materials may have influenced the results. Finally, the ethanolic extract of *S.exigua* had good activity against methicillin-resistant *S. aureus* while flavanone isolated from *S.exigua* also inhibited this microbe in the previous study (Sato *et al.*, 1995). Although the aqueous extract of Kheaw-Hom remedy had no activity the ethanolic extract had moderate activity against *S. aureus*, Methicillin-resistant *S. aureus* and *S. epidermidis* that are causes of skin infection in chickenpox.The results relate to Thai traditional usage that is recommended to both ingest and apply medicine on skin for secondary infection caused by bacteria from chickenpox. Some plants in this remedy have an astringent taste such as *M. siamensis*. It may be astringent, heal the blisters, pustules and prevent scarring. *M.siamensis* is a member of Guttiferae family (Wutthithammawet, 2002).

Plants in this family always show good antimicrobial activity (Linuma *et al.,* 1996) such as mangosteen peel. Thus this plant is used in many in Thai traditional remedies and should be continuously studied to isolate antibacterial compounds instead of commercial antibiotics.

Anti-inflammatory activities of the ethanolic and aqueous extracts of Kheaw-Hom remedy and its plant ingredients were tested by measuring their inhibitory effects on LPS-induced nitric oxide (NO) release from murine macrophages cell lines (RAW 264.7). The aqueous extract of Kheaw-Home remedy (KHA) showed anti-inflammatory activity with IC_{50} value of 46.86±0.82 μg/ml which was higher than the ethanolic extract (KHE) which showed IC_{50} value of 59.77±3.76 μg/ml. However, both of the Kheaw-Hom remedy extracts were lower than Prednisolone (positive control) with IC_{50} value of 1.31±0.05μg/ml. Especially, the ethanolic extract of *M.siamensis* (MSE) showed the higestest anti-inflammatory activity with IC_{50} value of 11.55±2.70 μg/ml. *S.exigua* (SEA) and *K. galanga* (KGA) showed the high anti-inflammatory activity with IC_{50} values of 3.17±0.68 and 10.30±0.99 μg/ml. However, all the extracts were lower than Prednisolone (IC_{50} = 1.31±0.05 μg/ml) which is a positive anti-inflammatory drug.

M. ferrea, D. loureiri, K. galanga, M. siamensis, M. elengi, and *G. malaccensis* were reported in the previous study for anti-inflammatory activity by inhibition of nitric oxide (NO) production (Makchuchit, 2010; Anuthakoengkun and Itharat, 2014) but the ethanolic extract of *M. siamensis* in this study showed highest anti-inflammatory activity. In addition, the stillbenoid isolated from stem wood of *D. loureiri* showed anti-inflammatory activity by inhibition COX-1 and COX-2 production (Likhitwitayawuid *et al.,* 2002). Ethyl-*p*-methoxycinnamate (EMPC) isolated from *K. galanga* showed anti-inflammatory activity by inhibition IL-1, TNF-α production (Umar *et al.,* 2014), COX-1 and COX-2 (Umar *et al.,* 2012) and patchouli alcohol isolated from *P. cablin* inhibited the over-expression of iNOS, IL-1, IL-6, TNF-α, and PGE_2 (Jeong *et al.,* 2013; Yu *et al.,* 2011).

The ethanolic extract of Kheaw-Hom remedy was stable in antimicrobial activity against MRSA with MIC values of 0.625 mg/ml for at least 8 months and the aqueous extract of Kheaw-Hom remedy was stable in antiviral activity against 25 $TCID_{50}$ of EV71 for at least 1 year and 8 months.

Kheaw-Hom remedy was analyzed by using gas chromatography-mass spectrometry (GC-MS). There are forty-seven components found in the ethanolic extract of Kheaw-Hom remedy. The highest content was ethyl p-methoxycinnamate (18.64%) and the second highest was patchouli alcohol (16.38%). The previous study reported that the major chemical constituents of volatile oil from *Kaempferia galangal* were identified as ethyl p-methoxycinnamate (Tewtrakul *et al.,* 2005). Ethyl p-methoxycinnamate showed antimicrobial activity against *S.aureus* and *C.albican* with MIC values of 0.333 and 0.111 mg/ml (Omar *et al.,* 2014) and showed anti-inflammatory activity on inhibited NO, COX-1 and COX-2 production (Umar *et al.,* 2014; Umar *et al.,* 2012) but there has been no report on antiviral activity. In addition, the previous study report that *Pogostemon cablin* collected from different regions contained patchouli alcohol found by using GC-MS (Hu *et al.,* 2005). Patchouli alcohol showed antimicrobial activity against MRSA (Wan *et al.,* 2016), showed anti-inflammatory activity by inhibiting TNF-α, IL-1, PGE_2 and NO (Yu *et al.,* 2011) and showed antiviral activity against H1N1 (Kiyaahara *et al.,* 2012).

In summary, the ethanolic extract of Kheaw-Hom remedy showed good antimicrobial activity against gram positive bacteria *S. aureus,* MRSA, *S. epidermidis* and *S. pyogenes*. The aqueous extract of Kheaw-Hom remedy showed good anti-inflammatory effects by inhibiting NO production and showed antiviral activities against EV71. Therefore, the product development of Kheaw-Hom remedy should include combination of the ethanolic and aqueous extracts of Kheaw-Hom or increasing the ratios of its plant ingredients which show high activity such as the ethanolic extract of *M. siamensis* and the aqueous extracts of *S. exigua* and *T. chantrieri.*

In addition, some plant ingredients in Kheaw-Hom remedy were reported in previous studies on antipyretic activity in rat induced pyrexia by brewer's yeast injection such as *D. loureiri* (Reanmongkol *et al.,* 2003), *M. elengi* (Purnima *et al.,* 2010) and *N. nucifera* (Mukherjee *et al.,* 1996). Some plant ingredients were reported in previous studies on immumodulatory activity such as *M. ferrea* (Chahar *et al.,* 2012) and *N. nucifera* (Mukherjee *et al.,* 2010). Therefore, this Kheaw-Hom remedy may have antipyretic and immumodulatory properties to treat fever and increase antibodies. Kheaw-Hom remedy and its plant ingredients should be continuously

studied to define antipyretic and immumodulatory properties and should be continuously studied for acute or subchronic toxicity in the rat to find the median lethal dose (LD_{50}) for comparing the dose for use in humans.

All of these fingings support the traditional use of Kheaw-Hom remedy for treating exanthematous fever and skin infection complications such as chickenpox, measles, Herpes zoster, and hand, foot and mouth disease (HFMD) because the aqueous extract of Kheaw-Hom remedy exhibited antiviral and anti-inflammatory activity. These results support the use of aqueous extract of Kheaw-Hom remedy for the treatment of inflammation-related diseases such as exanthematous fever. Moreover these results relate to Thai traditional usage practices. In addition, the ethanolic extract of Kheaw-Hom remedy exhibited antimicrobial activities against gram positive bacteria that cause skin infection complications in exanthematous fever. Kheaw-Hom remedy should be further to investigated for the active compound and for development of herbal medicine for exanthematous fever and skin infection complications in exanthematous fevers.

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APPENDICES

APPENDIX A

Chemical Reagents for Laboratory Experiments

1. Reagent for Antiviral activity based CPE assay

1.1 EMEM (Eagle's minimum essential medium)

1.1.1 Stock solution (10X)

EMEM powder	95.3 g
Sterile deionized water	1000 ml

Dissolve 95.3 g of EMEM powder in 1000 ml sterile deionized water and sterile through filtration with 0.2 μm membrane filter. Aliquot 50 ml per sterile bottle and kept at -20^{o}C.

1.1.2 Working solution (1X)

EMEM 10X	50 ml
5%$NaHCO_3$	10 ml
Penicillin (40,000 U/ml)	2.5 ml
Gentamicin (4000 μg/ml)	2.5 ml
Fungizone (1 mg/ml)	0.5 ml
L-glutamin	5 ml
Sterile deionized water	450 ml

Kept at 4^{o}C

1.1.3 Growth media (10% FBS in EMEM)

EMEM (Working solution 1X)	180 ml
Fetal bovine serum (FBS)	20 ml

Kept at 4^{o}C

1.1.4 Growth media (4% FBS in EMEM)

EMEM (Working solution 1X)	192 ml
Fetal bovine serum (FBS)	8 ml

Kept at 4^{o}C

1.2 Phosphate buffer sterile (PBS) without Ca+ and Mg2+, pH 7.2

1.2.1 PBS Stock solution (10X)

NaCl	80 ml
KCl	2 ml
KH_2PO_4 (anhydrous)	1.2 ml
Na_2HPO_4 (anhydrous)	9.1 ml
Deionized water	450 ml

Adjust pH to 7.2 by 1N NaOH or 1N HCl. Sterilize by autoclaving at 121 ^oC for 15 minutes and kept at 4^oC

1.2.2 PBS working solution (1X)

PBS Stock solution (10X)	10 ml
Sterile deionized water	90 ml

Kept at 4^oC

1.2.3 5% NaHCO3

$NaHCO_3$	25 g
Sterile deionized water	1000 ml

Sterile through filtration with 0.2 μm membrane filter and kept at 4^oC .

1.3 Antibiotics

1.3.1 Penicillin 40,000 U/ml (10X)

Penicillin	1,000,000 Unit/bottle
Sterile triple distilled water	25 ml

Aliquot 5 ml and kept at 4^oC

1.3.2 Gentamicin 4000 μg/ml (10X)

Gentamycin	80 mg
Sterile triple distilled water	18 ml

Aliquot 5 ml and kept at 4^oC

1.2.3 Fungizone 1mg/ml (10X)

Fungizone (Amphotericin B)	50 mg
Sterile triple distilled water	50 ml

Aliquot 1 ml and kept at 4^{o}C

2. Reagent for Antimicrobial activity

2.1 Resazurin solution (1mg/ml)

Resazurin sodium salt	10 mg
Sterile deionized water	10 ml

Dissolve 1 mg of resazurin sodium salt in 10 ml sterile deionized water and sterile through filtration with 0.2 μm membrane filter. Aliquot 1 ml per sterile eppendorf and kept at 2-8^{o}C. and protect from the light.

2.2 Nutrient Agar (NA)

NA powder	28 g
Deionized water	1000 ml

Suspend 28 g of NA powder in 1000 ml of deionized water, mix well and sterilize in autoclave at 121^{o}C for 15 minutes. Allow it to cool but not solidify, pour NA into each sterile plastic petri dish and replace the lid immediately. Place NA plates on a counter top to cool and set at room temperature and kept at 2-8^{o}C.

2.3 Mueller Hinton Agar (MHA)

MHA powder	38 g
Deionized water	1000 ml

Suspend 38 g of MHA powder in 1000 ml of deionized water, mix well and sterilize in autoclave at 121^{o}C for 15 minutes. Allow it to cool but not solidify, pour MHA into each sterile plastic petri dish and replace the lid immediately. Place MHA plates on a counter top to cool and set at room temperature and kept at 2-8^{o}C.

3. Reagent for inhibitory of Nitric oxide (NO) 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 1,000 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 membrane filter and keep in sterile bottle.

The complete media is mixture of 200 ml of RPMI, 20 ml fetal bovine serum and 2 ml penicillin/streptomycin. The medium is stored at 4 ^{o}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 bovineserum (FBS)

Slowly thaw the FBS (inactivate), heat 56 ^{o}C, 60 mins

(Aliquot, kept at -20 ^{o}C)

3.5 Phosphate 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 ^{o}C till completely thawed

(Aliquot, kept at -20 ^{o}C)

3.7 Trypsin-EDTA

Slowly thaw the frozen 0.5% trypsin-EDTA, 37 ^{o}C, 60 minutes till

Completely thawed (Aliquot, stored at -20 ^{o}C)

3.8 Griess reagent

Sulfanilamide	1.0	g
N-(1-naphthyl) ethylenediamine dihydrochloride	0.1	g
Phosphoric acid (H_3PO_4)	2.5	g
Distilled water to	1,000	ml

The reagent was protected from light with aluminum foil and stored at 4^{o}C

3.9 MTT

3-(4,5-Dimethyl-2-thaizolyl)-2,5-dipheyl-2*H*-Tetrazolium bromide or Thiazolyl blue tetrazolium bromide	200	mg
Phosphate-buffered saline (PBS)	40	ml

The reagent was protected from light with aluminum foil and stored at 4 ^{o}C

3.10 0.04 HCl in Isopropanol

Conc.HCl	0.83	ml
Isopropanol to	250	ml

APPENDIX B

ยาเขียวหอม

ยาผง ยาเม็ด (รพ.) ยาผง (รพ.)

สูตรตำรับ

ในผงยา 90 กรัม ประกอบด้วย ใบพิมเสน ใบผักกระโฉม ใบหมากผู้ ใบหมากเมีย ใบสันพร้าหอม รากแฝกหอม หัวเปราะหอม แก่นจันทน์เทศหรือจันทร์ชะมด แก่นจันทน์แดง ว่านกีบแรด ว่านร่อนทอง เนระพูสี พิษนาศน์ มหาสดำ ดอกพิกุล ดอกบุนนาค ดอกสารภี เกสรบัวหลวง หนักสิ่งละ 5 กรัม

คำแนะนำ

1. บรรเทาอาการไข้ร้อนในกระหายน้ำ

2. แก้พิษหัด พิษอีสุกอีใส (บรรเทาอาการไข้จากหัดและอีสุกอีใส)

ขนาดและวิธีใช้

ชนิดผง

ผู้ใหญ่ : รับประทานครั้งละ 1 กรัม ละลายน้ำกระสายยา ทกุ 4 – 6 ชั่วโมง เมื่อมีอาการ

เด็ก อายุ6 – 12 ปี : รับประทานครั้งละ 500 มิลลิกรัม ละลายน้ำกระสายยา ทกุ 4 – 6 ชั่วโมง เมื่อมีอาการ

น้ำกระสายยาที่ใช้

• กรณีบรรเทาอาการไข้ร้อนในกระหายน้ำ ใช้น้ำสกุ หรือน้ำดอกมะลิเป็นน้ำกระสายยา

• กรณีแก้พิษหัด พิษอีสุกอีใส ละลายน้ำรากผักชีต้ม เป็นน้ำกระสายยาทั้งรับประทานและชโลม

หมายเหตุการชโลมใช้ยาผงละลายน้ำ 1 ต่อ 3 แล้วชโลม (ประพรม) ทั่วตามตัวบริเวณที่ตุ่มใสยังไม่แตก

ชนิดเม็ด

ผู้ใหญ่ : รับประทานครั้งละ 1 กรัม ทกุ 4 – 6 ชั่วโมง เมื่อมีอาการ

เด็ก อายุ6 - 12 ปี : รับประทานครั้งละ 500 มิลลิกรัม ทกุ 4 – 6 ชั่วโมง เมื่อมีอาการ

คำเตือน

- ควรระวังการใช้ยาในผู้ป่วยที่แพ้ละอองเกสรดอกไม้

- ไม่แนะนำให้ใช้ในผู้ที่สงสัยว่าเป็นไข้เลือดออก เนื่องจากอาจบดบังอาการของไข้เลือดออก

- หากใช้ยาเป็นเวลานานเกิน 3 วัน แล้วอาการไม่ดีขึ้น ควรปรึกษาแพทย์

BIOGRAPHY

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