ANTIVIRAL ACTIVITY AGAINST HERPES SIMPLEX VIRUS TYPE 2 OF KHEAW-HOM REMEDY EXTRACT AND ITS PLANT INGREDIENTS

BY

WISANSAYA INTAWONG

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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ABSTRACT

Herpes simplex virus type 2 (HSV-2) occurs on the skin as a common infection to patients of all genders and all ages. Acyclovir (Zovirax), famciclovir (Famvir), and valacyclovir (Valtrex) are medications of choice to be used in treating HSV-2 infection. However, resistance to acyclovir is frequently found in hospitals. Interestingly in alternative drugs, including natural products and herbal medicine, are increased recently. Kheaw-Hom as a Thai traditional remedy is commonly used to relieve fever, treat aphthous ulcers, and regarding to treat symptoms from measles and chickenpox, but there are a few report for treating HSV-2. It consists of 18 herbs: Bua-luang (*Nelumbo nucifera* Gaertn.), Bun-nak (*Mesua ferrea* Linn.), Chan-dang (*Dracaena cochinchinensis*), Chan-thet (*Myristica fragrans* Houtt.), Faek-hom (*Vetiveria zizanioides* (L.) Nash ex Small), Ma-has-sa-dam (*Cyathea gigantea* Holtt.), Mak-mia (*Cordyline fruticose* (L.) A.Chev (Green leaves)), Mak-phu (*Cordyline fruticosa* (L.) A.Chev (Red leaves)), Nae-ra-phu-sri (*Tacca chantrieri* Andre.), Phak-kra-chom (*Limnophila rugosa* (Roth) Merr.), Phi-kul (*Mimusops elengi* Linn.), Phim-sen-ton (*Pogostemon cablin* (Blanco) Benth.), Phit-sa-nat (*Sophora exigua* Craib.), Proh-hom

(Kaempferia galanga Linn.), San-phra-hom (*Eupatorium stoechadosmum* Hance.), Sa-ra phi (*Mammea siamensis* Kosterm.), Wan-kep-rat (*Angiopteris evecta* (G.Forst) Hoffm.), and Wan-ron-thong (*Globba malaccensis* Ridl.). The aims of this study were to investigate antiviral activity against HSV-2 by using aqueous and ethanolic extracts of Khaew-Hom, its plant ingredients, and its fractions.

In vitro cytotoxic and antiviral activities of aqueous and ethanolic extracts of Kheaw-Hom, its plant ingredients and fractions revealed high potency exhibition against HSV-2. First, cytotoxicity of all extracts was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. These extracts were investigated for antiviral activity by plaque reduction assay, including HSV-2 preincubation, pretreatment, and posttreatment by plaque reduction assay. Then, the most effective extract of Kheaw-Hom was isolated into five fractions by column chromatography to test all experiments. Finally, all extracts were screened for chemical compounds by thin layer chromatography (TLC).

Results were found that the aqueous and ethanolic extracts showed no toxicity to Vero cells. The aqueous extract of Khaew-Hom exhibited against HSV-2 with IC_{50} values of 94.24±1.26 µg/mL and ethanolic extract showed no inhibition of HSV-2 by pre-incubation. In addition, aqueous extract of 18 plant ingredients in Khaew-Hom exhibited potent antiviral activity against HSV-2 by pre-incubation: *C. gigantea* (IC_{50} = 10.84±2.68 µg/mL), *P. cablin* (IC_{50} = 30.72±7.66 µg/mL), *N. nucifera* (IC_{50} = 37.08±9.72 µg/mL), *M. ferrea* (IC_{50} = 39.23±4.29 µg/mL), *M. elengi* (IC_{50} = 60.19±9.08 µg/mL), *D.cochinchinensis* (IC_{50} = 61.83±7.37 µg/mL) and *M. siamensis* (IC_{50} = 67.1±13.28 µg/mL). Fractionally, four examples of potent antiviral activity were seen against HSV-2 by pre-incubation: FA2 (IC_{50} = 18.86±6.02 µg/mL), FA3 (IC_{50} = 20.44±5.09 µg/mL), FA4 (IC_{50} = 31.41±6.71µg/mL), and FA5 (IC_{50} = 85.43±7.69 µg/mL). These extracts exhibited direct inactivation virus effect against HSV-2. Acyclovir as a positive control exhibited against HSV-2 with IC_{50} values of 11.61±2.71µg/mL by pre-incubation. In pre-treatment and post-treatment, all extracts had no HSV-2 inhibition. The preliminary phytochemical investigation of Kheaw-Hom aqueous extract, its plant ingredients, and its fractions was performed using the thin layer chromatography technique. The results indicated that fraction 2 and fraction 3 consist of orange and

purple spots that were not found in Kheaw-Hom aqueous extract and plant ingredients. Thus, the synergistic effect of phytochemicals may cause new compounds that were found in fractions.

These results suggest that the aqueous extract of Khaew-Hom, 7 of 18 plant ingredients and fractions 2-5 showed potent antiviral activity by pre-incubation. The mechanism of aqueous extract may be considered for antiviral activity by direct contact. Therefore, fractions should be considered to isolate pure compounds to identify a Khaew-Hom marker related to traditional Thai medical practice. Khaew-Hom may be developed as a topical drug suitable for treating HSV-2.

Keywords: Khaew-Hom, Remedy, Herpes simplex virus type-2 (HSV-2), Antiviral activity, Cytotoxic activity.

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TABLE OF CONTENTS

	Page
ABSTRACT	(1)
ACKNOWLEDGEMENTS	(4)
LIST OF TABLES	(10)
LIST OF FIGURES	(12)
LIST OF ABBREVIATIONS	(15)
CHAPTER 1 INTRODUCTION	1
1.1 Introduction	1
1.2 Objectives of this study	3
1.2.1 Overall objectives	3
1.2.2 Specific objectives	3
1.3 Conceptual Framework of thesis	4
CHAPTER 2 REVIEW OF LITERATURE	5
2.1 Herpes simplex virus	5
2.1.1 Symptoms and Transmission	5
2.1.1.1 Herpes simplex type-1	5
2.1.1.2 Herpes simplex type-2	6
2.1.2 Structure of herpes simplex virus	6
2.1.3 Replication cycle of herpes simplex virus	7
2.1.4 Pathophysiology of herpes simplex virus	8
2.1.4.1 Pathophysiology of herpes simplex virus type-1	8

Page

2.1.4.2 Pathophysiology of herpes simplex virus type-2 8
2.1.5 Pathogenesis of herpes simplex virus 8
2.1.6 Treatment 9
2.1.6.1 Acyclovir 9
(1) Administration 10
(2) Adverse Drug Reaction (ADR) 10
2.1.6.2 Famciclovir 10
(1) Administration 11
(2) Adverse Drug Reaction (ADR) 11
2.1.6.3 Valaciclovir 12
(1) Administration 12
(2) Adverse Drug Reaction (ADR) 13
2.1.7 Herpes simplex virus of Thai traditional medicine 14
2.2 Kheaw-Hom remedy 15
2.2.1 Composition (90 grams of powder) 15
2.2.2 Properties 15
2.2.3 Dosage and Administration 15
2.2.3.1 Powder 15
2.2.3.2 Tablet 15
2.2.4 Contraindication 16
2.2.5 Precautions 16
2.2.6 Side effects 16
2.2.7 More information 16
2.2.8 Antiviral activities of Kheaw-Hom remedy 16
2.2.9 Biological activities of Kheaw-Hom remedy 17
2.2.10 Phytochemicals and/or Bioactive compounds 17
2.3 Its plant ingredients of Kheaw-Hom remedy 18
2.3.1 Wan-gieb-rad (*Angiopteris evecta* (G.Forst) Hoffm.) 18

Page

2.3.2 Maak-mia (*Cordyline fruticosa* (L.) A.Chev (Green leaves)) 20
2.3.3 Maak-phu (*Cordyline fruticosa* (L.) A.Chev (Red leaves)) 21
2.3.4 Ma-has-sa-dam (*Cyathea gigantea* Holtt.) 23
2.3.5 Chan-dang (*Dracaena cochinchinensis*) 25
2.3.6 San-phra-hom (*Eupatorium stoechadosmum* Hance.) 28
2.3.7 Wan-ron-thong (*Globba malaccensis* Ridl.) 30
2.3.8 Proh-hom (*Kaempferia galanga* Linn.) 32
2.3.9 Phak-kra-chom (*Limnophila rugosa* (Roth) Merr.) 34
2.3.10 Sa-la-pii (*Mammea siamensis* Kosterm.) 36
2.3.11 Boon-nak (*Mesua ferrea* Linn.) 38
2.3.12 Phi-kul (*Mimusops elengi* Linn.) 40
2.3.13 Chan-thet (*Myristica fragrans* Houtt.) 42
2.3.14 Bua-luang (*Nelumbo nucifera* Gaertn.) 44
2.3.15 Phim-sen-ton (*Pogostemon cablin* (Blanco) Benth.) 48
2.3.16 Phit-sa-naat (*Sophora exigua* Craib.) 51
2.3.17 Na-era-phu-sri (*Tacca chantrieri* Andre.) 53
2.3.18 Faek-hom (*Vetiveria zizanioides* (L.) Nash ex Small) 55

CHAPTER 3 RESEARCH METHODOLOGY 59

3.1 Chemicals and reagents 59
3.1.1 Chemicals and reagents of extraction 59
3.1.2 Chemicals and reagents of antiviral activity 59
3.1.3 Chemicals and reagents of Colunm Chromatography (CC) and Thin-layer Chromatography (TLC) 60
3.2 Instruments, platic wares and glasswares 60
3.3 Plant materials 62
3.3.1 Preparation of crude extracts 65

Page

3.3.1.1 Maceration 65

3.3.1.2 Decoction 65

3.4 Cytotoxicity activity 66

3.4.1 Animal cell lines 66

3.4.2 Preparation of sample solution 66

3.4.3 Cytotoxicity activity by MTT assay 66

3.5 Antiviral activities 67

3.5.1 Animal cell lines 67

3.5.2 Propagation and titration of virus 67

3.5.3 Preparation of sample solution 68

3.5.4 Antiviral activity by plaque reduction assay 69

3.5.4.1 Pre-incubation assay 69

3.5.4.2 Pre-treatment assay 69

3.5.4.3 Post-treatment activity 70

3.6 Separation of five fractions of the aqueous extract of Kheaw-Hom remedy using column chromatography 73

3.7 Thin-layer Chromatography (TLC) 74

CHAPTER 4 RESULTS AND DISCUSSION 75

4.1 Preparation of crude extracts 75

4.2 *In vitro* assay for cytotoxic activity and antiviral activities of Kheaw-Hom extracts, and its plant ingredients on Vero cells 78

4.3 Thin-layer Chromatography (TLC) of Kheaw-Hom remedy and its plant ingredients 95

4.4 *In vitro* assay for cytotoxic activity and antiviral activities of fractions on Vero cells 101

Page

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS 111

REFERENCES 114

APPENDICES

APPENDIX A 124

APPENDIX B 125

BIOGRAPHY 127

LIST OF TABLES

Tables	Page
2-1 Costs of an antiviral drug in Thailand	13
2-2 Antiviral and biological activity of Kheaw-Hom remedy and its plant ingredients	57
3-1 List of chemicals and reagents of extraction	59
3-2 List of chemicals and reagents of antiviral activity	59
3-3 List of chemicals and reagents of Column chromatography (CC) and Thin-layer Chromatography (TLC)	60
3-4 List of instruments, plastic wares, and glasswares	60
3-5 Description and voucher specimen number of plant ingredients in Kheaw-Hom remedy	63
3-6 Fraction of Colunm Chromatography (CC)	73
4-1 The percentage yields of the aqueous and ethanolic extracts of Kheaw-Hom remedy and its plant ingredients.	76
4-2 Cytotoxicity of the Kheaw-Hom remedy extracts on Vero cell line by using MTT assay with incubated 2 hours and 3 days (n=3)	79
4-3 Cytotoxicity of acyclovir on Vero cell line by using MTT assay with incubated 2 hours and 3 days (n=3)	79
4-4 The half maximal inhibitory concentration (IC_{50}) of Kheaw-Hom remedy and acyclovir against HSV-2 on Vero cell line by pre-incubation (n=3)	83
4-5 The half maximal inhibitory concentration (IC_{50}) of Kheaw-Hom remedy and acyclovir against HSV-2 on Vero cell line by pre-treatment (n=3)	83
4-6 The half maximal inhibitory concentration (IC_{50}) of Kheaw-Hom remedy and acyclovir against HSV-2 on Vero cell line by post-treatment (n=3)	84

Tables Page

4-7 Cytotoxicity of aqueous extract of its plant ingredients on Vero cell line by using MTT assay with incubated 2 hours (n=3) 87

4-8 Cytotoxicity of aqueous extract of its plant ingredients on Vero cell line by using MTT assay with incubated 3 days (n=3) 89

4-9 The half maximal inhibitory concentration (IC_{50}) of aqueous extract of its plant ingredients against HSV-2 on Vero cell line by pre-incubation (n=3) 91

4-10 The half maximal inhibitory concentration (IC_{50}) of aqueous extract of its plant ingredients against HSV-2 on Vero cell line by pre-treatment (n=3) 93

4-11 The half maximal inhibitory concentration (IC_{50}) of aqueous extract of its plant ingredients against HSV-2 on Vero cell line by post-treatment (n=3) 94

4-12 The Percentage yields of Fraction by Column chromatography (CC) 101

4-13 Cytotoxicity of Fraction of the aqueous Kheaw-Hom remedy extract on Vero cell line by using MTT assay with incubated 2 hours and 3 days (n=3) 104

4-14 The half maximal inhibitory concentration (IC_{50}) of the fraction of the aqueous Kheaw-Hom remedy extract against HSV-2 on Vero cell line by pre-incubation (n=3) 105

4-15 The half maximal inhibitory concentration (IC_{50}) of the fraction of the aqueous Kheaw-Hom remedy extract against HSV-2 on Vero cell line by pre-treatment (n=3) 106

4-16 The half maximal inhibitory concentration (IC_{50}) of the fraction of the aqueous Kheaw-Hom remedy extract against HSV-2 on Vero cell line by post-treatment (n=3) 106

LIST OF FIGURES

Figures	Page
2-1 Structure of herpes simplex virus	6
2-2 Replication cycle of herpes simplex virus	7
2-3 Primary infection (a) and Recurrent infection (b)	9
2-4 Chemical Structure of acyclovir	10
2-5 Chemical Structure of famciclovir	11
2-6 Chemical Structure of valacyclovir	12
2-7 Mechanism of herpes disease by Thai traditional medicine theory	14
2-8 *Angiopteris evecta* (G.Forst) Hoffm. (MARATTIACEAE)	18
2-9 *Cordyline fruticosa* (L.) A.Chev (Green leaves) (AGAVACEAE)	20
2-10 *Cordyline fruticosa* (L.) A.Chev (Red leaves) (AGAVACEAE)	21
2-11 *Cyathea gigantea* Holtt. (CYATHEACEAE)	23
2-12 *Dracaena cochinchinensis* (DRACANACEAE)	25
2-13 *Eupatorium stoechadosmum* Hance. (COMPOSITAE)	28
2-14 *Globba malaccensis* Ridl. (ZINGIBERACEAE)	30
2-15 *Kaempferia galanga* Linn. (ZINGIBERACEAE)	32
2-16 *Limnophila rugosa* (Roth) Merr. (SCROPHULARIACEAE)	34
2-17 *Mammea siamensis* Kosterm. (GUTTIFERAE)	36
2-18 *Mesua ferrea* Linn. (GUTTIFERAE)	38
2-19 *Mimusops elengi* Linn. (SOPOTACEAE)	40
2-20 *Myristica fragrans* Houtt. (MYRISTICACEAE)	42
2-21 *Nelumbo nucifera* Gaertn. (NELUMBONACEAE)	44
2-22 *Pogostemon cablin* (Blanco) Benth. (LABIATAE)	48
2-23 *Sophora exigua* Craib. (FABACEAE)	51
2-24 *Tacca chantrieri* Andre. (TACCACEAE)	53
2-25 *Vetiveria zizanioides* (L.) Nash ex Small (GRAMINEAE)	55

Figures Page

3-1 Antiviral activity at three different stages of pre-incubation (**1**), pre-treatment (**2**), and post-treatment (**3**) on Vero cells by plaque reduction assay 72

3-2 Isolation of the aqueous extract of Kheaw-Hom remedy by Diaion HP 73

3-3 Thin-layer Chromatography (TLC) 74

4-1 Plaques reduction of the aquoes extract of Kheaw-Hom remedy by pre-incubation assay 80

4-2 Antiviral activity of aqueous extract of Kheaw-Hom, its plant ingredients, and acyclovir against HSV-2 by pre-incubation 95

4-3 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and its plant ingredients using $CHCl_3$: EtOAc : MeOH (1:3:1) solvent system 96

4-4 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and its plant ingredients using $CHCl_3$: MeOH (3:2) solvent system 97

4-5 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and its plant ingredients using EtOAC : MeOH : water (3.5:1:0.5) solvent system 98

4-6 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and pure compounds using 3 solvents namely $CHCl_3$: EtOAC : MeOH (1:3:1), $CHCl_3$: MeOH (2:3), and EtOAC : MeOH : water (3.5:1:0.5) 100

4-7 Isolation of the aqueous extract of Kheaw-Hom remedy by Diaion HP-20 101

Figures Page

4-8 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and isolated fractions from the aqueous extract of Kheaw-Hom remed using $CHCl_3$: EtOAC : MeOH (1:3:1) solvent system 107

4-9 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and isolated fractions from the aqueous extract of Kheaw-Hom remed using $CHCl_3$: MeOH (2:3) solvent system 108

4-10 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and isolated fractions from the aqueous extract of Kheaw-Hom remedy using EtOAC : MeOH : water (3.5:1:0.5) solvent system 109

4-11 Reversed-phase of TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and isolated fractions from the aqueous extract of Kheaw-Hom remedy 110

LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
%	Percent
/	Per
<	Less than
>	More than
=	Equal
µg	Microgram
µg/mL	Microgram per milliliter
µL	Microliter
°C	Degree celsius
ATTC	American type culture collection
CC	Column chromatography
$CHCl_3$	Chloroform
cm	Centimeter
CMC	Carboxy Methyl Cellulose
CO_2	Carbon dioxide
Conc.	Concentration
EtOAc	Ethyl acetate
DMSO	Dimethylsulfoxide
et al.	Et alii, and others
FBS	Fetal bovine serum
g	Gram
HSV-1	Herpes simplex virus type 1
HSV-2	Herpes simplex virus type 2
IC_{50}	Concentration causing 50% inhibition effect
LPS	Lipopolysaccharide

Symbols/Abbreviations	Terms
m	Meter
MEM	Minimal essential medium
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-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromid
NaOH	Sodium hydroxide
$NaHCO_3$	Sodium bicarbonate
NO	Nitric oxide
NT	Not tested
OD	Optical density
PFU	Plaque-forming unit
PBS	Phosphate buffer saline
P/S	Penicillin-Streptomycin
TLC	Thin-layer chromatography
w/w	Weight by weight
WHO	World Health Organization

CHAPTER 1
INTRODUCTION

1.1 Introduction

Herpes simplex virus (HSV) is a double-stranded DNA virus that is grouped to the genus Simplexvirus within the family of Herpesviridiae, subfamily Alphaherpesvirinae (Koelle et al., 2003). There are two main Herpes simplex viruses referred to as Herpes simplex virus type 1 (HSV-1) and Herpes simplex virus type 2 (HSV-2). HSV-1 is transmitted by oral to oral, skin contract infection, and oral to genital. At the same time, HSV-2 is transmitted by genital to genital contraction during sex (James et al., 2020). Common presentations of HSV include blistering sores, itching, the pain of urination, fever, headaches, swollen lymph nodes, lack of appetite, and tiredness (Archananupab., 2010). After infection, HSV establishes latency in the nervous system for the host's lifetime (Khadr et al., 2019). Many factors include menstrual periods, stress, and sun exposure involved in HSV reactivation (Cynthia et al., 2021). Globally, there were approximately 3,752 million people and 491.5 million with HSV-1 and HSV-2. The HSV-1 disease occurs in people below 49 years (66.6%), while HSV-2 is prevalent among populations aged 15-49 years (13.2%) (WHO, 2020).

DNA polymerase inhibitor is the group of drugs that are used to treat HSV. Examples of this group of drugs include acyclovir, famciclovir, and valacyclovir (WHO, 2020). However, antiviral drugs have many side effects: headaches, diarrhea, nausea, and vomiting. In addition, they show a low efficacy for HSV treatment (Zhong M-G et al., 2013). Therefore, new effective drugs and herbal medicines have been developed as alternative drugs to treat Herpes simplex.

Thai traditional practitioners have used herbal medicines to treat illness for a long time. Kheaw-Hom remedy is a remedy in the Thai National List of Essential Medications 2012. It is used to treat aphthous ulcers and relieve fever from measles

and chickenpox (Chusri et al., 2014). There are eighteen plant ingredients in Kheaw-Hom remedy include Bua-luang (*Nelumbo nucifera* Gaertn.), Bun-nak (*Mesua ferrea* Linn.), Chan-dang (*Dracaena cochinchinensis*), Chan-thet (*Myristica fragrans* Houtt.), Faek-hom (*Vetiveria zizanioides* (L.) Nash ex Small), Ma-has-sa-dam (*Cyathea gigantea* Holtt.), Mak-mia (*Cordyline fruticosa* (L.) Goeppert.), Mak-phu (*Cordyline fruticosa* (L.) Goeppert.), Nae-ra-phu-sri (*Tacca chantrieri* Andre), Phak-kra-chom (*Limnophila rugosa* Merr.), Phi-kul (*Mimusops elengi* Linn.), Phim-sen-ton (*Pogostemon cablin* (Blanco) Benth.), Phit-sa-nat (*Sophora exigua* Craib), Proh-hom (*Kaempferia galanga* Linn.), San-phra-hom (*Eupatorium stoechadosmum* Hance), Sa-ra phi (*Mammea siamensis* Kosterm.), Wan-kep-rat (*Angiopteris evecta* (G.Forst) Hoffm.), and Wan-ron-thong (*Globba malaccensis* Ridl.).

Kheaw-Hom remedy has been reported on anti-microbial, anti-inflammatory, and antiviral activities. Its ethanolic extract could inhibit gram-positive bacteria such as *Staphylococcus epidermidis* (MIC = 1.25 mg/mL, MMC = 2.5 mg/mL), *Staphylococcus aureus* (MIC = 0.625 mg/mL, MMC = 1.25 mg/mL) and methicillin-resistant *Staphylococcus aureus* (MIC = 0.625 mg/mL, MMC = 0.625 mg/mL) In addition, the aqueous extract of Kheaw-Hom remedy showed anti-inflammationactivity with IC_{50} value of 46.86±0.82 μg/mL while the ethanolic extract showed IC_{50} value of 59.77±3.76 μg/mL by effects on lipopolysaccharide (LPS) induced nitric oxide (NO) release from RAW 264.7 cells (Sukkasem el at., 2016). For the report to antiviral activities, the ethanolic extract of Ya-Kheaw remedy showed antiviral activity against varicella zoster virus (VZV) at a concentration of 250 μg/ml (Sanguansermsri et al., 2005). The aqueous extract of Kheaw-Hom remedy exhibited antiviral activity against 25 $TCID_{50}$ enterovirus 71 with cytopathic effect less than 50% at a concentration of 400 μg/mL (Sukkasem el at., 2016). However, there have been no reports of Kheaw-Hom remedy against HSV.

In Thai traditional theory, Herpes simplex virus could be triggered by the imbalance of four elements. There are many factors of element imbalance, such as insufficient rest, stress, and overwork. Factors increased the fire element (Pha-ri thai-huk-Kee) and then stimulated the wind element (Ang-ka-mang-ka-nu-sa-ri-va-ta) to

increase fever. After that, the water element is decreased (Lo-hit-tang) and can not nourish the soil element. Finally, the elemental soil (Mang-sang and Ta-jo) loses its function and leads to sores on the skin and blisters. Kheaw-Hom remedy is also used to treat fever rashes such as measles and chickenpox. Thus, HSV and the symptoms associated with HSV may be treated by this remedy.

However, there is no report of Kheaw-Hom remedy on antiviral activity against Herpes simplex virus directly. Therefore, this study aims to investigate the antiviral activity of Kheaw-Hom remedy extracts against HSV to be used as the basic knowledge for developing the antiviral herbal drug in the future.

1.2 Objectives of this study

1.2.1 Overall objective

1.2.1.1 The overall objective of this research were to investigate the antiviral activities of Kheaw-Hom remedy extracts, its plant ingredients, and fractions against Herpes simplex virus type-2.

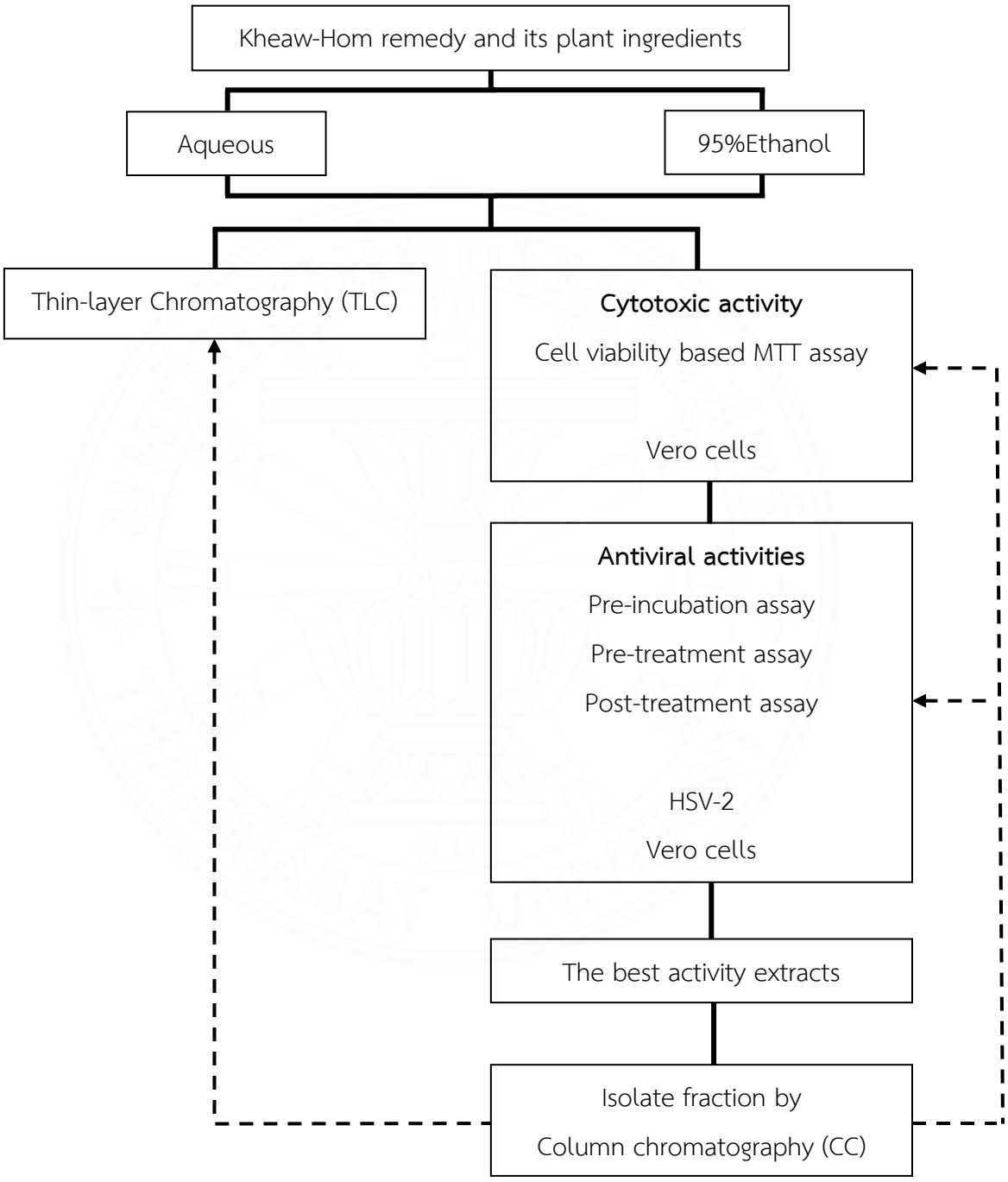
1.2.2 Specific objectives

1.2.2.1 To investigate antiviral activities of the ethanolic and aqueous extracts of Kheaw-Hom remedy against Herpes simplex virus type-2.

1.2.2.2 To investigate the antiviral activities of plant ingredient extracts of Kheaw-Hom remedy against Herpes simplex virus type-2.

1.2.2.3 To investigate the antiviral activities of fractions from Kheaw-Hom remedy extract against Herpes simplex virus type-2.

1.3 Conceptual Framework of thesis



CHAPTER 2
REVIEW OF LITERATURE

2.1 Hesper simplex virus

Herpes simplex virus (HSV) is a double-stranded DNA virus belonging to the *Herpesviridae* family, subfamily *Alphaherpesvirinae*, genera *Simplexvirus* (Koelle et al., 2003). The serotypes are HSV-1 and HSV-2 that transmitted from person to person. HSV-1 is transmitted by oral to oral or skin contract infection and mother to neonatal. However, HSV-1 can be infected through oral to genital. HSV-2 is transmitted by genital to genital that contraction during sex (James et al., 2020). The primary HSV target cells are epithelial cells and keratinocytes infected by neuronal and immune cells (Guan et al., 2019). The mild symptoms of HSV include blistering sores, itching, and pain during urination (Genital Herpes) (Archananupab, 2010). Providing HSV infected into the body causing a latent infection that is infectious for a lifetime (Khadr et al., 2019). Although it does not have symptoms, the viral DNA of HSV will continue within sensory neurons in the absence of detectable infectivity (Lachmann, 2004). However, some stimuli can stimulate, such as menstrual periods, stress, and sun exposure.

2.1.1 Symptoms and Transmission

2.1.1.1 Herpes simplex virus type-1

HSV-1 can cause mild to server disease that mostly asymptomatically and subclinical primary HSV-2 target cells by infectious for a lifetime (Khadr et al., 2019). Symptoms of HSV-1 include blistering sores around the mouth. In addition, some patients have symptoms such as fever, headaches, lack of appetite, and tiredness (WHO., 2020). Oral to oral is mainly transmitted by the HSV-1 infection that contacts the HSV-1 virus in sores and saliva around the mouth. However, HSV-1 can also be transmitted by oral to skin surfaces, from mother to neonatal, and oral to genital from sores of the HSV-1 infection (Archananupab., 2010).

2.1.1.2 Herpes simplex virus type-2

HSV-2 can cause mild symptoms that blistering sores around the genitals, fever, body aches, swollen lymph nodes, and shooting pain in the legs, hips, and buttocks (WHO., 2020). genital to genital is mainly transmitted by the HSV-2 infection that contacts genital surfaces, skin, and fluid or sores (Archananupab, 2010).

2.1.2 Structure of herpes simplex virus

Herpes simplex virus has an entity four-layered structure. First, the core containing the large consists of a single linear molecule of a double-stranded DNA genome. Second, the capsid is icosapentahedral around the core with a 100 nm diameter constructed of 162 capsomeres. Next, between capsid and envelope is an amorphous protein coat called the tegument. Finally, envelopes are covered in a glycoprotein-bearing lipid bilayer (Whitley RJ, 1996).

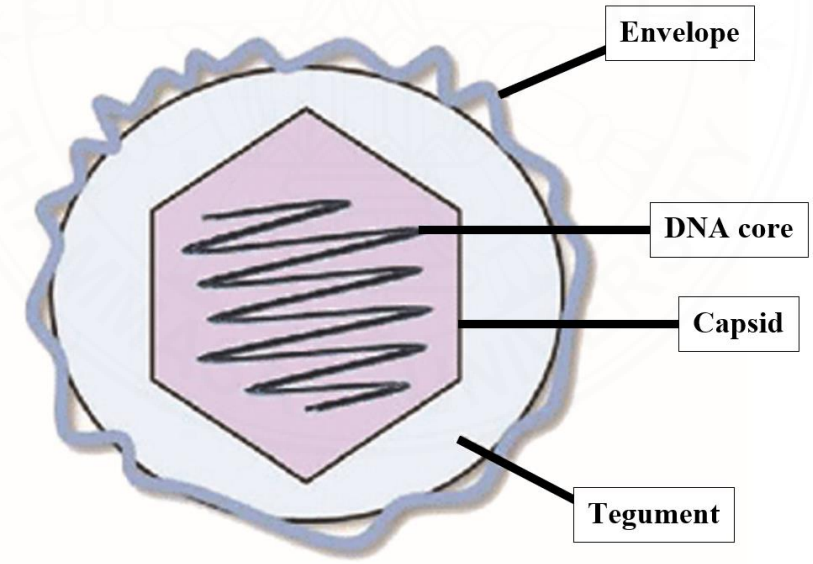


Figure 2-1 Structure of herpes simplex virus (Xu et al., 2019).

2.1.3 Replication cycle of herpes simplex virus

Step of Herpes simplex virus replication cycle after the cell was infected HSV. In the first step, HSV attaches to the host cell's surface by proteins that glycoproteins C (gC) and B (gB). Second, the viral entry into the host cell by a process called penetration. Third, the viral fuses the outer envelope with the plasma membrane by interacting with four glycoproteins, including gD, gB, and the heterodimer gH/gL. Then, the viral particle comes apart and releases the viral genome called uncoating. After that, the viral genome replication and transcription synthesized viral mRNA by the host cell RNA-polymerase II. The capsid assembly and viral genome packaging occur in the nucleus when the structural proteins and newly synthesized RNA bud out from the ER. The vial mature and convert to its infectious form. Finally, the mature viral is released from the host cell and infects other cells (Kukhanova et al., 2014).

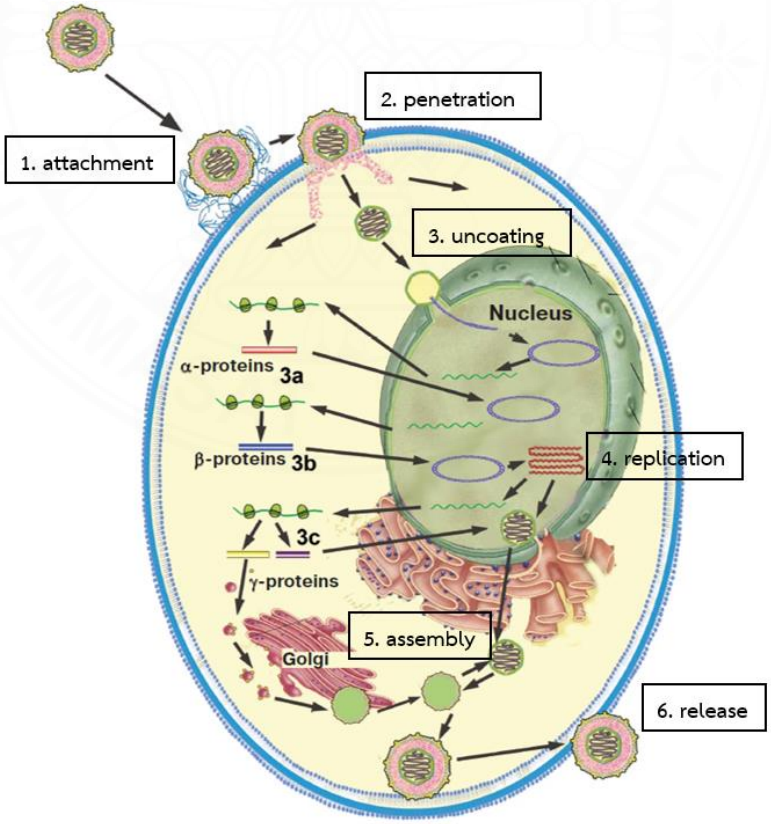


Figure 2-2 Replication cycle of the herpes simplex virus (Kukhanova et al., 2014).

2.1.4 Pathophysiology of herpes simplex virus

The herpes simplex virus infection can cause infections of the skin, mouth, genitals, and mucous membranes and spread disease via skin to the mucosa, mucosa to the skin, and skin to skin contact by the virus that is transmitted through the mucous membranes via direct exposure to an infected person.

2.1.4.1 Pathophysiology of herpes simplex virus type-1

The incubation period for the primary infection is around 2-3 days or can be up to 20 days. The patients' primary infection symptoms are fever, headaches, lack of appetite, and tiredness, and symptoms usually resolve on their own within 7-10 days. When symptoms improve, the virus hides within the trigeminal ganglion, but the virus is stimulated, it may replicate of virus that calls recurrent. It often occurs around the lips (herpes labialis or fever blisters). There are often small blisters on the lips. Then burst into scabs for 2-4 days, before blistering a few hours to days that may be a burning pain or itching around the lesion (Archananupab, 2010).

2.1.4.2 Pathophysiology of herpes simplex virus type-2

The incubation period for the primary infection is around 2-10 days. The patients' primary infections are fever and blistering sores around the genitals. In addition, there may be pain or itching and scabs. It was recovered by itself for 2-3 weeks, and the virus hides within the trigeminal ganglion. Then there will be frequent recurrent infections, especially during the first 6 months after the first infection. Recurrently, the patient will have a burning, itching at the lesion. Then, The patient blisters in the genital area and scabs within 4-5 days. It was then recovered by itself for 10 days (Archananupab, 2010).

2.1.5 Pathogenesis of herpes simplex virus

The spread of herpes simplex virus (HSV) infection depends on intimate personal contact with someone infecting HSV. The virus must connect with the wound skin or mucosal surfaces for virus infection to the body. The primary infection, viral replication at the site, is transported retrogradely by neurons to the

dorsal root ganglia. The latency is established that more severe the primary infection by another round of viral replication. The more likely it is that recurrences that reflected by the number, size, and extent of lesions. When a proper stimulus causes reactivation, the virus becomes evident at appearing as skin blisters, mucocutaneous sites, or mucosal ulcers by the latency. (Whitley et al., 2007).

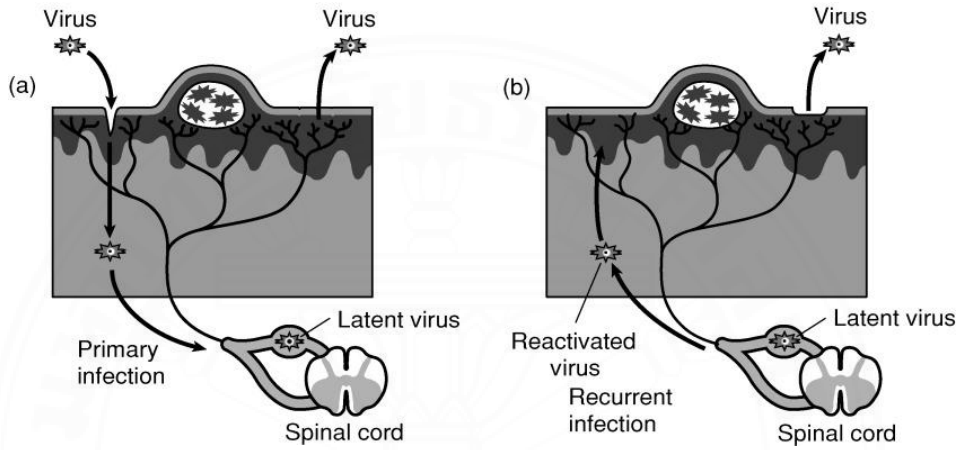


Figure 2-3 Primary infection (a) and Recurrent infection (b) (Whitley et al., 2007).

2.1.6 Treatment

Medications could be administered intravenously, applied topically, or taken orally. For treatment of HSV, using antiviral medications include acyclovir, famciclovir, and Valaciclovir (Archananupab, 2010). But cannot directly against the HSV infection. There can help to decrease the frequency and severity of symptoms.

2.1.6.1 Acyclovir

Acyclovir, the viral-induced enzyme thymidine kinase, is the notable safety related to initial activation. It is mainly used to treat the Herpes simplex virus (HSV) and Herpes varicella virus (VZV) (Kimberlin et al., 2007).

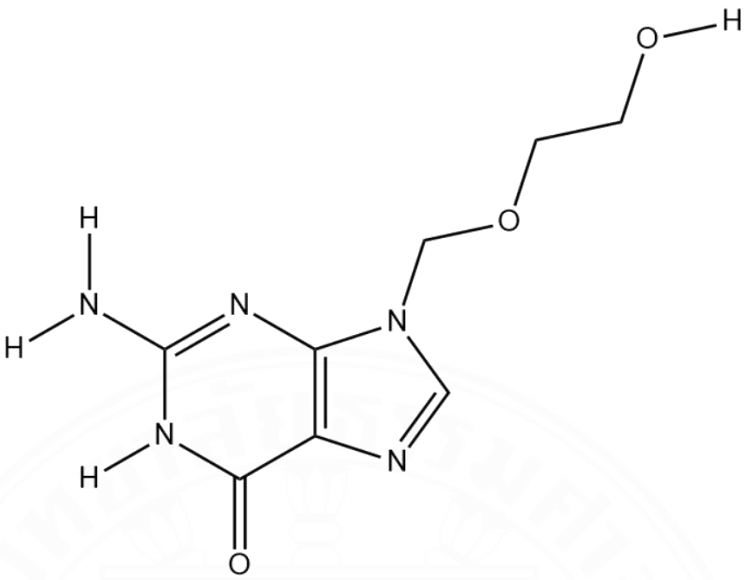


Figure 2-4 Chemical Structure of acyclovir

(1) Administration

- Genital herpes simplex virus (Frist clinical episode) that treat orally for 7-10 days using by 200 mg 5x/days or 400 mg 3x/days
- Genital herpes simplex virus (Episodic, recurrent infection) that treat orally for 5 days using 200 mg 5×/day or 800 mg 2×/day
- Oral suppressive therapy is 400 mg 2×/day or 1000 mg 1×/day (Kimberlin et al., 2007).

(2) Adverse Drug Reaction (ADR)

The most common drug adverse reaction include nausea and vomiting when used short-term oral acyclovir, headache, diarrhea, nausea, and vomiting when used 6 months (Arndt, 1988).

2.1.6.2 Famciclovir

An acyclic nucleoside analog and penciclovir is the inactive diacetyl ester prodrug of famciclovir that rapidly oxidized and deacetylated

(Kimberlin et al., 2007). It is used to treat the herpes simplex virus (HSV) and acute herpes varicella-zoster virus. (VZV) (Sacks, 1997).

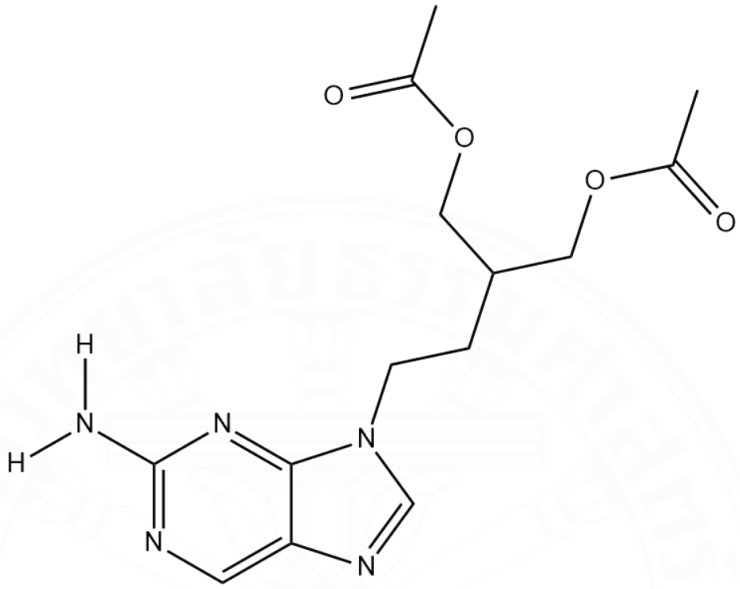


Figure 2-5 Chemical Structure of famciclovir

(1) Administration

Famciclovir should be administered shortly after the signs and symptoms.

- Genital herpes simplex virus (Frist clinical episode) is treated orally for 7-10 days using 1000 mg 2x/days.
- Genital herpes simplex virus (Episodic, recurrent infection) that treat orally for 5 days using 500 mg 2×/day or 1000 mg 1×/day
- Oral suppressive therapy is 500 mg 1×/day or 1000 mg 1×/day (Kimberlin et al., 2007).

(2) Adverse Drug Reaction (ADR)

The most common drug adverse reaction include Hyper/hypopigmented scars, Dyspepsia (indigestion), Headache, Nausea, and Constipation by patients taking famciclovir (Semaan et al., 2020).

2.1.6.3 Valaciclovir

Valacyclovir is an L-valyl ester, a nucleoside analog antiviral agent and prodrug of acyclovir used to treat herpes simplex virus and varicella-zoster virus infections (Valacyclovir, 2012). It is rapidly converted to acyclovir after oral administration by the first-pass metabolism in the liver (Kimberlin et al., 2007).

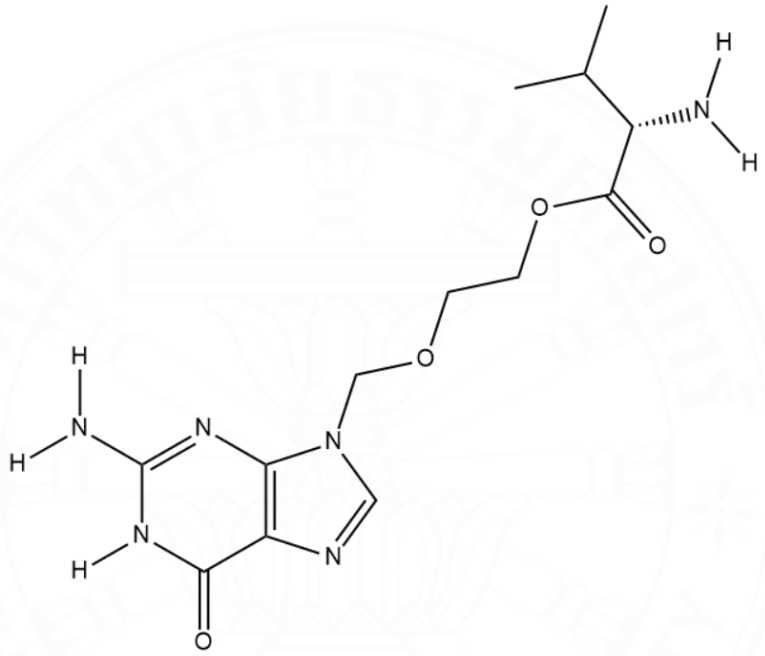


Figure 2-6 Chemical Structure of valacyclovir

(1) Administration

- Genital herpes simplex virus (Frist clinical episode) that treat orally for 7-10 days using by 200 mg 5x/days or 400 mg 3x/days
- Genital herpes simplex virus (Episodic, recurrent infection) that treat orally for 5 days using 200 mg 5×/day or 800 mg 2×/day
- Oral suppressive therapy is 400 mg 2×/day or 1000 mg 1×/day (Kimberlin et al., 2007).

(2) Adverse Drug Reaction (ADR)

Several of the most common side effects include headache, sickness, and diarrhea (Durglishvili et al., 2009).

Table 2-1 Costs of an antiviral drug in Thailand

Product	Dosage form	Unit	Medium price / packing size (Inc. VAT) (Bath)	Referent
Acyclovir	Tablet 200 mg	25	44.94	According to the announcement of the National Drug System Development Committee
	Tablet 400 mg	25	64.20	According to the announcement of the National Drug System Development Committee
	Tablet 800 mg	35	240.75	According to the announcement of the National Drug System Development Committee
Famciclovir	Tablets 250 mg	21	2,140	According to the announcement of the National Drug System Development Committee
Valaciclovir	Tablets 500 mg	42	2910.40	According to the announcement of the National Drug System Development Committee

HSV vaccines would decrease the severity of symptoms, infectivity to other individuals and reduce virus replication. However, preventative vaccines might also preclude symptoms of disease induced by wild-type virus and decease or prevent virus replication (Whitley et al., 2018). But HSV can reach sensory neurons where the latent infection is challenging to go against directly HSV 1-2 (Tajpara et al., 2019). Therefore, HSV vaccines are still to be developed continuously.

2.1.7 Herpes simplex virus of Thai traditional medicine

The body was to a stimulus, such as insufficient rest, stress, or overwork. Then, the affects increased the fire element (Pha-ri thai- huk-Kee), and next stimulated the wind element (Ang-ka-mang-ka-nu-sa-ri-va-ta) increased, resulting in fever. After that, the water element's impact decreased (Lo-hit-tang), causing the water to nourish the soil element not to work fully. Finally, the elemental soil (Mang-sang and Ta-jo) loses its original function, causing sores on the skin and blisters.

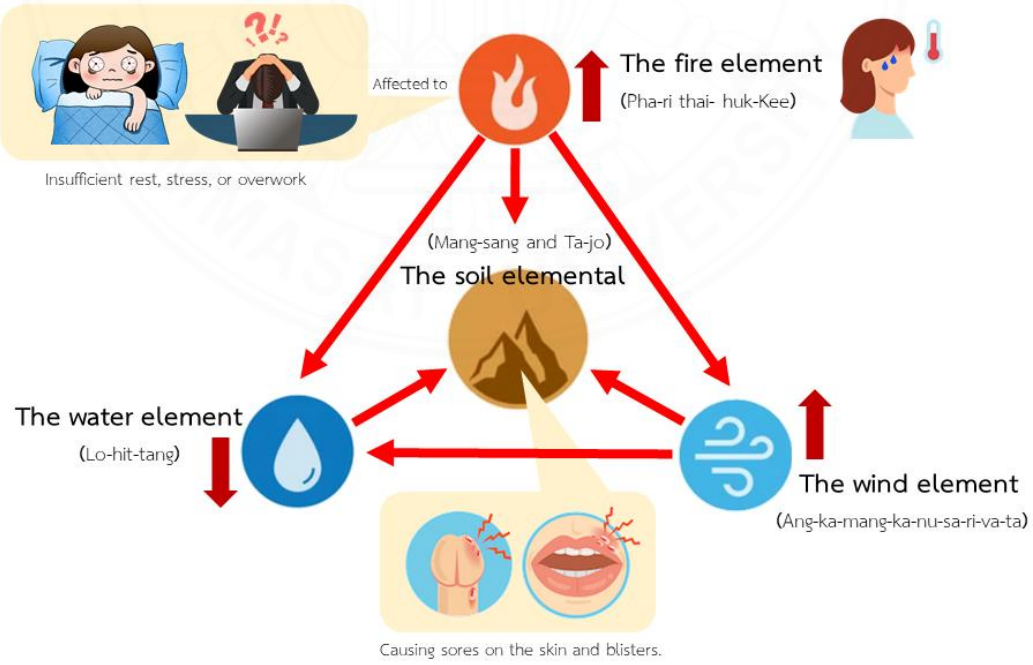


Figure 2-7 Mechanism of herpes disease by Thai traditional medicine theory

2.2 Kheaw-Hom remedy

2.2.1 Composition (90 grams of powder)

There are consists of eighteen herbals medicine include Bua-luang (*Nelumbo nucifera* Gaertn.), Bun-nak (*Mesua ferrea* Linn.), Chan-dang (*Dracaena cochinchinensis*), Chan-thet (*Myristica fragrans* Houtt.), Faek-hom (*Vetiveria zizanioides* (L.) Nash ex Small), Ma-has-sa-dam (*Cyathea gigantea* Holtt.), Mak-mia (*Cordyline fruticosa* (L.) Goeppert.), Mak-phu (*Cordyline fruticosa* (L.) Goeppert.), Nae-ra-phu-sri (*Tacca chantrieri* Andre), Phak-kra-chom (*Limnophila rugosa* Merr.), Phi-kul (*Mimusops elengi* Linn.), Phim-sen-ton (*Pogostemon cablin* (Blanco) Benth.), Phit-sa-nat (*Sophora exigua* Craib), Proh-hom (*Kaempferia galanga* Linn.), San-phra-hom (*Eupatorium stoechadosmum* Hance), Sa-ra phi (*Mammea siamensis* Kosterm.), Wan-kep-rat (*Angiopteris evecta* (G.Forst) Hoffm.), and Wan-ron-thong (*Globba malaccensis* Ridl.). that weighs 5 grams each.

2.2.2 Properties

2.2.2.1 Relief of fever and treating aphthous ulcers.

2.2.2.2 Relief of fever from measles and chickenpox

2.2.3 Dosage and Administration

2.2.3.1 Powder

(1) **Adult:** Take 1 g/time, dilute in water, quaque 4-6 hora

(2) **Children aged 6-12 years:** Take 500 mg/time, dilute in water, quaque 4-6 hora

2.2.3.2 Tablet

(1) **Adult:** Take 1 g/time, dilute in water, quaque 4-6 hora

(2) **Children aged 6-12 years:** Take 500 mg/time, dilute in water, quaque 4-6 hora

2.2.4 Contraindication

There is no report for contraindication for Kheaw-Hom remedy.

2.2.5 Precautions

2.2.5.1 Caution should be exercised in patients allergic to pollen.

2.2.5.2 It is not recommended for use in a person with suspected dengue fever because it may obscure the symptoms.

2.2.5.3 If the drug is used for more than 3 days and the symptoms do not improve, consult a doctor.

2.2.6 Side effects

There is no report for side effect of Kheaw-Hom remedy.

2.2.7 More information

2.2.7.1 Thai traditional medicine advises patients with measles and chickenpox not to eat seafood, eggs, and cold water as they are wrong.

2.2.7.2 In the recipe, *Aristolochia pierei* (family ARISTOLOCHIA) was cut out because research data indicates that the affiliates used and are available in the market. Plants in the genus *Aristolochia* have been reported to cause nephrotoxicity, and in 2002 the World Health Organization declared the genus *Aristolochia* to be a human carcinogen. (Thai National List of Essential Medications, 2012).

2.2.8 Antiviral activities of Kheaw-Hom remedy

- ***Varicella zoster* virus (VZV):** Antiviral activity against *varicella-zoster* virus (VZV) that pre-treatment test with the 20% ethanolic extract of Ya-Kheaw remedy at concentration 250 µg/ml significantly decreases varicella-zoster virus infection (Sanguansermsri et al., 2005).
- **Enterovirus 71 (EV71):** The aqueous extract of Kheaw-Hom remedy (KHA) showed that KHA had antiviral activity against 25 $TCID_{50}$ EV71 with a cytopathic effect of less than 50% at a concentration of 400 µg/mL (Sukkasem et al., 2016).

2.2.9 Biological activities of Kheaw-Hom remedy

- **Antibacterial activity:** The ethanolic extract of Kheaw-Hom remedy against bacteria such as *S.epidermidis* (MIC = 1.25 mg/mL, MMC = 2.5 mg/mL), *S.aureus* (MIC = 0.625 mg/mL, MMC = 1.25 mg/mL) and methicillin-resistant *S.aureus* (MIC = 0.625 mg/mL, MMC = 0.625 mg/mL) (Sukkasem et al., 2016).
- **Antibacterial activity:** The ethanolic extract of Kheaw-Hom remedy exhibited antimicrobial activity against *S.aureus*, methicillin-resistant *S.aureus,* and *S.epidermic* with MIC values of 31, 125 and 31 μg/mL (Chusri et al., 2014).
- **Anti-inflammation:** the aqueous extract of Kheaw-Hom remedy showed anti-inflammationactivity with IC_{50} value of 46.86±0.82 μg/mL. In contrast, the ethanolic extract showed IC_{50} value of 59.77±3.76 μg/mL by effects on lipopolysaccharide (LPS) induced nitric oxide (NO) release from RAW 264.7 cells (Sukkasem et al., 2016).

2.2.10 Phytochemicals and/or Bioactive compounds

- Ethyl p-methoxycinnamate, patchouli alcohol, Heptadecanoic acid, Isocurcumenol, and Linolein by GC-MS (Sukkasem et al., 2016)

2.3 Its plant ingredients of Kheaw-Hom remedy

2.3.1 Wan-gieb-rad (*Angiopteris evecta* (G.Forst) Hoffm.)

Figure 2-8 *Angiopteris evecta* (G.Forst) Hoffm. (MARATTIACEAE)

Scientific name: *Angiopteris evecta* (G.Forst) Hoffm.

Family: MARATTIACEAE

Common name: Wan-gieb-rad, King fern, Giant fern

Botanical characteristics

Angiopteris evecta (G.Forst) Hoffm is a tree growing high of 0.5-1 meters. Either side of the petiole insertion, the rhizome bears two flat, rounded, stipule-like outgrowths, leathery, dark brown, the caliber long of 10-15 centimeters. The leaves have tinged with green, ovate characteristics, long of 5-15 centimeters and wide of 1.5-3 centimeters (BGO, 2013).

Usage: Thai Traditional used rhizomes of *Angiopteris evecta* (G.Forst) Hoffm. to relieve fever, diarrhea, diuretic, and wound healing (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- Alkaloids, Flavonoids, and Tannins (Molla et al., 2014)

- Polyphenols, Monoterpenoid and sesquiterpenoids, Quinone and Saponin (Rahmawati et al., 2018)
- Stigmast-5-en-3-β-ol ,4,5-dihydro-4-hydroxy-5-methyl-1H-pyran-1-one, Derivative 4,5-dihydro-4-acetyl-5-methyl-1H-pyran-1-one and Angiopteroside (Taveepanich et al., 2005)

Antiviral activities of *Angiopteris evecta* (G.Forst) Hoffm.

- **HIV-1:** The structure of angiopteroside, which was isolated from rhizome of *Angiopteris evecta* Hoffm., was confirmed using X-ray crystallography. Angiopteroside showed significant activity for inhibition of HIV-1 Reverse Transcriptase (IC_{50} at 0.91 mM) as compared to the IC_{50} for ddI, a positive control, of 0.87 mM (Taveepanich et al., 2005)
- **Enterovirus 71 (EV71):** The aqueous extract of *Angiopteris evecta* (G.Forst) Hoffm. Showed that the aqueous extract of *A.evecta* had antiviral activity against 25 $TCID_{50}$ EV71 with a cytopathic effect of less than 50% at a concentration of 200 µg/mL. Morphological changes of Vero cells that were infected with EV71 at 25 $TCID_{50}$ were observed after incubated for 5 days (Sukkasem et al., 2016).

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

- **Anti-inflammation:** the aqueous extract of *A.evecta* showed anti-inflammation with IC_{50} value of 82.98±3.08 µg/mL (Sukkasem et al., 2016).
- **Antibacterial activity:** the ethanolic extract of *A.evecta* inhibited *S.pyogenes* with an inhibition zone of 9.33±1.53 mm. by disc diffusion method (Sukkasem et al., 2016).

2.3.2 Maak-mia (*Cordyline fruticose* (L.) A.Chev (Green leaves))

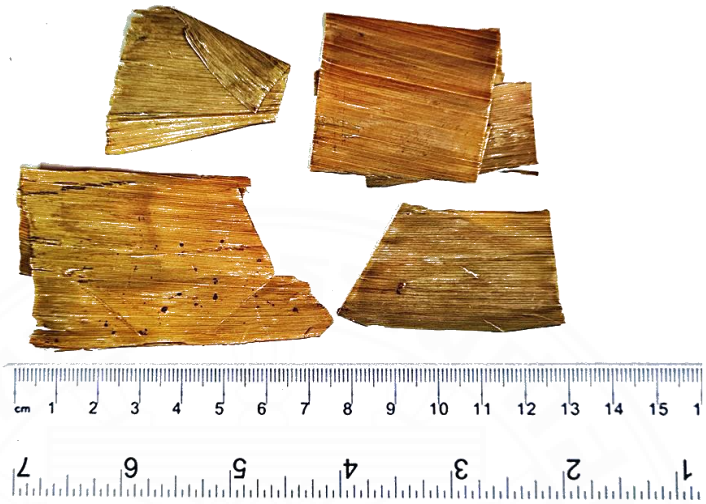


Figure 2-9 *Cordyline fruticose* (L.) A.Chev (Green leaves) (AGAVACEAE)

Scientific name: *Cordyline fruticose* (L.) A.Chev (Green leaves)

Family: AGAVACEAE

Common name: Maak-mia

Botanical characteristics

Cordyline fruticose (L.) A.Chev (Green leaves) is an erect growing high around 1 to 3 meters from tuberous roots. Leaves are usually tinged with green, lanceolate to oblanceolate that long with 30 to 50 centimeters. The panicles are terminal, purplish, slender, and branches up to 30 centimeters in length. The flowers are pink (Stuartxchange, 2014).

Usage: Thai Traditional used leaves of *Cordyline fruticose* (L.) A.Chev (Green leaves) for relieve cough, whooping cough, and relieve the common cold (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- **Steroidal saponins**: Fruticoside H, Fruticoside I and Fruticoside J (Fouedjou et al., 2013)

2.3.3 Maak-phu (*Cordyline fruticosa* (L.) A.Chev (Red leaves))

Figure 2-10 *Cordyline fruticosa* (L.) A.Chev (Red leaves) (AGAVACEAE)

Scientific name: *Cordyline fruticosa* (L.) A.Chev (Red leaves)

Family: AGAVACEAE

Common name: Maak-phu

Botanical characteristics

Cordyline fruticosa (L.) A.Chev (Red leaves) is an erect growing high around 1 to 3 meters from tuberous roots. Leaves are usually tinged with red or purple, lanceolate to oblanceolate that long with 30 to 50 centimeters. The panicles are terminal, purplish, slender, and branches up to 30 centimeters in length. The flowers are pink (Stuartxchange, 2014).

Usage: Thai Traditional used leaves of *Cordyline fruticosa* (L.) A.Chev (Red leaves) for relieving cough, whooping cough, and relieve the common cold (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- **Steroidal saponins**: Fruticoside H, Fruticoside I and Fruticoside J (Fouedjou et al., 2013)

Biological activities of ***Cordyline fruticosa*** **(L.) A.Chev (red leaves)**

- **Antibacterial activity:** the ethanolic extract of *C. fruticose* inhibited *S.pyogenes* with an inhibition zone of 9.00±1.73 mm. by disc diffusion method (Sukkasem et al., 2016).

2.3.4 Ma-has-sa-dam (*Cyathea gigantea* Holtt.)

Figure 2-11 *Cyathea gigantea* Holtt. (CYATHEACEAE)

Scientific name: *Cyathea gigantea* Holtt.

Family: CYATHEACEAE

Common name: Ma- has-sa-dam

Botanical characteristics

Cyathea gigantea Holtt. is a fern was growing up to 2 m high. Petioles are dark brown to nearly black, 50-100 cm long, and pinnately compound leaf. (Royal Botanic Garden Edinburgh, 2015)

Usage: Thai Traditional used the stem of *Cyathea gigantea* Holtt. to relieve cough, relieve fever, and reduce pain. Wood is used to treat diarrhea and fever (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- Alkaloids and Glycosides (Kale et al., 2015)
- Oleaonic acid (Juneja et al., 1990)

- Isophytol, N-Hexadecanoic acid, Phytol; N-Tetracosanol-1, 1-heptacosanol, Octacosanol, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Octadecanoic acid, 1-eicosanol Cycloheptasiloxane, Tetradecamethyland and Neophytadiene (Nath et al., 2019)
- Dryocrassyl formate, 12a-hydroxyfern-9(11)-ene, and sitostanyl formate (Arai et al., 2003).

Biological activities of *Cyathea gigantea* Holtt.

- **Antibacterial activity:** The ethanolic extract of Kheaw-Hom remedy inhibited gram-positive bacteria such as *S.aureus* (MIC = 5 mg/mL, MMC = 5 mg/mL), methicillin-resistant *S.aureus* (MIC = 2.5 mg/mL, MMC = 2.5 mg/mL) and *S.epidermidis* (MIC = 5 mg/mL, MMC = 5 mg/mL) (Sukkasem et al., 2016).

2.3.5 Chan-dang (*Dracaena cochinchinensis*)

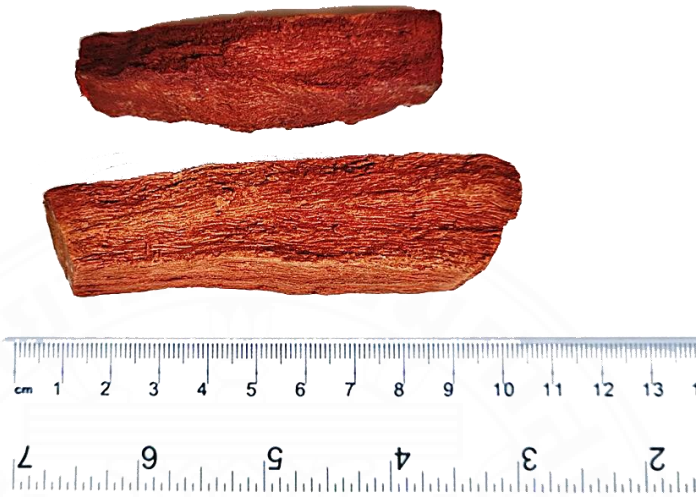


Figure 2-12 *Dracaena cochinchinensis* (DRACANACEAE)

Scientific name: *Dracaena cochinchinensis*

Family: DRACANACEAE

Common name: Chan-dang, Chan-par, Cinnabaris, Dragon's blood tree

Botanical characteristics

Dracaena cochinchinensis is a tree growing 5 - 15 meters tall with boles that can be 1 meter in diameter by grayish-white or brown. Leaves crowded at the apex of the sessile, branches, leathery, and sword-shaped. Fruits are orange sub-globose berries with 1-3 seeds (Sukkasem et al., 2016).

Usage: Thai Traditional used the stem of *Dracaena cochinchinensis* to relieve common cold, promote the lymphatic system and blood tonic (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- Terpenes, Steroids and Steroidal Saponin (Fan et al., 2014)
- Lignans (Fan et al., 2014)

- **Phenol**: resveratrol (Fan et al., 2014)
- **Flavonoids:** Chalcones such as 2,4,4'-trihydroxychalcone, 2'-methoxy-4,4'-dihydroxychalcone and 2-methoxy- 4,4'-dihydroxychalcone and Dihydrochalcones such as loureirin A, B, C, D and cochinchinenin A (Fan et al., 2014)
- **Chalcane–stilbene conjugates:** Cochinchinnenes G and H (Hao et al., 2015)

Antiviral activities of *Dracaena cochinchinensis*

- **HIV-1:** The ethanol and aqueous extracts of the heartwood of *Dracaena cochinchinensis* showed anti-HIV-1 integrase activity by the multiple integration assay (MIA) with IC_{50} values of 28.0 and 22.1 μg/mL, respectively (Bunluepuech et al., 2009).

Biological activities of *Dracaena cochinchinensis*

- **Antipyretic:** *in Vivo*, the antipyretic activity of *Dracaena cochinchinensis* was measured by slightly modifying in rats with yeast-induced. Pyrexia was induced by subcutaneously injecting 20% (w/v) brewer's yeast suspension (10 mL/kg) into the animals' dorsum region. The temperature of the only rat increased at least 0.7°C for testing the experiments. After drug administration was measured at 1, 2, 3, 4, and 5 hours, shown that *Dracaena cochinchinensis* was extracted with the methanol fraction to reduce yeast-induced fever in rats (Wantana et al., 2003).
- **Antibacterial activity:** the ethanolic extract of *D.cochinchinensis* inhibited bacteria such as *S.aureus* (MIC = 2.5 mg/mL, MMC = 2.5 mg/mL), methicillin-resistant *S.aureus* (MIC = 2.5 mg/mL, MMC = 2.5 mg/mL), *S.epidermidis* (MIC = 1.25 mg/mL, MMC = 5 mg/mL), *S.pyogenes* (MIC = 1.25 mg/mL, MMC = 2.5 mg/mL), and *C.albicans* (MIC = 2.5 mg/mL, MMC = 5 mg/mL) (Sukkasem et al., 2016).

- **Anti-inflammation:** the ethanolic extract of *D.cochinchinensis* showed anti-inflammation with IC_{50} value of 40.73±4.99 μg/mL (Sukkasem et al., 2016).

2.3.6 San-phra-hom (*Eupatorium stoechadosmum* Hance.)

Figure 2-13 *Eupatorium stoechadosmum* Hance. (COMPOSITAE)

Scientific name: *Eupatorium stoechadosmum* Hance.

Family: COMPOSITAE (ASTERACEAE)

Common name: San-phra-hom

Botanical characteristics

Eupatorium stoechadosmum Hance. is a smooth herb that high of 60 to 90 meters. The fragrant leaves are long, up to 19 centimeters. Segments are elliptic-ovate or elliptic-lanceolate and toothed at the margins up to 13 centimeters long. The inflorescence is terminal up to 14 centimeters across. Flowering heads are 3 to 4 millimeters across. Flowers are fragrant and tinged white (Sukkasem et al., 2016).

Usage: Thai Traditional used leaves of *Eupatorium stoechadosmum* Hance. for relieving fever, ulcer, headaches, and fractures (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- **Essential oil:** thymohydroquinone dimethyl ether, β-caryophyllene and selina-4,11-diene (Dung et al., 1993).
- **Coumarin and ayapin:** 6,7-methylenedioxycomarin (Trang et al., 1993)
- 2-Hydroxy-4-methylacetophenone, 8, 10-Epoxy-9-acetoxythymol angelate, 9- Isobutyryloxy-8, 10-dihydroxythymol and 9-Angeloyloxy-8, 10-dihydroxythymol. (Trang et al., 1993)

Biological activities of *Eupatorium stoechadosmum*

- **Anti-inflammation:** the ethanolic extract of *E.stoechadosmum* showed anti-inflammation with IC_{50} value of 78.12±7.86 μg/mL (Sukkasem et al., 2016).
- **Antibacterial activity:** the ethanolic extract of *E.stoechadosmum* inhibited bacteria such as *S.aureus* (MIC = 1.25 mg/mL, MMC = 1.25 mg/mL), methicillin-resistant *S.aureus* (MIC = 2.5 mg/mL, MMC = 2.5 mg/mL), *S.epidermidis* (MIC = 1.25 mg/mL, MMC = 1.25 mg/mL), *S.pyogenes* (MIC = 0.625 mg/mL, MMC = 0.625 mg/mL), and *C.albicans* (MIC = 1.25 mg/mL, MMC = 1.25 mg/mL) (Sukkasem et al., 2016).

2.3.7 Wan-ron-thong (*Globba malaccensis* Ridl.)

Figure 2-14 *Globba malaccensis* Ridl. (ZINGIBERACEAE)

Scientific name: *Globba malaccensis* Ridl.

Family: ZINGIBERACEAE

Common name: Wan-ron-thong

Botanical characteristics

Globba malaccensis Ridl. is a perennial plant growing high to 1 meter. The leaves are alternate and arranged in rows. The stalk leaves are short and green that measuring 5-7 centimeters. The leaves are lanceolate, petioled, long, smooth, and measuring 10-15 centimeters wide, 30-35 centimeters long. The roots have a spicy fragrance and aromatic. The flowers are tinged yellow.

Usage: Thai Traditional used Rhizomes of *Globba malaccensis* Ridl. to treat diarrhea and scorpion bite or centipede. (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- Curcumenol (Kumar et al., 2012)

Antiviral activities of *Globba malaccensis* Ridl.

- **Enterovirus 71 (EV71):** The aqueous extract of *Globba malaccensis* Ridl. showed that the aqueous extract of *G.malaccensis* had antiviral activity against 100 $TCID_{50}$ EV71 with a cytopathic effect of less than 50% at a concentration of 400 µg/mL. Morphological changes of Vero cells that were infected with EV71 at 100 $TCID_{50}$ were observed after incubated for 5 days (Sukkasem, 2016).

Biological activities of *Globba malaccensis* Ridl.

- **Anti-inflammation:** the ethanolic extract of *G.malaccensis* showed anti-inflammation with IC_{50} value of 24.11±4.82 µg/mL (Sukkasem et al., 2016).
- **Antibacterial activity:** the ethanolic extract of *G.malaccensis* inhibited bacteria such as *S.aureus* (MIC = 2.5 mg/mL, MMC = 2.5 mg/mL), methicillin-resistant *S.aureus* (MIC = 2.5 mg/mL, MMC = 5 mg/mL), *S.epidermidis* (MIC = 2.5 mg/mL, MMC = 5 mg/mL), *S.pyogenes* (MIC = 1.25 mg/mL, MMC = 1.25 mg/mL), and *C.albicans* (MIC = 2.5 mg/mL, MMC = 5 mg/mL) (Sukkasem et al., 2016).

2.3.8 Proh-hom (*Kaempferia galanga* Linn.)

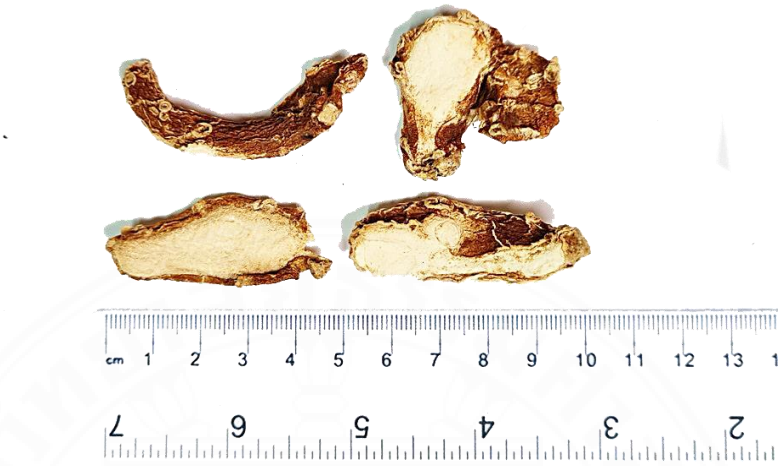


Figure 2-15 *Kaempferia galanga* Linn. (ZINGIBERACEAE)

Scientific name: *Kaempferia galanga* Linn.

Family: ZINGIBERACEAE

Common name: Proh-hom

Botanical characteristics

Kaempferia galanga Linn. is a stemless herb arising from fibrous cylindric roots by the tuberous aromatic rootstocks. The leaves are rounded base, horizontally spreading, orbicular to broadly ovate, measuring up to 7-15 centimeters long. Flowers are few with lanceolate bracts about 3.5 centimeters long. The staminal crest is a 2-lobed and quadrate (Sukkasem et al., 2016).

Usage: Thai Traditional used rhizomes of *Kaempferia galanga* Linn. to relieve cough and urticaria (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- Saponin, tannin, terpenoid, steroid, phlobatinin, alkaloid, flavonoid, coumarin, and phenol (Rao & Dowluru, 2014)
- Ethyl *p*-methoxy cinnamate and 4-methoxy cinnamic acid (Chowdhury at el., 2014).

Antiviral activities of *Kaempferia galanga* Linn.

- **Hepatitis C (HCV):** the aqueous extracts of *Kaempferia galangal* Linn. showed that a concentration 200 mg/ml of the aqueous extracts of *K.galangal* had inhibited HCV protease more than 70% (Sookkongwaree et al., 2006).

Biological activities of *Kaempferia galanga* Linn.

- **Anti-inflammation:** the aqouse extract of *K.galanga* showed anti-inflammation with IC_{50} value of 10.30±0.99 μg/mL while the ethanolic extract IC_{50} value of 46.15±5.39 μg/mL (Sukkasem et al., 2016).
- **Antimicrobial activity:** the ethanolic extract of *K.galanga* inhibited bacteria such as *S.pyogenes* (MIC = 0.625 mg/mL, MMC = 0.625 mg/mL), and *C.albicans* (MIC = 0.625 mg/mL, MMC = 2.5 mg/mL) (Sukkasem et al., 2016).

2.3.9 Phak-kra-chom (*Limnophila rugosa* (Roth) Merr.)

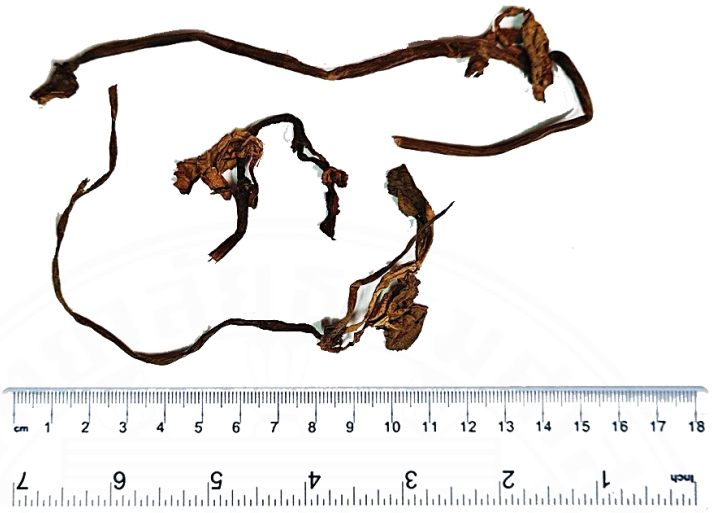


Figure 2-16 *Limnophila rugosa* (Roth) Merr. (SCROPHULARIACEAE)

Scientific name: *Limnophila rugosa* (Roth) Merr.

Family: SCROPHULARIACEAE

Common name: Phak-kra-chom

Botanical characteristics

Limnophila rugosa (Roth) Merr. is an erect herb, up to 50 centimeters high. The leaves are oblong-ovate, opposite with 3 to 10 centimeters long, 1.5 to 4 centimeters wide, and toothed at the margins. The upper surface of the leaves is rough. Flowers are about 1 centimeter long, clustered on stems and purplish found in the leaves' axils that terminate the leafy branches (Sukkasem et al., 2016).

Usage: Thai Traditional used leaves of *Limnophila rugosa* (Roth) Merr. to relieve cough and relieve the common cold (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- Alkaloid, Flavonoid, Phenols, Tannin, and Triterpenoids (Steroid) (Acharya at el., 2014)

Biological activities of *Limnophila rugosa* (Roth) Merr.

- **Antibacterial activity:** the ethanolic extract of *L.rugosa* inhibited *S.aureus* with MIC = 5 mg/mL and MMC = 5 mg/mL (Sukkasem et al., 2016).

2.3.10 Sa-la-pii (*Mammea siamensis* Kosterm.)

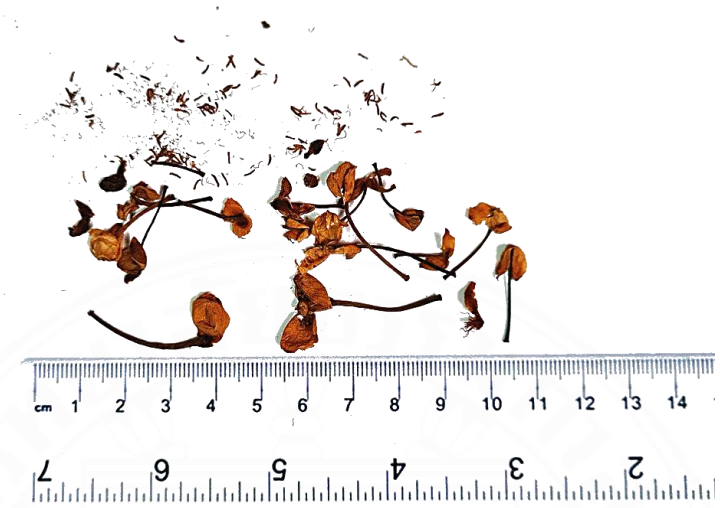


Figure 2-17 *Mammea siamensis* Kosterm. (GUTTIFERAE)

Scientific name: *Mammea siamensis* Kosterm.

Family: GUTTIFERAE

Common name: Sa-la-pii

Botanical characteristics

Mammea siamensis Kosterm. is a small evergreen that up to 15 centimeters high. The leaves are opposite, oblong-obovate with 4-5 centimeters wide, 10-15 centimeters long, coriaceous, and glabrous. Flowers are fragrant, ramiflorous or cauliflorous, solitary or few-flowered fascicle, and tinged with white. The stamens numerous are yellow. Fruit is drupe and ellipsoid. The fruit is an ellipsoid drupe and 1 seeded (Sukkasem et al., 2016).

Usage: Thai Traditional used flowers of *Mammea siamensis* Kosterm. for cardiotonic, blood tonic, and relieve the common cold (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- β-sitosterol, friedelin, and stigmasterol (Subhadhirasakul et al., 2005)

Antiviral activities of *Mammea siamensis* Kosterm.

- **Enterovirus 71 (EV71):** the aqueous extract of *Mammea siamensis* Kosterm. Showed that the aqueous extract of *M.siamensis* had antiviral activity against 25 $TCID_{50}$ EV71 with a cytopathic effect of less than 50% at a concentration of 100 µg/mL. Morphological changes of Vero cells that were infected with EV71 at 25 $TCID_{50}$ were observed after incubated for 5 days (Sukkasem et al., 2016).

Biological activities of *Mammea siamensis* Kosterm.

- **Anti-inflammation:** the ethanolic extract of *M.siamensis* showed anti-inflammation with IC_{50} value of 11.55±2.70 µg/mL (Sukkasem et al., 2016).
- **Antibacterial activity:** the ethanolic extract of *M.siamensis* inhibited bacteria such as *S.aureus* (MIC = 0.005 mg/mL, MMC = 0.005 mg/mL), methicillin-resistant *S.aureus* (MIC = 0.005 mg/mL, MMC = 0.005 mg/mL), *S.epidermidis* (MIC = 0.039 mg/mL, MMC = 0.039 mg/mL), and *S.pyogenes* (MIC = 0.019 mg/mL, MMC = 0.195 mg/mL) (Sukkasem et al., 2016).

2.3.11 Boon-nak (*Mesua ferrea* Linn.)

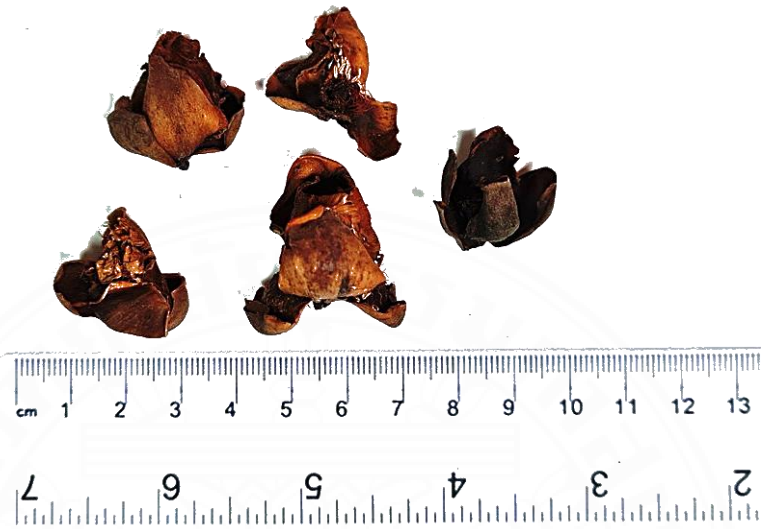


Figure 2-18 *Mesua ferrea* Linn. (GUTTIFERAE)

Scientific name: *Mesua ferrea* Linn.

Family: GUTTIFERAE

Common name: Boon-nak, Indian rose chestnut

Botanical characteristics

Mesua ferrea Linn. is a tree that up to 15 meters high. The young shoots are white or red. The leaves are opposite, oblong-lanceolate or lanceolate, measuring around 2-4 centimeters wide, 7-12 centimeters long. The solitary flower, terminal or leaf-axil, fragrant, tinged with white. The stamens numerous are yellow. Fruit is an ellipsoid and drupe (Sukkasem et al., 2016).

Usage: Thai Traditional used flowers of *Mesua ferrea* Linn. for cardiotonic, blood tonic, carminative and astringent (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- Mesuol (Chahar et al., 2012)

- Mesuaferrone B, quercitrin, quercetin, kaempferol-3- O-rhamnoside,5,6,6'-trihydroxy[1.1'-biphenyl]-3,3'-dicarboxylic acid, rhusflavanone, gallic acid, 3-amino-4-hydroxybenzoic acid, procatechuic acid ethyl and procatechuic acid ester **N. nucifera**
- **Xanthones:** 4-methoxypyranojacareubin, 4-hydroxy-3-prenylpyranoxanthone, 1-hydroxy-5,7-dimethoxyxanthone, 5-hydroxy-1,6,7-trimethoxyxanthone and 2-hydroxy-1,5-dimethoxyxanthone (Chukaew et al., 2019)

Biological activities of *Mesua ferrea* Linn.

- **Anti-inflammation:** the ethanolic extract of *M.ferrea* showed anti-inflammation with IC_{50} value of 65.71±1.09 μg/mL (Sukkasem et al., 2016)
- **Antibacterial activity:** the ethanolic extract of *M.ferrea* inhibited bacteria such as *S.aureus* (MIC = 0.156 mg/mL, MMC = 0.625 mg/mL), methicillin-resistant *S.aureus* (MIC = 0.625 mg/mL, MMC = 0.625 mg/mL), *S.epidermidis* (MIC = 0.625 mg/mL, MMC = 0.625 mg/mL), and *S.pyogenes* (MIC = 1.25 mg/mL, MMC = 1.25 mg/mL) (Sukkasem et al., 2016).

2.3.12 Phi-kul (*Mimusops elengi* Linn.)

Figure 2-19 *Mimusops elengi* Linn. (SOPOTACEAE)

Scientific name: *Mimusops elengi* Linn.

Family: SOPOTACEAE

Common name: Phi-kul, Sang dong, Bullet Wood

Botanical characteristics

Mimusops elengi Linn. is a medium-sized evergreen tree that reaches up to 25 meters high. The leaves are alternate simple, ovate or elliptic that measure around 3-6 centimeters wide, 5-12 centimeters long. The flowers are tinged with white turning brown, solitary or 2-6 flowered fascicle, axillary and fragrant. The fruits are yellow or orange, ovoid, and berry. The seeds are dark brown (Sukkasem et al., 2016).

Usage: Thai Traditional used flowers of *Mimusops elengi* Linn. for cardiotonic, treatment of sore and muscular pain (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- Triterpenes, Saponins, and Steroids (Arifin et al., 2019)

Biological activities of *Mimusops elengi* Linn.

- **Anti-inflammation:** the aqueous extract of *M.elengi* showed anti-inflammation with IC_{50} value of 48.25±5.02 μg/mL (Sukkasem et al., 2016)
- **Antibacterial activity:** the aqueous extract of *M.elengi* inhibited bacteria such as *S.aureus* (MIC = 5 mg/mL, MMC = 5 mg/mL), methicillin-resistant *S.aureus* (MIC = 5 mg/mL, MMC = 5 mg/mL), and *S.epidermidis* (MIC = 5 mg/mL, MMC = 5 mg/mL), (Sukkasem et al., 2016).

2.3.13 Chan-thet (*Myristica fragrans* Houtt.)

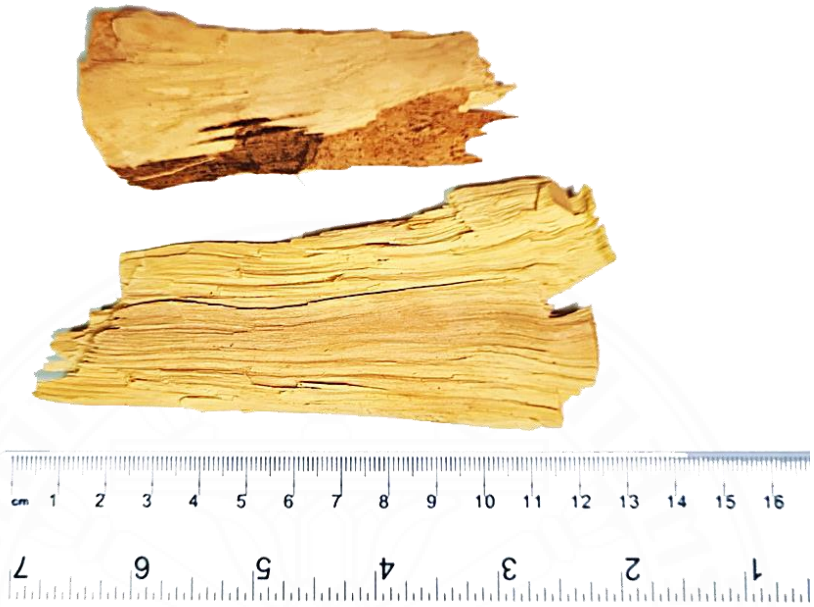


Figure 2-20 *Myristica fragrans* Houtt. (MYRISTICACEAE)

Scientific name: *Myristica fragrans* Houtt.

Family: MYRISTICACEAE

Common name: Chan-thet, Nutmag tree

Botanical characteristics

Myristica fragrans Houtt. is an aromatic evergreen tree that high up 9-12 meters. There are spreading branches and a yellow fleshy fruit similar in appearance to peach or apricot. The flowers are small in axillary racemes and dioecious. The fruit is a globose drupe. The seed or nutmeg is firm, whitish and juicy. There are transversed by red-brown veins and abounding in oil.

Usage: Thai Traditional used the stem of *Myristica fragrans* Houtt. for cardiotonic and carminative (Wutthithammawet, 2002)

Phytochemicals and/or bioactive compounds

- Flavonoids, alkaloids, terpenoids, steroids, saponins, phenols, diterpenes, glycosides, tannins, phlobatannins and quinones (Sattar et al., 2019)
- Methane, 3,4-dichlorophenethylamine and octadecane (Balakrishnan et al., 2017)
- Maceneolignan I K A D F and H, Mirislignanometin E, Myrifralignan C, Licarin B, *Threo*-austrobailignan-5, Nectandrin B, Verrucosin, Myristicin, Anthriscinol, 4-Allyl-2,6-dimethoxyphenol and Malabaricone C (Morikawa et al., 2018)

Biological activities of *Myristica fragrans* Houtt.

- **Anti-inflammation:** the ethanolic extract of *M. fragrans* showed anti-inflammation with IC_{50} value of 88.67±5.21 μg/mL (Sukkasem et al., 2016)
- **Antibacterial activity:** the ethanolic extract of *M. fragrans* inhibited bacteria such as *S.aureus* (MIC = 5 mg/mL, MMC = 5 mg/mL), and *S.pyogenes* (MIC = 0.156 mg/mL, MMC = 0.156 mg/mL) (Sukkasem et al., 2016).

2.3.14 Bua-luang (*Nelumbo nucifera* Gaertn.)

Figure 2-21 *Nelumbo nucifera* Gaertn. (NELUMBONACEAE)

Scientific name: *Nelumbo nucifera* Gaertn.

Family: NELUMBONACEAE

Common name: Bua-luang, Sacred lotus

Botanical characteristics

Nelumbo nucifera Gaertn. is an aquatic perennial with creeping rootstocks. The leaves are rounded, peltate large, and raised above the water. The leaves measure around 50 to 90 centimeters wide. The flowers are attractive and tinged with white, pink, or red. The flowers measure approximately 15 to 25 centimeters in diameter and stand out of the water. The pink petals have around 20 measures 7 to 15 centimeters in length. The large structure is shaped like an inverted cone, located by the flower's center in ovules. The numerous stamens are yellow around the inverted cone. The rich carpels are 13 millimeters long, with bony smooth pericarp and black (Sukkasem et al., 2016).

Usage: Thai Traditional used the pollen of *Nelumbo nucifera* Gaertn. for cardiac tonic, faintness, and vertigo (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- **Kaempferol:** trifolin, astragalin, kaempferol 3-O-gln and kaempferol 3-O-gln methyl ester (Chen et al., 2019)
- **Quercetin:** quercitrin, isoquercitrin, quercetin 3-O-gln and quercetin 3-O-ara-gal (Chen et al., 2019)
- **Myricetin:** myricetin 3-O-glu, myricetin 3-O-gln, myricetin 3-O-gal and syringetin 3-O-glu (Chen et al., 2019)
- **Diosmetin:** diosmetin and diosmetin 7-O-hexose (Chen et al., 2019)
- **Anthocyanin:** delphinidin 3-O-glu (Chen et al., 2019)
- **Tannins:** leucocyanidin, 5,7,30,50-tetrahydroxy flavanone (Chen et al., 2019)

Antiviral activities of *Nelumbo nucifera* Gaertn.

- **HIV:** the isolate compounds of the leaves from Nelumbo nucifera were (+)-1(R)-Coclaurine and (-)-1(S)-norcoclaurine for test experiment anti-HIV. Showed that (+)-1(R)-Coclaurine and (-)-1(S)-norcoclaurine demonstrated potent anti-HIV activity with EC_{50} values of 0.8 and <0.8 microg/mL, respectively, and therapeutic index (TI) values of >125 and >25, respectively. Liensinine, negferine, and isoliensinine compounds were isolated from the embryo and leaves of N.nucifera, showed that potent anti-HIV activities with EC_{50} values of <0.8 microg/mL and TI values of >9.9, >8.6, and >6.5, respectively (Kashiwada et al., 2005).

- **Enterovirus 71 (EV71):** the aqueous extract of *Nelumbo nucifera* Gaertn. Showed that the aqueous extract of *N.nucifera* had antiviral activity against 25 $TCID_{50}$ EV71 with a cytopathic effect of less than 50% at a concentration of 100 µg/mL. Morphological changes of Vero cells that were infected with EV71 at 25 $TCID_{50}$ were observed after incubated for 5 days (Sukkasem et al., 2016).
- **Rotaviruses:** the aqueous extract of fruit from *Nelumbo nucifera* was found to have strong significant antiviral activity with a 50% inhibitory concentration (IC_{50}) <300 µg/mL. by *in vitro* on MA-104 cells for against rhesus rotavirus (RRV) (Knipping et al., 2012).

Biological activities of *Nelumbo nucifera* Gaertn.

- **Anti-inflammation**: the aqouse extract of *N.nucifera* showed anti-inflammation with IC_{50} value of 43.91±2.60 µg/mL (Sukkasem et al., 2016).
- **Antibacterial activity**: the aqouse extract of *N.nucifera* inhibited bacteria such as *S.aureus* (MIC = 1.25 mg/mL, MMC = 1.25 mg/mL), methicillin-resistant *S.aureus* (MIC = 1.25 mg/mL, MMC = 1.25 mg/mL), and *S.epidermidis* (MIC = 1.25 mg/mL, MMC = 2.5 mg/mL), (Sukkasem et al., 2016).
- **Antipyretic activity**: *In Vivo*, the stalks of Nelumbo nucifera were extracted with ethanol for a test experiment of antipyretic potential on yeast-induced pyrexia and normal body temperature in rats. The extract showed produce significant lowering of normal body temperature up to 3 hours at a dose of 200 mg/kg and at a dose of 400 mg/kg showed significant lowering of body temperature up to 6 hours after administration of the extract. Yeast-induced pyrexia, the extract showed dose-dependent reduction of body

temperature up to 4 hours at both the doses and compared to paracetamol (Sinha et al., 2000).

2.3.15 Phim-sen-ton (*Pogostemon cablin* (Blanco) Benth.)

Figure 2-22 *Pogostemon cablin* (Blanco) Benth. (LABIATAE)

Scientific name: *Pogostemon cablin* (Blanco) Benth.

Family: LABIATAE

Common name: Phim-sen-ton, Pachouli

Botanical characteristics

Pogostemon cablin (Blanco) Benth. is an erect, aromatic, branched, and hairy herb that grows up to 0.5 to 1 meters high. The leaves are oval, measuring around 10 centimeters long and wide. The leaves are serrated with doted glands beneath, sitting on a petiole around 8 centimeters long. The flowers are tiny, crowded, terminal, borne in hairy, tinged with white and light purple. The corolla is 8 millimeters long, with obtuse lobes. The axillary spikes 2 to 8 cm centimeters, 1 to 1.5 centimeters in diameter. The calyx is around 6 millimeters long (Sukkasem et al., 2016).

Usage: Thai Traditional used leaves of *Pogostemon cablin* (Blanco) Benth. for reliving common cold, carminative, and diuretic. (Ramya *et al*., 2013)

Phytochemicals and/or bioactive compounds

- Triterpenoids and Steroids, Mono- and Sesquiterpenoids, Alkaloids, Flavonoids and Phenylpropanoid glycosides (Liu et al., 2016)
- **Flavonoids:** 4′,5-Dihydroxy-3′,7-dimethoxyflavanone, 5-Hydroxy-7,3′,4′-trimethoxyflavanone, 5,4′-Dihydroxy-3,7,3′-trimethoxyflavone, 5-Hydroxy-3,7,4′-tetramethoxyflvone (Li et al., 2011)
- **Polyphenolics:** 5, 7-dihydroxy-8-((2R)-2-methylbutan-1-onyl)-phenylacetic acid 7-O-β-D-glucopyranoside and 5, 7-dihydroxy-8-(2-methylbutan-1-onyl)-ethyl phenylmethyl ester. (Liu et al., 2016)

Antiviral activities of *Pogostemon cablin* (Blanco) Benth.

- **Influenza virus:** the twenty polyphenolic compounds were isolated from *Pogostemon cablin* (Blanco) Benth. For their potential ability to inhibit neuraminidase of influenza A virus. Showed that better inhibitory activity against NA. The significant potent compounds of this series were compounds 2 (IC_{50} = 3.87 ± 0.19 μ mol/mL), 11, 12, 14, 15, 19 and 20 (IC_{50} was in 2.12 to 3.87 μ mol/mL) (Liu et al., 2016).

Biological activities of *Pogostemon cablin* (Blanco) Benth.

- **Anti-inflammation**: the ethanolic extract of *P.cablin* showed anti-inflammation with IC_{50} value of 37.16±2.12 μg/mL (Sukkasem et al., 2016)
- **Antibacterial activity**: the ethanolic extract of *P.cablin* inhibited bacteria such as *S.aureus* (MIC = 0.625 mg/mL, MMC = 0.625 mg/mL), methicillin-resistant *S.aureus* (MIC = 1.25

mg/mL, MMC = 2.5 mg/mL), *S.epidermidis* (MIC = 0.625 mg/mL, MMC = 1.25 mg/mL), *S.pyogenes* (MIC = 0.156 mg/mL, MMC = 0.156 mg/mL), and *C.albicans* (MIC = 2.5 mg/mL, MMC = 5 mg/mL) (Sukkasem et al., 2016).

2.3.16 Phit-sa-naat (*Sophora exigua* Craib.)

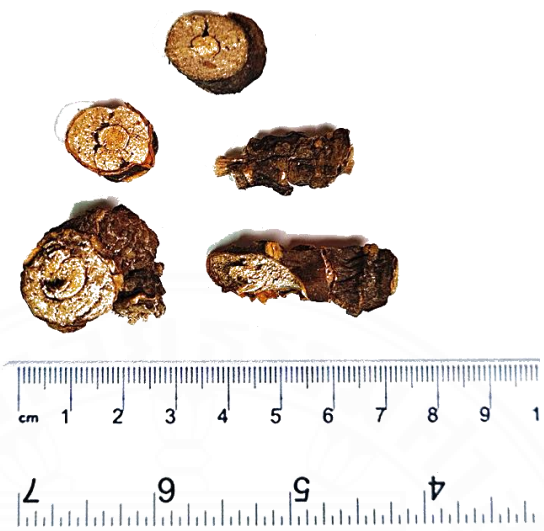


Figure 2-23 *Sophora exigua* Craib. (FABACEAE)

Scientific name: *Sophora exigua* Craib.

Family: FABACEAE

Common name: Phit-sa-naat

Botanical characteristics

Sophora exigua Craib. is a shrub that grows to 15-30 centimeters high. The stem is very short. Leaves are pinnately compound, alternate, and basal. The shape of leaves are elliptic, ovate or ovate-oblong. The leaves' ends are obovate with 1.5-3 centimeters wide, 2-5 centimeters long. The surface of the leaves is white pubescent. The inflorescence is a raceme that many florets. The flowers are purple, with the corollas are long peduncles. The fruits are a white pubescent, parallel-margin pod, and single seed (Sukkasem et al., 2016).

Usage: Thai Traditional used the trunk of *Sophora exigua* Craib. for relieve common cold, reduce cough, and increase breast milk (Wutthithammawet, 2002)

Phytochemicals and/or bioactive compounds

-

Biological activities of *Sophora exigua* Craib.

- **Anti-inflammation**: the aqueous extract of *S.exigua* showed anti-inflammation with IC_{50} value 3.17±0.68 μg/mL while the ethanolic extract IC_{50} value of 22.84±3.95 μg/mL (Sukkasem et al., 2016)
- **Antibacterial activity**: the ethanolic extract of *S.exigua* inhibited bacteria such as *S.aureus* (MIC = 0.156 mg/mL, MMC = 0.313 mg/mL), methicillin-resistant *S.aureus* (MIC = 0.156 mg/mL, MMC = 0.313 mg/mL), *S.epidermidis* (MIC = 0.156 mg/mL, MMC = 0.313 mg/mL), *S.pyogenes* (MIC = 0.156 mg/mL, MMC = 0.156 mg/mL), and *C.albicans* (MIC = 0.625 mg/mL, MMC = 0.625 mg/mL) (Sukkasem et al., 2016).

2.3.17 Na-era-phu-sri (*Tacca chantrieri* Andre.)

Figure 2-24 *Tacca chantrieri* Andre. (TACCACEAE)

Scientific name: *Tacca chantrieri* Andre.

Family: TACCACEAE

Common name: Na-era-phu-sri

Botanical characteristics

Tacca chantrieri Andre. is a monocotyledon that has long-stalked. The broad leaves of an olive- (dark) green grow up to 70 centimeters wide. The inflorescences are maroon or black, dark purple that grows up to 50 centimeters wide, sometimes consisting of around 25 flowers. The inflorescence also has smaller black flowers with 5 petals that hang like berries, giving it a bat-shaped appearance (Sukkasem et al., 2016).

Usage: Thai Traditional used rhizomes of *Tacca chantrieri* Andre. for treat burns, stomachaches, gastric ulcers, and incised wounds (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

-

Antiviral activities of *Tacca chantrieri* Andre.

- **Enterovirus 71 (EV71):** the aqueous extract of *Tacca chantrieri* Andre. Showed that the aqueous extract of *T.chantrieri* had antiviral activity against 100 $TCID_{50}$ EV71 with a cytopathic effect of less than 50% at a concentration of 50 µg/mL. Morphological changes of Vero cells that were infected with EV71 at 100 $TCID_{50}$ were observed after incubated for 5 days (Sukkasem et al., 2016).

Biological activities of *Tacca chantrieri* Andre.

- **Antibacterial activity**: the ethanolic extract of *T.chantrieri* inhibited bacteria such as *S.aureus* (MIC = 2.5 mg/mL, MMC = 2.5 mg/mL), methicillin-resistant *S.aureus* (MIC = 2.5 mg/mL, MMC = 2.5 mg/mL), and *S.epidermidis* (MIC = 5 mg/mL, MMC = 5 mg/mL), (Sukkasem et al., 2016).

2.3.18 Faek-hom (*Vetiveria zizanioides* (L.) Nash ex Small)

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

Scientific name: *Vetiveria zizanioides* (L.) Nash ex Small

Family: GRAMINEAE

Common name: Faek-hom, Vetiver grass

Botanical characteristics

Vetiveria zizanioides (L.) Nash ex Small is an erect, tufted perennial, coarse that grows 1 to 2 meters high. The roots are fragrant and fibrous. The leaves are arranged in two rows, about 1 meter long, 1 centimeters or less in width, and folded. Panicles are terminal, erect, purple or greenish, about 20 centimeters long; the branches are slender, whorled, spreading, or ascending, 5 to 12 centimeters long. Sessile spikelets are about 4 mm long and muricate (Sukkasem et al., 2016).

Usage: Thai Traditional used roots of *Vetiveria zizanioides* (L.) Nash ex Small for dissolving or break kidney stones and relieve common cold (Wutthithammawet, 2002).

Phytochemicals and/or bioactive compounds

- Khusimene, α-amorphene, *cis*-β-guaiene, δ-amorphene, γ-cadinene, cis-eudesm-6-en-11-ol, khusimone, ziza-6(13)-en-3-one, khusinol, khusian-2-ol, vetiselinenol, cyclocopacamphan-12-ol, 2-epi-ziza-6(13)-3α-ol, isovalencenal, β-vetivone khusimol, nootkatone, α-vetivone, isovalencenol, bicyclovetivenol, zizanoic acid, hydrocarbons, alcohols, carbonyl compounds and carboxylic acids (Martinez et al., 2004)

Biological activities of *Vetiveria zizanioides* (L.) Nash ex Small

- **Antibacterial activity**: the ethanolic extract of *V.zizanioides* inhibited *S.pyogenes* with MIC = 1.25 mg/mL, and MMC = 1.25 mg/mL (Sukkasem et al., 2016).

Table 2-2 Antiviral and biological activity of Kheaw-Hom remedy and its plant ingredients

Scientific name	Family	Antiviral activity						Antipyretic activity	Antimicrobial activity	Anti-inflammatory
		VZV	E71	HIV	HCV	Rota virus	Influenza virus			
A. evecta	MARATTIACEAE	-	✓	✓	-	-	-	-	✓	✓
C. fruticosa (Green leaves)	AGAVACEAE	-	-	-	-	-	-	-	-	-
C. fruticosa (Red leaves)	AGAVACEAE	-	-	-	-	-	-	-	✓	-
C. gigantea	CYATHEACEAE	-	-	-	-	-	-	-	✓	-
D. cochinchinensis	DRACANACEAE	-	-	✓	-	-	-	✓	✓	✓
E. stoechadosmum	COMPOSITAE	-	-	-	-	-	-	-	✓	✓
G. malaccensis	ZINGIBERACEAE	-	✓	-	-	-	-	-	✓	✓
K. galanga	ZINGIBERACEAE	-	-	-	✓	-	-	-	✓	✓
L. rugosa	SCROPHULARIACEAE	-	-	-	-	-	-	-	✓	-
M. siamensis	GUTTIFERAE	-	✓	-	-	-	-	-	✓	✓
M. ferrea	GUTTIFERAE	-	-	-	-	-	-	-	✓	✓
M. elengi	SOPOTACEAE	-	-	-	-	-	-	-	✓	✓
M. fragrans	MYRISTICACEAE	-	-	-	-	-	-	-	✓	✓

Table 2-2 Antiviral and biological activity of Kheaw-Hom remedy and its plant ingredients (Continuce)

Scientific name	Family	Antiviral activity						Antipyretic activity	Antimicrobial activity	Anti-inflammatory
		VZV	E71	HIV	HCV	Rota virus	Influenza virus			
N. nucifera	NELUMBONACEAE	-	✓	✓	-	✓	-	✓	✓	✓
P. cablin	LABIATAE	-	-	-	-	-	✓	-	✓	✓
S. exigua	FABACEAE	-	-	-	-	-	-	-	✓	✓
T. chantrieri	TACCACEAE	-	✓	-	-	-	-	-	✓	-
V. zizanioides	GRAMINEAE	-	-	-	-	-	-	-	✓	-
Kheaw-Hom remedy	-	✓	✓	-	-	-	-	-	✓	✓

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Chemicals and reagents

3.1.1 Chemicals and reagents of extraction

Table 3-1 List of chemicals and reagents of extraction

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

3.1.2 Chemicals and reagents of antiviral activity

Table 3-2 List of chemicals and reagents of antiviral activity

Name	Source
Acyclovir ($C_8H_{11}N_5O_3$)	TCI, Japan
Carboxy Methyl Cellulose (CMC)	Sigma, USA
Crystal violet	Tokyo Chemical Industry, Japan
Deionized water	Milford, USA
Dimethyl Sulfoxide (DMSO)	RCL Labscan, Thailand
Fetal Bovine Serum (FBS)	Gibco, USA
Formaldehyde 37%	EMSURE, Thailand
Minimum Essential Medium (MEM)	Gibco, USA
Penicillin-Streptomycin (P/S)	Gibco, USA
Phosphase-buffered saline (PBS)	Gibco, USA
Sodium bicarbonate ($NaHCO_3$)	Amresco, India
Sodium Pyruvate	Gibco, USA
Thiazolyl blue tetrazolium bromide (MTT)	Sigma, USA
Trypan Blue Stain (0.4%)	Gibco, USA

Table 3-2 List of chemicals and reagents of antiviral activity (Continued)

Name	Source
Trypsin-EDTA	Gibco, USA

3.1.3 Chemicals and reagents of Colunm Chromatography (CC) and Thin-layer Chromatography (TLC)

Table 3-3 List of chemicals and reagents of Column chromatography (CC) and Thin-layer Chromatography (TLC)

Name	Source
Deionized water	Milford, USA
Chloroform Grade AR	RCI Labscan, Thailand
Diaion HP-20	Supelco, USA
Ethyl acetate	RCI Labscan, Thailand
Methanolic Grade AR	QReC, New Zealand
TLC Silica gel 60 F_{254}	Merck KGaA, Germany

3.2 Instruments, platic wares and glasswares

Table 3-4 List of instruments, plastic wares, and glasswares

Name	Source
75 cm^2 plastic tissue culture flasks	Costar Corning, USA
12-well microplates flat, bottom with lid	Costar Corning, USA
24-well microplates flat, bottom with lid	Costar Corning, USA
96-well microplates flat, bottom with lid	Costar Corning, USA
Autoclave	Hirayama, Japan
Centrifuge tube 15, 50 ml	Corning, China
CO_2 humidified incubator	Shel lab, USA
Crucibles	Coorstex, USA
Disposable pipatte 5, 10, 25 mL	SLP Life Sciences, Korea

Table 3-4 List of instruments, plastic wares, and glasswares (Continued)

Name	Source
Eppendrofs 1.5 mL	Costar Corning, USA
Erlenmeyer flasks	Schott Duran, Germany
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	Hausser Scientific, USA
Hot air oven	Memmert, Germany
Inverted microscope	Nikon, Japan
Laminar airflow	Boss tech, Thailand
Lipophilizer	Telster, Spain
Membran filter with pore-size rating of 0.22 µm	Acordish, UK
Micropipettes 20 µL, 200 µL, 1000 µL	Gibson, USA
Microplate reader	Bio Tek, USA
Multi-channels pipette 200 µL	Costar Corning, USA
Multi-channels pipette 20 µL	Gilson, France
Pipette tips 100 µL, 1000 µL	Costar Corning, USA
Pipetteboy	Integra biosciences, Switzerland
Reagent reservoir (Sterile)	Costar Corning, USA
Refrigerrator (4°C)	Sharp, Japan
Refrigerrator (-20°C)	Sanyo, Japan
Sonicator	Elma, Germany
Syringes 5 mL, 10 mL	Nipro, Thailand
Vacuum pump	Rocker, Taiwan
Vortex mixer	Scientific industries, USA
Water bath	Memmert, Germany
Water purification machine	Elga, UK

3.3 Plant materials

Kheaw-Hom remedy consists of eighteen herbs. Each herb was purchased from Charoensuk Osot Pharmacy. All plant materials were identified by comparison with authentic herbarium specimens at the herbarium of Thai Medicinal Plants at Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla Province, Thailand. The voucher specimens number of each plant are shown in Table 3-5.

Table 3-5 Description and voucher specimen number of plant ingredients in Kheaw-Hom remedy

Scientific Name	Family Name	Genetic name	Voucher specimen number	Part used	Flavor	%in remedy
Angiopteris evecta (G.Forst) Hoffm.	MARATTIACEAE	Wan-gieb-rad	SKP 110-1 01 05 01	Rhizome	Flavorless	5.56
Cordyline fruticosa (L.) A.Chev (Green leaf)	AGAVACEAE	Maak-mia	SKP 005 03 06 01	Leaf	Flavorless	5.56
Cordyline fruticosa (L.) A.Chev (Red leaf)	AGAVACEAE	Maak-phu	SKP 005 03 06 01	Leaf	Flavorless	5.56
Cyathea gigantea Holtt.	CYATHEACEAE	Ma- has-sa-dam	SKP 059 03 07 01	Stem	Cool	5.56
Dracaena cochinchinensis	DRACAENACEAE	Chan-dang	SKP 065 04 12 01	Stem	Bitter	5.56
Eupatorium stoechadosmum Hance	COMPOSITA	San-phra-hom	SKP 051 05 19 01	Leaf	Cool&Flavorless	5.56
Globba malaccensis Ridl.	ZINGIBERACEAE	Wan-ron-thong	SKP 206 07 13 01	Rhizome	Hot& Fragrant	5.56
Kaempferia galanga Linn.	ZINGIBERACEAE	Proh-hom	SKP 206 11 07 01	Rhizome	Hot& Fragrant	5.56
Limnophila rugosa Merr.	SCROPHULARIACEAE	Phak-kra-chom	SKP 177 12 18 01	Leaf	Cool& Fragrant	5.56
Mammea siamensis Kosterm.	GUTTIFERAE	Sa-la-pii	SKP 083 13 19 01	Flower	Cool& Fragrant	5.56
Mesua ferrea Linn.	GUTTIFERAE	Boon-nak	SKP 083 13 06 01	Flower	Cool& Fragrant	5.56

Table 3-5 Description and voucher specimen number of plant ingredients in Kheaw-Hom remedy (Continued)

Scientific Name	Family Name	Genetic name	Voucher specimen number	Part used	Flavor	%in remedy
Myristica fragrans Houtt.	MYRISTICACEAE	Chan-thet	SKP 121 13 06 01	Stem	Hot& Fragrant	5.56
Mimusops elengi Linn.	SAPOTACEAE	Phi-kul	SKP 171 13 05 01	Flower	Cool& Fragrant	5.56
Nelumbo nucifera Gaertn.	NELUMBONACEAE	Bua-luang	SKP 125 14 14 01	Pollen	Astringent& Fragrant	5.56
Pogostemon cablin (Blanco) Benth.	LABIATAE	Phim-sen-ton	SKP 095 16 03 01	Leaf	Cool& Fragrant	5.56
Sophora exigua Craib	FABACEAE	Phit-sa-naat	SKP 072 19 05 01	Trunk	Bitter	5.56
Tacca chantrieri Andre	TACCACEAE	Na-era-phu-sri	SKP 189 20 03 01	Rhizome	Astringent	5.56
Vetiveria zizanioides (L.) Nash ex Small	GRAMINEAE	Faek-hom	SKP 081 22 26 01	Root	Cool& Fragrant	5.56
Kheaw-Hom remedy	-	Kheaw-Hom	-	-	Bitter&Cool	100

3.3.1 Preparation of crude extracts

The extracts of Kheaw-Hom remedy and plant ingredients were obtained from Miss Kanmanee Sukkasem. Briefly, all ingredients were cleaned with water, sliced into small pieces, and dried in a hot air oven at 45-50°C. Then, they were ground to crude powder. Each plant ingredient was weighed and mixed, followed by the Kheaw-Hom formula, as showed in Table 3-5. Finally, the preparation and each plant were macerated in 95% ethanol and decocted in distilled water.

3.3.1.1 Maceration

The crude powder of Kheaw-Hom remedy and ingredients were macerated in 95% ethanol for three days and filtered through a Whatman No.1 filter paper. A rotary evaporator concentrated the filtrate. The maceration was repeated twice with residue and dried again by vacuum drying. Percentage yields of all the ethanolic extracts were calculated as the formula below.

3.3.1.2 Decoction

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

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

The crude extracts were kept in a freezer (-20°C) until use.

3.4 Cytotoxicity activity

Base on the measurement of the viability of cells. The yellow water-soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) is metabolically reduced to a blue-violet insoluble formazan by viable cells. The number of viable cells correlates to the color intensity determined after dissolving the formazan by photometric measurements (ISO 2009). The cytotoxic of all samples were investigated before the antiviral activity was evaluated.

3.4.1 Animal cell lines

African green monkey kidney epithelial cell line (Vero cell) obtained from American Type Culture Collection (ATCC CCL-81). This cell line will be cultured in a Minimal essential medium (MEM) containing 1% Pyruvate, 1% penicillin-streptomycin (P/S), and supplemented with 10% heat-inactivated fetal bovine serum (FBS). The cells were maintained at 37°C in a 5% CO_2 incubator and sub passage every four days.

3.4.2 Preparation of sample solution

The aqueous extracts were dissolved in sterile distilled water (DI water) and adjusted to a 50 mg/mL concentration. Then, the stock solution of aqueous extract was filtered through a 0.22 µm sterile filter. The 95% ethanolic extracts and fractions from CC were dissolved in sterile dimethyl sulfoxide (DMSO) and adjusted to a 50 mg/mL concentration. Acyclovir was dissolved in sterile distilled water, adjusted to a 40 mg/mL concentration, and used as a positive control. Each stock solution was stored at -20°C until use.

3.4.3 Cytotoxicity activity by MTT assay

The cytotoxicity of Kheaw-Hom extracts and its plant ingredients on Vero cells were determined using the MTT assay. Firstly, a confluent monolayer of Vero cells was seeded in a 96-well plate at 4 x 10^5 cell/mL and incubated for 24 hours at 37°C in a 5% CO_2 incubator. Second, the various concentration of Kheaw-Hom and its plant ingredient extracts were added into each well (100 µL/well). All experiments were performed in triplicate and incubated for 2 hours or 72 hours at

37°C in a 5% CO_2 incubator. After incubation, the supernate (100 µL/well) was removed from each well. Then 10 µL of MTT solution (5 mg/mL) was added into each well and incubated for 3 hours at 37°C in a 5% CO_2 incubator. Finally, the supernate was removed and dissolved with 100 µL of DMSO per well. The absorbance values were measured at 570 nm using a microplate reader. Data were represented as mean ± standard error of the mean (SEM). The percentage of cell viability calculated using the following equation:

$$\%\text{cell viability} = \frac{OD_{samble}}{OD_{control}} \times 100$$

If %cell viability is reduced to less than 70 % of the cell controls, it has a cytotoxic potential (ISO 2009).

3.5 Antiviral activities

Kheaw-Hom remedy extracts, the water extracts of plant ingredients, and five fractions from Kheaw-Hom extract were determined antiviral activity against Herpes Simplex virus.

3.5.1 Animal cell lines

African green monkey kidney epithelial cell line (Vero cell) obtained from American Type Culture Collection (ATCC® CCL-81). It was cultured in a Minimal essential medium (MEM) supplement with 1% pyruvate, 1% penicillin-streptomycin (P/S), and 10% heat-inactivated fetal bovine serum (FBS). The cells were maintained at 37°C in a 5% CO_2 incubator and sub passage every four days.

3.5.2 Propagation and titration of virus

The herpes simplex virus (ATCC® VR-734) was propagated in a monolayer of Vero cells and incubated for two days at 37°C in a 5% CO_2 incubator. After that, cells were harvested and stored at -80°C. For viral titration, a monolayer of the Vero cells was performed in a 12-well plate at 3.5 x 10^5 cell/mL and incubated

for two days at 37°C in a 5% CO_2 incubator. Then, the viruses were diluted by 10-fold dilution. After that, 250 µL/well of the virus dilution was infected on the Vero cell in triplicate. Vero cells and viruses were incubated for two hours at 37°C in a 5% CO_2 incubator and mixed every 15 minutes. After incubation, the supernate was removed, then 2 mL of 1.5% CMC overlay was added to the well and incubated for three days at 37°C in a 5% CO_2 incubator. After three days, the cell was fixed with 10% formaldehyde (1 mL/well) and allowed to air dry at room temperature for 2 hours. Then, each well was washed and stained with 0.1%crystal violet (2 mL/well) for 30 minutes. Finally, the plate was washed and air-dry at room temperature. The plaque-forming unit (PFU) was calculated using the following equation

$$\text{plaque-forming unit (PFU/mL)} = \frac{\text{Average of plaque number in triplicate wells x Dilution factor}}{\text{Virus load inserted}}$$

3.5.3 Preparation of sample solution

The aqueous extracts were dissolved in sterile distilled water (DI water) and adjusted to a 10 mg/mL concentration. Then, the stock solution of aqueous extract was filtered through a 0.22 µm sterile filter. The 95% ethanolic extracts and fraction from CC were dissolved in sterile dimethyl sulfoxide (DMSO) and adjusted to a 50 mg/mL concentration. Acyclovir was dissolved in sterile distilled water and adjusted to a 40 mg/mL concentration as a positive control. Each stock solution was stored at -20°C until use. All samples were prepared at various concentrations and diluted with 1X MEM or 2X MEM to obtain final concentrations at 200, 100, 50, 25, and 12.5 µg/ml for tested experiments.

3.5.4 Antiviral activity by plaque reduction assay

All samples were investigated antiviral activity at three different stages of pre-incubation, pre-infection, and post-infection (**Figure 3-1**). Cell-infection without sample was used as a control, and all experiments were performed in triplicate.

3.5.4.1 Pre-incubation assay

The pre-incubation assay was used to investigate the effect of the sample on virus inactivation before infection. Firstly, the Vero cells were seeded in a 12-well plate at 3.5 x 10^5 cell/mL and incubated for two days at 37°C in 5% CO_2. Second, the 100 PFU/well of virus (500 µL/well) was co-incubated with the various concentrations of extracts (500 µL/well) in a 24-well plate for 1 hour at 37°C 5% CO_2 and mixing well every 15 minutes. Then, the supernatant (250 µL/well) was transferred to the Vero cells in a 12-well plate for infection and incubated for two hours at 37°C in a 5% CO_2 and mixing well every 15 minutes. After incubation, the supernate was removed, added 1.5%CMC overlay (2 mL/well), and incubated for three days at 37oC in a 5% CO_2. Finally, cells were fixed with 10% formaldehyde and stained with 0.1% crystal violet. After staining, the plaque number was counted and the percentage of inhibition calculated using the equation below. The experiment was performed in triplicate and reported as mean ± SEM. The IC_{50} was calculated using the GraphPad Prism 5.01 GraphPad Software, USA.

$$\%\text{Inbition of virus} = \frac{\text{Average of plaque number}_{\text{Control virus}} - \text{Average of plaque number}_{\text{Sample}}}{\text{Average of plaque number}_{\text{Control virus}}} \times 100$$

3.5.4.2 Pre-treatment assay

A pre-treatment assay was used to determine the effect of extracts on the virus before entering the Vero cells. First, the Vero cells were seeded in a 12-well plate at 3.5 x 10^5 cell/mL and incubated for 2 days at 37°C in a 5% CO_2 incubator. Second, the Vero cell was treated with various concentrations of extracts (250 µL/well) and incubated for 2 hours at 37°C in a 5% CO_2 incubator. Then, the supernate was removed and infected by 100 PFU/well of virus (250 µL/well) for 2

hours at 37°C in a 5% CO_2 incubator and mixed well every 15 minutes. After incubation, the excess virus was removed and overlaid 1.5%CMC (2 mL/well). Next, the plate was incubated for 3 days at 37°C in a 5% CO_2 incubator. After that, cells were fixed with 10% formaldehyde (1 mL/well) at room temperature for 2 hours. Then, the solution was removed and washed. Next step, the plate was stained with 0.1%crystal violet (2 mL/well) for 30 minutes. Finally, the plate was washed and dried at room temperature. The number of plaques was counted and calculated using the following equation. The experiment was performed in triplicate and reported as the percentage of plaque reduction. The experiment was performed in triplicate and reported as mean ± SEM. The IC_{50} was calculated using the GraphPad Prism 4.03 GraphPad Software, USA.

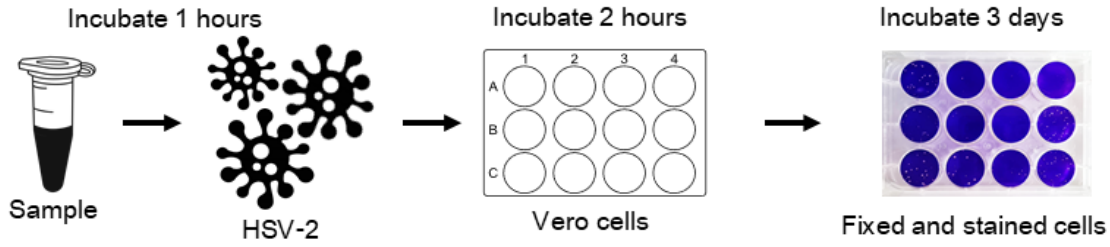
$$\%\text{Inbition of virus} = \frac{\text{Average of plaque number}_{\text{Control virus}} - \text{Average of plaque number}_{\text{Sample}}}{\text{Average of plaque number}_{\text{Control virus}}} \times 100$$

3.5.4.3 Post-treatment activity

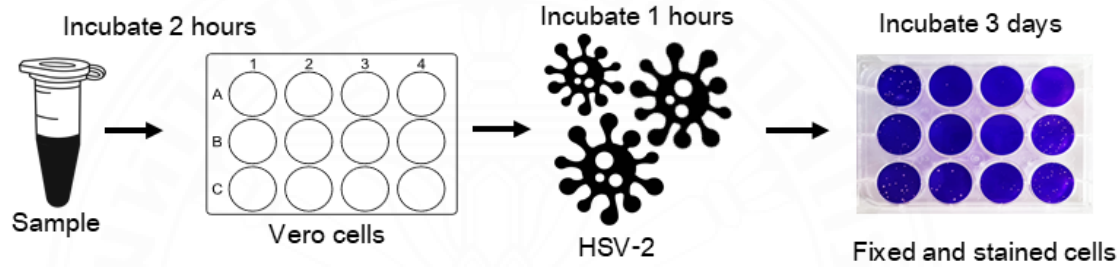
A post-treatment assay was used to observe the effect of extracts on viral replication. Firstly, the Vero cells were seeded into a 12-well plate at 3.5 x 10^5 cells/well and incubated for 2 days at 37°C in a 5% CO_2 incubator. Secondly, the Vero cells were infected by 100 PFU/well of HSV (250 µL/well), then incubated for 2 hours at 37°C in a 5% CO_2 and mixing well every 15 minutes. After that, the excess virus was removed, and 2 mL/well of various concentrations of extracts in 1.5% CMC was added into each well. Next, the plate was incubated for 3 days at 37°C in a 5% CO_2 incubator. After incubation, cells were fixed with 10%Fomaldehyde (1 mL/well) at room temperature for 2 hours. Then, the supernate was removed and washed with water. After washing, the plate was stained with 0.1% crystal violet 2 mL/well at room temperature for 30 minutes. Finally, the plate was washed and dried at room temperature. The number of plaques was counted and calculated using the following equation. The experiment was performed in triplicate and reported as mean ± SEM. The IC_{50} was calculated using the GraphPad Prism 5.01 GraphPad Software, USA.

$$\%\text{Inbition of virus} = \frac{\text{Average of plaque number}_{\text{Control virus}} - \text{Average of plaque number}_{\text{Sample}}}{\text{Average of plaque number}_{\text{Control virus}}} \times 100$$

(1) Pre-incubation



(2) Pre-treatment



(3) Post-treatment

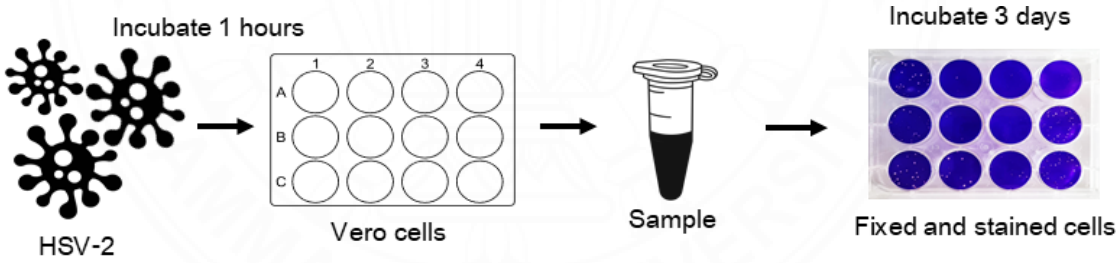


Figure 3-1 Antiviral activity at three different stages of pre-incubation (**1**), pre-treatment (**2**), and post-treatment (**3**) on Vero cells by plaque reduction assay

3.6 Separation of five fractions of the aqueous extract of Kheaw-Hom remedy using column chromatography

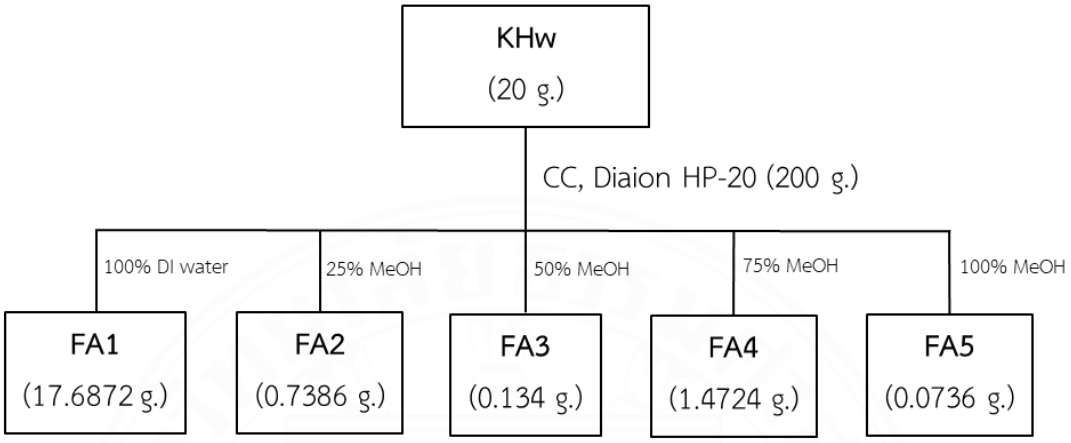


Figure 3-2 Isolation of the aqueous extract of Kheaw-Hom remedy by Diaion HP

The diaion HP-20 (200 g) was packed in a column width of 5 centimeters by the dry pack with a resin height of around 9 inches. The 20 g aqueous extract was weighed and dissolved in deionized water (DI). The sample solution was loaded into a column with 100% DI water and sequentially eluted with 25% MeOH, 50% MeOH, 75% MeOH, and 100% MeOH, respectively, to give five fractions. (FA1, FA2, FA3, FA4, and FA5, respectively), as shown in Table 3-6.

Each fraction was detected by TLC fingerprint

Table 3-6 Fraction of Column chromatography (CC)

Fraction	Volume
Fraction 1 100%DI water	2 L
Fraction 2 75%DI water:25%MeOH	1.6 L
Fraction 3 50%DI water:50%MeOH	1.2 L
Fraction 4 25%DI water:75%MeOH	1.3 L
Fraction 5 100%MeOH	800 mL

All fractions were kept in a freezer (-20°C) until use. Percentage yields of all the fractions were calculated using the following equation:

$$\%\text{yields} = \frac{\text{Weight of the fraction extract (g)}}{\text{Weight of the extract (g)}} \times 100$$

3.7 Thin-layer Chromatography (TLC)

Thin-layer Chromatography is a highly versatile separation method that is widely used for both qualitative and quantitative sample analysis. First, the aqueous extract of Kheaw-Hom remedy, its plant ingredient, and fractions was dissolved with solution (100%methanol), and then prepared samples were at 50 mg/mL concentration. Then, the Auto TLC spotter detected 5 µL of each sample on TLC Silica gel 60 F_{254}. The mobile phase used three solvent systems viz $CHCl_3$: EtOAC : MeOH (1:3:1), $CHCl_3$: MeOH (2:3), and EtOAC : MeOH : water (3.5:1:0.5). Then, TLC was evaluated in UV light at λ = 254 and 365 nm. After that, sprayed the acidic anisaldehyde reagent and heated it at 110-130°C on a TLC plate heater for around 5 minutes. Finally, TLC was evaluated in visible light. Retardation Factor (R_f) were calculated using the following equation:

$$R_f = \frac{D_s}{D_f}$$

D_s = the migration distance of the substance

D_f = the migration distance of the solvent front

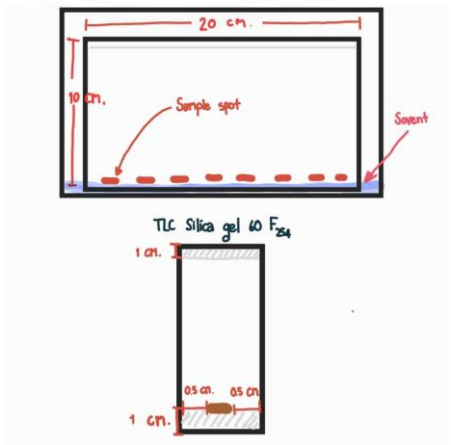


Figure 3-3 Thin-layer Chromatography (TLC)

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Preparation of crude extracts

The aqueous and ethanolic extracts of Kheaw-Hom remedy and its plant ingredients were obtained from Kanmanee Sukkasem. The percent yield of Kheaw-Hom remedy and its plant ingredients are shown in **Table 4-1**.

The percentage yields of the aqueous extracts of Kheaw-Hom remedy (13.36%) were higher than ethanolic extracts (8.75%). The results showed that the percentage yields of aqueous extracts of plant ingredients ranged from 1.70% to 41.13%. The aqueous extract of *V. zizanioides* showed the maximum percent yield (41.13%), while *M. fragrans* showed the minimum percent yield (1.70%). The percent yields of ethanolic extracts of the plant ingredients were ranged from 1.05% to 19.49%. The yield of ethanolic extract of *D. cochinchinensis* was 19.49%, while *M. fragrans* showed the lowest yield (Sukkasem et al., 2016).

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

Sample	Thai name	Ethanolic extract		Aqueous extract	
		Code	%Yield	Code	%Yield
Kheaw-Hom remedy	Kheaw-Hom	KH95e	8.75	KHw	13.36
Angiopteris evecta (G.Forst) Hoffm.	Wan-gieb-rad	AE95e	1.27	AEw	10.77
Cordyline fruticosa (L.) A.Chev (Green leaf)	Maak-mia	CF95e	10.15	CFw	16.82
Cordyline fruticosa (L.) A.Chev (Red leaf)	Maak-phu	CO95e	9.09	COw	18.01
Cyathea gigantea Holtt.	Ma- has-sa-dam	CG95e	1.53	CGw	4.41
Dracaena cochinchinensis	Chan-dang	DC95e	19.49	DCw	2.25
Eupatorium stoechadosmum Hance	San-phra-hom	ES95e	7.70	ESw	20.29
Globba malaccensis Ridl.	Wan-ron-thong	GM95e	7.38	GMw	7.29
Kaempferia galanga Linn.	Proh-hom	KG95e	7.38	KGw	13.41
Limnophila rugosa Merr.	Phak-kra-chom	LR95e	3.52	LRw	20.88
Mammea siamensis Kosterm.	Sa-la-pii	MS95e	8.45	MSw	30.56
Mesua ferrea Linn.	Boon-nak	MF95e	17.50	MFw	13.36
Mimusops elengi Linn.	Phi-kul	ME95e	8.40	MEw	13.61
Myristica fragrans Houtt.	Chan-thet	MyF95e	1.05	MyFw	1.70
Nelumbo nucifera Gaertn.	Bua-luang	NN95e	8.40	NNw	19.59
Pogostemon cablin (Blanco) Benth.	Phim-sen-ton	PC95e	5.67	PCw	16.25

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

Sample	Thai name	Ethanolic extract		Aqueous extract	
		Code	%Yield	Code	%Yield
Sophora exigua Craib	Phit-sa-naat	SE95e	11.60	SEw	9.11
Tacca chantrieri Andre	Na-era-phu-sri	TC95e	3.27	TCw	14.25
Vetiveria zizanioides (L.) Nash ex Small	Faek-hom	VZ95e	2.73	VZw	41.13

4.2 *In vitro* assay for cytotoxic activity and antiviral activities of Kheaw-Hom extracts, its plant ingredients on Vero cells

The cytotoxicity of Kheaw-Hom and plant ingredient extracts was determined by MTT assay in Vero cells. The treatment was divided in 2 hours and 3 days of treatment with plant extracts. All extracts were tested for cytotoxicity by measuring the percentage of cell viability. If cell viability is reduced to less than 70% of the cell control, it has a cytotoxic potential (ISO, 2009).

The aqueous and ethanolic extracts of Kheaw-Hom remedy were tested at five concentrations ranging from 100, 50, 25, 12.5, and 6.25 µg/mL. After 2 hours of incubation, the aqueous and ethanolic extracts of Kheaw-Hom exhibited no cytotoxicity at all concentrations. After three days of incubation, the aqueous extract of Kheaw-Hom had no toxicity on Vero cells, while the ethanolic extracts of Kheaw-Hom showed toxicity on cells at 100, 50, and 25 µg/mL, as shown in **Table 4-2**. However, the previous work has reported that the aqueous extract had no cytotoxic effects on the Vero cells. On the other hand, the ethanolic extract had cytotoxic effects on the Vero cells by MTT assay (Sukkasem et al., 2016). These accorded results could be due to cognate experimental methods and the obtained extract from Kanmanee Sukkasem. Therefore, Kheaw-Hom remedy and acyclovir at maximum non-toxic concentration were evaluated for antiviral activities. Acyclovir, which was used as a positive control, showed low toxicity to Vero cells at 2 hours and 3 days after incubation, as shown in **Table 4-3.**

Table 4-2 Cytotoxicity of the Kheaw-Hom remedy extracts on Vero cell line by using MTT assay with incubated 2 hours and 3 days (n=3)

	Code.	%cell viability at Concentration (µg/mL)				
		100	50	25	12.5	6.25
incubated 2 hours	KHw	98.92±3.00	104.24±3.91	99.25±0.40	103.44±1.44	117.14±2.59
	KH95e	85.06±5.67	95.79±4.50	99.45±3.69	97.81±2.92	99.28±3.17
incubated 3 day	KHw	85.96±2.62	85.77±1.22	84.64±2.36	99.21±2.47	116.34 2.31
	KH95e	7.75±0.49*	5.04±0.10*	5.83±2.39*	74.90±3.05	81.37±2.89

*If %cell viability is reduced to less than 70 % of the cell controls, it has a cytotoxic potential (ISO 2009).

Table 4-3 Cytotoxicity of acyclovir on Vero cell line by using MTT assay with incubated 2 hours and 3 days (n=3)

	%cell viability at Concentration (µg/mL) of acyclovir								
	100	50	25	12.5	6.25	1.56	0.78	0.39	0.19
incubated 2 hours	98.28±1.61	99.22±0.84	100.95±0.80	101.23±1.83	106.62±1.65	NT	NT	NT	NT
incubated 3 day	85.37±1.36	88.20±0.89	86.46±2.23	89.44±3.39	97.59±2.09	86.38±3.84	88.48±4.21	90.71±3.71	94.06±2.10

*NT = Not tested

The anti-Herpes simplex type-2 activities of extracts were evaluated in 3 different experiments: pre-incubation, pre-treatment, and post-treatment by plaque reduction assay. The aqueous extract of Kheaw-Hom remedy (KHw) exhibited potent antiviral activity against HSV-2 with the half-maximal inhibitory concentration (IC_{50}) value of 94.24 ± 1.26 µg/mL by pre-incubation, while the Kheaw-Hom ethanolic extract was without influence, as shown in **Table 4-4.** For pre-treatment and post-treatment, the KHw had less anti-HSV activity than 50% at 100 µg/mL (**Table 4-5 and Table 4-6**). At the same time, the KH95e had no anti-HSV effect at 100 µg/mL in all experiments.

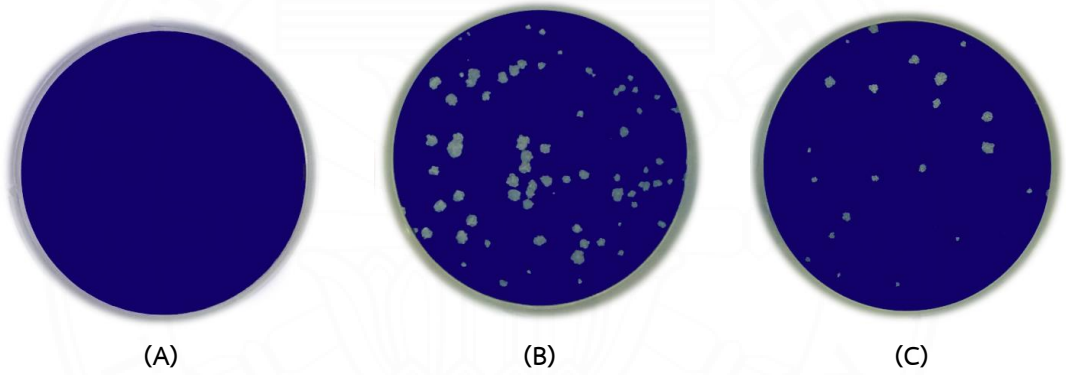


Figure 4-1 Plaques reduction of the aquoes extract of Kheaw-Hom remedy by pre-incubation assay: (**A**) Uninfected Vero cells, (**B**) Vero cells infected with HSV-2, and a concentration at 100 µg/mL of KHw incubated with HSV-2 before infected Vero cells.

Kheaw-hom remedy is a traditional Thai herb that was used to treat thirst and relieve fever from chickenpox and measles (Chusri, et al., 2014). The previous report showed that Kheaw-Hom remedy had an anti-inflammationand anti-bacterial activity (Sukkasem, 2016). Most importantly, the Kheaw-Hom remedy has never been reported against HSV-2. Therefore, this study is the first report on antiviral activity against HSV-2. Furthermore, this study found that the aqueous extract of Kheaw-Hom remedy exhibited potent antiviral activity against HSV-2, while the ethanolic extract did not show anti-HSV type 2 activity.

Moreover, a previous study demonstrated the antiviral activity against the varicella-zoster virus (VZV) (Family: Herpesviridae) of Ya-Kheaw. The results showed that the 20% ethanolic extract of Ya-Kheaw had antiviral activity against varicella-zoster virus (VZV) at a concentration of 250 µg/mL (Sanguansermsri et al., 2005). Ya-Kheaw is a traditional Thai remedy to treat fever from chickenpox and measles and has some ingredients identical to the Kheaw-Hom remedy (Sukkasem, 2016). In this study, aqueous extract of Kheaw-Hom inhibited HSV-2, but the ethanolic extract showed no anti-HSV activity. The polarity of solvent extraction may cause it. The solvent chemical properties such as polarity can affect bioactive compounds extraction (Dhanani et al., 2017; Ngo et al., 2017). Therefore, bioactive compounds with high polarity may be active compounds from the aqueous extract of Kheaw-Hom.

Although aqueous extract of Kheaw-Hom remedy (KHw) exhibited antiviral activity in the present study, this extract has been shown different results. KHw had inhibited enterovirus 71 (EV71) with cytopathic effect less than 50% at concentration 400 µg/mL that no activity against EV71 (Sukkasem el at., 2016). Enterovirus (EV) and herpes simplex virus 1 and 2 (HSV-1 and HSV-2) are the primary etiologic agents of central nervous system (CNS) infections (Sarquiz-Martínez et al., 2017). EV71 is non-enveloped viruses, while HSV is envelope viruses (Whitley RJ, 1996). The enveloped viruses were more sensitive to anti-viral agents than non-enveloped viruses (Firquet et al., 2015). Therefore, the antiviral effect of KHw against HSV-2 was more potent than the antiviral effect of KHw against EV71.

A previous study, Kheaw-Hom aqueous extract showed anti-inflammation activity with IC_{50} value of 46.86±0.82 µg/mL by effects on lipopolysaccharide (LPS) induced nitric oxide (NO) release from RAW 264.7 cells (Sukkasem et al., 2016). Therefore, KHw has affected anti-HSV-2 and anti-inflammation that supported use for treating the blister of herpes.

However, both Kheaw-Hom remedy extracts were less affected than the positive control. Acyclovir exhibited the highest potent against HSV-2 with IC_{50} value of 11.61 ± 2.71, and 0.42 ± 0.10 µg/mL by pre-incubation and post-treatment, as

shown in **Table 4-4** and **Table 4-6.** In contrast, pre-treatment with acyclovir had less than 50% at 100 µg/mL (**Table 4-5**). The previous report showed that acyclovir inhibited HSV-1 and HSV2 infection by interfering with the viral DNA polymerase and viral genome replication (Kukhanova et al., 2014). The parent compound is a prodrug that requires the action of the HSV-specified enzyme, thymidine kinase, to begin the activation process. After mono-phosphorylation, further anabolism is accomplished by cellular enzymes (Sacks, 1987). Therefore, acyclovir is effective only after already infected with HSV.

Table 4-4 The half maximal inhibitory concentration (IC_{50}) of Kheaw-Hom remedy and acyclovir against HSV-2 on Vero cell line by pre-incubation (n=3)

Code.	extract	%Inhibition					IC_{50} (µg/mL)
		100	50	25	12.5	6.25	
KHw	Aqueous	54.47±3.60	31.49±9.78	35.46±6.04	30.11±8.07	NT	94.24±1.26
KH95e	95%Ethanol	42.80±3.00	21.93±5.82	3.94±7.63	11.57±9.68	NT	>100
Acyclovir		96.09±0.86	86.88±2.18	71.07±4.01	52.62±7.62	28.58±6.77	11.61±2.71

*NT = Not tested

Table 4-5 The half maximal inhibitory concentration (IC_{50}) of Kheaw-Hom remedy and acyclovir against HSV-2 on Vero cell line by pre-treatment (n=3)

Code.	extract	%Inhibition					IC_{50} (µg/mL)
		100	50	25	12.5	6.25	
KHw	Aqueous	3.93±0.73	NT	NT	NT	NT	>100
KH95e	95%Ethanol	-5.06±0.87	NT	NT	NT	NT	>100
Acyclovir		21.91±0.45	NT	NT	NT	NT	>100

*NT = Not tested

Table 4-6 The half maximal inhibitory concentration (IC_{50}) of Kheaw-Hom remedy and acyclovir against HSV-2 on Vero cell line by post-treatment (n=3)

Code.	extract	%Inhibition						IC_{50} (µg/mL)
		100	12.5	1.56	0.78	0.39	0.19	
KHw	Aqueous	9.71±2.13	NT	NT	NT	NT	NT	>100
KH95e	95%Ethanol	NT	13.03±2.61	NT	NT	NT	NT	>12.5
Acyclovir		NT	NT	96.43±0.97	65.47±5.75	47.52±5.85	22.97±4.25	0.42±0.10

*NT = Not tested

Kheaw-Hom aqueous extract showed highly potent anti-herpes simplex virus type-2 (HSV-2). Thus, the aqueous extracts of plant ingredients of Kheaw-Hom remedy were performed cytotoxic and antiviral activities. The aqueous extracts of plant ingredients have investigated the cytotoxicity of Vero cells at a varied concentration from 100, 50, 25, 12.5, and 6.25 μg/mL. The results showed that aqueous extracts of all plant ingredients were non-toxic on Vero cells when incubated at 2 hours, as shown in **Table 4-7.** However, three days after incubation, *L. rugosa* (LRw) showed toxicity to Vero cells at 100 and 50 μg/mL. In addition, the other aqueous extract of plant ingredients was non-toxic on Vero cells, as shown in **Table 4-8**. Then, the aqueous extracts at maximum non-toxic concentration were evaluated for antiviral activity.

Its plant ingredients were investigated the sample's effect on virus inactivation before infection. As a result, seven plants from all plant ingredients exhibited potent antiviral activity against HSV-2 by pre-incubation, namely *C. gigantea* , *P. cablin, N. nucifera, M. ferrea, M. elengi, D. cochinchinensis* and *M. siamensis* with IC_{50} value of 10.84 ± 2.68, 30.72 ± 7.66, 37.08 ± 9.72, 39.23 ± 4.29, 60.19 ± 9.08, 61.83±7.37 and 67.11 ± 13.28 μg/mL, respectively (**Table 4-9**). On the other hand, other plant ingredients in Kheaw-Home remedy had less effect than 50% at 100 μg/mL by pre-incubation. For pre-treatment and post-treatment, all aqueous plant ingredients extracts had an effect of less than 50% at concentrations of 100 μg/mL (**Table 4-10 and 4-11**).

The seven plants in Kheaw-Hom remedy exhibited against HSV-2 have not been reported on anti-HSV. However, some isolated compounds found in plant ingredients Kheaw-Hom showed anti-HSV. In the previous study, the Oleaonic acid that was isolated from *C. gigantea* exhibited potent against HSV-1 and HSV-2 with EC_{50} values of 6.8 ± 1.24 and 7.8 ± 1.4 μg/mL, respectively (Juneja et al., 1990; Mukherjee et al., 2013). Resveratrol is found in *D. cochinchinensis* (Fan et al., 2014). Resveratrol inhibited both HSV-1 and HSV-2 replication in a dose and time-dependent manner by inhibiting protein ICP-4 expression (Annunziata et al., 2018). Gallic acid that is found in *M. ferrea* reduced HSV-2 replication in a concentration-

dependent manner when incubated with the virus prior to incubating with cells after infection with an IC_{50} value of 64.35 μM (Kratz et al., 2008; Zhang et al., 2019). In addition, Quercetin that was found in *N. nucifera* exhibited anti-HSV-2 with EC_{50} = 86.7 ± 7.4 mg/L (Chiang et al., 2003; Chen et al., 2019).

Moreover, other phytochemical compounds have been reported on anti-HSV activity. Terpene could inhibit viral replication of HSV decrease glycosylation of viral polypeptides. Phenolic compounds and alkaloids inhibited HSV with an EC_{50} value of 15.3 μg/mL and IC_{50} values of 26.8 μm. Alkaloid compounds can prevent the penetration of the virus into the cell. Flavonoid compounds showed highly potent inhibitory activity against HSV infection (Hassan et al., 2015). In this thesis, the phytochemicals compound in some plants is related to the previous report. *P. cablin* contain triterpenoid, flavonoid, and polyphenolic compound (Liu et al., 2016). *M. ferrea* and *N. nucifera* contained flavonoid compounds (Zhang et al., 2019; Chen et al., 2019). *M. elengi* contains triterpenes, saponins, and Steroids (Arifin et al., 2019). Therefore, these compounds may be active compounds of plant ingredients of Kheaw-Hom.

In brief, an order of potency of all extract to potent inhibited HSV-2 by pre-incubation was CGw > Acyclovir > PCw > NNw > MFw > MEw > DCw > MSw > KHw, respectively as shown in **Figure 4-2.** Although *C. gigantea* was higher potent against HSV-2 than Kheaw-Hom remedy, the folk healers believe that combining plants can increase the effectiveness of the medicines and reduce herbal drug toxicity. Therefore, a combination of many plants is used to treat many diseases (Chusri et al., 2014). Kheaw-Hom aqueous extract has been reported on anti-inflammationand anti-bacterial activity (Sukkasem et al., 2016). Moreover, thesis results are related to the used traditional Thai medical practice. Then, the aqueous extract of Kheaw-Hom remedy and its plant ingredients were performed chemical fingerprint by TLC. Finally, the aqueous extract of Kheaw-Hom remedy was isolated fraction.

Table 4-7 Cytotoxicity of aqueous extract of its plant ingredients on Vero cell line by using MTT assay with incubated 2 hours (n=3)

Code.	Scientific Name	Thai name	%cells viability				
			100	50	25	12.5	6.25
AEw	*A. evecta*	Wan-gieb-rad	83.24±1.96	90.45±3.26	90.88±6.40	83.58±5.97	95.65±3.02
CFw	*C. fruticosa (Green leaf)*	Maak-mia	80.98±4.75	87.20±6.11	94.47±6.27	82.33±9.18	95.04±6.47
COw	*C. fruticosa (Red leaf)*	Maak-phu	91.91±1.90	88.56±2.58	83.14±1.18	85.31±3.26	85.29±2.42
CGw	*C. gigantea*	Ma-has-sa-dam	89.67±5.91	85.42±3.73	80.88±1.48	83.27±3.52	83.56±1.90
DCw	*D. cochinchinensis*	Chan-dang	80.73±0.93	88.69±4.03	84.08±4.97	83.27±6.28	92.60±5.07
ESw	*E. stoechadosmum*	San-phra-hom	88.83±7.75	86.90±3.41	86.11±4.14	93.00±7.03	100.39±2.42
GMw	*G. malaccensis*	Wan-ron-thong	85.23±5.16	90.11±3.74	94.81±4.10	91.09±6.35	103.00±4.53
KGw	*K. galanga*	Proh-hom	89.40±1.99	82.73±3.47	88.72±6.81	87.03±6.42	80.28±6.34
LRw	*L. rugosa*	Phak-kra-chom	76.70±4.09	90.22±7.94	90.83±2.94	91.53±6.94	103.03±3.71
MSw	*M. siamensis*	Sa-la-pii	90.15±6.05	92.02±1.58	87.73±5.41	88.06±6.74	96.24±3.85
MFw	*M. ferrea*	Boon-nak	82.41±5.02	82.77±2.03	85.27±9.12	81.86±4.30	81.74±2.83
MEw	*M. elengi*	Phi-kul	89.90±3.17	85.51±9.70	86.14±5.47	85.53±6.45	85.67±6.32

Table 4-7 Cytotoxicity of aqueous extract of its plant ingredients on Vero cell line by using MTT assay with incubated 2 hours (n=3) (Continued)

Code.	Scientific Name	Thai name	%cells viability				
			100	50	25	12.5	6.25
MyFw	*M. fragrans*	Chan-thet	92.29±4.25	88.48±6.94	83.71±5.21	89.97±1.97	90.62±6.23
NNw	*N. nucifera*	Bua-luang	81.93±3.10	84.74±6.07	85.64±3.67	89.51±5.18	98.93±5.79
PCw	*P. cablin*	Phim-sen-ton	80.94±5.07	85.99±5.84	87.51±4.29	82.95±6.71	95.12±8.99
SEw	*S. exigua*	Phit-sa-naat	96.43±6.89	92.88±8.00	87.06±2.56	92.28±8.73	97.26±4.87
TCw	*T. chantrieri*	Na-era-phu-sri	99.13±4.90	95.66±5.30	90.38±3.41	95.07±3.83	92.90±7.21
VZw	*V. zizanioides.*	Faek-hom	88.83±7.42	95.17±5.38	91.32±3.10	90.59±5.05	101.78±2.49

Table 4-8 Cytotoxicity of aqueous extract of its plant ingredients on Vero cell line by using MTT assay with incubated 3 days (n=3)

Code.	Scientific Name	Thai name	%cells viability				
			100	50	25	12.5	6.25
AEw	*A. evecta*	Wan-gieb-rad	86.82±3.49	90.49±6.98	91.97±1.71	94.38±0.49	100.20±8.76
CFw	*C. fruticosa (Green leaf)*	Maak-mia	90.43±5.30	85.59±2.95	85.63±1.45	89.55±4.52	88.77±1.44
COw	*C. fruticosa (Red leaf)*	Maak-phu	87.83±2.29	90.62±2.92	91.20±3.03	88.99±4.78	93.76±6.64
CGw	*C. gigantea*	Ma-has-sa-dam	96.44±2.11	95.23±5.62	94.48±2.32	96.43±2.84	96.59±2.80
DCw	*D. cochinchinensis*	Chan-dang	91.88±8.14	88.47±1.95	84.88±1.99	87.23±5.35	88.86±1.37
ESw	*E. stoechadosmum*	San-phra-hom	87.98±4.03	93.25±8.44	91.08±4.24	92.62±6.42	95.21±10.40
GMw	*G. malaccensis*	Wan-ron-thong	82.28±5.63	85.74±5.05	86.75±4.95	81.01±1.34	96.09±1.87
KGw	*K. galanga*	Proh-hom	93.12±1.11	88.20±4.48	88.79±6.96	90.25±7.21	91.21±5.19
LRw	*L. rugosa*	Phak-kra-chom	9.41±3.05*	58.67±5.21*	86.78±8.89	94.56±8.14	98.73±7.78
MSw	*M. siamensis*	Sa-la-pii	95.56±3.70	89.77±4.66	86.74±2.88	89.51±1.21	87.13±8.61
MFw	*M. ferrea*	Boon-nak	98.30±7.67	90.73±4.42	91.01±3.72	93.78±3.30	94.90±1.74
MEw	*M. elengi*	Phi-kul	97.76±2.52	94.64±8.41	90.44±7.42	94.95±4.76	92.15±10.68

Table 4-8 Cytotoxicity of aqueous extract of its plant ingredients on Vero cell line by using MTT assay with incubated 3 days (n=3) (Continued)

Code.	Scientific Name	Thai name	%cells viability				
			100	50	25	12.5	6.25
MyFw	*M. fragrans*	Chan-thet	94.98±3.21	92.97±4.64	92.01±1.61	93.95±1.76	93.95±7.28
NNw	*N. nucifera Gaertn.*	Bua-luang	97.45±7.34	92.78±3.33	94.10±1.29	96.94±3.27	92.08±4.63
PCw	*P. cablin*	Phim-sen-ton	89.03±2.09	88.85±2.35	89.36±1.91	89.12±1.80	97.49±4.29
SEw	*S. exigua*	Phit-sa-naat	84.49±6.83	89.45±6.22	88.34±3.73	93.67±9.83	98.44±9.37
TCw	*T. chantrieri*	Na-era-phu-sri	98.97±0.95	95.09±4.34	88.08±0.59	91.69±1.73	88.57±5.35
VZw	*V. zizanioides.*	Faek-hom	84.75±4.21	87.46±5.75	87.96±6.25	81.88±2.43	97.99±4.81

*If %cell viability is reduced to less than 70 % of the cell controls, it has a cytotoxic potential (ISO 2009).

Table 4-9 The half maximal inhibitory concentration (IC_{50}) of aqueous extract of its plant ingredients against HSV-2 on Vero cell line by pre-incubation (n=3)

Code.	Scientific Name	%inhibition					(IC_{50}) (µg/mL)
		100	50	25	12.5	6.25	
CGw	*C. gigantea*	99.70±0.52	93.55±4.95	82.58±7.83	56.19±3.65	28.04±11.67	10.84±2.68
PCw	*P. cablin*	82.59±8.72	72.10±4.14	40.08±8.92	20.77±11.77	NT	30.72±7.66
NNw	*N. nucifera*	95.45±2.18	61.32±2.16	35.98±11.07	27.60±7.53	NT	37.08±9.72
MFw	*M. ferrea*	93.19±8.11	77.10±9.80	22.43±9.08	20.53±4.19	NT	39.23±4.29
MEw	*M. elengi*	74.61±8.75	41.94±3.29	15.28±9.46	23.54±13.20	NT	60.19±9.08
DCw	*D. cochinchinensis*	77.70±7.35	40.44±6.48	17.20±8.82	7.22±4.22	NT	61.83±7.37
MSw	*M. siamensis*	76.60±6.38	39.78±9.56	26.33±10.51	17.08±3.85	NT	67.11±13.28
AEw	*A. evecta*	28.58±3.08	NT	NT	NT	NT	>100
CFw	*C. fruticosa (Green leaf)*	37.29±5.76	NT	NT	NT	NT	>100
COw	*C. fruticosa (Red leaf)*	18.45±3.48	NT	NT	NT	NT	>100
ESw	*E. stoechadosmum*	32.03±9.78	NT	NT	NT	NT	>100
GMw	*G. malaccensis*	17.63±4.63	NT	NT	NT	NT	>100

Table 4-9 The half maximal inhibitory concentration (IC_{50}) of aqueous extract of Kheaw-Hom remedy, its plant ingredients and acyclovir against HSV-2 on Vero cell line by pre-incubation (n=3) (Continued)

Code.	Scientific Name	%inhibition					(IC_{50}) (µg/mL)
		100	50	25	12.5	6.25	
KGw	*K. galanga*	16.86±3.50	NT	NT	NT	NT	>100
LRw	*L. rugosa*	36.83±3.63	NT	NT	NT	NT	>100
MyFw	*M. fragrans*	33.67±15.89	NT	NT	NT	NT	>100
SEw	*S. exigua*	24.62±4.30	NT	NT	NT	NT	>100
TCw	*T. chantrieri*	20.17±6.05	NT	NT	NT	NT	>100
VZw	*V. zizanioides*	28.46±8.60	NT	NT	NT	NT	>100
KHw	Kheaw-Hom remedy	54.47±3.60	31.49±9.78	35.46±6.04	30.11±8.07	NT	94.24±1.26
Acy	Acyclovir	96.09±0.86	86.88±2.18	71.07±4.01	52.62±7.62	28.58±6.77	11.61±2.71

*NT = Not tested

Table 4-10 The half maximal inhibitory concentration (IC_{50}) of aqueous extract of its plant ingredients against HSV-2 on Vero cell line by pre-treatment (n=3)

Code.	Scientific Name	%inhibition					(IC_{50})
		100	50	25	12.5	6.25	(µg/mL)
CGw	*C. gigantea*	28.32±0.76	NT	NT	NT	NT	>100
PCw	*P. cablin*	34.71±4.56	NT	NT	NT	NT	>100
NNw	*N. nucifera*	21.86±4.88	NT	NT	NT	NT	>100
MFw	*M. ferrea*	15.10±6.58	NT	NT	NT	NT	>100
MEw	*M. elengi*	32.67±2.56	NT	NT	NT	NT	>100
DCw	*D. cochinchinensis*	32.06±3.43	NT	NT	NT	NT	>100
MSw	*M. siamensis*	27.17±9.58	NT	NT	NT	NT	>100
AEw	*A. evecta*	13.95±4.89	NT	NT	NT	NT	>100
CFw	*C. fruticosa (Green leaf)*	10.73±3.82	NT	NT	NT	NT	>100
COw	*C. fruticosa (Red leaf)*	23.57±8.89	NT	NT	NT	NT	>100
ESw	*E. stoechadosmum*	20.08±3.96	NT	NT	NT	NT	>100
GMw	*G. malaccensis*	20.46±6.24	NT	NT	NT	NT	>100
KGw	*K. galanga*	5.33±1.20	NT	NT	NT	NT	>100
LRw	*L. rugosa*	22.07±2.93	NT	NT	NT	NT	>100
MyFw	*M. fragrans*	22.48±5.75	NT	NT	NT	NT	>100
SEw	*S. exigua*	17.01±5.20	NT	NT	NT	NT	>100
TCw	*T. chantrieri*	23.05±1.17	NT	NT	NT	NT	>100
VZw	*V. zizanioides.*	23.90±6.36	NT	NT	NT	NT	>100

*NT = Not tested

Table 4-11 The half maximal inhibitory concentration (IC_{50}) of aqueous extract of its plant ingredients against HSV-2 on Vero cell line by post-treatment (n=3)

Code.	Scientific Name	%inhibition					IC_{50}
		100	50	25	12.5	6.25	(µg/mL)
CGw	*C. gigantea*	20.74±6.46	NT	NT	NT	NT	>100
PCw	*P. cablin*	8.88±6.74	NT	NT	NT	NT	>100
NNw	*N. nucifera*	15.54±4.71	NT	NT	NT	NT	>100
MFw	*M. ferrea*	11.08±4.87	NT	NT	NT	NT	>100
MEw	*M. elengi*	9.76±3.99	NT	NT	NT	NT	>100
DCw	*D. cochinchinensis*	8.05±5.20	NT	NT	NT	NT	>100
MSw	*M. siamensis*	16.70±7.62	NT	NT	NT	NT	>100
AEw	*A. evecta*	15.92±7.58	NT	NT	NT	NT	>100
CFw	*C. fruticosa (Green leaf)*	5.87±2.49	NT	NT	NT	NT	>100
COw	*C. fruticosa (Red leaf)*	8.45±6.14	NT	NT	NT	NT	>100
ESw	*E. stoechadosmum*	15.21±2.76	NT	NT	NT	NT	>100
GMw	*G. malaccensis*	9.70±0.09	NT	NT	NT	NT	>100
KGw	*K. galanga*	11.22±2.80	NT	NT	NT	NT	>100
LRw	*L. rugosa*	NT	NT	12.95±1.59	NT	NT	>25
MyFw	*M. fragrans*	24.86±0.62	NT	NT	NT	NT	>100
SEw	*S. exigua*	10.12±5.59	NT	NT	NT	NT	>100
TCw	*T. chantrieri*	5.36±5.87	NT	NT	NT	NT	>100
VZw	*V. zizanioides.*	15.33±3.87	NT	NT	NT	NT	>100

*NT = Not tested

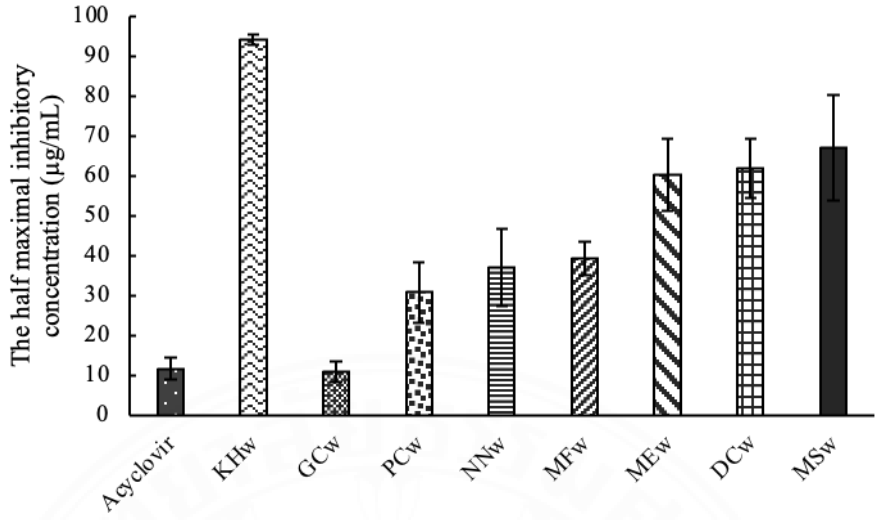
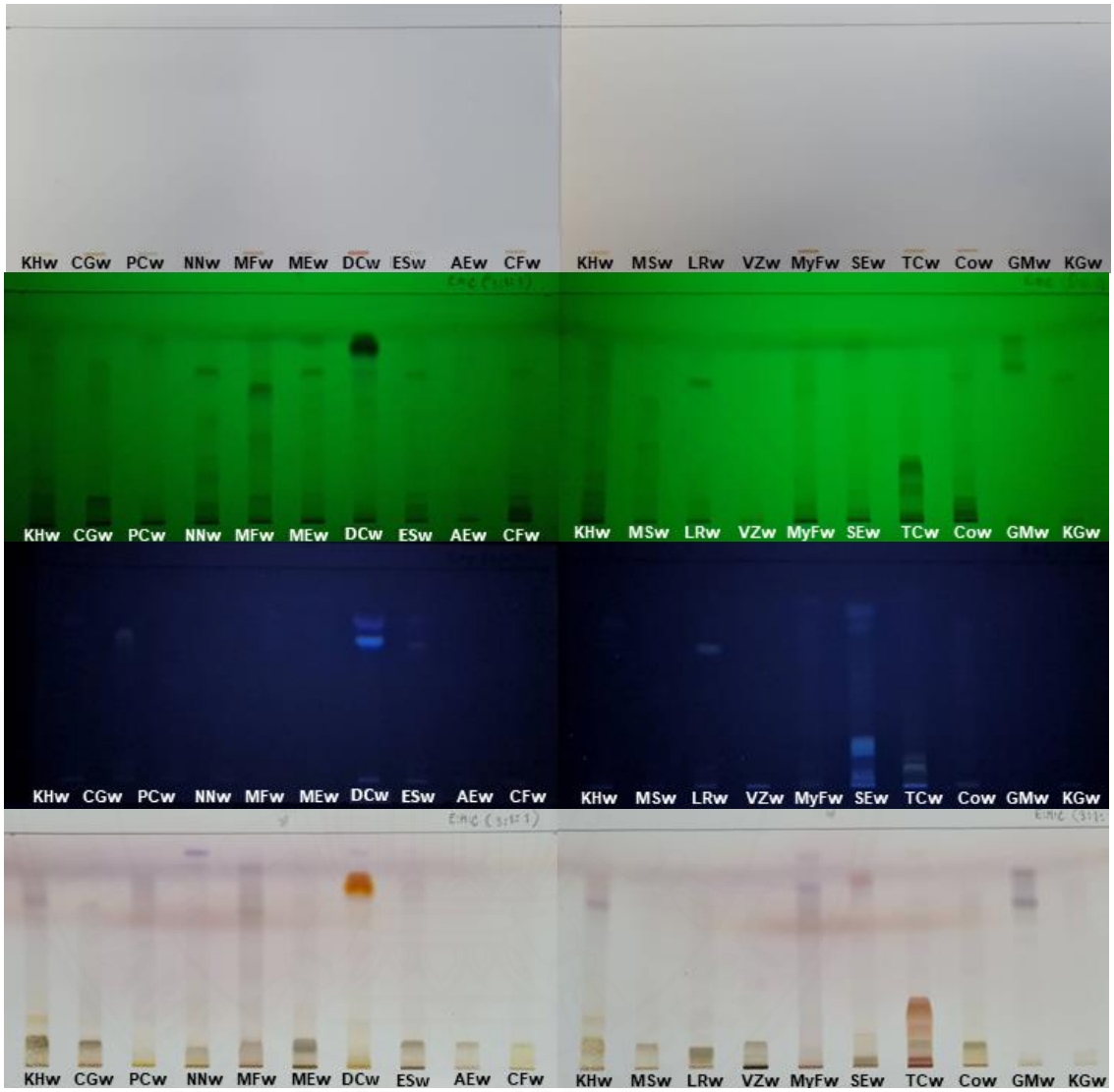


Figure 4-2 Antiviral activity of aqueous extract of Kheaw-Hom, its plant ingredients, and acyclovir against HSV-2 by pre-incubation

4.3 Thin-layer Chromatography (TLC) of Kheaw-Hom remedy and its plant ingredients

TLC fingerprints of the aqueous of Kheaw-Hom remedy (KHw) and its plant ingredient were carried out using silica gel by normal phase and three solvent systems such as $CHCl_3$: EtOAc : MeOH (1:3:1), $CHCl_3$: MeOH (2:3), and EtOAc : MeOH : water (3.5:1:0.5). After spraying TLC with acidic anisaldehyde reagent and heat, the TLC-separated bands of KHw represented a green, orange and purple spot. An orange and purple spot represented only TLC-separated bands of KHw while a green spot is represented on all plant ingredients, as shown in **Figures 4-3, 4-4, and 4-5**. A purple and green spots in TLC fingerprints of the aqueous of Kheaw-Hom remedy expected to be terpenoid or steroid compounds. Because of spraying with acidic anisaldehyde reagent represented purple, blue, and green spot that detected the terpenoid or steroid compounds (Soonthornchareonnon et al., 2008). In addition, the green color spot may be triterpenoid or steroid compounds. Therefore, liebermann-burchard's can be used to confirm. After Liebermann-burchard's spraying, triterpenoid compounds represented the pink spot, and steroid compounds represented the green spot (Soonthornchareonnon et al., 2008).

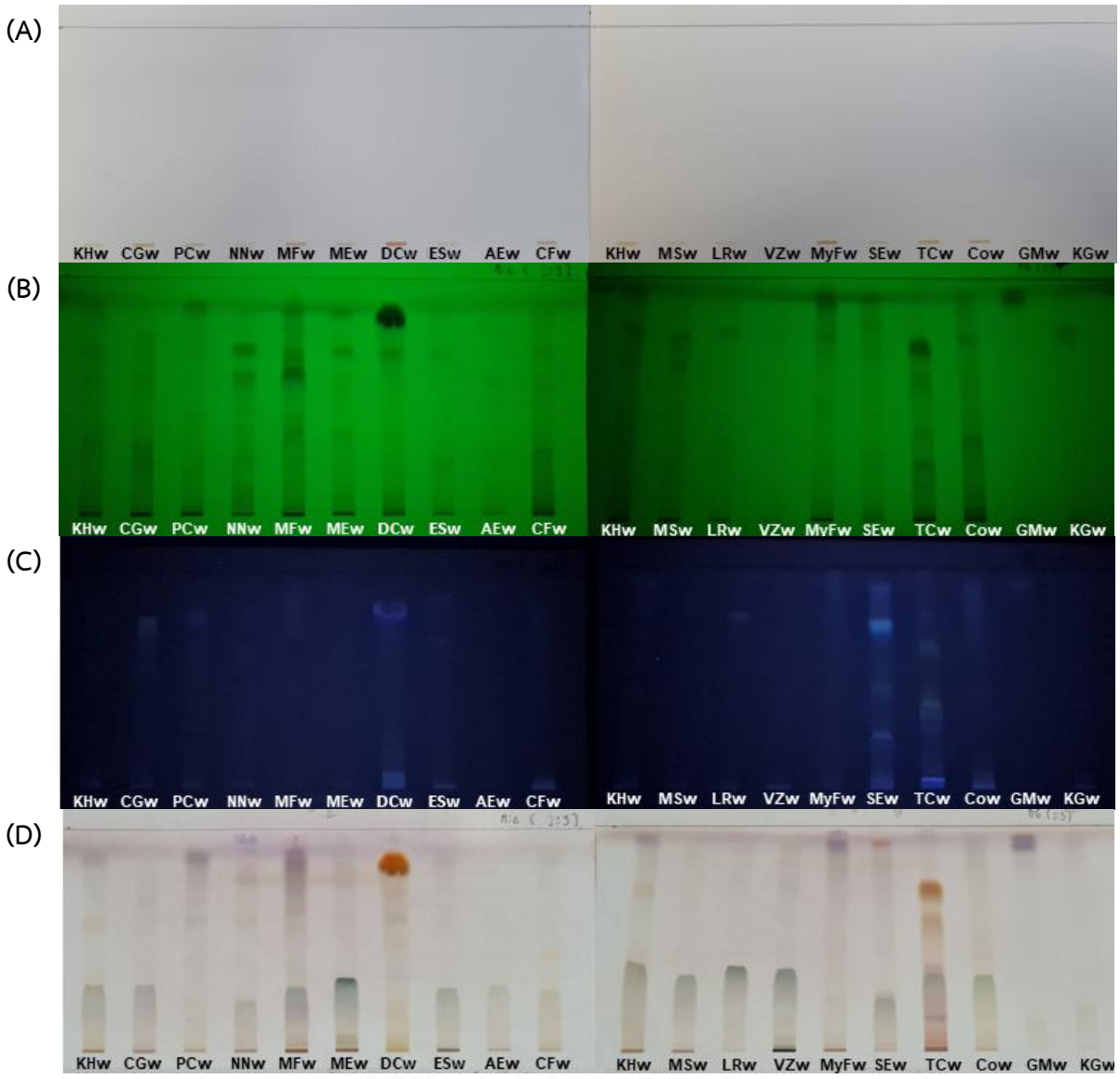
$CHCl_3$: EtOAc : MeOH (1:3:1)



Note: TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and its plant ingredients were detected under visible light (**A**), UV light at 254 nm (**B**), 365 nm (**C**), and by heating at 110-150°C after spraying with acidic anisaldehyde reagent (visible light) (**D**).

Figure 4-3 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and its plant ingredients using $CHCl_3$: EtOAc : MeOH (1:3:1) solvent system

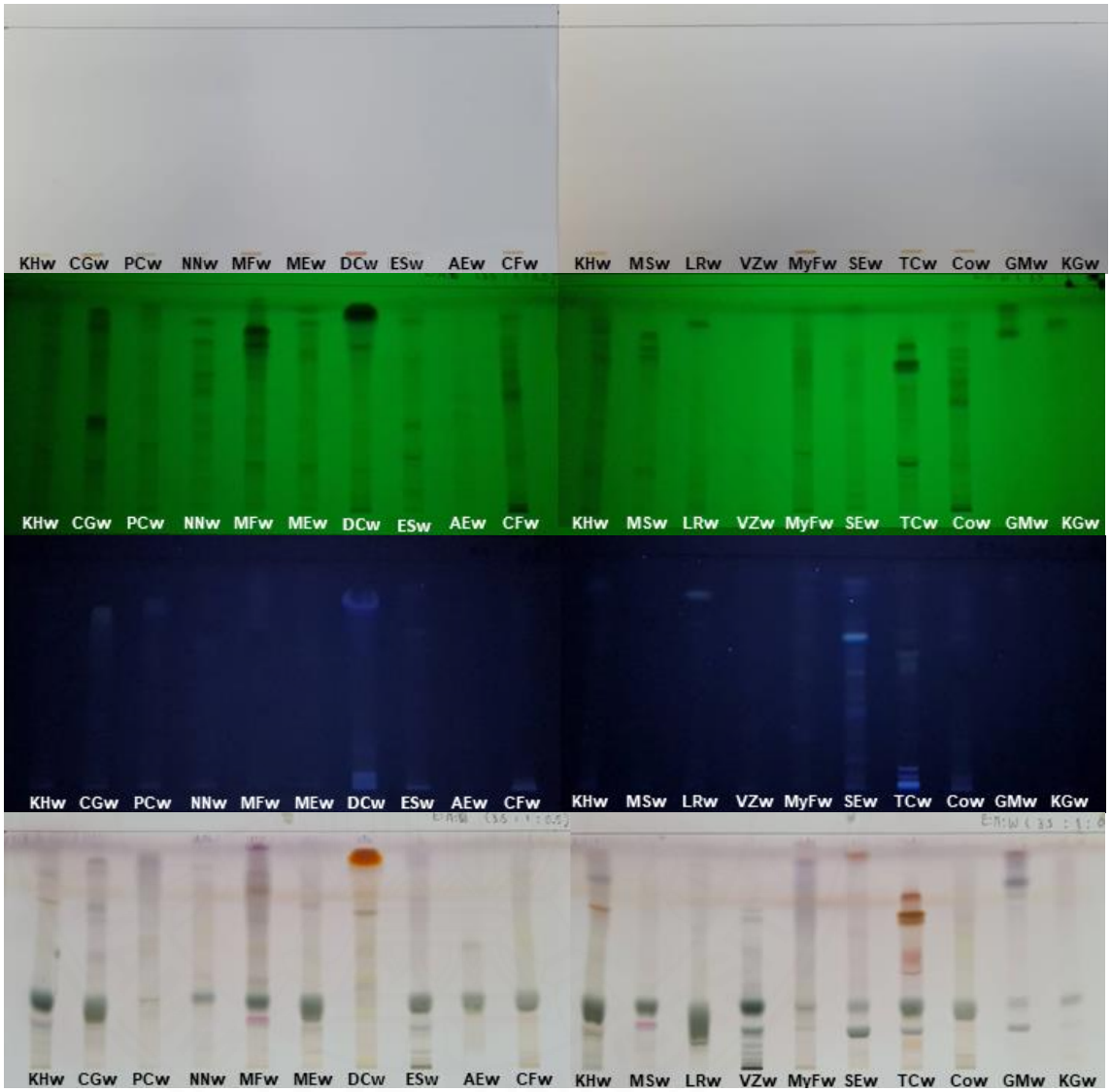
$CHCl_3$: MeOH (2:3)



Note: TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and its plant ingredients were detected under visible light (**A**), UV light at 254 nm (**B**), 365 nm (**C**), and by heating at 110-150°C after spraying with acidic anisaldehyde reagent (visible light) (**D**).

Figure 4-4 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and its plant ingredients using $CHCl_3$: MeOH (3:2) solvent system

EtOAc : MeOH : water (3.5:1:0.5)

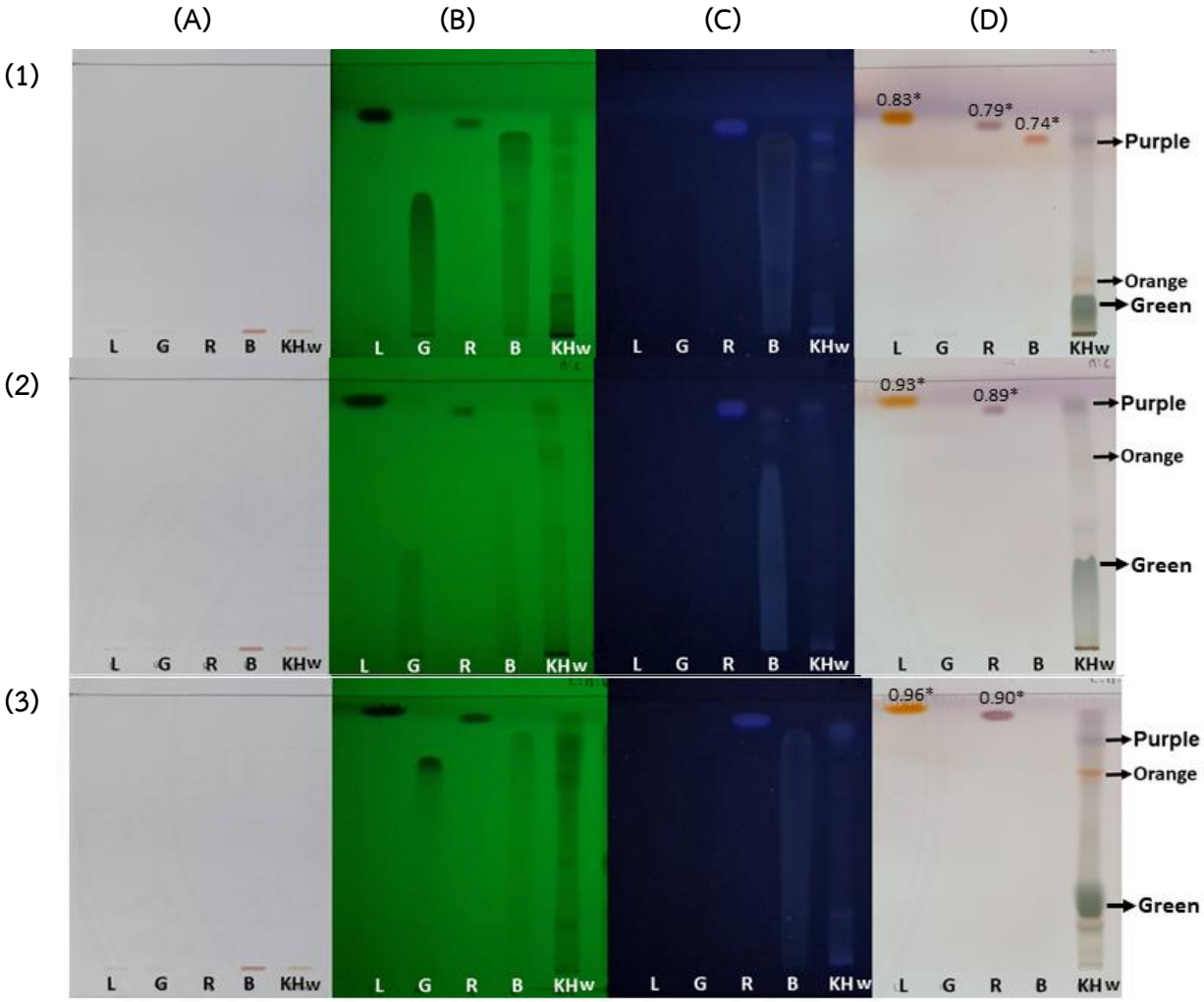


Note: TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and its plant ingredients were detected under visible light (**A**), UV light at 254 nm (**B**), 365 nm (**C**), and by heating at 110-150°C after spraying with acidic anisaldehyde reagent (visible light) (**D**).

Figure 4-5 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and its plant ingredients using EtOAc : MeOH : water (3.5:1:0.5) solvent system

Therefore, pure compounds that was reported as isolated compounds from plant ingredients of Khaew-Hom were spotted on silica gel. Loureirin B and resveratrol are the phytochemical compounds of *D. cochinchinensis* (Fan et al., 2014). Gallic acid is a phytochemical compound of *M.ferrea* (Zhang et al., 2019). Four pure compounds; namely loureirin B, gallic acid, resveratrol, and brazilin were spotted to compare with the Kheaw-Home aqueous extract. The results of TLC fingerprints showed that all pure compounds were not found in the Kheaw-Home aqueous extract, as shown in **Figure 4-6**. Kheaw-Hom remedy consisted of 18 plants in an equal ratio or 5.5% w/w of each ingredient. Therefore, a small amount of each plant may affect the yield of pure compound, so pure compounds in plant ingredients of Kheaw-Hom remedy were not found in Khaew-Hom extract by TLC.

Then, the aqueous extract of Kheaw-Home remedy was isolated fractions to investigate cytotoxic, antiviral activity, and chemical fingerprint.



Note: TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and pure compounds were detected under visible light (**A**), UV light at 254 nm (**B**), 365 nm (**C**), and by heating at 110-150°C after spraying with acidic anisaldehyde reagent (visible light) (**D**). using 3 solvent namely $CHCl_3$: EtOAc : MeOH (1:3:1) (**1**), $CHCl_3$: MeOH (2:3) (**2**), and EtOAc : MeOH : water (3.5:1:0.5) (**3**). Tracks: L= Loureirin B; G=Gallic acid; R=Resverratrol; B=Brazilin; KHw=aqouse extract of Kheaw-Home remedy

Figure 4-6 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and pure compounds using 3 solvents namely $CHCl_3$: EtOAc : MeOH (1:3:1), $CHCl_3$: MeOH (2:3), and EtOAc : MeOH : water (3.5:1:0.5)

4.4 *In vitro* assay for cytotoxic activity and antiviral activities of fractions on Vero cells

The 20 g of crude aqueous extract was separated by column chromatography (CC) to obtain five fractions (FA1-FA5). The diaion HP-20 (200 g) was packed in a column by the dry pack. The sample solution was loaded into a column with 100% DI water and sequentially eluted with 25% MeOH, 50% MeOH, 75% MeOH, and 100% MeOH, respectively (**Figure 4-7**), to give five fractions such as FA1, FA2, FA3, FA4, and FA5. The highest percent yield was FA1, followed by FA4, FA2, FA3 and FA5, as shown in **Table 4.12**. After that, all fractions were tested for cytotoxic and antiviral activities.

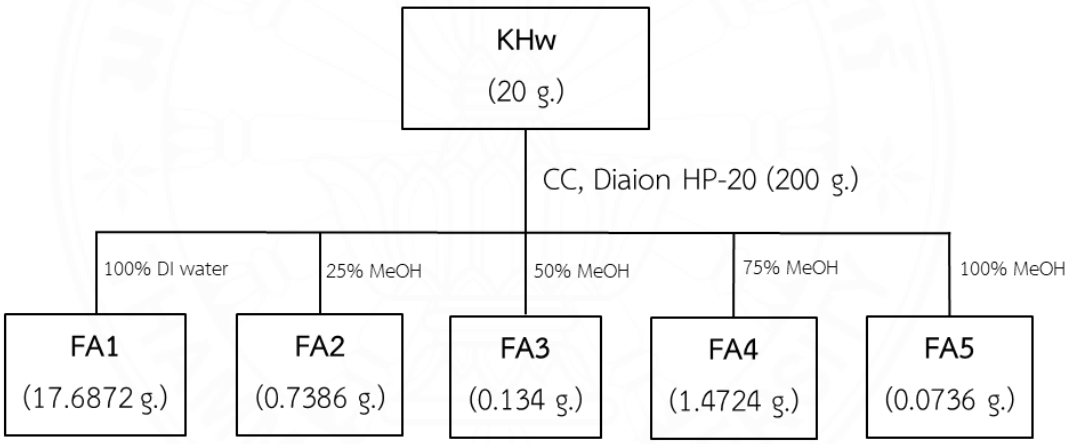


Figure 4-7 Isolation of the aqueous extract of Kheaw-Hom remedy by Diaion HP-20

Table 4-12 The Percentage yields of Fraction by Column chromatography (CC)

Code.	Fraction	Volume	%Yields (w/w)
FA1	Fraction 1 100%DI water	2 L	88.44
FA2	Fraction 2 75%DI water:25%MeOH	1.6 L	3.69
FA3	Fraction 3 50%DI water:50%MeOH	1.2 L	0.67
FA4	Fraction 4 25%DI water:75%MeOH	1.3 L	7.36
FA5	Fraction 5 100%MeOH	800 mL	0.37

According to the cytotoxic and antiviral activities, the fractions of aqueous extracts of Kheaw-Hom remedy were investigated cytotoxicity on Vero cells at varying concentrations from 100, 50, 25, 12.5, and 6.25 μg/mL. All fractions (FA1-FA5) were non-toxic on Vero cells after 2 hours of incubation. However, at the end of 3 days incubation, fraction 5 showed a toxic effect at 100, 50, and 25 μg/mL, while other fractions showed no cytotoxicity on cells, as shown in **Table 4-13**. Therefore, the fractions of aqueous extracts of Kheaw-Hom remedy at maximum non-toxic concentration were evaluated for antiviral activity.

Investigating the fractions was tested for antiviral activities against Herpes simplex type-2 (HSV-2). As a result, FA2, FA3, FA4, and FA5 exhibited potent antiviral activity against HSV-2 by pre-incubation with IC_{50} values of 18.86 ± 6.02, 20.44 ± 5.09, 31.41 ± 6.71 and 85.43 ± 7.69 μg/mL, respectively (**Table 4-14**). However, FA1 had an effect of less than 50% at concentrations 100 μg/mL. Whereas, all fractions of aqueous extract of Kheaw-Hom remedy exhibited antiviral activity against HSV-2 with effect less than 50% at a concentration 100 μg/mL by pre-treatment and post-treatment, as shown in **Table 4-15**, and **4-16**.

Regarding normal-phase of TLC fingerprints of five fractions (FA1-FA5) using three solvent systems, namely $CHCl_3$: EtOAC : MeOH (1:3:1), CHCl3 : MeOH (2:3), and EtOAC : MeOH : water (3.5:1:0.5). After spraying TLC with acidic anisaldehyde reagent and heat. An orange and purple spot was detected in FA2 and FA3. FA2 and FA3 had high potent anti-HSV-2. The green spot was detected in FA1, while FA1 showed no antiviral activity, as shown in **Figures 4-8, 4-9,** and **4-10**. The green spot may be assigned to the presence of terpenoid or steroid compound (Soonthornchareonnon et al., 2008). Plant-derived terpenoid and steroid has been reported with anti-inflammationactivity (Prakash, 2017; Yuan et al., 2006). Therefore, FA1 may be an anti-inflammationagent for patients with HSV-2.

In addition, reversed-phase of TLC fingerprints using three solvent systems, namely MeOH : Acetonitrile : water (2:2:6), MeOH : water (6:4), and $CHCl_3$: MeOH : water (1:6.5:2.5) After spraying TLC with acidic anisaldehyde reagent and

heat. The green spot was detected in FA1, and orange and purple spots were detected in FA2 and FA3. (**Figure 4-11**) Both TLC- fingerprint techniques have similar spots of all fractions by normal and reverse polarity. FA2 and FA3 had high potent anti-HSV-2, but their percent yield were low (FA2 = 3.69% and FA3 = 0.67%). Therefore, This partially isolated active fraction is a high anti-HSV-2 activity in fractions 2 and 3. These fractions can be further developed, possibly combining fractions 2 and 3, and their respective ratios can be used for further development.

As a result, The orange and purple spots may be active compounds of these fractions. However, the purple and orange spots may be synergistic compounds because these cannot be detected in plant ingredients of Khaew-Hom. Therefore, this thesis expects purple and green spots to be terpenoid compounds against HSV-2.

Table 4-13 Cytotoxicity of Fraction of the aqueous Kheaw-Hom remedy extract on Vero cell line by using MTT assay with incubated 2 hours and 3 days (n=3)

	Concentretion (µg/mL)	%cells viability				
		FA1	FA2	FA3	FA4	FA5
incubated 2 hours	100	93.83±6.00	79.97±4.35	92.62±6.82	79.84±2.55	81.23±8.69
	50	88.64±7.78	87.54±6.18	94.40±7.18	91.25±4.85	82.49±7.99
	25	83.72±4.88	90.30±3.02	85.46±5.34	86.89±5.26	83.15±7.01
	12.5	90.49±6.74	83.65±9.87	89.24±7.98	84.57±9.19	90.40±6.44
	6.25	90.04±7.45	89.82±5.34	92.86±7.83	97.70±6.82	93.19±4.53
incubated 3 days	100	90.73±7.15	91.35±8.61	94.08±1.53	82.88±2.90	18.94±4.90*
	50	88.65±7.32	98.02±3.97	94.64±8.95	89.98±4.74	43.26±6.30*
	25	90.34±3.07	97.94±7.83	92.45±2.43	91.91±2.23	64.08±2.88*
	12.5	83.32±5.92	91.57±4.05	86.69±1.63	87.21±4.34	93.60±5.27
	6.25	91.22±4.57	105.00±2.65	91.98±4.51	100.58±5.59	99.73±5.72

*****If %cell viability is reduced to less than 70 % of the cell controls, it has a cytotoxic potential (ISO 2009).

Table 4-14 The half maximal inhibitory concentration (IC_{50}) of the the aqueous extract of Kheaw-Hom remedy, fractions and acyclovir against HSV-2 on Vero cell line by pre-incubation (n=3)

Code.	%Yield	%inhibition					IC_{50} (µg/mL)
		100	50	25	12.5	6.25	
FA1	88.44	10.70±3.31	5.30±2.06	NT	NT	NT	>100
FA2	3.69	97.72±1.16	74.45±2.16	59.28±10.83	39.23±10.69	19.71±9.61	18.86±6.02
FA3	0.67	96.78±3.35	89.59±7.14	62.45±2.37	32.58±11.82	19.07±3.07	20.44±5.09
FA4	7.36	98.72±1.14	80.31±3.73	38.09±2.23	20.41±8.60	NT	31.41±6.71
FA5	0.37	69.10±7.91	30.38±5.07	34.07±9.91	19.11±8.33	NT	85.43±7.69
KHw	13.36	54.47±3.60	31.49±9.78	35.46±6.04	30.11±8.07	NT	94.24±1.26
Acyclovir	-	96.09±0.86	86.88±2.18	71.07±4.01	52.62±7.62	28.58±6.77	11.61±2.71

*NT = Not tested

Table 4-15 The half maximal inhibitory concentration (IC_{50}) of the fraction of the aqueous Kheaw-Hom remedy extract against HSV-2 on Vero cell line by pre-treatment (n=3)

Code.	%inhibition					IC_{50} (µg/mL)
	100	50	25	12.5	6.25	
FA1	29.96±1.52	NT	NT	NT	NT	>100
FA2	23.79±5.36	NT	NT	NT	NT	>100
FA3	42.30±6.18	NT	NT	NT	NT	>100
FA4	10.73±0.54	NT	NT	NT	NT	>100
FA5	14.06±5.26	NT	NT	NT	NT	>100

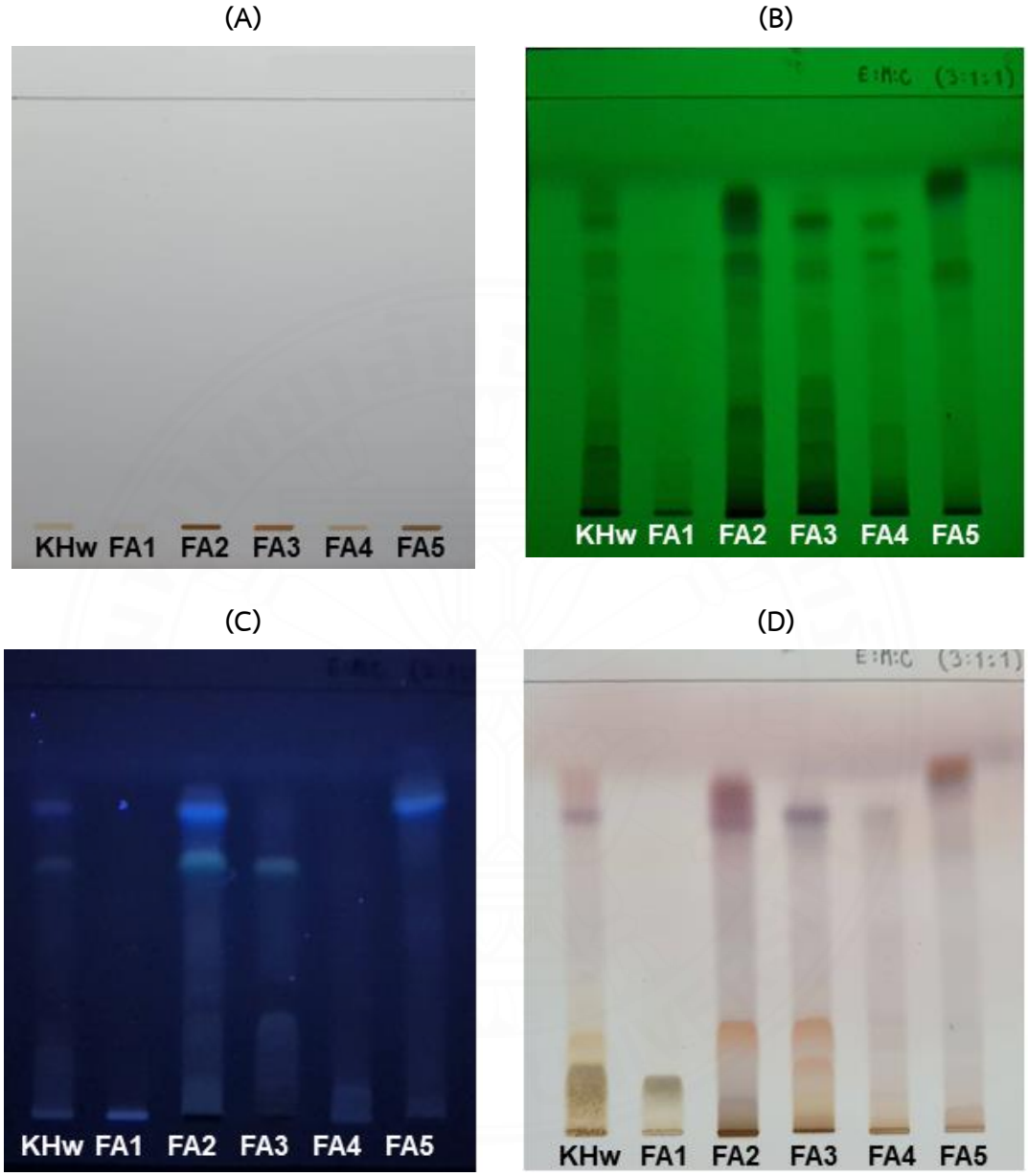
*NT = Not tested

Table 4-16 The half maximal inhibitory concentration (IC_{50}) of the fraction of the aqueous Kheaw-Hom remedy extract against HSV-2 on Vero cell line by post-treatment (n=3)

Code.	%inhibition					IC_{50} (µg/mL)
	100	50	25	12.5	6.25	
FA1	6.63±3.35	NT	NT	NT	NT	>100
FA2	13.17±3.55	NT	NT	NT	NT	>100
FA3	19.36±0.25	NT	NT	NT	NT	>100
FA4	17.03±3.36	NT	NT	NT	NT	>100
FA5	NT	NT	NT	4.55±3.52	NT	>12.5

*NT = Not teste

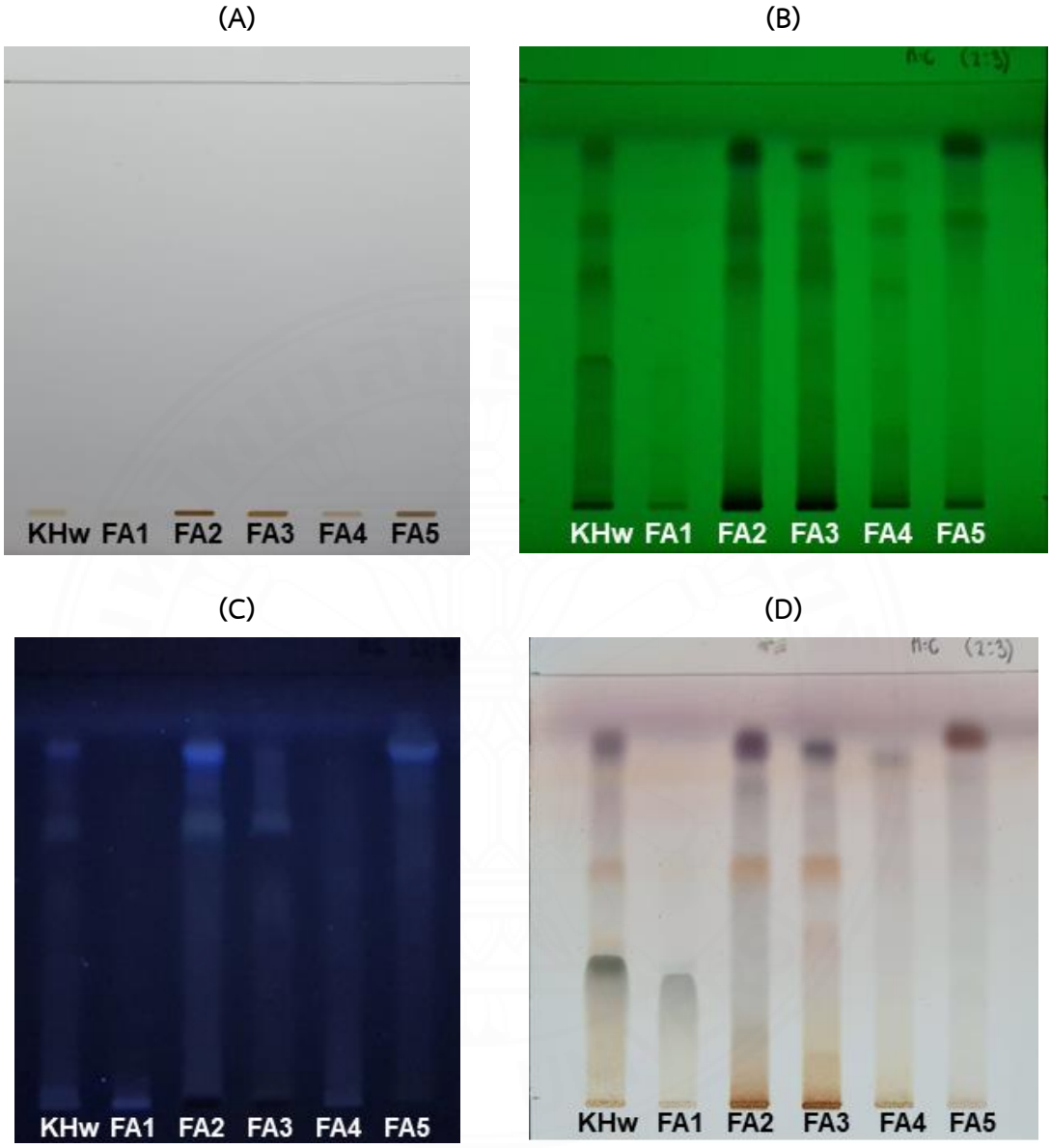
$CHCl_3$: EtOAc : MeOH (1:3:1)



Note: TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and fractions from the aqueous extract of Kheaw-Hom remedy (FA1, FA2, FA3, FA4, and FA5) were detected under visible light, (**A**) UV light at 254 nm (**B**), 365 nm (**C**), and by heating at 110-150°C after spraying with acidic anisaldehyde reagent (visible light) (**D**).

Figure 4-8 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and isolated fractions from the aqueous extract of Kheaw-Hom remed using $CHCl_3$: EtOAc : MeOH (1:3:1) solvent system

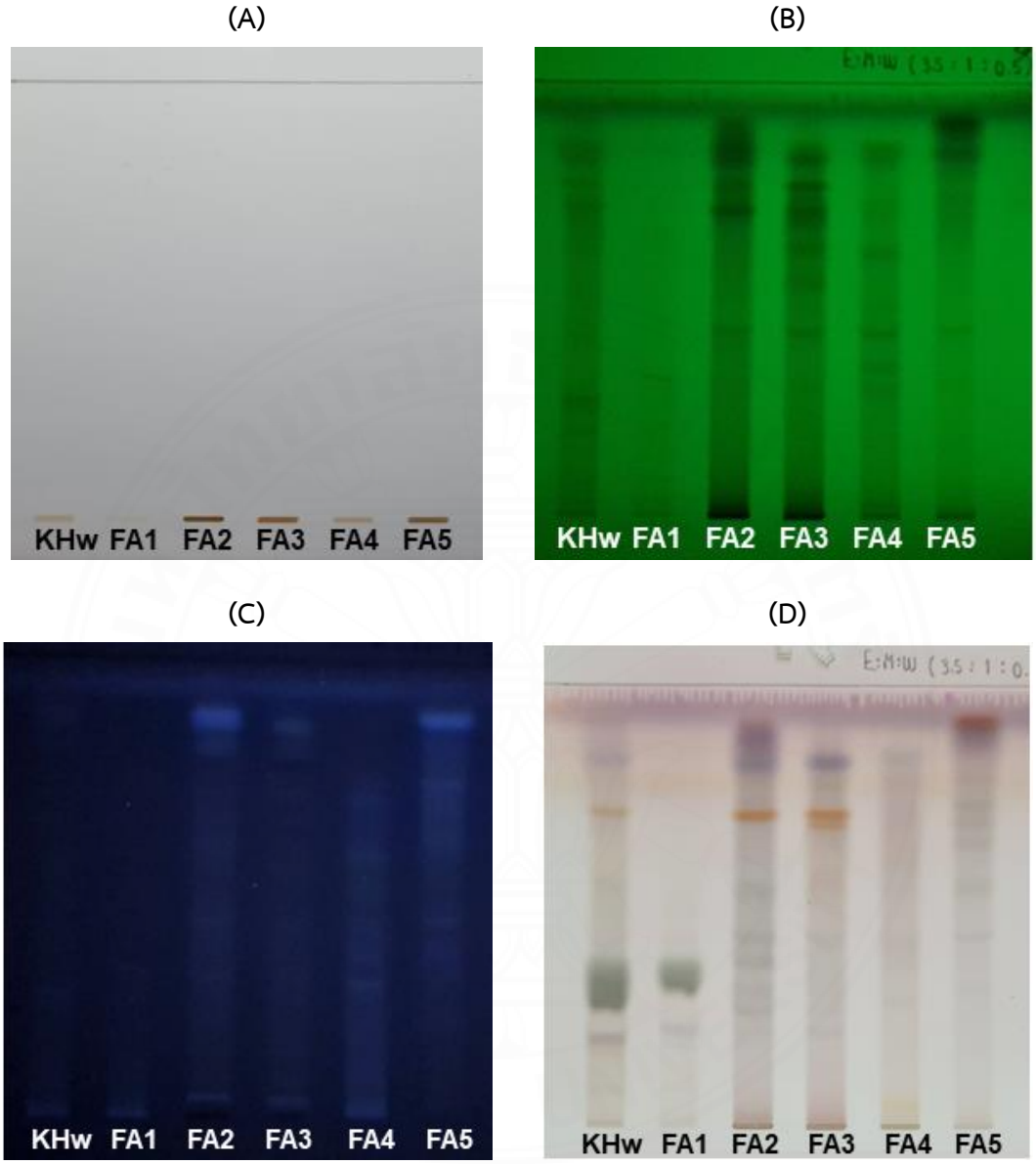
$CHCl_3$: MeOH (2:3)



Note: TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and fractions from the aqueous extract of Kheaw-Hom remedy (FA1, FA2, FA3, FA4, and FA5) were detected under visible light, (**A**) UV light at 254 nm (**B**), 365 nm (**C**), and by heating at 110-150°C after spraying with acidic anisaldehyde reagent (visible light) (**D**).

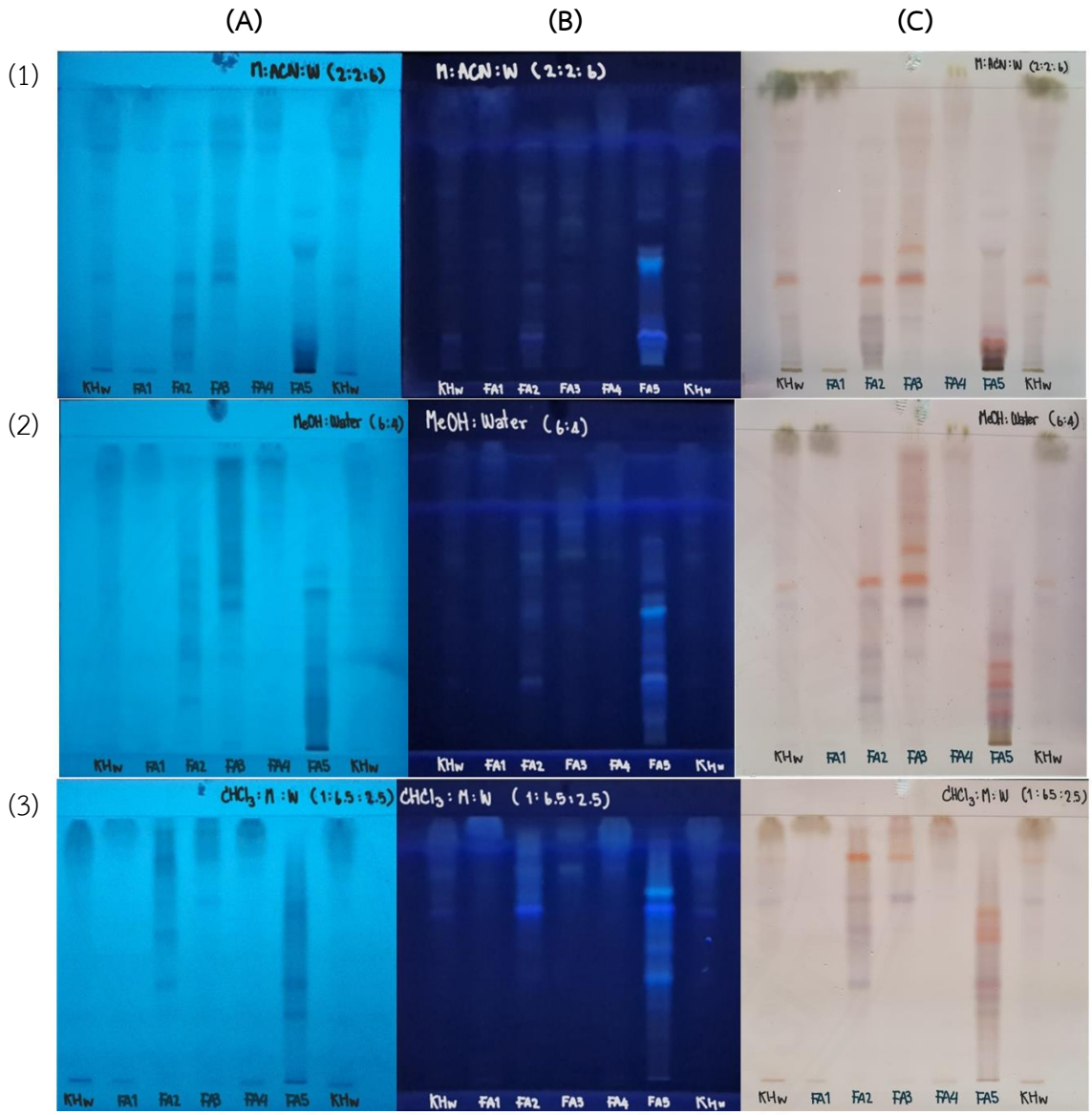
Figure 4-9 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and isolated fractions from the aqueous extract of Kheaw-Hom remed using $CHCl_3$: MeOH (2:3) solvent system

EtOAc : MeOH : water (3.5:1:0.5)



Note: TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and fractions from the aqueous extract of Kheaw-Hom remedy (FA1, FA2, FA3, FA4, and FA5) were detected under visible light, (**A**) UV light at 254 nm (**B**), 365 nm (**C**), and by heating at 110-150°C after spraying with acidic anisaldehyde reagent (visible light) (**D**).

Figure 4-10 TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and isolated fractions from the aqueous extract of Kheaw-Hom remedy using EtOAc : MeOH : water (3.5:1:0.5) solvent system



Note: TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and pure compounds were detected under UV light at 254 nm (A), 365 nm (B), and by heating at 110-150°C after spraying with acidic anisaldehyde reagent (visible light) (C). using 3 solvent namely MeOH : Acetonitrile : water (2:2:6) (1), MeOH : water (6:4) (2), and $CHCl_3$: MeOH : water (1:6.5:2.5) (3).

Figure 4-11 Reversed-phase of TLC fingerprints of the aqueous extract of Kheaw-Hom remedy (KHw) and isolated fractions from the aqueous extract of Kheaw-Hom remedy

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

Kheaw-Hom remedy is commonly used to treat fever and skin infections in Thai traditional medicine. It consists of 18 herbs as follow: Bua-luang (*Nelumbo nucifera* Gaertn.), Bun-nak (*Mesua ferrea* Linn.), Chan-dang (*Dracaena cochinchinensis*), Chan-thet (*Myristica fragrans* Houtt.), Faek-hom (*Vetiveria zizanioides* (L.) Nash ex Small), Ma-has-sa-dam (*Cyathea gigantea* Holtt.), Mak-mia (*Cordyline fruticosa* (L.) Goeppert.), Mak-phu (*Cordyline fruticosa* (L.) Goeppert.), Nae-ra-phu-sri (*Tacca chantrieri* Andre), Phak-kra-chom (*Limnophila rugosa* Merr.), Phi-kul (*Mimusops elengi* Linn.), Phim-sen-ton (*Pogostemon cablin* (Blanco) Benth.), Phit-sa-nat (*Sophora exigua* Craib), Proh-hom (*Kaempferia galanga* Linn.), San-phra-hom (*Eupatorium stoechadosmum* Hance), Sa-ra phi (*Mammea siamensis* Kosterm.), Wan-kep-rat (*Angiopteris evecta* (G.Forst) Hoffm.), and Wan-ron-thong. The administration of Kheaw-Hom remedy is powder and it is diluted in water to prepare for oral and topical administation, especially chickenpox lesions. Chickenpox is in the same family as herpes simplex. Therefore, the objective of this research was to investigate the antiviral activity of Kheaw-Hom remedy extracts, its plant ingredients, and fractions against Herpes simplex virus type-2. This is the first report of aqueous and ethanol extract of Kheaw-Hom remedy, its plant ingredients, and fraction for their cytotoxic and antiviral activity against HSV-2.

Cytotoxic activity of all extract on Vero cell lines by MTT assay. For incubate 2 hours, The aqueous and ethanolic extracts of Kheaw-Hom remedy, the aqueous extracts of Kheaw-Hom ingredients, fraction, and acyclovir are non-toxic on Vero cells. For 3 days incubation, the ethanolic extracts of Kheaw-Hom remedy, the aqueous extracts of *L. rugosa,* and fraction 5 showed toxicity to Vero cells.

All extracts were investigated for antiviral activity against HSV-2 by pre-incubation, pre-treatment, and post-treatment. The purpose of each experiment is to investigate the effect of the extract on virus inactivation, determine the effect of extracts on the virus before entering the Vero cells, and observe the effect of

extracts on viral replication. The results showed that the aqueous extracts of Kheaw-Hom remedy against HSV-2 by pre-incubation when was incubated with the virus before infection on Vero cells. Furthermore, the aqueous extracts of the Kheaw-Hom remedy affect virus inactivation before infected to new host cells. In contrast, acyclovir showed the highest antiviral effect against HSV-2 by pre-incubation and post-treatment. Acyclovir inhibited HSV2 infection by interfering with the viral DNA polymerase and genome replication. Therefore, acyclovir treatment is more effective on HSV-infected cells than Kheaw-Hom remedy.

In addition, the plants have also been used as a folk medicine to treat fever, skin infections, and other diseases. There are 7 of 18 plant ingredients against HSV-2 by pre-incubation, namely *C. gigantea, P. cablin, N. nucifera, M. ferrea, M. elengi, D. cochinchinensis,* and *M. siamensis.* In this study, *C. gigantea* is the highest anti-viral activity against HSV-2 when was compared with acyclovir. The folk healers believe that combination of herbs showed more effective treatment than single plant. Therefore, a combination of herbs also used to treat on many diseases more than single plant. *C. gigantea* is often an ingredient in fever remedies.

The TLC fingerprint of the Kheaw-hom remedy represented three spots: green, orange, and purple. A green and purple spot excepted to be terpenoid compounds because of these terpenoid group compounds were sprayed byacidic anisaldehyde reagent showing in purple, blue, and green spot. However, almost all plants represented a green spot in the same position as the Kheaw-Hom remedy. In contrast, orange and purple spots are detected in only the TLC fingerprint of Kheaw-Hom. Moreover, the pure compound including loureirin B, gallic acid, resveratrol, and brazilin are not represented in the TLC fingerprint of Kheaw-Hom. Therefore, the three spots of the TLC fingerprint of the Kheaw-Hom remedy are excepted to be compound in terpenoid group.

The aqueous extract of Kheaw-Hom remedy was isolated fraction and tested antiviral activities against HSV-2. Fraction 2 to fraction 5 exhibited against HSV-2 with IC_{50} range of 18.86±6.02 to 85.43±7.69 µg/mL by pre-incubation. In addition, the TLC fingerprint of fractions 1 represented a green spot but fraction 1 no antiviral

activity. In contrast, TLC-fingerprint of fractions 2 and 3 represented an orange and purple spot compared with the aqueous extracts of Kheaw-Hom remedy. It's expected to be caused by a synergistic effect.

In summary, our research indicates that the aqueous extracts of Kheaw-Hom remedy, seven plant ingredients, and fraction 2- fraction5 are highly effective in combating HSV-2 infection when the extract is incubated with the virus directly. In contrast, the extract is not affected by viruses entering the host cells and viral replication. These results also support this remedy for the treatment of herpes simplex and general support use because people and folk medicine commonly use the aqueous extract to prepare oral and topical drugs. This study offers the experimental basis for extracts as a potential drug in the treatment of HSV-2. Therefore, we suggest that the extraction method of this remedy should be decoction with water because this remedy extract demonstrated high against HSV-2.

For the recommendation of this study, Khaew-Hom may be developed as a topical drug suitable for treating HSV-2 by cream or gel. The products should be tested pre-formulation such as stability in physicochemical properties (pH, oxidant, temperature, and moisture condition), physicomechanical properties, and *in vitro* availability properties before investigation on product development. Furthermore, fractions 2 and 3 should be isolated pure compounds to find the active marker of Kheaw-Hom remedy. Separation of active compound of its water extract should be performed using the reverse phase technique.

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APPENDICES

APPENDIX A

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 ทุก 4–6 ชั่วโมง เมื่อมีอาการ

เด็ก อายุ 6–12 ปี : รับประทานครั้งละ 500 มิลลิกรัม ละลายน้ำกระสายยา ทุก 4–6 ชั่วโมง เมื่อมีอาการ

น้ำกระสายยาที่ใช้

- กรณีบรรเทาอาการไข้ร้อนในกระหายน้ำ ใช้น้ำสุก หรือน้ำดอกมะลิเป็นน้ำกระสายยา
- กรณีแก้พิษหัด พิษอีสุกอีใส ละลายน้ำรากผักชีต้ม เป็นน้ำกระสายยาทั้งรับประทานและชโลม

หมายเหตุ การชโลมใช้ยาผงละลายน้ำ 1 ต่อ 3 แล้วชโลม (ประพรม) ทั่วตามตัวบริเวณที่ตุ่มใสยังไม่แตก

ชนิดเม็ด ผู้ใหญ่ : รับประทานครั้งละ 1 กรัม ทุก 4–6 ชั่วโมง เมื่อมีอาการ

เด็ก อายุ 6-12 ปี : รับประทานครั้งละ 500 มิลลิกรัม ทุก 4–6 ชั่วโมง เมื่อมีอาการ

คำเตือน

- ควรระวังการใช้ยาในผู้ป่วยที่แพ้ละอองเกสรดอกไม้
- ไม่แนะนำให้ใช้ในผู้ที่สงสัยว่าเป็นไข้เลือดออก เนื่องจากอาจบดบังอาการของไข้เลือดออก
- หากใช้ยาเป็นเวลานานเกิน 3 วัน แล้วอาการไม่ดีขึ้น ควรปรึกษาแพทย์

APPENDIX B

Chemical Reagents for Laboratory Experiments

Reagent for cytotoxic and antiviral activity

1. **MEM (Minimum Essential Medium)**

1.1 stock solution (1X)

MEM powder	9.5	g
Sterile deionized water	1000	mL
10% hydrochloric acid	0.5-0.7	µL
5%$NaHCO_3$	2	g

Dissolve 9.5 g of MEM powder in 1000 mL sterile deionized water and sterile through filtration with 0.2 µm membrane filter. Adjust pH 7.2 to 7.4 with 10% hydrochloric acid. Aliquot 400, 400, and 200 mL in sterile duran and kept at -20°C.

1.2 Complete media (10%FBS in MEM)

MEM (stock solution (1X)	358	mL
Fetal bovine serum	40	mL
P/S	1	mL
Sodium Pyruvate	1	mL

1.3 Maintain media (3%FBS in MEM)

MEM (stock solution (1X)	386	mL
Fetal bovine serum	12	mL
P/S	1	mL
Sodium Pyruvate	1	mL

1.4 stock solution (2X)

MEM powder	9.5	g
Sterile deionized water	500	mL
10% hydrochloric acid	0.5-0.7	µL

5%$NaHCO_3$	2	g

Dissolve 9.5 g of MEM powder in 500 mL sterile deionized water and sterile through filtration with 0.2 μm membrane filter. Adjust pH 7.2 to 7.4 with 10% hydrochloric acid. Kept at -20°C.

1.5 Maintain media (6%FBS in MEM)

MEM (stock solution (2X)	468	mL
Fetal bovine serum	30	mL
P/S	1	mL
Sodium Pyruvate	1	mL

2. Phosphate buffer saline (PBS)

PBS	1	Tablet
Distilled water	500	mL
Sterilize by autoclave before use		

3. 10% Formaldehyde

Formaldehyde 37%	54	mL
PBS	145	mL

4. 0.1% Crystal violet

Crystal violet	0.1	mg
Absolute Ethanal	10	mL
Distilled water	90	mL

5. 3% Carboxy Methyl Cellulose

Carboxy Methyl Cellulose	15	mg
Distilled water	500	mL
Sterilize by autoclave before use		

BIOGRAPHY

Name	Wisansaya Intawong
Educational Attainment	Academic Year 2018: Bachelor of Applied Thai Traditional Medicine, College of Allied Health Sciences, Suan Sunandha Rajabhat University

Publications

Intawong, W., Panthong, S., Sukkasem, K., and Itharat, A. (2021). Inhibition of herpes simplex virus type-2 by Khaew-Hom remedy extracts. *Proceeding of Traditional Thai Medicine in Aging Society, The 3rd National Conference in Traditional Thai Medicine (NC-TTM3)* (pp. 146-154).Thailand, Prince of Songkla University, ISBN (e-book): 978-616-271-632-4

Conferences and Presentations

Intawong, W., Panthong, S., Sukkasem, K., and Itharat, A. (2021). Inhibition of herpes simplex virus type-2 by Khaew-Hom remedy extracts. *The 3rd National Conference in Traditional Thai Medicine (NC-TTM3).* 5-6 May 2021, Thailand, Prince of Songkla University (Poster presentation).