# ANTIVIRAL ACTIVITY OF BENCHALOKAWICHIAN, PRASACHANDAENG AND ITS PLANT COMPONENTS EXTRACTS AGAINST HERPES SIMPLEX VIRUS

BY

NAPHALAI MAKHON

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE
IN APPLIED THAI TRADITIONAL MEDICINE
FACULTY OF MEDICINE
THAMMASAT UNIVERSITY
THE ACADEMIC YEAR 2021

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## ABSTRACT

Herpes simplex virus (HSV) causes severe illness, including neurological disease. HSV consists of two types: HSV-1 and HSV-2, associated with oral-facial and oral-genital infection. However, HSV-2 infection is the commonest cause of herpes. Antibiotics are used to treat herpes, but antibiotic misuse can promote drug resistance to herpes simplex virus. For this reason, herbal medicines may be investigated to provide new antiviral agents. Benchalokawichian (BLW) and Prasachandaeng (PJD) are included on Thailand's National List of Essential Drugs. They are used to treat fever and lower body temperature. In addition, folk healers use them to treat skin diseases. BLW consists of five bases: *Capparis micracantha*, *Tiliacora triandra*, *Harrisonia perforate*, *Clerodendrum indicum*, and *Ficus racemose.* Prasachandaeng consists of twelve plants: *Bouea macrophylla* Griff, *Caesalpinia sappan*, *Citrus aurantifolia* ( Christm) Swingle, *Dracaena cochinchinensis* ( Lour. ) S. C. Chen, *Heliciopsis terminalis* Kurz (Sleumer) , *Jasminum* sambac L. (Aiton) , *Kaempferia galanga* L,

*Ligusticum Chuanxiang* Hort, *Mammea siamensis* T.Andersin, *Mesua ferrea* L, *Myristica fragrans* Hout, *and Nelumbo nucifera* Gaertn. Inhibition effect of BLW and PJD against HSV-2 has not yet been investigated for HSV. Therefore, this study aimed to examine the antiviral activity of BLW, PJD, and their plant component extracts against HSV-2. First, cytotoxic activity of all extracts on Vero cells was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay at two hours and after three days. The results found that all samples showed no cytotoxicity for antiviral activity by plaque reduction assay for pre-incubation assay, pre-treatment assay, and pre-treatment assay.

Ethanolic and aqueous BLW extracts revealed that Vero cell had non-cytotoxicity at 100 µg/mL after two hours and three days of incubation. BLW extracts were pre-incubated with HSV-2 for two hours before infection. After pre-incubation, the inhibition effect of BLW extracts against HSV-2 was below 50% at 100 µg/mL. Similarly, BLW extracts showed less effect against HSV-2 with pre-treatment assay and post-treatment assay.

Ethanolic extract of PJD showed no Vero cell toxicity at 100 µg/mL after two hours of incubation. However, it was toxic to Vero cells at 100 µg/mL after three days of incubation. An inhibitory effect could be observed when the ethanolic extract of PJD was incubated with HSV-2 before infection to Vero cells ($IC_{50}$ = 24.46±6.05 µg/mL). For pre-treatment assay and post-treatment assay, the inhibition percentage of ethanolic extract of PJD against HSV-2 was under 50% at the highest concentration, with no toxic effect on cells. Ethanolic extract of *D. cochinensis*, the main ingredient of PJD, showed potent anti-HSV-2 with $IC_{50}$ of 37.23±4.15 µg/mL. *C. sappan* and *K. galangal*, plant ingredients of PJD, observed by pre-incubation assay. *C. sappan* had the highest antiviral activity against HSV-2 with an $IC_{50}$ value of 1.91±0.13 µg/mL, while *K. galangal* did not inhibit HSV-2. Brazilin and Ethyl-*p*-methoxycinnamate (EPMC) were found in PJD extract to have an inhibitory effect against HSV-2 with $IC_{50}$ values of 4.28±1.64 and >100 µg/mL in the pre-incubation process.

Aqueous extract of PJD revealed no anti-HSV-2. However, after hydrolysis, anti-HSV-2 activity existed with $IC_{50}$ value of 28.03±6.44 µg/mL. To compare the

chemical fingerprint between acid hydrolysis extract and PJD aqueous extract, thin-layer chromatography (TLC) was used to monitor the PJD chemical compound. Many different chemical compounds were made between PJD acid hydrolysis and aqueous extraction. The acid hydrolysis process may cause chemical changes in the aqueous extract.

These findings suggest that PJD ethanolic and acid hydrolysis extracts eliminated HSV-2 directly. Its plant components, including *D. cochinensis, C. sappan*, and brazilin, a pure compound of PJD, were effective against HSV-2. However, PJD was suitable for development as an external drug due to its direct effect on herpes viruses.

**Keywords:** Herpes simplex virus type-2, Benchalokawichian, Prasachandaeng, Plaque reduction assay, Pre-incubation assay.

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# LIST OF ABBREVIATIONS

| Symbols/Abbreviations | Terms |
|---|---|
| % | Percent |
| > | More than |
| < | Less than |
| = | Equal |
| ≥ | More than or equal |
| ≤ | Less than or equal |
| & | And |
| ß | Beta |
| α | Alpha |
| µg | Microgram |
| µm | Micrometer |
| µM | Micromolar |
| µg/mL | Microgram per milliliter |
| ๐ | Degree Celsius |
| ATCC | American type culture collection |
| $CHCl_3$ | Chloroform |
| cm | Centimeter |
| $CO_2$ | Carbon dioxide |
| conc. | Concentration |
| CPE | Cytopathic effect |
| DMSO | Dimethyl Sulfoxide |
| $IC_{50}$ | Concentration causing 50%inhibition |
| MEM | Minimal essential mediumet |
| al. | Et alii, and colleagues |
| FBS | Fetal bovine serum |

| Symbols/Abbreviations | Terms |
|---|---|
| h | Hour |
| m | Meter |
| Vero | African green monkey cell line |
| MTT | 3-(4, 5-dimethyl-5-thaizolyl)-2, 5 diphenyl tetrazolium bromine |
| ml | Milliliter |
| mm | Millimeter |
| mg | Milligram |
| $NaHCO_3$ | Sodium bicarbonate |
| PBS | Phosphate buffer saline |
| OD | Optical density |
| PFU | Plaque-forming unit |
| PBS | Phosphate buffer saline |
| P/S | Penicillin-Streptomycin |
| LPS | Lipopolysaccharide |
| TLC | Thin-layer chromatography |
| HSV-1 | Herpes simplex virus type-1 |
| HSV-2 | Herpes simplex virus type-2 |
| ATCC | American type culture collection |

# CHAPTER 1
# INTRODUCTION

## 1.1 Background

Herpes simplex virus (HSV), a member of the Alphaherpesvirinae subfamily, is classified into Herpes simplex virus type-1 (HSV-1) and Herpes simplex virus type-2 (HSV-2) (Saleh, Yarrarapu & Sharma, 2020). Herpes simplex virus is a DNA virus with the characteristics of the enveloped virus and cell division replication from the nucleus (Puttawattana, 2016). It can infect various cell types, including epithelial cells, mucus membranes, and neuronal cells, and hide in nerve cells. Herpes simplex virus can cause pain, skin rash, inflammation, keratitis, and trigeminal neuralgia (Miranda-Saksena, Denes, Diefenbach & Cunningham, 2018; Horbul, Schmechel, Miller, Rice & Southern, 2011). Herpes simplex virus infect pregnancy or immunocompromised people may be severe and lead to death (Achananuphan, 2010). Herpes simplex virus type-1 is typically transmitted by oral-oral route, while Herpes simplex virus type-2 is transmitted via genital contact (Sauerbrei, 2016). In 2016, the prevalence of Herpes simplex virus type-2 infection worldwide was 491.5 million people. Africa region has the highest rate of Herpes simplex virus type-2 infection in the world, followed by the Western Pacific, South-East Asia, and Americas Regions. While the number of Herpes simplex virus type-1 infection patients was the largest in the South-East Asia region, the Africa region has the lowest rate of Herpes simplex virus type-1 infection. Global patients were infected between the ages of 15-49 (James *et al.*, 2020). In Thailand 2019, the genital Herpes simplex virus infection report found that women were more infected with Herpes simplex virus than men, with the most common infections being between the ages of 15-24. (Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, 2019).

Acyclovir (ACV) is an antiviral drug that is effective in treating herpes. However, the misuse of antibiotics can promote the development of resistance to antiviral drugs by the virus (Institute of Medicine (US) Committee on implementation

of Antiviral Medication Strategies for an Influenza Pandemic, 2008). However, acyclovir has low bioavailability and many adverse events such as nausea and headaches (Bomgaars *et al*., 2008; De, Hart, & Breuer, 2015). Thus, the investigation of traditional medicines or herbal medicine is probably to provide new antiviral agents (Hassan, 2015; Hornig & McGregor, 2014).

Benchalokawichian (Harak) and Prasachandaeng are mentioned in Thai traditional medicine texts and the National List of Essential Medicines, which are used to treat fever.

Benchalokawichian was mentioned in Thai traditional medicine classical texts such as Takshila that are Khireimnakhang and Khireimnakhaw (Thai Medical Promotion Rehabilitation Foundation Ayurveda College (Chiwokkomarapat), 1992). Benchalokawichian consist of the five roots of medicinal plants such as Ching-chi (*Capparis micracantha* DC), Ya-nang (*Tiliacora triandra* (Colebr.) Diels), Khon-tha (*Harrisonia perforate* (Blanco) Merr), Thao-yai-mom (*Clerodendrum indicum* (L.) *Kuntze*), and Ma-duea-chumphon (*Ficus racemose* L). It is used to treat skin diseases such as herpes simplex, herpes zoster and allergic rashes by traditional Thai practitioners. Benchalokawichian was reported on antipyretic, antinociceptive, anti-inflammatory, anti-allergic, and anti-human immunodeficiency virus (HIV) (Jongchanapong, Singharachai, Palanuvej, Ruangrungsi, & Towiwat, 2010; Juckmeta & Itharat, 2012; Juckmeta *et al*., 2014, Palo *et al*., 2017, Booranasubkajorn *et al.,* 2017).

Prasachandaeng is mentioned in the Thai medical education textbook Volume 1 of Phraya Phitsanu Prasatwet. It has been used for the treatment of fever and thirst (Phraya Phitsanu Prasatwet, 1908). It consists of 12 plant ingredients such as Ma-prang-wan (*Bouea macrophylla* Griff), Fang (*Caesalpinia sappan* L.), Ma-naw (*Citrus aurantifolia* (Christm)Swingle), Chan-daeng (*Dracaena cochinchinensis* (Lour.)S.C. Chen), Mueng-kon (*Heliciopsis terminalis* (Kurz)Sleumer), Ma-li (*Jasminum* sambac (L.) Aiton), Proh-*horm (Kaempferia galanga* L), Kot-hua-bua (*Ligusticum Chuanxiang* Hort), Sa-ra-pee (*Mammea siamensis* T.Andersin), Boon-nag (*Mesua ferrea* L), Chan-tet (*Myristica fragrans* Hout), and Bua-luang (*Nelumbo nucifera* Gaertn). Some plant ingredients of Prasachandaeng, including *C. sappan*, *D. cochinchinensis*, *J. sambac,* and

*N. nucifera*, were reported on antiviral activity. *C. sappan* contained sappanchalcone, oxysappanchalcone, quercetin, and flavonoids. These compounds showed antiviral activity against influenza virus (H3N2), dengue virus type -2, HSV-1 and HSV-2 (Liu *et al*., 2009; Johari *et al*., 2012; Zandi *et al.,* 2011; Lyu *et al.,* 2005). The water extract of *J. sambac* could inhibit adenoviruses (Chiang *et al*., 2003). The leaves and seed of *N. nucifera* contained (+)-1(R)-coclaurine, (–)-1(S)-norcoclaurine and NN-B-4 that have the ability of anti-HIV and anti-HSV-1 (Kashiwada, 2005; Kuo, 2005). Other plant components in the Prasachandaeng remedy have been reported as anti-inflammatory, anti-allergy, antinociceptive, and antipyretic. However, there is no reports on antiviral activity against the Herpes simplex virus of Benchalokawichian and Prasachandaeng extracts.

## 1.2 Research question

Did Benchalokawichian, Prasachandang, and plant components extract against Herpes Simplex type-2?

## 1.3 The objectives of this study

### 1.3.1 Overall objectives of this study

The overall objective of this research was to investigate the antiviral activity of Benchalokawichian, Prasachandang, and their plant components extracts against Herpes Simplex Virus Type-2.

### 1.3.2 Specific objectives of this study

1.3.2.1 To investigate the antiviral activity of the extracts of Benchalokawichian, Prasachandaeng against Herpes Simplex Virus Type-2.

1.3.2.2 To investigate the antiviral activity of plant components extracts of Benchalokawichian, Prasachandaeng against Herpes Simplex Virus Type-2.

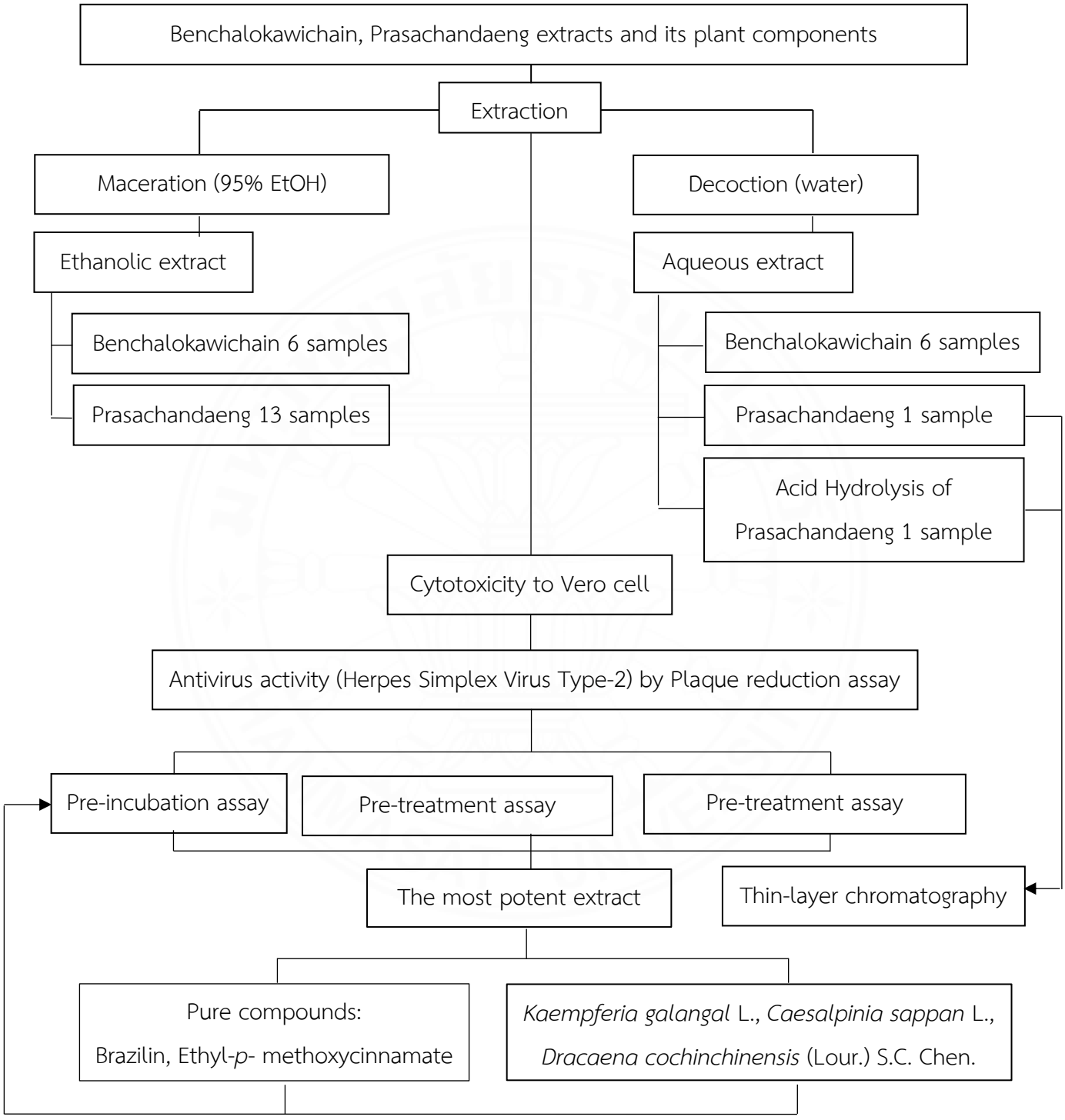
1.3.2.3 To investigate the antiviral activity of active marker in Benchalokawichian or Prasachandaeng extract against Herpes Simplex Virus Type-2.

1.3.2.4 To investigate the chemical fingerprints of antiviral extracts using thin-layer chromatography (TLC).

**1.4 Expected benefits from research**

The basic knowledge of the antiviral activity of Benchalokawichian extract, Prasachandang extract, and their plant components extract against Herpes simplex virus type-2 to deverlop the product of two these remedies for herpes treatment in the future.

**1.5 Conceptual framework of this thesis**



# CHAPTER 2
# REVIEW OF LITERATURE

## 2.1 Taxonomy of Herpes Simplex Virus

Family Herpesviridae is a big family. It has nearly a hundred members, and many types of humans and animals are infected. Herpes viruses have a double-stranded genome and a direct line. Molecular weight is 80-150 x 106 kd and length of about 124-235 kbp. It is big enough to produce 80-100 proteins and without virion transcriptase in the molecule. Herpes simplex virus has icosahedral nucleocapsid surrounded by a matrix of matrix proteins covered with a lipid envelope. It increases the number in the nucleus. The complete particle has a diameter of 102-200 nm. The structure is shown in **Figure 2.1** (Puttawattana, 2016).

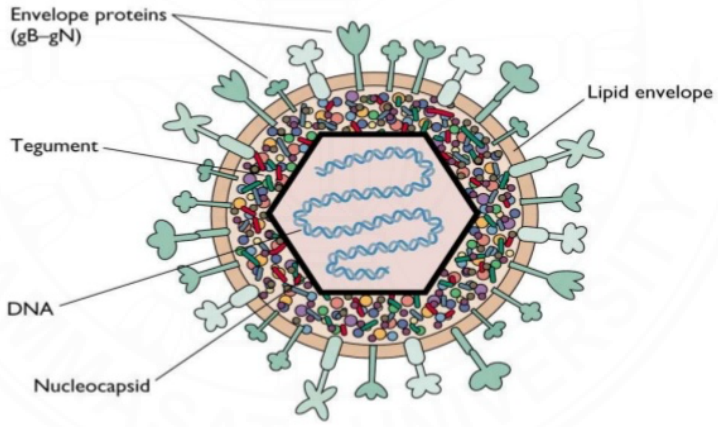



**Figure 2.1** Structure of Herpes Simplex Virus
(Available from https://www.onlinebiologynotes.com/herpes-simplex-virushsv-structure-genome-mode-transmission-pathogenesis-infection-laboratory-diagnosis-treatment/)

Herpes simplex virus has spread in humans contains 2 types i.e., Herpes simplex virus type -1 and Herpes simplex virus type -2. Herpes simplex virus belongs to the family Herpesviridae (Subfamily Alphaherpesvirinae). Herpes simplex virus type -1 infects the body above the navel, such as encephalitis, conjunctivitis, acute gingivostomatitis, and herpes labialis. Herpes simplex virus type-2 infects the body

below the navel, such as genital herpes and neonatal herpes. Both types of herpes cause vesicular eruption on the skin ( Puttawattana, 2016).

## 2.2 Herpes Simplex Virus

### 2.2.1 Epidemiology of Herpes Simplex Virus

In 2016, 187 million people aged 15–49 years had at least one episode of Herpes simplex virus: 5.0% of the world's population. Of these, 178 million (95% of those with HSV-related GUD) had HSV-2 compared with 9 million (5%) with HSV-1 (Looker *et al*., 2020).

### 2.2.2 Signs and Symptoms of Herpes Simplex Virus Type-1

Herpes infection type-1 causes oral sores. There will be blisters around the mouth, often called cold sores. Patients will have pain, itching, and burning around the mouth before the wound spreads to other areas. The wound shows in **Figure 2.2**. The frequency of recurrence varies from person to person, depending on the immune system. Infection with genital herpes type-1 may be asymptomatic and less common than genital herpes virus type-2 (World Health Organization, 2020).

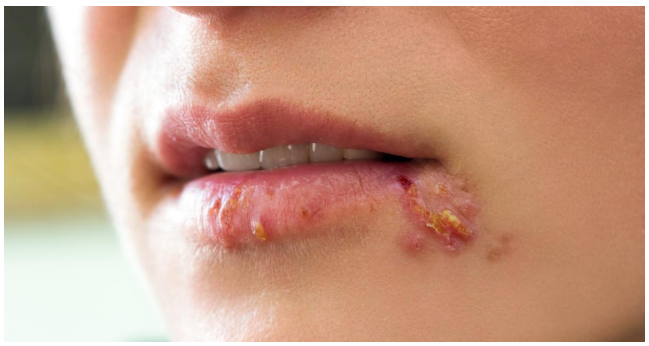


**Figure 2.2** Herpes Simplex Virus Type-1

(Available from https://www.findatopdoc.com/Healthy-Living/herpes-simplex-virus)

### 2.2.3 Transmission of Herpes Simplex Type -1

Herpes simplex virus type-1 is mainly transmitted by oral contact and saliva sores. The herpes virus type-1 can be transmitted to the genital area through

oral and sexual contact. Patients with herpes virus type-1 can be transmitted from a genitally infected mother to their baby during labor, causing neonatal herpes. Immunocompromised patients, such as people with HIV and Herpes, Herpes are more severe. Herpes infection leads to more severe complications, such as encephalitis or keratitis (World Health Organization, 2020).

### 2.2.4 Treatment of Herpes Simplex Virus Type -1

Anti-viral drugs, such as acyclovir, famiclovir, and valacyclovir, are the most effective drugs in treating herpes infection. In addition, these drugs can help decrease the severity and frequency of symptoms (World Health Organization, 2020).

### 2.2.5 Signs and Symptoms of Herpes Simplex Virus Type -2

Genital or rectal sores characterize genital herpes. The wound is shown in **Figure 2.3**. In addition to genital sores, there are also fever, body aches, and swollen lymph nodes. The second infection is less severe than the first infection. Patients will have leg and hip pain before the onset of symptoms (World Health Organization, 2020).

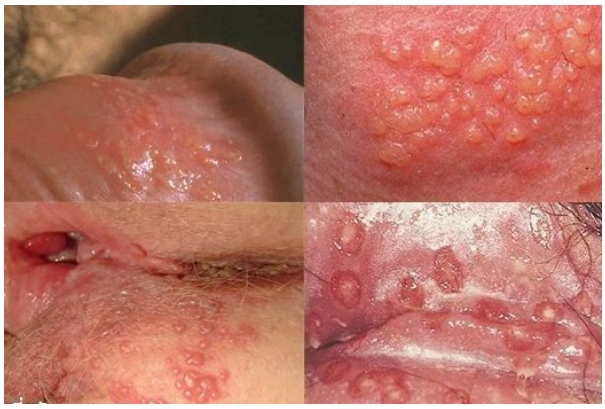


**Figure 2.3** Genital Herpes Type -2
(Available from https://medthai.com/)

### 2.2.6 Transmission of Herpes Simplex Virus Type -2

Herpes simplex virus type-2 is mostly sexually transmitted by touching the genitals, skin, sores, or fluids of someone infected with the virus. Herpes simplex virus type -2 can be transmitted from mother to infant during birth leading to

neonatal death. Infection with Herpes simplex virus type-2 increases the risk of contracted HIV approximately three times. Herpes simplex virus type-2 infection in HIV patients is common in approximately 60-90% of all infected individuals and can lead to severe complications (World Health Organization, 2020).

### 2.2.7 Treatment of Herpes Simplex Virus Type -2

The treatment uses the same medications as the treatment for Herpes simplex virus type-1. Anti-viral drugs are effective. They help decrease the severity and frequency (World Health Organization, 2020). The type that has the best treatment results is acyclovir because it is effective and causes the least resistance, high selectivity, and low toxicity (Birkmann & Zimmermann, 2016). The forms of acyclovir include oral medication, skin cream, and injection, which depends on the symptoms and the severity of the disease.

## 2.3 Replication of Herpes Simplex Virus

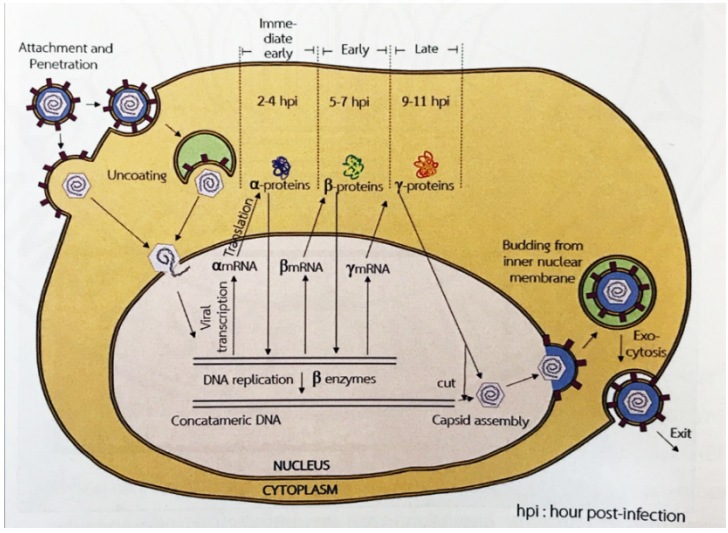



**Figure 2.4** Replication Cycle of Herpes Simplex Virus (Sanguansermsri,2015)

### 2.3.1 Attachment

Viruses use viral attachment protein to bind to a receptor on the cell surface. Attachment is an energy-free and important step in the multiplication of viruses, which is essential for the development of anti-viral drugs.

**2.3.2 Penetration**

After the attachment to the receptor, it passes the lipid bilayer of the plasma membrane or the nuclear membrane into the cell. The process of penetrating cells is energy-based. There are 3 mechanisms to enter into cells.

**2.3.2.1 Direct penetration (Translocation)**

The virus through the cytoplasmic membrane of cells, most of which occur with the naked virus.

**2.3.2.2 Endocytosis**

Virus particles bind to a plasma membrane that is clathrin or caveolin coated, which forces the membrane cells to bend to surround the virus particles.

**2.3.2.3 Fusion**

It is found only in enveloped viruses that the envelope of the virus is fused with the cell membrane on the surface of the cell.

**2.3.3 Uncoating**

The uncoating phase is the procedure for removing the capsid to release the viral genome. The uncoating phase is the procedure for removing the capsid to release the viral genome. This process can occur on the cell surface, inside the cytoplasm, nuclear pore, or nucleus. The uncoating phase occurs with penetration or occurs immediately after the virus enters the cell.

**2.3.4 Replication**

The multiplication of the virus's genetic material will vary depending on the virus type.

**2.3.5 Assembly**

Proteins and viral genomes should be insufficient quantities to form complete viral particles.

**2.3.6 Maturation**

This process changes the structure of the virus particles, which the virus becomes more stable until the complete virus particle

### 2.3.7 Release

cell lysis: The cell ruptures, causing the virus to leave the cell.

Budding: Viruses need cell walls to build into an envelope.

Cell to cell: Some enveloped viruses can pass from cell to cell without leaving the cell. The cycle is shown in **Figure 2.4** (Sanguansermsri, 2015; Bhattarakosol, 2018).

## 2.4 The inflammatory process of the lesion

Herpes virus multiplies and moves along nerves to infect epithelial cells. When a cell is infected with a virus, the immune system attacks cells. Dendrites and macrophages destroy pathogens that enter the body. It stimulates the activity of B cells, T cells, NK cells as well as CD4+ and CD8+ to move to the infected tissue and destroy the infected cells in the area, causing pain, swelling, redness around the lesion, shown in **Figure 2.5** (Puthavathana, 2016).

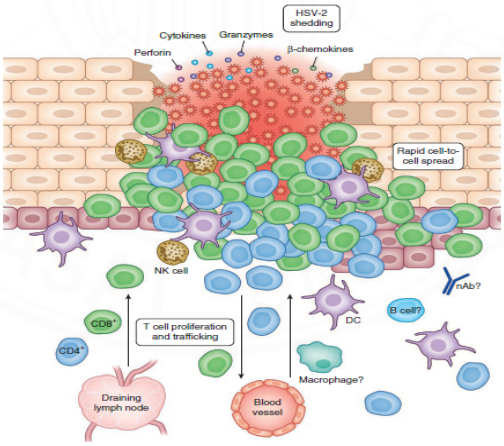



**Figure 2.5** Host immunity in the genital mucosa (Schiffer & Corey, 2013*)*

## 2.5 Latent Infection Virus

The condition of the cell is infected virus does not increase in number the virus particles, but virus genome contains latent inside the cell. The viral genome

lies within the cell. When the body is stimulated by factors such as stress, the virus multiplies and causes disease. In the latent infection stage, viral proteins are little produced. It only has proteins necessary for the survival of the virus in the cell. The herpes virus will infect and multiply in the epithelial cell, but latent infections will occur in the trigeminal ganglion neurons (Puttawattana, 2016).

## 2.6 Recurrent Infection

After the latency stage, when the environment stimulates the body, the virus can replicate in nerve cells, move along the axon nerve, and infect the epithelial cells where the lesion or other epithelium occurred. Recurrent Infection explains in **Figure 2.6** (Puttawattana, 2016; Bhattarakosol, 2018). Herpes simplex virus type -2 infection is more severe and recurring than Herpes simplex virus type -1 infection, with approximately 60-80% recurrence of Herpes simplex virus type -2 infection in patients with Herpes simplex virus type-1 found 10-20 % (Phattharakosol, 2018; Zimmerli, 2020).

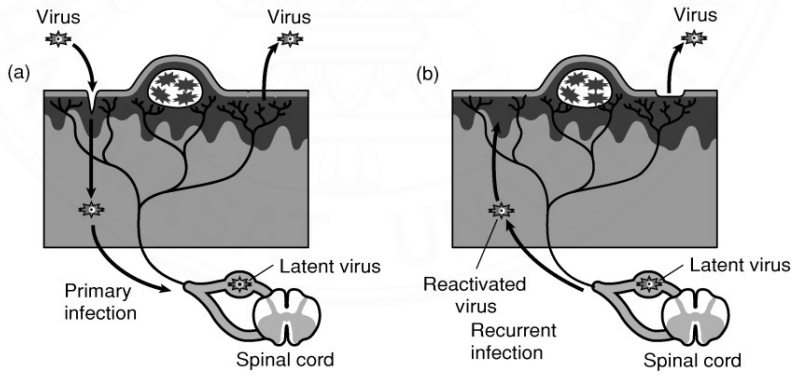



**Figure 2.6** (a) Primary Infection (b) Recurrent Infection (Whitley *et al*., 2007)

## 2.7 Route of infection

Contact from one person to another through direct contact, sexual contact, mother to child during pregnancy or labor, use sharing items, and immunocompromised patient (Phattarakosol, 2018).

## 2.8 Herpes treatment

When there is a burning sensation, use a cold compress, maintaining the body's hygiene and appliance, including the use of medicines. The drug used to treat Herpes simplex is acyclovir, which is effective against replicating the virus. It is available in oral, topical, and injectable forms depending on the severity of the disease. The side effects of acyclovir include nausea, vomiting, dizziness, and prolonged use leading to drug-resistant infections resulting in higher dosages and expensive medicine imports (Bruminhent, 2015).

## 2.9 Mechanism of Acyclovir

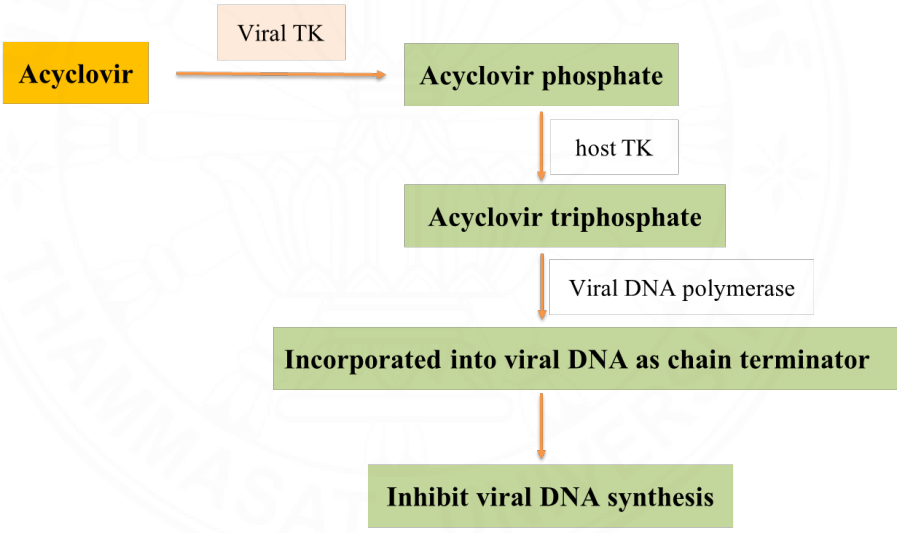



**Figure 2.7** Mechanism of Acyclovir (Sangusermsri, 2015)

Acyclovir was the first drug used to treat herpes. Acyclovir begins inactive form. It is converted to acyclovir monophosphate with the virus thymidine kinase enzyme. Then, It will be changed to acyclovir triphosphate with cellular kinase. Acyclovir triphosphate is an inhibitor of the DNA polymerase herpes virus. Acyclovir triphosphate presents a competitive inhibitor that increases deoxyguanosine triphosphate (pppdG) to the DNA, competing with pppdG binding with DNA polymerase. Acyclovir triphosphate competing with pppdG binding with DNA

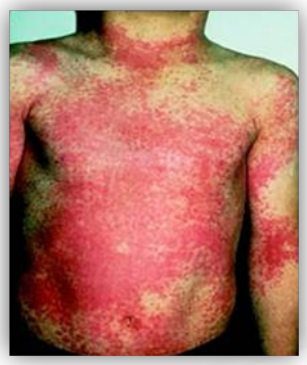
polymerase. Acyclovir prevents DNA virus synthesis and virus replication by inhibiting viral DNA polymerase. A mechanism shows in **Figure2.7** (Sangusermsri, 2015). Medicines work best on cells infected with the virus (Johnston & Corey, 2016).

## 2.10 Herbal medicines to treat Herpes simplex

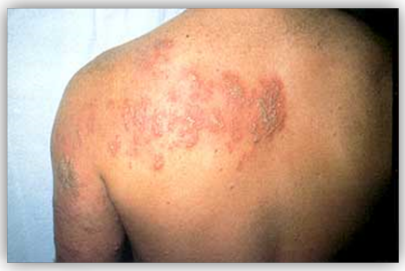
Currently, the search for new or natural substances to replace antibiotics is a matter of interest to researchers. According to the research, it is found that in China, the herbal formula for the treatment of Herpes simplex is JieZe-1 and Long Dan Xie Gan Tan (LDXGT). It is researched to be effective against Herpes simplex (Shao *et al*., 2020; Cheng *et al*., 2008). In Thailand, the popular herb used to treat herpes is *Clinacanthus nutans*, the active ingredient in the herb that can inhibit the proliferation of herpes simplex types 1 and 2 (Pongmuangmul., 2016).

## 2.11 Explanation of pathogenesis in Thai traditional medicine

The study in Thai traditional medicine textbooks found that the Tukkasila mentioned the types and characteristics of fever, namely, venom and meningococcal fever. Khi-pid are a fever and then a rash that is red, black, green. Khi-karn is a fever and a purulent rash on the skin. The skin will peel when the symptoms are severe, causing severe burning pain, shown in **Figure 2.8.**



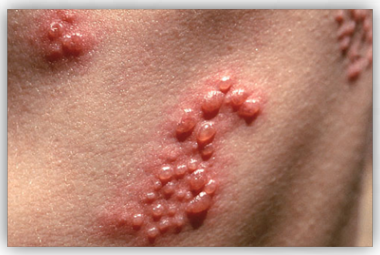
**Khi-pid**



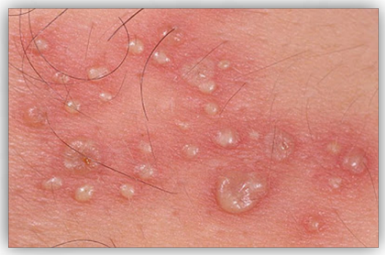
**Khikarn**

**Figure 2.8** Character of Khipid and Khikarn in Thai traditional medicine (Available from https://phuketthaitraditionalmedicinecenter.blogspot.com/2013/06/blog-post_7757.html)

Tukkasila scripture is mentioned as Khikarn which has many symptoms similar to herpes, such as namely Khireimnakhang with blisters appearing on the skin and Khireimnakhaw with cloudy blisters appearing on the skin, shown in **Figure 2.9.** (Chiwokkomarapat, 1992).



**Khireimnakhang**



**Khireimnakhaw**

**Figure 2.9** Character of Khireimnakhang and Khireimnakhaw in Thai traditional medicine (Available from https://phuketthaitraditionalmedicinecenter.blogspot.com/2013/06/blog-post_7757.html)

The mechanism of herpes disease in Thai traditional medicine is believed to be caused by a virus or kimichat that stimulates the fire element (San-tab-pak-kee) to exacerbate. Patients will have symptoms of fever. After stimulating the wind element (Ang-ka-mang-ka-nu-sa-ri-va-ta), patients will have headaches, pain, and itching around the wound. When the wind element is increased, resulting in the increase of the water element (Lo-hit-tang), the patient will have blood and lymph seeping around the wound. When the three elements increase, the soil element (Skin, Mang-sang, Ta-jo) is inflamed. The pathogenic cycle is shown in **Figure 2.10.**

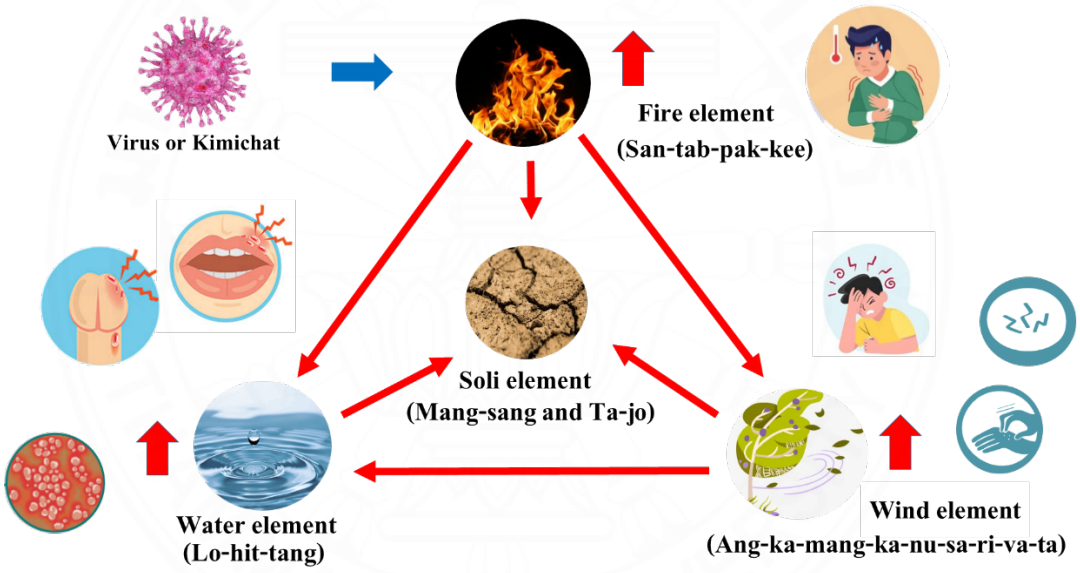



**Figure 2.10** Mechanisms of drug formulas on pathogenesis in Thai traditional medicine 1

Treatment of the disease have to reduce the influence of the fire element. The herbal formulas which can reduce heat have to bitter taste . Thus Benchalokawichian and Prasachandaeng which are bitter taste remedy have properties to treat fever. Benchalokwichian is a medicine that has a cooling effect. Prasachandaeng has a calm and cool taste. It can help reduce heat or fire elements, which is the first factor of fever and hot skin. In Thai traditional medicine, it is believed that the virus is the Kimichart that activates the fire element. Therefore, this recipe will

also affect eliminating Kimichart this the cause of this disease. If fire elemental is reduced, soli elemental's expression will improve and existing skin diseases would gradually disappear, shown in **Figure 2.11.**

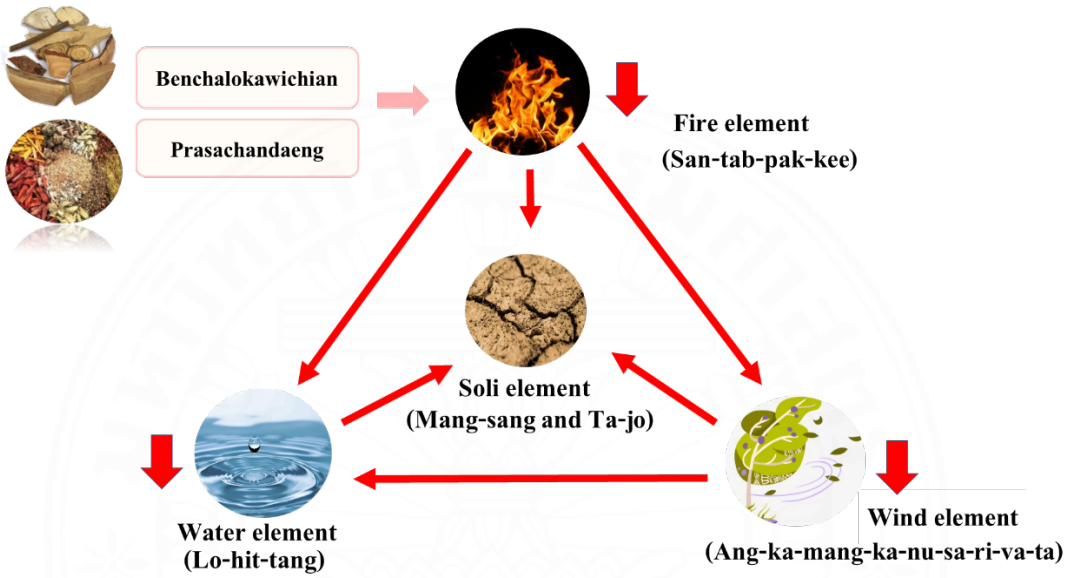



**Figure 2.11** Mechanisms of drug formulas on pathogenesis in Thai traditional medicine 1

## 2.12 Data of the plant ingredients in Benchalokawichian or Harak remedy

### 2.12.1 Benchalokawichian remedy

#### 2.12.1.1 General data of Benchalokawichian remedy

Benchalokawichian remedy is an antipyretic drug in Thai traditional medicine. Benchalokawichian is roots of five herbs in equal proportions from *Ficus racemose* Linn., *Capparis micracantha* DC., *Clerodendrum indicum* (L.) Kuntze ., *Harrisonia perforate* (Blanco) Merr., *Tiliacora triandra* (Colebr.) Diels (Samitinan ,2006). Benchalokawichian remedy (BLW) is a pharmacopeia of the National Drug List and a remedy for fever in Takkasila that has properties to neutralize fever (Thai Medical Promotion Rehabilitation Foundation Ayurveda College (Chiwokkomarapat,1992). Folk healers treat external diseases such as herpes simplex and skin rash.

**2.12.1.2 Biological activity of Benchalokawichian remedy**

In Thai traditional medicine, Benchalokawichian is a drug that can be used both internal and external the body. Internal treatments are used to treat fever and Ya-Kra-Tung-Pid. External treatment is used to decrease heat in the skin, rash, herpes, and shingles. Folk healers have been used to treat patients for a long time that found a research study on Benchalokawichian medicine for anti-inflammatory, anti-allergic, anti-nociceptive, and antipyretic effects.

**Table 2.1** Biological activity of Benchalokawichian remedy

| Activities | Results | References |
|---|---|---|
| Anti-inflammatory | Ethanolic extract of Benchalokawichian inhibits activity on the release of inhibitory activities against lipopolysaccharide ( LPS) induced nitric oxide ( NO) production in RAW 264. 7 cell lines with $IC_{50}$ value of 40.36 µg/mL. | Juckmeta & Itharat, 2012 |
| Anti-allergic | Pectolinarigenin and *O*- methylalloptaeroxylin showed the highest Inhibited effect on the release *β*-hexosaminidase from RBL-2H3 cell with $IC_{50}$ values of 6.3 µg/mL and 14.16 µg/mL, respectively. | Juckmeta *et al*., 2014 |
| Anti-allergic | Ethanolic extract of Benchalokawichian inhibited effect on the release *β*- hexosaminidase from RBL- 2H3 cells with $IC_{50}$ values of 39.8 µg/mL | Juckmeta *et al*., 2014 |

**Table 2.1** Biological activity of Benchalokawichian remedy (Cont.)

| Activities | Results | References |
|---|---|---|
| Anti-inflammatory | Male Wistar rats ( 190- 250 g) were orally treated with Benchalokawichian for 14 days before induced with LPS (6 mg/kg, i.v.). The markers of organ injury/dysfunction and pro-inflammatory cytokines were measured at 6 hours after LPS administration. Benchalokawichian trends to protect against endotoxemia-induced organ injuries and pro-inflammatory cytokines. | Booranasubkajorn *et al*., 2017 |
| Antipyretic | The animals were pretreated orally with 2% Tween 80 solutions, ASA (300 mg/kg) or various doses of Benchalokawichian (25, 50, 100, 200, and 400 mg/kg) for 1 hour before injection of 50 µg/kg of LPS injected intramuscularly into the right thigh of the rat. Rectal temperature was measured before the pre-treatment of animals and at 1 hour intervals for 7 hours after the administration. Benchalokawichian 100 and 200 mg/kg were found to be as potent as ASA. | Jongchanapong *et al*., 2010 |

**Table 2.1** Biological activity of Benchalokawichian remedy (Cont.)

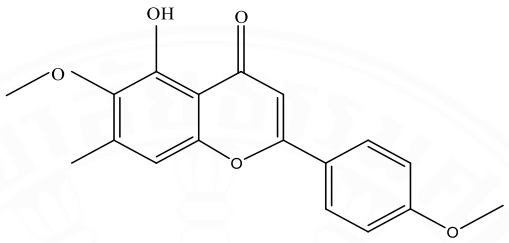
| Activities | Results | References |
|---|---|---|
| Antipyretic | The animals were pretreated orally with 2% Tween 80 solutions, ASA (300 mg/kg) or various doses of Benchalokawichian (25, 50, 100, 200, and 400 mg/kg) for 1 hour before injection of 50 µg/kg of LPS injected intramuscularly into the right thigh of the rat. Rectal temperature was measured before the pre-treatment of animals and at 1 hour intervals for 7 hours after the administration. Benchalokawichian 100 and 200 mg/kg were found to be as potent as ASA. | Jongchanapong *et al*., 2010 |
| Anti-nociceptive | The ethanolic extract of Benchalokawichian decreased pain of rats by Mouse Hot-Plate, Mouse Tail-Flick, Acetic acid-induced writhing test found that %MPE-min Benchalokawichian all dose ($p<0.05$) decrease pain of rat. | Jongchanapong *et al*., 2010 |

**Table 2.1** Biological activity of Benchalokawichian remedy (Cont.)

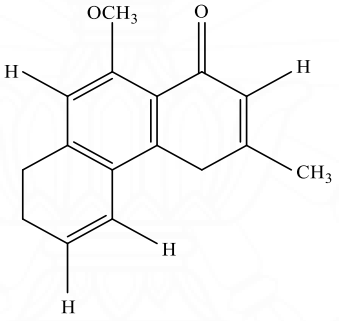
| Activities | Results | References |
|---|---|---|
| Anti-inflammatory | The 80% ethanolic Benchalokawichian and its components Inhibited effects on COX mRNA expression. IL-1β (1 ng/mL) significantly increased the COX-1 and COX-2 mRNA expressions relative to the untreated group of human umbilical vein endothelial cells ($p < 0.05$). Treatment with or without indomethacin (100 µg/mL) in the IL-1B-induced human umbilical vein endothelial cell significantly attenuated the mRNA expressions of COX-1 and COX-2 ($p < 0.05$). | Palo *et al*., 2017 |

**2.12.1.3 Chemical constituents of Benchalokawichian or Harak remedy**

Benchalokawichian has many important chemical components, including pectolinarigenin, *O*-methylalloptaeroxylin **(Figure 2.12)** (Juckmeta *et al*., 2014).




Pectolinarigenin




*O*- methylalloptaeroxylin

**Figure 2.12** Chemical structure of Benchalokawichian (Juckmeta *et al*., 2014)

### 2.12.2 *Ficus racemose* L.

#### 2.12.2.1 General data of *F. racemose*

*F. racemosa* (Moraceae) has different dialects, such as Maduexutthumphon, Duexkeliyng, Maduex, Duexnum, and Maduexkhong. **(Figure 2.13)** Description of *F. racemosa* has small to medium-sized trees and a height 5-20 meters. The branches are covered with reddish-brown hair. The leaf has an alternate, simple leaf, ovate shape, acute apex, and obtuse base. Leaf are arranged alternately, sharp leaf and petiole length 10.5 cm. Leaf has shown in **Figure 2.15**. These flowers are small and cluster. Fruits have an oval shape when ripe turns dark red **(Figure 2.14)** (Phargarden Faculty of Pharmaceutical Sciences Ubon Ratchathani University, 2010).

**Part used:** Root

**Traditional use:** Reduce fever

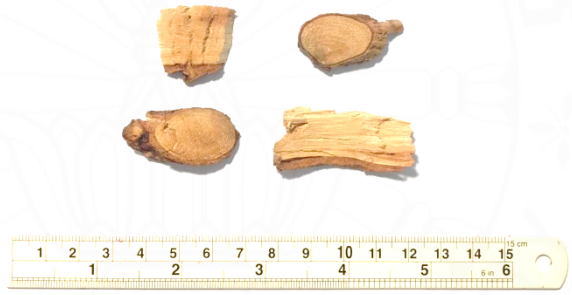


**Figure 2.13** Root of *F. racemosa*

**Figure 2.14** Fruits of *F. racemosa* (Shah.S.K., *et al*.)

**Figure 2.15** Leaves and fruits of *F. racemosa*

(Available from https://pfaf.org/user/Plant.aspx?LatinName=Ficus+racemosa)

#### 2.12.2.2 Biological activity of *F. racemose*

**Table 2.2** Biological activity of *F. racemose*

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *F. racemose* | Root | Anti-allergic | The ethanolic extract exhibited *β*- hexosaminidase effect, $IC_{50}$ value of 15.92 µg/ml. | Thapphung *et al.,* 2009 |
| *F. racemose* | Root | Anti-allergic | The ethanolic extract shows the anti- allergic activity with $IC_{50}$ values of 27.1 µg/mL. | Juckmeta *et al*., 2014 |
| *F. racemose* | Root | Anti-inflammatory | The ethanolic extract exhibited an anti-inflammatory effect exhibited NO with $IC_{50}$ value of 70.15 µg/ml. | Suranat *et al.,* 2009 |
| *F. racemose* | Root | Anti-inflammatory | The ethanolic extract inhibited activity on the release of inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines with $IC_{50}$ value >100 µg/mL. | Juckmeta & Itharat, 2012 |

**Table 2.2** Biological activity of *F. racemose* (Cont.)

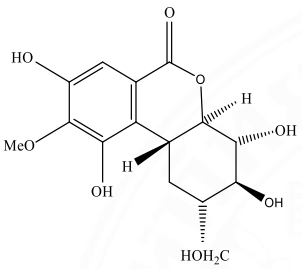
| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *F. racemose* | Wood | Anti-HIV | The ethanolic extract and water extract exhibited HIV-1 IN activity with $IC_{50}$ values of 7.8 µg/ml and 29.5 µg/ml, respectively. | Bunluepuech & Supinya, 2009 |
| *F. racemose* | Root | Antipyretic | The ethanolic extract was tested for Antipyretic activity in rats with a lipopolysaccharide (LPS) value of 50µg/kg. Extract injected acetylsalicylic acid concentration 50, 100, 200, and 400 mg/kg under the skin of the right thigh in the rat. The fever reduced to 36.99 ± 0.16 ℃, 36.94 ± 0.14 ℃, 36.89 ± 0.12 ℃, 37.04 ± 0.18 ℃, respectively. | Chomchuen *et al*., 2010 |

**Table 2.2** Biological activity of *F. racemose* (Cont.)

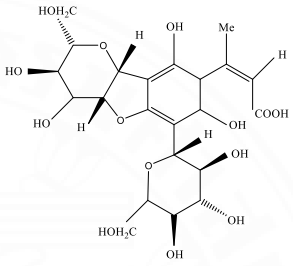
| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *F. racemose* | Bark | Anti-inflammatory | The value of $IC_{50}$ of cold water and hot water extract was 3,691. 97 and 4,207. 1 μg/ ml, respectively. The value of $IC_{50}$ of ibuprofen and prednisolone was 13,377.5 and 11,812.18 μg/ml, respectively. | Dharmadeva, Galgamuwa, Prasadinie, & Kumarasinghe, 2018 |
| *F. racemose* | Leaf | Anti-inflammatory | The extract (400 mg: kg) exhibited maximum anti-inflammatory effect, that is 30. 4, 32. 2, 33. 9, and 32. 0% at the end of 3 hours with carrageenin, serotonin, histamine, dextran- induced rat paw edema, respectively compared phenylbutazone. | Mandal, Maity, Das, Saba, & Pal, 2000 |

### 2.12.2.3 Chemical constituents of *F. racemose*

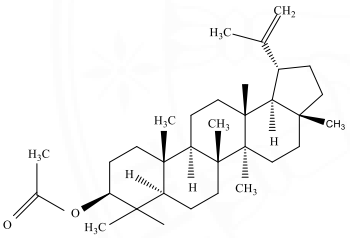
*F. racemose* has many important chemical components, including bergenin, racemosic acid, lupeol, lupeol acetate, α-amyrin, β-amyrin, β-sitosterol, stigmasterol, coumarin, friedelin, kaempherol, ergbenin, bergapten, gallic acid, ellagic acid, rutin, arabinose, racemosic acid, show in **Figure 2.16** (Li *et al*., 2004; Ahmed & Urooj, 2010).



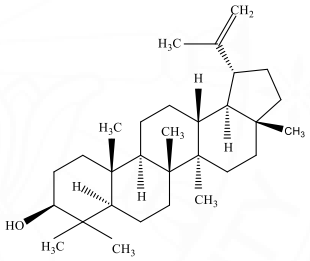
Bergenin



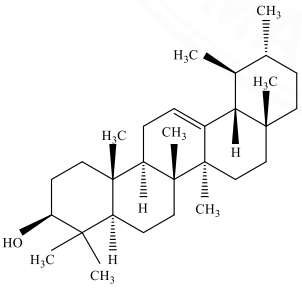
Racemosic acid



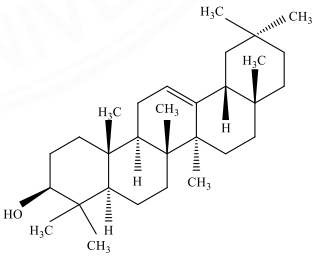
Lupeol



Lupeol acetate

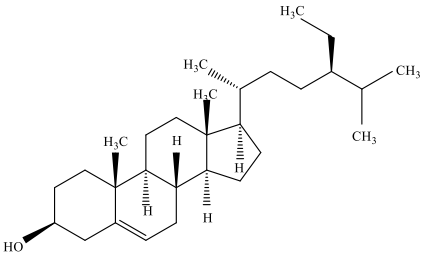


α-amyrin

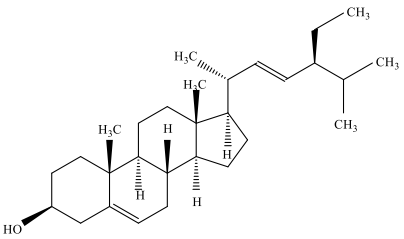


β-amyrin

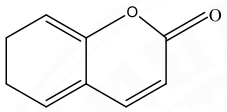
**Figure 2.16** Structures of phytochemicals identified of *F. racemose* (Li *et al*., 2004; Ahmed & Urooj, 2010)



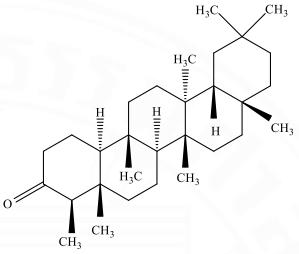
Stigmasterol



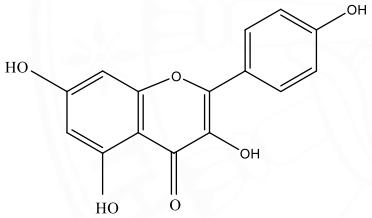
Stigmasterol



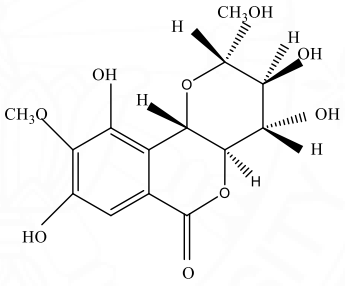
Coumarin



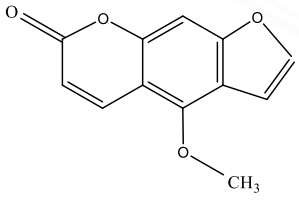
Friedelin



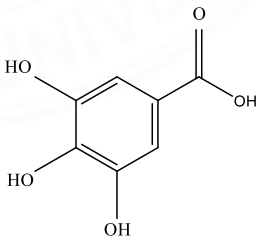
Kaempherol



Bergenin



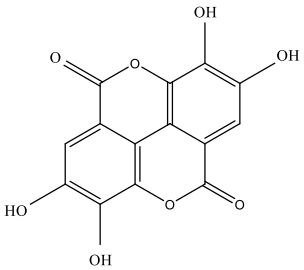
Bergapten



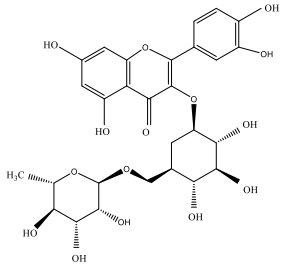
Gallic acid

**Figure 2.16** Structures of phytochemicals identified of *F. racemose* (Cont.)

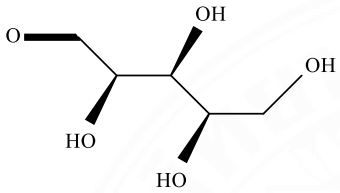
(Li *et al*., 2004; Ahmed & Urooj, 2010)



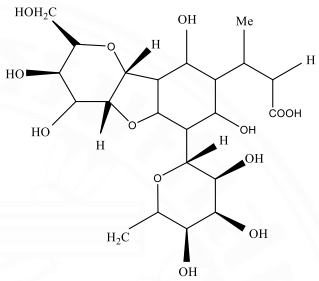
Gallic acid



Rutin



Arabinose



Racemosic acid

**Figure 2.16** Structures of phytochemicals identified of *F. racemose* (Cont.)

(Li *et al*., *2004*; Ahmed & Urooj, 2010)

### 2.12.3 *Capparis micracantha* DC.

#### 2.12.3.1 General data of *C. micracantha*

*C. micracantha* (Capparaceae) **(Figure 2.17)** has different dialects, such as rakkrarokyai, rakkonkong raksamaysor, and rakmark. Description of *C. micracantha* has shrub. The simple leaf has an ovate shape. The leaf shows in figure 2.20. The flower is tongue-shaped. The outer lobe has 2 petals, and petals have yellow or red. The flower is shown in **Figure 2.18**. The stamens have small, white, round, when ripe sweet flesh (Phargarden Faculty of Pharmaceutical Sciences Ubon Ratchathani University, 2010).

**Part used:** Root

**Traditional use:** Reduce fever, Gastritis, Cancer

**Figure 2.17** Root of *C. micracantha*

**Figure 2.18** Leaf and flower of *C. micracantha*

(Available from http://www.dnp.go.th/botany/mindexdictdetail.aspx?runno=1827)

#### 2.12.3.2 Biological activity of *C. micracantha*

**Table 2.3** Biological activity of *C. micracantha*

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *C. micracantha* | Root | Anti-allergic | The ethanolic extract has the effect of reducing allergenic by inhibiting *β*- hexosaminidase with $IC_{50}$ value of 9.50 µg/mL. | Thapphung *et al.,* 2009 |
| *C. micracantha* | Root | Anti-inflammatory | The ethanolic extracted inhibit activity on the release against lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264. 7 cell lines with $IC_{50}$ value of 61.35 µg/mL. | Juckmeta & Itharat, 2012 |
| *C. micracantha* | Wood | Anti-HIV | The extract of water and ethanolic inhibited the activity of HIV-1 IN found that the $IC_{50}$ value of > 100 µg/mL. | Bunluepuech & Supinya, 2009 |

### 2.12.4 *Clerodendrum indicum* (L.) Kuntze

#### 2.12.4.1 General data of *C. indicum*

*C. indicum* (Lamiaceae) (**Figure 2.19)** has different dialects names such as mitheayaymom, mitheayase, phayarakdaiw, yarlingjon, kasalong. Description of *C. indicum* has a small shrub with a height of 1-3 meters**.** The simple leaf has a narrow lanceolate shape, acute leaf apex, and arrangement alternately arranged opposite. The wheel-shaped leaves are the appearance of a small bouquet blooming on the leaves. The base of the oval petals has 5 pointed petals, with a saucer of 5 green petals when falling from the ground, the petals are red. Flower show in **Figure 2.20.** The fruit is round (Phargarden Faculty of Pharmaceutical Sciences Ubon Ratchathani University, 2010).

**Part used:** Root

**Traditional use:** Reduce fever

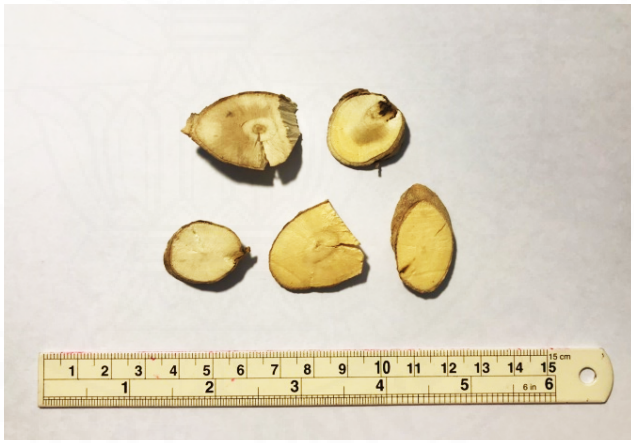


**Figure 2.19** Root of *C. indicum*

**Figure 2.20** Flower of *C. indicum*

(Available from

http://www.qsbg.org/database/botanic_book%20full%20option/search_detail

.asp?botanic_id=911)

**2.12.4.2 Biological activity of *C. indicum***

**Table 2.4** Biological activity of *C. indicum*

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *C. indicum* | Root | Anti-allergic | The ethanolic extract inhibited *β*-hexosaminidase show $IC_{50}$ value of 90.01 µg / ml. | Thapphung *et al.,* 2009 |
| *C. indicum* | Root | Anti-allergic | The ethanolic extract inhibited the anti-allergic activity show $IC_{50}$ values of 57.8 µg/mL. | Juckmeta *et al*., 2014 |
| *C. indicum* | Root | Anti-inflammatory | The ethanolic extract inhibited NO show $IC_{50}$ value of 51.46 µg/mL. | Suranat *et al.,* 2009 |
| *C. indicum* | Root | Anti-inflammatory | The methanolic extract reduced the ear edema of rats at concentrations 1, 2, and 4 mg/ ml, which showed an inhibitory effect of 38. 61% , 53. 47% , and 64. 36% , respectively. | Panthong *et al*., 2003 |
| *C. indicum* | Root | Anti-inflammatory | The ethanolic extract inhibited activity on the release of inhibitory activities against lipopolysaccharide ( LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines with $IC_{50}$ value of 46.55 µg/mL. | Juckmeta & Itharat, 2012 |

**Table 2.4** Biological activity of *C. indicum* (Cont.)

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *C. indicum* | Whole plant | Anti-HIV | The water extract has inhibited the HIV-1 IN virus with $IC_{50}$ value of 43.5 µg/ml. | Bunluepuech & Supinya, 2009 |
| *C. indicum* | Root | Antipyretic | The methanolic extract of *C. indicum* at concentration 300 mg/kg reduced the rectal temperature (induced by subcutaneous injection of yeast) similarly to 300 mg/kg of aspirin. | Panthong *et al*., 2003 |

### 2.12.4.3 Chemical constituents of *C. indicum*

*C. indicum* has many important chemical components, including hispudidin, 5-*O*-ethylclerodendricin, lridiod diglucoside, bungein, clerodermic acid, 1(R) lucumin, uncinatone, 1(R) lucumin, acacetin-7-o-methylglucuronate, serratagenic acid, verbacoside, scutellarin, pectolinarigenin **(Figure 2.21)** (Shrivastava & Patel, 2007; Somwong & Suttisri, 2018).

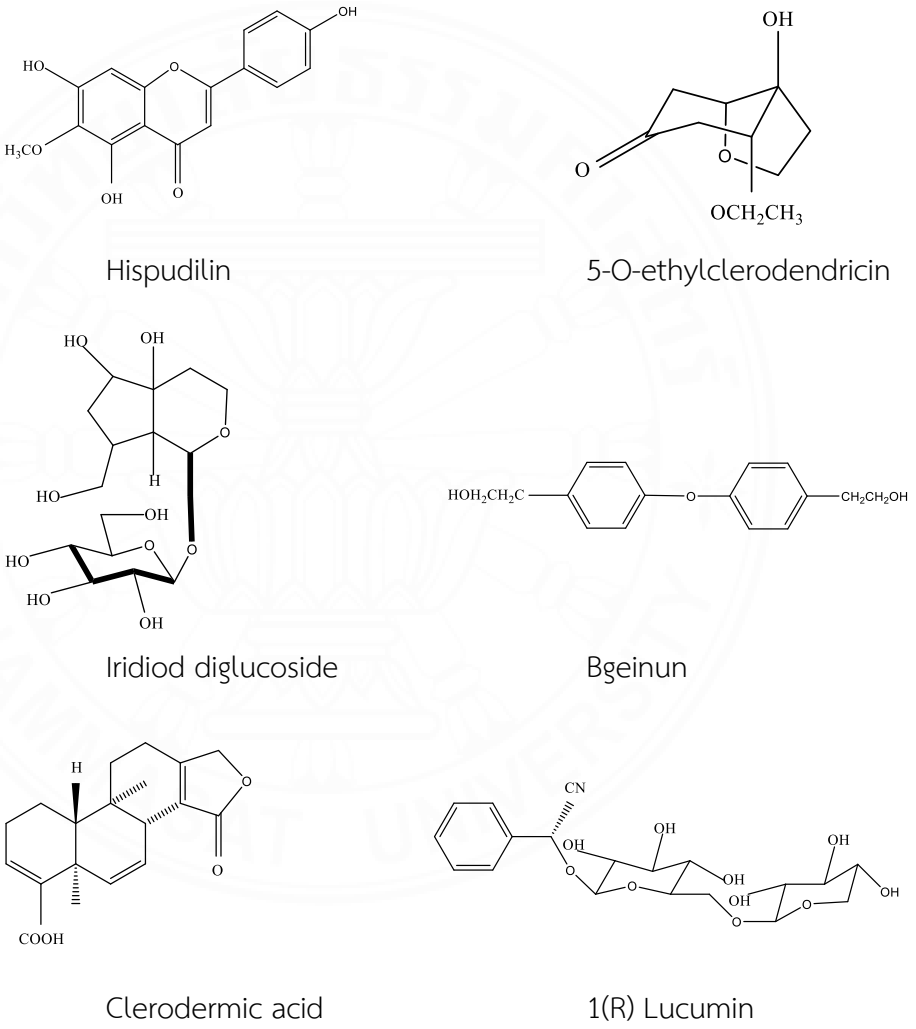
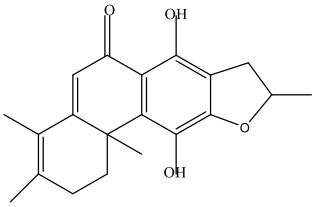
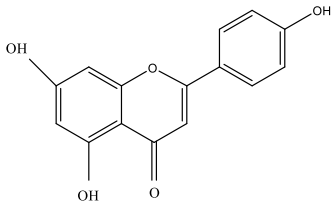


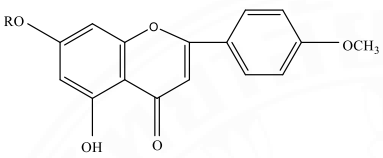

**Figure 2.21** Major chemical of *Clerodemdrum* genus
(Shrivastava & Patel, 2007; Somwong & Seruttisri, 2018)



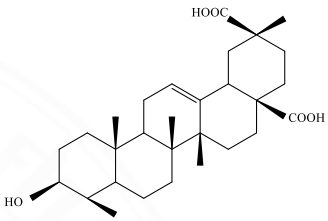
Uncinatone



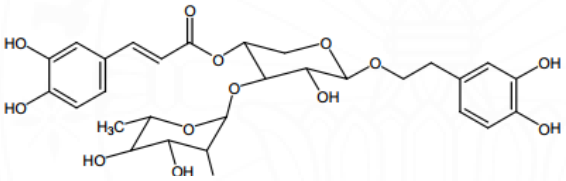
1(R) Lucumin



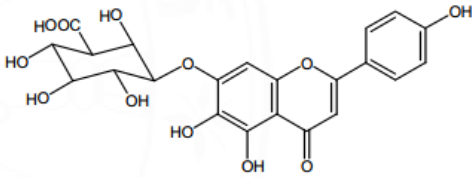
Acacetin-7-o-methylglucuronate



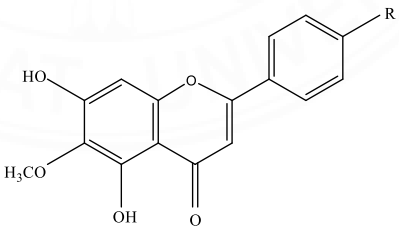
Serratagenic acid



Verbacoside



Scutellarin



Pectolinarigenin and Hispidurin

**Figure 2.21** Major chemical of *Clerodemdrum* genus (Cont.)

(Shrivastava & Patel, 2007; Somwong & Suttisri, 2018)

### 2.12.5 *Harrisonia perforate* (Blanco) Merr.

#### 2.12.5.1 General data of *H. perforate*

*H. perforate* (Simaroubaceae) (**Figure 2.22)** has different dialects names such as rakkontha, rakkalantha, rakkotha, raksifan and raknamcie. Description of *H. perforate* has shrub. The trunk has gray and spikes at the base. The leaf has an odd-pinnately compound leaf, obovate shape, obtuse apex, attenuate base, and serrated margin. The inflorescence from the trunk, the leaves near the end of the branches, 5 rounded petals, slender, red on the outside show in **Figure 2.24**. The fruit is round, shown in **Figure 2.23** (Phargarden Faculty of Pharmaceutical Sciences Ubon Ratchathani University, 2010).

**Part used:** Root

**Traditional use:** Reduce fever, Diarrhea

**Figure 2.22** Root of *H. perforate*

**Figure 2.23** Fruit of *H. perforate*
(Available from http://www.qsbg.org/Database/plantdb/mdp/medicinal-specimen.asp?id=770)

**Figure 2.24** Flower of *H. perforate*
(Available from
https://www.teaoilcenter.org/%E0%B8%84%E0%B8%99%E0%B8%97%E0%B8%B2/)

#### 2.12.5.2 Biological activity of *H. perforate*

**Table 2.5** Biological activity of *H. perforate*

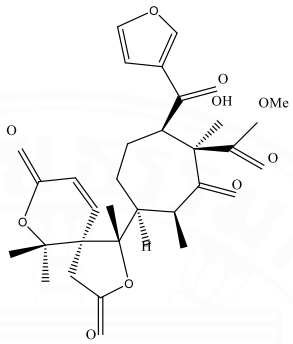
| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *H. perforate* | Root | Anti-allergic | The ethanolic extract inhibited ***β***- hexosaminidase show $IC_{50}$ value of 84.40 µg/mL. | Thapphung *et al.,* 2009 |
| *H. perforate* | Root | Anti-allergic | The ethanolic extract shows the anti- allergic activity with $IC_{50}$ values of 14.5 µg/mL. | Juckmeta *et al*., 2014 |
| *H. perforate* | Root | Anti-inflammatory | The ethanolic extract inhibited activity on the release of inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines with $IC_{50}$ value of 53.16 µg/mL. | Juckmeta & Itharat, 2012 |
| *H. perforate* | Fruits and roots | Anti-inflammatory | Harperfolide exhibited potent anti- inflammatory activity by suppressing nitric oxide ( NO) production from activated macrophages with $IC_{50}$ value of 6.51 µM. | Choodej, Sommit, & Pudhom, 2013 |

**Table 2.5** Biological activity of *H. perforate* (Cont.)

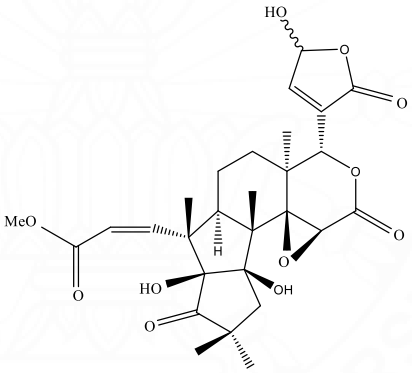
| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *H. perforate* | Wood | Anti-HIV | The water extract has the effect of suppressing the HIV-1 IN virus with $IC_{50}$ value of 2.3 µg/mL. | Bunluepuech & Supinya, 2009 |
| *H. perforate* | Root | Anti-inflammatory | The ethanolic extract inhibited response to carrageenan occurred at the second hour after *H. perforata* at 50, 100, 200, 400 mg/ kg administration. The percentage of Inhibition of 28.49, 31.18, 47.85, and 65.05, respectively, while indomethacin 5 mg/kg caused 37.10% inhibition of rat paw edema. | Somsil, Ruangrungsi, Limpanasitikul, & Itthipanichpong, 2012 |

### 2.12.5.3 Chemical constituents of *H. perforate*

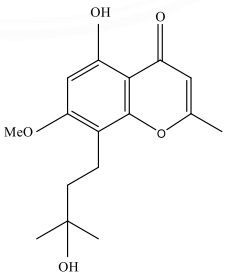
Root bark *H. perforate* has many important chemical components, including harperforatin, herperfolide, and harperamone **(Figure 2.25)** (Choodej *et al*., 2013).




Harperforatin




Harperfolide




Harperamonethat

**Figure 2.25** Structure of *H. perforate* (Choodej *et al*., 2013)

### 2.12.6 *Tiliacora triandra* (Colebr.) Diels

#### 2.12.6.1 General data of *T. triandra*

*T. triandra* ( Menispermaceae) **(Figure 2.26)** has different dialects names such as and chynang (Chiang Mai), Theawaikheyw(Middle), Puceakheakheyw, Yapakani, Theayanang, Wanya (Suratthani) . Description of *T. triandrai* is creeping stem and rhizomes. Wood wrapped around trees or twigs. The vine is green and has a length of 10-15 meters. The branches of the dish have a scar. The stalk is hairy. The leaf has a compound leaf, that is ovate leaf. The leaf arrangement is an alternate distichous. The leaf is shown in **Figure 2.27.** The inflorescence is raceme that into a bouquet of small. The aggregate fruit is hard seeds. The seed is horseshoe-shaped. The fruit shows in **Figure 2.28** (Phargarden Faculty of Pharmaceutical Sciences Ubon Ratchathani University, 2010).

**Part used:** Root

**Traditional use:** Reduce fever, Chicken pox, Constipation

**Figure 2.26** Root of *T. triandra*

**Figure 2.27** Leaf of *T. triandra*

(Available from http://www.phargarden.com/main.php?action=viewpage&pid=148)

**Figure 2.28** Fruit of *T. triandra*

(Available from http://www.phargarden.com/main.php?action=viewpage&pid=148**)**

#### 2.12.6.2 Biological activity of *T. triandra*

**Table 2.6** Biological activity of *T. triandra*

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *T. triandra* | Root | Anti-allergic | The ethanolic extract inhibited *β*-hexosaminidase with $IC_{50}$ value of 10.33 µg/mL. | Thapphung *et al.*, 2009 |
| *T. triandra* | Root | Anti-inflammatory | The ethanolic extract inhibited activity on the release of activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines with $IC_{50}$ value of 54.65 µg/mL | Juckmeta & Itharat, 2012 |
| *T. triandra* | Stem | Anti-HIV | The ethanolic extract and water extract exhibited HIV-1 IN activity with $IC_{50}$ value of >100 µg/ml. | Bunluepuech & Supinya, 2009 |

### 2.12.6.3 Chemical constituents of *T. triandra*

*T. triandra* has many important chemical components, including alkaloids such as tiliacorinine, 2'-nortiliacorinine, tiliacorine, 13'-bromo-tiltacorinine (**Figure 2.29**) (Sureram *et al*., 2012)**.**

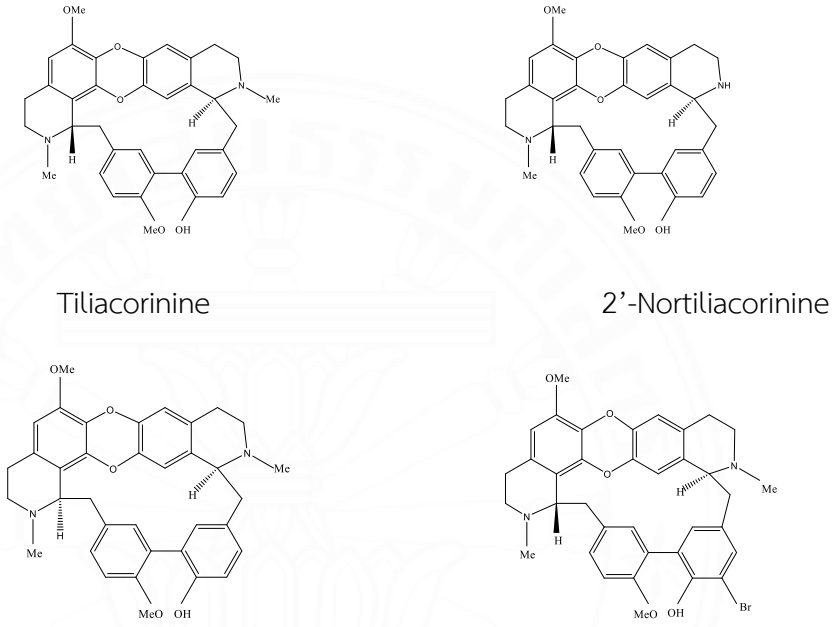



**Figure 2.29** Chemical structure of *T. triandra* (Sureram *et al.*, 2012)

## 2.13 Data of the plant ingredients in Prasachandaeng remedy

### 2.13.1 Prasachandaeng remedy

#### 2.13.1.1 General data of Prasachandaeng remedy

Prasachandaeng remedy is a recipe used by traditional healers to treat ailments for a long time. Prasachandaeng contains 12 kinds of herbs from root of *Bouea macrophylla* Griff., stem of *Caesalpinaia sappan* L., root of *Citrus aurantifolia* Swing., stem of *Dracaena cochinchinensis* Gagnep., flower of *Jasminum sambac* L., root of *Helicia terminalis* Kurz., rhizome of *Kaempferia galangal* L., rhizome of *Ligusticum chuanxiong* Hort., flower of *Mammea siamensis* Kosterm., flower of *Mesua ferrea* L., stem of *Myristica fragrans* Houtt., and flower of *Nelumbo nucifera* Gaertn. Prasachandaeng contains *D. cochinchinensis* of 50 percent and other herbs in equal proportions of 50 percent. It has been recorded in the Thai traditional medical textbook, namely Thai medical education textbook Volume 1 of Phraya Phitsanu Prasatwet (Phraya Phitsanu Prasatwet, 1908). Announcements of the National Drug System Development Committee are drug formulas in the National List of Essential Medicine 2019 and mentioned properties in treating fever thirst (National List Essential Medicines, 2019).

### 2.13.1.2 Biological activity of Prasachandaeng

**Table 2.7** Biological activity of Prasachandaeng

| Activities | Results | References |
|---|---|---|
| Antipyretic | The ethanolic extract of Prasachandaeng inhibited antipyretic activity on the production of NO and PGE with the $IC_{50}$ values of 42.40 ± 0.72 and 4.65 ± 0.76 µg/mL, respectively. It compares Acetaminophen (ACP), A standard drug that decreases fever, inhibits NO and PGE activity, with the $IC_{50}$ values of 99.50 ± 0.43 and 6.110 ± 0.661 µg/mL, respectively. | Prommee *et al*, 2021 |
| Anti-inflammatory | The extract inhibited Nitric Oxide (NO) is induced by Lipopolysaccharide (LPS). The results found that the water extract of prasachandaeng inhibited the ethanolic extract of prasachandaeng with $IC_{50}$ value of 16.87±2.51 and 39.70±1.48 µg/ml, respectively. | Sangphum, 2016 |

### 2.13.2 *Bouea macrophylla* Gritt.

#### 2.13.2.1 General data of *B. macrophylla*

*B. macrophylla* (Anacardiaceae) (**Figure 2.31**) has different dialects names such as Maprang, Bak Prang (Northeast), Mapang (North), Prang (South). Description of *B. macrophylla* is a medium-sized tree, tall 20-30 m, rough, scaly bark, light brown and white latex. The leaves are single, alternating, arranged, hard, lanceolate, smooth on both sides, undulate, acuminate leaf tip, obtuse base, 7-9 cm wide, 20-28 cm long, 1-2 cm long petioles. The flower is an inflorescence of small branches, yellow, with 3-5 petals and calyx, usually behind the leaves. The fruit is soft, green, and yellow. The fruit is shown in **Figure 2.30**. The peel is smooth and glossy (Faculty of Natural Resources Prince of Songkla University, 2020).

**Part used:** Root

**Traditional use:** Reduce fever, Detoxify

**Figure 2.30** Fruit of *B. macrophylla*
(Available from
http://www.qsbg.org/Database/Botanic_Book%20full%20option/search_
detail.asp?botanic_id=3131)

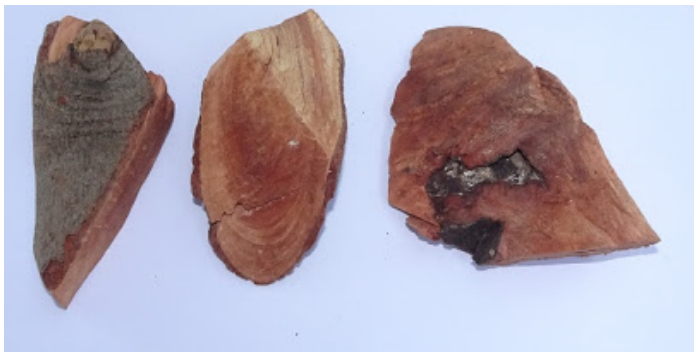


**Figure 2.31** Root of *B. macrophylla*
(Available from http://hinfo.su.ac.th/~kuiherb/kui-herb-web/details.php?cat=1&id=8&herb_id=503)

### 2.13.3 *Caesalpinia sappan* L.

#### 2.13.3.1 General data of *C. sappan*

*C. sappan* (Leguminosae) has different dialects names such as keang, fangsom, fangsen, mankhong (Vudhithammavej, 2009). Description of *C. sappan* is the high tree is 5-13 m. The secondary rachis leaf; oblong width 5-10 mm. length 8-20 mm., emarginated leaf, truncate leaf, entire leaf, short petioles. The yellow flower is 5 sepals and 5 petals. The legume fruit is brown and width 3-4 cm. length 5-8.5 cm. oval seeds are 2-4 seeds, wide is 0.8-1 cm, long is 1.5-1.8 cm, found in limestone forest, limestone forest, dry evergreen and bloom between June to December, The legume fruit is shown in **Figure 2.32** (Faculty of Natural Resources Prince of Songkla University, 2010).

**Part used:** Stem **(Figure 2.33)**

**Traditional use:** Nourish the blood, Reduce fever, Treat diarrhea

**Figure 2.32** Seed of *C. sappan*

(Available from https://www.shutterstock.com/th/search/caesalpinia+sappan+l)

**Figure 2.33** Stem of *C. sappan*

(Available from http://www.thaicrudedrug.com/main.php?action=viewpage&pid=88)

#### 2.13.3.2 Biological activity of *C. sappan*

**Table 2.8** Biological activity of *C. sappan*

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *C. sappan* | Heartwood | Anti-influenza virus (H3N2) | Sappanchalcone inhibited the activity of influenza virus (H3N2) with CPE reduction assay that found $IC_{50}$ values of 2.06 μg/mL. It compares oseltamivir acid and ribavirin of positive control with $IC_{50}$ values of 0.065 and 9.17 μg/mL, respectively. | Liu *et al*., 2009 |
| *C. sappan* | Heartwood | Anti-influenza virus (H3N2) | Oxysappanchalcone inhibited the influenza virus (H3N2) activity with CPE reduction assay that found $IC_{50}$ values of 1.06 μg/mL. It compares oseltamivir acid and ribavirin of positive control with $IC_{50}$ values of 0.065 and 9.17 μg/mL, respectively. | Liu *et al.,* 2009 |
| *C. sappan* | Pure compound | Anti-JEV effects | Quercetin exhibited anti-JEV effects with an $IC_{50}$ value of 212.1 μg/mL that the JEV were treated with the compound after virus adsorption. The SI value of 1.2. | Johari *et al*., 2012 |

**Table 2.8** Biological activity of *C. sappan* (Cont.)

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *C. sappan* | Pure compound | Anti HSV-1 and HSV-2 | Group of flavonoids inhibited HSV-1, HSV-2 in Vero cell by virus-induced cytopathic effect (CPE) inhibitory assay, plaque reduction assay, and yield reduction assay that result in high inhibited HSV-1 and HSV-2. | Lyu *et al.,* 2005 |
| *C. sappan* | Pure compound | Anti-dengue virus type -2 (DENV-2) | Quercetin inhibited the activity of dengue virus type -2 ( DENV- 2) , and pre- treatment with the compounds showed that 50 μg $mL^{-1}$ of quercetin could decrease the number of DENV-2 foci by 14% ± 1.5 when compared to the non- treated cells. In the post- adsorption assay, quercetin exhibited the most significant anti-viral activity against DENV-2 amongst the bioflavonoids tested with $IC_{50}$ value of 35.7 μg $mL^{-1}$. The SI value for quercetin in the post-adsorption assay was relatively high at 7.07. | Zandi *et al*., 2011 |

**Table 2.8** Biological activity of *C. sappan* (Cont.)

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *C. sappan* | Pure compound | Anti-inflammatory | Brazilin and protosappanin D inhibited NO production $IC_{50}$ values of 3.7 μM and 9.6 μM, respectively. Protosappanin A and protosappanin E showed inhibited NO production with 11.2 μM and 25.6 μM. | Washiyama *et al.*, 2009 |
| *C. sappan* | Pure compound | Anti-inflammatory | Sappanchalcone and protosappanin D showed Inhibition of $PGE_2$ production by J774.1 with the $IC_{50}$ values of 7.7 μM and 7.8 μM, respectively, and then protosappanin C and protosappanin E showed Inhibition with 22.6 μM and 22.9 μM, respectively. | Washiyama *et al*., 2009 |
| *C. sappan* | Pure compound | Anti-rhinovirus | A concentration of 10 μM quercetin attenuated both RV( rhinovirus) and UV-RV-stimulated IL-8 responses by 75–80% | Ganesan *et al*., 2012 |

**Table 2.8** Biological activity of *C. sappan* (Cont.)

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *C. sappan* | Pure compound | Anti-equid herpesvirus 1 | Quercetin has Inhibited anti- equid herpesvirus 1 in virucidal action and the viral replication cycle. It showed Inhibition of anti-viral at 0 and 1 hour. | Gravina *et al*., 2011 |
| *C. sappan* | Roots and heartwood | Anti-allergic | Dichloromethane extract of the roots and heartwood exhibited *β*- hexosaminidase in the rat. It shows inhibition of 98.7% and 87.5% at the concentration of 1 0 0 µg/ ml, respectively. The compounds of sappanchalcone possessed the most potent effect against allergic reaction in RBL- 2 H3 cells with an inhibitory concentration ($IC_{50}$) value of 7.6 µM, followed by 3-deoxysappanchalcone $IC_{50}$ value of 15.3 µM. | Yodsaoue *et al*., 2009 |

**Table 2.8** Biological activity of *C. sappan* (Cont.)

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *C. sappan* | Pure compound | Anti-inflammatory | TNF-a mRNA expression, protosappanin A, protosappanin D and protosappanin E showed Inhibition with the $IC_{50}$ values of 12.3 μM, 12.6 μM and 14.2 μM, respectively. Regarding IL-6 mRNA expression, protosappanin D and sappanchalcone showed Inhibition with the $IC_{50}$ values of 3.0 μM and 17.4 μM, respectively. Regarding COX-2 mRNA expression, protosappanin D showed Inhibition with the $IC_{50}$ value of 21.4 μM. Regarding iNOS mRNA expression, brazilin, protosappanin D, and sappanchalcone showed Inhibition with the $IC_{50}$ values of 3.6 μM, 13.2 μM and 16.6 μM, respectively. | Washiyama *et al*., 2009 |

**Table 2.8** Biological activity of *C. sappan* (Cont.)

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *C. sappan* | Pure compound | Anti-influenza A virus | The cytopathic effect (CPE) of quercetin against influenza A virus A/Puerto Rico/8/34 (H1N1), A/FM-1/47/1 (H1N1), A/Aichi/2/68 (H3N2) at 48 hours post-infection with $IC_{50}$ value of 7.76, 6.23, 2.74 μg/mL, respectively. | Wu *et al*., 2015 |
| *C. sappan* | Stem | Anti-inflammatory | Anti-inflammatory activity was evaluated by inhibiting Nitric Oxide (NO), induced by Lipopolysaccharide (LPS). The ethanolic and water extracts of *C. sappan* stem inhibited anti-inflammatory activity with $IC_{50}$ value of 5.42±0.24 μg/ml and 7.60±0.28 μg/ml, respectively. | Sangphum *et al*., 2016 |

### 2.13.3.3 Chemical constituents of *C. sappan*

*C. sappan* has many important chemical components, including flavonoid, brazilin, protosappanin A, protosappanin D, sappanchalcone, protosappanin B, protosappanin C, and protosappanin E **(Figure 2.34)** (Lyu *et al*., 2005; Washiyama *et al.,* 2009).

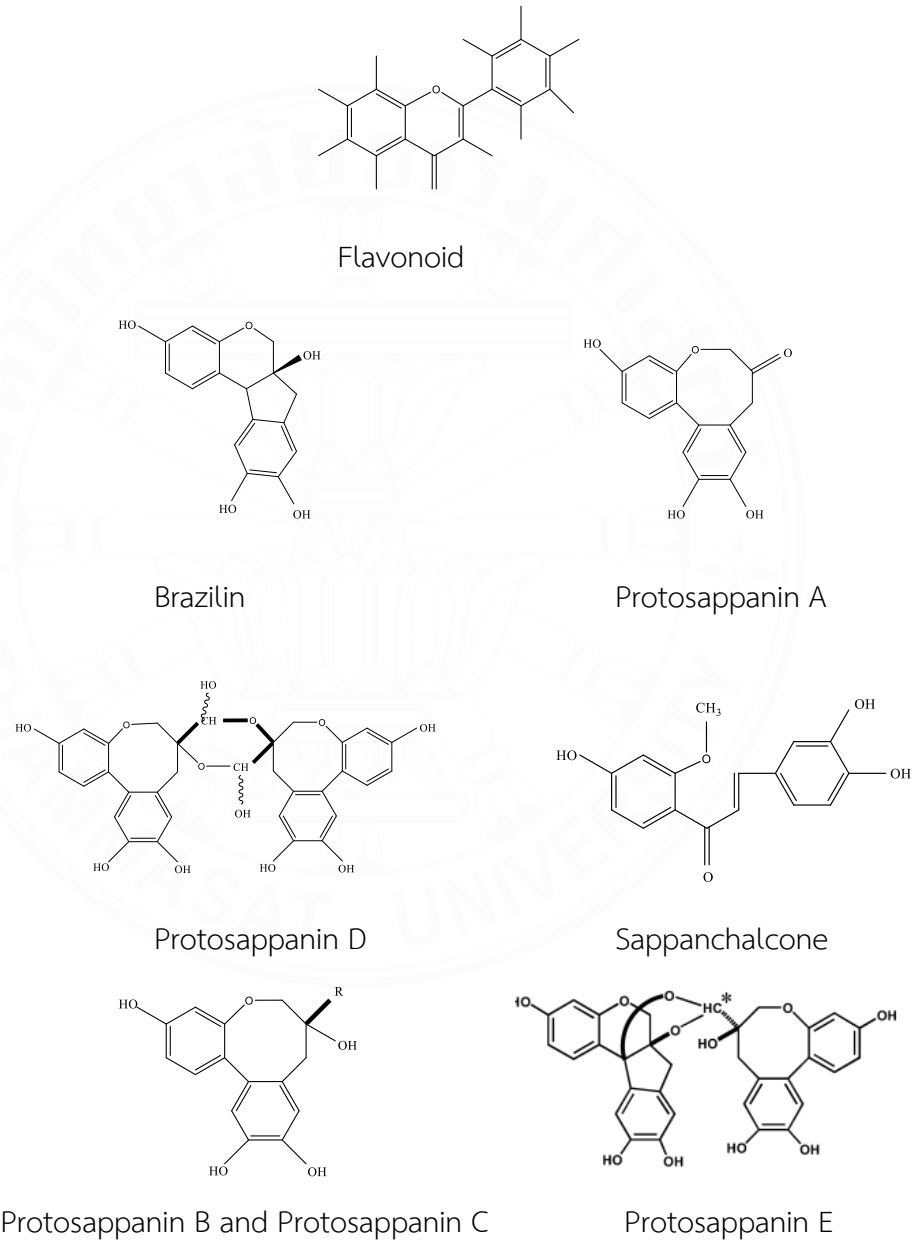



**Figure 2.34** Chemical structure of *C. sappan* (Lyu *et al*., 2005; Washiyama *et al.,* 2009)

### 2.13.4 *Citrus aurantifolia* (Christm.) Swing.

#### 2.13.4.1 General data of *C. aurantiifolia*

*C. aurantiifolia* (Rutaceae) has different dialects names such as sommanow (Central), Som Now (South), Kroksa Ma (Khmer-Surin), Mak Fah (Thai Yai - Mae Hong Son) (Wud Vudhithammavej, 2009). Description of *C. aurantiifolia* is a shrub 2-4 meters high. The trunk bark is gray-brown, the branches are light green on the trunk. The branches have hard spines. The leaves are composed of a leaf, alternate, light green, elongated or oval shape. The leaf apex has an acute leaf. The flowers are single flowers or inflorescences, forming the axillary area and the tips of the branches, white petals. The sepal is light green. The pistil is cylindrical-shaped. The fresh fruit is round and elongated or oval, with a width of 3-12 cm long. The shell surface is rough and has oil glands on the surface. The fruit is shown in **Figure 2.35**. The seeds are small, oval-shaped, with a pointed tip. Inside the seeds is white tissue (Faculty of Natural Resources Prince of Songkla University, 2010).

**Part used:** Root

**Traditional use:** Wound healing

**Figure 2.35** Fruit and leaf of *C. aurantiifolia*

(Available from

https://identify.plantnet.org/prosea/species/Citrus%20aurantifolia%20(Christm.)%

20Swingle,%20orth.%20var./data)

### 2.13.5 *Dracaena cochinchinensis* (Lour.) S.C. Chen.

#### 2.13.5.1 General data of *D. cochinchinensis*

*D. cochinchinensis* (Dracaenaceae) has different dialects names such as Chan-daeng, Chan-pha, Lakka-chan (Vudhithammavej, 2009). Description of *D. cochinchinensis* is shrub or tree, up to 1.5-4 m high. The mature tree is maybe up to 17 m high. The straight trunk is a crown tree up to 100 crowns. The smooth bark is gray and maybe branch out from the trunk. A simple leaf is alternately arranged at the apex (Phargarden Faculty of Pharmaceutical Sciences Ubon Ratchathani University, 2010). A small plant with a single leaf is clustered; acuminate leaf width 4-5 cm. length 45-80 cm., no petiole, coriaceous and entire leaves (Vudhithammavej, 2009). The flower is inflorescences that are a large bunch in the axillary and apex. Inflorescences are about 45-100 cm. The small flower is white or green; 6 petals, about 0.7-1 cm, 6 stamens, 3 pistils, calyx tube. The center of the flower has a bright red dot. The fruit is a large bunch of fruit, about 1 cm in size, round shape, greenish-brown. The surface of the fruit is smooth, usually 1 seed. The ripe fruit is dark red. It blooms around July to August and is found in tall limestone mountain forests and full sun. The old tree, the core turns from white to red, called Chan Daeng. The stem is shown in **Figure 2.36**, **2.37.** The tree will gradually become old and die when the core is full red (Phargarden Faculty of Pharmaceutical Sciences Ubon Ratchathani University, 2010).

**Part used:** Stem

**Traditional use:** Scurf, Cough, Antipyretic, Wound healing, Cardiotonic (Vudhithammavej, 2009)

**Figure 2.36** Stem of *D. cochinchinensis*

(Available from http://www.thaicrudedrug.com/main.php?action=viewpage&pid=48)


**Figure 2.37** Stem of *D. cochinchinensis*

(Available from http://www.thaicrudedrug.com/main.php?action=viewpage&pid=48)

#### 2.13.5.2 Biological activity of *D. cochinchinensis*

**Table 2.9** Biological activity of *D. cochinchinensis*

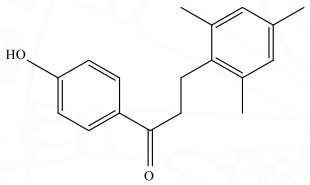
| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *D. cochinchinensis* | Pure compound | Anti-HSV | Resveratrol inhibits HSV-1 and HSV-2 replication, virus attachment and reduces the amount of ICP- 4 . Resveratrol inhibits HSV replication, ribonucleotide reductase, including ICP-4. | Docherty *et al*., 1999; Annunziata *et al*., 2018 |
| *D. cochinchinensis* | Pure compound | Anti-influenza A | Loureiriol inhibited effects against influenza A virus with an $IC_{50}$ value of 49.70 μM. | Pang et al., 2020 |
| *D. cochinchinensis* | Stem wood | Anti-nociceptive | The extracts and fractions from *D. cochinchinensis* inhibited anti- nociceptive activities in experimental animals. Oral administration of the methanol extract and methanol fraction of *D. cochinchinensis* (100-400 mg/kg) in animals decreased the number of writhings and stretchings induced by intraperitoneal acetic acid and licking activity of the late phase in the formalin test compate aspirin (200 mg/ kg). | Reanmongkol *et al.,* 2003 |

**Table 2.9** Biological activity of *D. cochinchinensis* (Cont.)

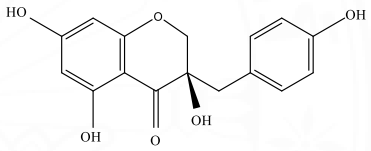
| | | | | |
|---|---|---|---|---|
| *D. cochinchinensis* | Stem wood | Antipyretic | The oral administration yeast induced fever in rats. The methanolic extract and methanol fraction of *D. cochinchinensis* ( 200- 400 mg/ kg) decreased temperature in rats. | Reanmongkol *et al.,* 2003 |
| *D. cochinchinensis* | Pure compound | Anti-inflammatory | Flavonoids and stilbenoids with COX- 1 and COX- 2 inhibitory activity from *D. cochinchinensis* inhibited activities of the enzymes COX- 1 and COX- 2: 4,3' ,5' - trihydroxystilbene ( $IC_{50}$ value of 2. 61 and 2. 16 μM,respectively); 4,3' - dihydroxy- 5' - methoxystilbene ( $IC_{50}$ value of 4. 92 and 2. 21 μM, respectively); 4- hydroxy- 3' ,5' - dimethoxystilbene ( $IC_{50}$ value of 4. 84 and 1.29 μM, respectively) | Likhitwitayawuid *et al.,* 2002 |

### 2.13.5.3 Chemical constituents of *D. cochinchinensis*

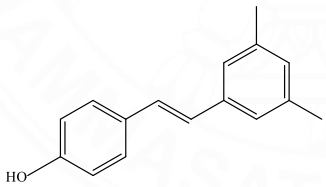
*D. cochinchinensis* has many important chemical components, including Cochinchinensisn D, cochinchinensisns B (94) and D (96), 4,40-dihydroxy-2,6-dimethoxydihydrochalcone ( 97) , 2,40- dihydroxy- 4,6dimethoxydihydrochalcone ( 98) ,3,5,7- trihydroxy- 40- methoxyhomoisoflavanone ( eucomol) , 5,7,40-trihydroxyhomoisoflavanone, and 7,40- dihydroxy- 5- methoxyhomoisoflavanone, Likhitwitayawuid also isolated cochinchinensisol ( 99) , 4,6,40- trihydroxy- 2-methoxydihydrochalcone,4,30,50-trihydroxystilbene (100), 4,30-dihydroxy-50-methoxystilbene ( 101) , and 4- hydroxy- 30,50- dimethoxy- stilbene, Homoisoflavans 69 and 7,10-dihydroxy-11-methoxydracaenone **(Figure 2.38)** (Thu *et al*., 2020).



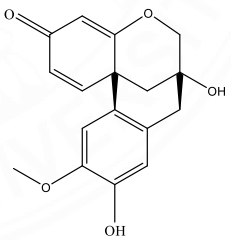

Compounds 93-98




Compounds 99




Compounds 100-102




Compounds 103

**Figure 2.38** Chemical of structure of *D. cochinchinensis* (Thu *et al*., 2020)

### 2.13.6 *Heliciopsis terminalis* (Kurz.) Sleumer.

#### 2.13.6.1 General data of *H. terminalis*

*H. terminalis* (Proteaceae) has different dialects, such as muedkonkhaw, muedkondong, muedkonphu, phrmkht, tikhwanchang. Description of *H. terminalis* is a medium to large tree, about 10-20 meters. The leaves are solitary, lanceolate or ovate, acute leaf apex, attenuate leaf base, 4-8 cm wide, 12-24 cm long, petioles 1.5-3 cm long. The flowers are 15-30 cm long, fragrant white flowers, 12-15 mm long, red pubescent peduncle about half the length. Petals are brown. The peduncle is oval. The fruit is oval, size 2-2.5 x 3-4 cm, smooth surface. The fruit is shown in **Figure 2.39** (Sanga Sabhasri Research and Development Department, The Botanical Garden Organization, 2011).

**Part used:** Root

**Traditional use:** Antipyretic, Pain, Edema, Measles, Chickenpox, Catarrh (Suchart Poovarat. Thai traditional medicine book, 2021)

**Figure 2.39** Fruit of *H. terminalis*

(Available from

http://www.qsbg.org/Database/Botanic_Book%20full%20option/search_detail.asp?botanic_id=864)

### 2.13.7 *Jasminum sambac* (L.) Aiton.

#### 2.13.7.1 General data of *J. sambac*

*J. sambac* (Oleaceae) has different dialects names such as malila, malison, maliphong, maliwan (Phargarden Faculty of Pharmaceutical Sciences Ubon Ratchathani University, 2010). Description of *J. sambac* has a high scandent shrub 1-2 meters. The leaves are single, opposite, oval, or oval. The width of the leaf is 3.5-4.5 cm long and 3.7-11.2 cm. The base is obtuse or acuminate. The flowers are white with a diameter of 2-3 cm. The flowers are single or compound dichasium. The flower is shown in **Figure 2-40 and 2.41.** The leaves are cuneate. Leaf width 0.5-2 mm. and the length of the leaf 0.4-1.2 cm. The fruit is round. The diameter of the fruit is about 6 mm (Picheansoonthon *et al,* 2004).

**Part used:** Flower

**Traditional use:** Cardiotonic, Fatigue, Antipyretic, Stomachache, Headache (Phargarden Faculty of Pharmaceutical Sciences Ubon Ratchathani University, 2010)

**Figure 2.40** Flower of *J. sambac*

(Available from https://www.florihana.com/us/absolutes/675-jasmine-sambac.html)


**Figure 2.41** Flower of *J. sambac*

(Available from http://www.thaicrudedrug.com/main.php?action=viewpage&pid=106)

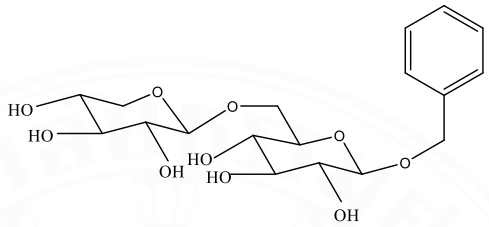
### 2.13.7.2 Biological activity of *J. sambac*

**Table 2.10** Biological activity of *J. sambac*

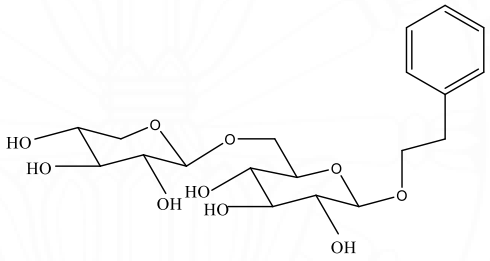
| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *J. sambac* | Roots | Anti-inflammatory | The ethanolic extract inhibited carrageenan-induced rat paw edema compared to diclofenac (10 mg/kg, p.o.). Maximum anti-inflammatory activity was observed at 4 hours. | Sengar *et al.,* 2015 |
| *J. sambac* | Roots | Antipyretic | The ethanolic extract inhibited Antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rats. The result of *J. sambac* (200 mg/kg, p.o.) after 4 hr showed significant ($p > 0.05$) and 400 mg/kg significant after $2^{nd}$ , $3^{rd}$ and $4^{th}$ hr compare paracetamol at $2^{nd}$, $3^{rd}$ and $4^{th}$ hr was substantial ($p>0.001$). | Sengar *et al*., 2015 |
| *J. sambac* | Flower | Anti-adenoviruses (ADV)-3 | The water extract of *J. sambac* inhibited anti-adenoviruses had $EC_{50}$ value between 100—150 mg/ml. | Chiang *et al*., 2003 |

### 2.13.7.3 Chemical constituents of *J. sambac*

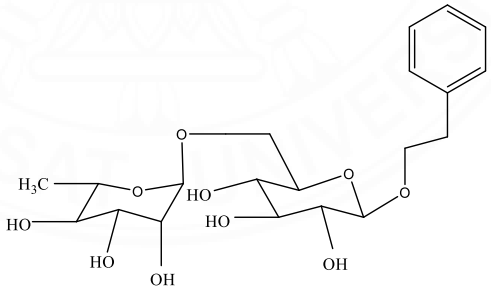
*J. sambac* has many important chemical components, including Benzyl *6-0-* **β** -o-xylopyranosyl- **β** -D-glucopyranoside (**β** -primeveroside), 2-phenylethyl **β** –primeveroside, 2-phenylethyl *6-0-* **α** -L-rhamnopyranosyl- **β** -D-glucopyranoside **(Figure 2.42)** (Inagaki *et al.,*1995).



Benzyl 6-0- **β** -o-xylopyranosyl- **β** -D-glucopyranoside (**β** -primeveroside),



2-phenylethyl **β** –primeveroside



2-phenylethyl 6-0- **α** -L-rhamnopyranosyl- **β** -D-glucopyranoside

**Figure 2.42** Chemical structure of *J. sambac* (Inagaki *et al.,* 1995)

### 2.13.8 *Kaempferia galangal* L.

#### 2.13.8.1 General data of *K. galangal*

*K. galangal* (Zingiberaceae) has different dialects names such as perahomdang, perahomkhaw, wanhom, hompera, wantindin, wanpandinyen (Vudhithammavej, 2009). Description of *K. galanga*l is an underground stem. The leaves are single, elliptic, and ovate. The leaf apex is acute, obtuse. The leaf margin is entire, width 5-10 cm long 7-15 cm. The petioles are long 1-3 cm The inflorescence flower is cymose that long 2-4 cm. The flower is white. The stamen and inferior ovary contain manyovules. The fruit is a capsule. The rhizome is a continuously growing horizontal underground stem shown in **Figure 2.43 and 2.44** (Vudhithammavej, 2009; Faculty of Natural Resources Prince of Songkla University, 2005).

**Part used:** Rhizome

**Traditional use:** Stuffy nose, Cure cold, Carminative, Diarrhea (Vudhithammavej, 2009)

**Figure 2.43** Rhizome of *K. galangal*

(Available from http://gernot-katzers-spice-pages.com/engl/Kaem_gal.html)

**Figure 2.44** Rhizome of *K. galangal*

(Available from http://gernot-katzers-spice-pages.com/engl/Kaem_gal.html)

### 2.13.8.2 Biological activity of *K. galangal*

**Table 2.11** Biological activity of *K. galangal*

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *K. galangal* | Pure compound | Anti-corona virus | Luteolin could also inhibit the 3CLPro of SARS-CoV2 with an $IC_{50}$ value of 20.2 μM. | Ryu *et al*., 2010 |
| *K. galangal* | Pure compound | Anti-corona virus | Kaempferol has been reported to exert anti-corona virus effects, indicating its potential in the treatment of COVID-19. | Schwarz *et al.,* 2014 |
| *K. galangal* | Rhizomes | Anti-allergic | The rhizomes of *K. galangal* in water extract inhibited effects on the release of β-hexosaminidase from RBL-2H3 cells with $IC_{50}$ value of 49.5 μg/mL. | Tewtrakul, 2007 |
| *K. galangal* | Rhizomes | Anti -inflammatory | The crude extract treated rats after 3$^{rd}$ h of carrageenan administration. The chloroform extract showed an inhibition value of 42.9% compared with indomethacin that the most active crude extract. | Umar *et al*., 2012 |

**Table 2.11** Biological activity of *K. galangal* (Cont.)

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *K. galangal* | Rhizomes | Anti -inflammatory | The fraction 3 (F-3) active chloroform extract in the fraction-treated rats after 3rd h of carrageenan administration. F-3 was found to anti-inflammatory activity for inhibition value of 51.9% when compared to indomethacin that most active fraction 3. ($p < 0.001$) | Umar *et al*., 2012 |
| *K. galangal* | Rhizomes | Anti -inflammatory | F-3 was further fractionated to afford sub-fractions 1. SF-1 showed inhibition value of 53.7% when compared with the indomethacin (45.6% inhibition, $p < 0.01$). | Umar *et al*., 2012 |
| *K. galangal* | Rhizomes | Anti -inflammatory | Ethyl-*p*-methoxycinnamate (EPMC) was found to inhibited cyclooxygenase enzymes 1 (COX-1) and 2 (COX-2) value of 42. 9% and 57. 82% , respectively compared with the indomethacin similarly inhibited COX-1 and COX-2 value of 82.8% and 54.6%, respectively. The $IC_{50}$ values of EPMC for COX-1 and COX-2 were estimated to be 1.12 μM and 0.83 μM, respectively, compared with the indomethacin were found to be 0.33 μM and 0.51 μM, respectively. | Umar *et al*., 2012 |

**Table 2.11** Biological activity of *K. galangal* (Cont.)

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *K. galangal* | Rhizomes | Anti-nociceptive | Anti-nociceptive activity in mice and rats using acetic acid-induced writhing, formalin, hot plate, and tail-flick tests. The extract at test doses of 50, 100, and 200 mg/ kg, p. o. clearly demonstrated anti- nociceptive activity in all tests. This activity was dose- and time-dependent. The extract administered at 200 mg/kg, p.o. had an anti- nociceptive effect than aspirin (100 mg/kg, p.o.) but less than morphine (5 mg/kg, s.c.). | Ridtitid *et al.*, 2008 |

### 2.13.8.3 Chemical constituents of *K. galanga*l

*K. galanga*l has many important chemical components, including ethyl- cinnamate, cymene, ethyl-*p*-methoxycinnamate, kaempferol, kaempferide, cinnamaldehyde **(Figure 2.45)** (Umar, M. I. *et al*., 2011).

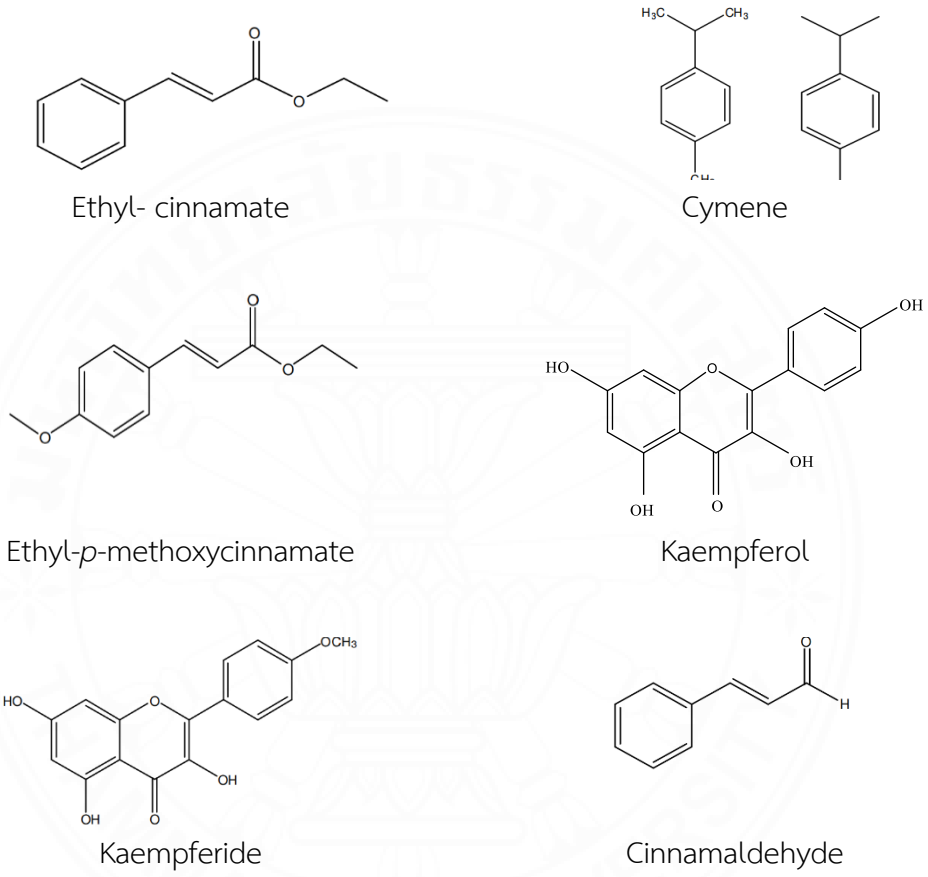



**Figure 2.45** Chemical structure of *K. galangal* (Umar, M. I. *et al*., 2011).

### 2.13.9. *Ligusticum chuanxiong* Hort.

#### 2.13.9.1 General data of *L. chuanxiong*

*L. chuanxiong* (Umbelliferae) has different dialects, such as Chuan Giang. Description of L. chuanxiong is the trunk is 30-60 cm high, underground rhizomes **(Figure 2.46)** straight stems. The leaves have pinnately compound leaves, long stem and triangular leaf frame, and acuminate. The flower has a cymose type. The flowers are white (Vudhithammavej, 2009).

**Part used:** Rhizome

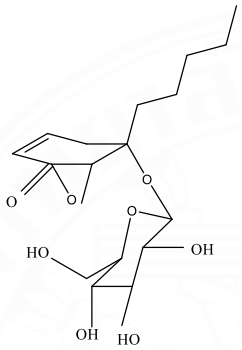
**Traditional use:** Flatulence (Vudhithammavej, 2009)


**Figure 2.46** Rhizome of *L. chuanxiong*

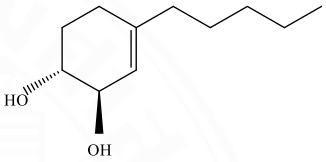
(Available from http://www.thaicrudedrug.com/main.php?action=viewpage&pid=33)

### 2.13.9.2 Chemical constituents of *L. chuanxiong*

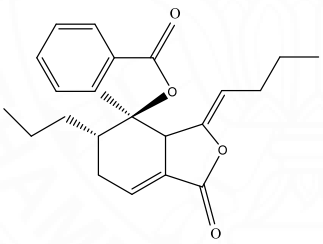
*L. chuanxiong* has many important chemical components, including (A) ligusticoside A, (B) 4-pentylcyclohex-3-ene-1a,2b-diol (C) (3′Z)-(3S,8S,3a′S,6′R)-4,5-dehydro-3.3a′,8.6′-diligustilide, (D) chuanxiongdiolide R3, (E(3′Z)-(3S,8R,3a′S,6′R)-4,5-dehydro-3.3a′,8.6′-diligustilide, (F) chuanxiongdiolide R1 **(Figure 2.47)** (Chang, 2009; Wei, 2006).



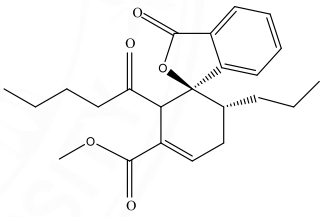

Ligusticoside A




4-pentylcyclohex-3-ene-1a,2b-diol




(3′Z)-(3S,8S,3a′S,6′R)-4,5-dehydro-3.3a′,8.6′-diligustilide




Chuanxiongdiolide R3

**Figure 2.47** Chemical structure of *L. chuanxiong* (Chang, 2009; Wei, 2006)

### 2.13.10. *Mammea siamensis* T. Anderson.

#### 2.13.10.1 General data of *M. siamensis*

*M. siamensis* (Calophyllaceae) has different dialects names such as dak sray pe (Wud Vudhithammavej, 2009). Description of *M. siamensis* is the tree 10-15 centimeters tall with reddish-brown inner bark. The leaves are single; oppositedecussate. The leaves are ovate. Leaf width 2.5-7 cm and leaf length 7.5-25 cm, with obtuse or emarginate, and entire. The flowers are solitary or cymose, and the stems and branches when blooming are 1.2-2.5 cm. The flower is shown in **Figure 2.48** The fruit is fusiform to elliptical, the length of the fruit is 2.5-5 cm, the fruit is light green and the fruit is yellow to the color (Picheansoonthon *et al*., 200; Chayamrit, 1997).

**Part used:** Flower

**Traditional use:** Antipyretic, Cardiotonic, Fatigue (Picheansoonthon *et al*., 2004)

**Figure 2.48** Flower of *M. siamensis*

(Available form http://www.phargarden.com/main.php?action=viewpage&pid=292)

**2.13.10.2 Biological activity of *M. siamensis***

**Table 2.12** Biological activity of *M. siamensis*

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *M. siamensis* | Flower | Anti-inflammatory | The methanolic extract inhibited LPS-activated NO production in RAW264.7 cells ($IC_{50}$ value of 28.9 lg/mL). The EtOAc-soluble fraction of bio-assay guided fraction inhibited LPS- activated NO production in RAW264.7 cells ($IC_{50}$ value of 8.3 μM). Kayeassamin G, the constituents from the flowers of *M. siamensis* on LPS-activated NO production in RAW264.7 cells ($IC_{50}$ value of 0.8 lg/mL). | Morikawa, 2012 |

### 2.13.10.3 Chemical constituents of *M. siamensis*

*M. siamensis* has many important chemical components, including cumarin, mammea A/AC, mammea A/AD, mammea A/AB cyclo D, mammea A/AC cyclo D, mammea B/AB cyclo D, mammea E/BB **(Figure 2.49)** (Mahidol *et al.*, 2002; Morikawa, 2012).

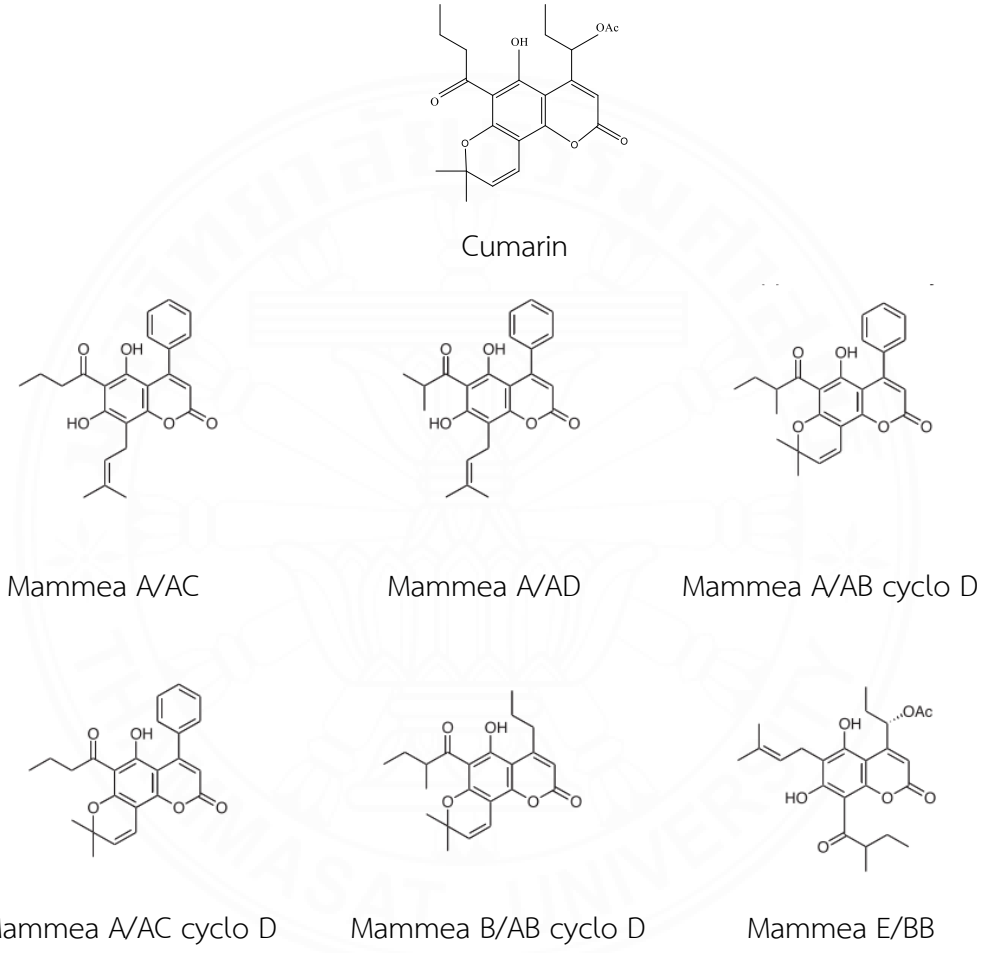



**Figure 2.49** Chemical structure isolated from the flowers of *M. siamensis* (Mahidol *et al*., 2002; Morikawa, 2012).

### 2.13.11 *Mesua ferrea* L.

#### 2.13.11.1 General data of *M. ferrea*

*M. ferrea* (Calophyllaceae) has different dialects names such as nakbut (Vudhithammavej, 2009). Description of *M. ferrea* Is tree up to 30 m. The bark is brown. The inner shell contains light yellow latex. The wood is dark red. The leaves are single, opposite, oblong, or lanceolate. The flowers are white-pink, solitary, or inflorescence flowers. The flower stalk is less than 1 cm long; more than 50 yellow-orange stamens, 2 ovaries, 4 sepals. **(Figure 2.50)** The fruit is a dry indehiscent. The fruit is dark orange or purple that wide 2 cm and long 2.5-3.5 cm. The seeds are flat and hard with 1-4 seeds, dark brown, found in the rain forest and dry evergreen forest (Picheansoonthon et al., 2004; Phargarden Faculty of Pharmaceutical Sciences Ubon Ratchathani University, 2010).

**Part used:** flower

**Traditional use:** antipyretic, cardiotonic, fatigue (Picheansoonthon *et al*., 2004)

**Figure 2.50** Flower of *M. ferrea*

(Available from http://www.phargarden.com/main.php?action=viewpage&pid=67)

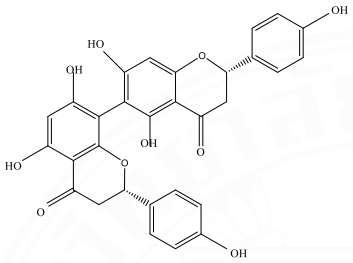
#### 2.13.11.2 Biological activity of *M. ferrea*

**Table 2.13** Biological activity of *M. ferrea*

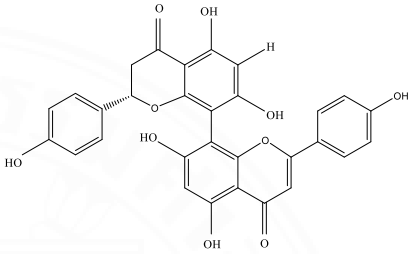
| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *M. ferrea* | Stem bark | Anti-iflammatory | The methanolic extract induced lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cell line. The inhibited of $IC_{50}$ value of 63.36 ± 3.791 µg/ml compared with diclofenac standard. | Murthuza, 2018 |
| *M. ferrea* | Stem bark | Anti-iflammatory | The methanolic extract using carrageenan-induced paw edema in Wistar albino rats. *M. ferrea* (0.6833 ± 0.01667) showed potent anti-inflammatory activity at 100 mg/kg. | Murthuza, 2018 |

### 2.13.11.3 Chemical constituents of *M. ferrea*

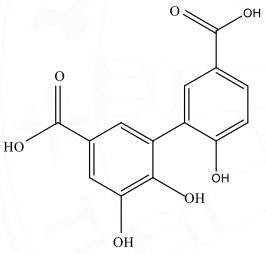
*M. ferrea* has many important chemical components, including rhuaflavanone, mesuaferrone, 5,6,6'-trihydroxy[1.1'-biphenyl]-3,3'-dicarboxylic acid, 3-amino-4-hydroxybenzoic acid, procatechuic acid, gallic acid, and procatechuic acid ethyl ester **(Figure 2.51)** (Zhang, 2019).



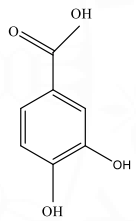
Rhuaflavanone



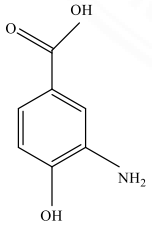
Mesuaferrone



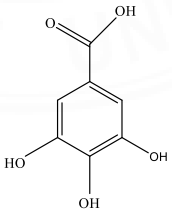
5,6,6'-trihydroxy[1.1'-biphenyl]-3,3'- dicarboxylic acid



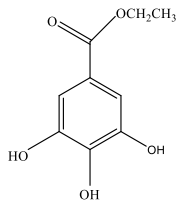
procatechuic acid



3-amino-4-hydroxybenzoic acid



gallic acid



procatechuic acid ethyl ester

**Figure 2.51** Chemical structure of *M. ferrea* (Zhang, 2019)

### 2.13.12. *Myristica fragrans* Houtt.

#### 2.13.12.1 General data of *M. fragrans*

*M. fragrans* (Myristicaceae) has different dialects names such as Nutmeg Tree, Nutmeg, Mace, Luk chan, chan ban (Vudhithammavej, 2009). Description of *M. fragrans* is leaves have a single and shape of ovate. Leaf apex is acute and the surface is smooth. The flowers are single. The flowers are the shape of urceolate and yellow flowers. The fruit is round or oval when ripe, yellow, and mature will sees the overgrown seed coat. The fruit show in **Figure 2.52** (Vudhithammavej, 2009).

**Part used:** Stem

**Traditional use:** Antipyretic, Cardiotonic (Vudhithammavej, 2009)

**Figure 2.52** Fruit of *M. fragrans*

(Available from http://www.thaicrudedrug.com/main.php?action=viewpage&pid=55)

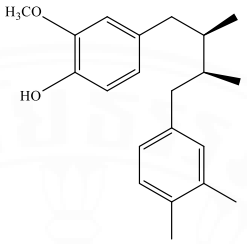
### 2.13.12.2 Biological activity of *M. fragrans*

**Table 2.14** Biological activity of *M. fragrans*

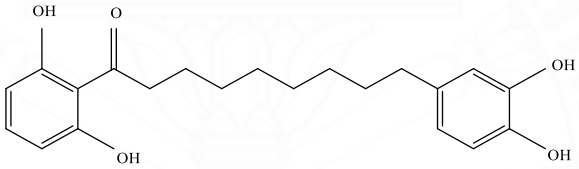
| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *M. fragrans* | Seeds | Anti-inflammatory | The $CHCl_3$ extract of licarin B (1), 30-methoxylicarin B (2), myrisfrageal A (3), isodihydrocainatidin (4), dehydrodiisoeugenol (5), and myrisfrageal B (6), that compound of *M. fragrans* Houtt inhibited nitric oxide production in lipopolysaccharide with $IC_{50}$ values of 53.6, 48.7, 76.0, 36.0, 33.6, and 45.0 μM, respectively. | Cao, 2013 |
| *M. fragrans* | Seeds | Anti-inflammatory | The $IC_{50}$ values of myrislignan and machilin D were 21.2 and 18.5 μM. Their inhibitory activity was more than L-N(6)-(1-iminoethyl)-lysine with $IC_{50}$ value of 27.1 μM. | Cao, 2015 |
| *M. fragrans* | Aril (mace) | Anti-inflammatory | The ethanolic extracts inhibited the anti-inflammatory activity of aril of *M. fragrans*. It was determined by the LPS-induced nitric oxide (NO) in a RAW264.7 cell line. The result effect of anti-inflammatory activity with $IC_{50}$ values of 82.19 μg/ml. | Suthisamphat, 2020 |

### 2.13.12.3 Chemical constituents of *M. fragrans*

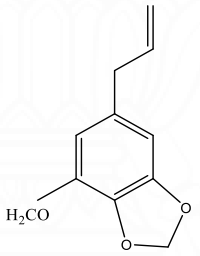
*M. fragrans* has many important chemical components, including macelignan, meso- dihydroguaiaretic acid, myristicin, malabaricone C, myristicin (Ha, M. T., 2020), β-sitosterol, fragransin $B_1$, grandisin, raphidecursinol B, surinamensin, (Francis, S. K., 2019) myrislignan, and machilin D (Cao, 2015). **(Figure 2.53)**



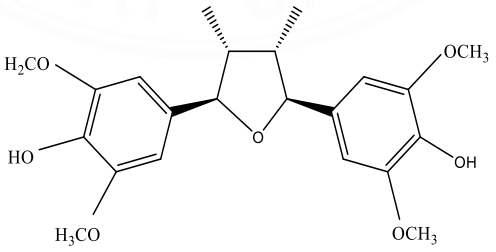

Macelignan and meso-dihydroguaiaretic acid




Malabarocone C

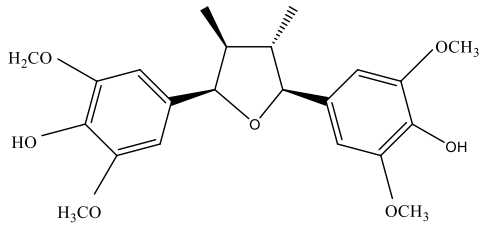



Miristicin

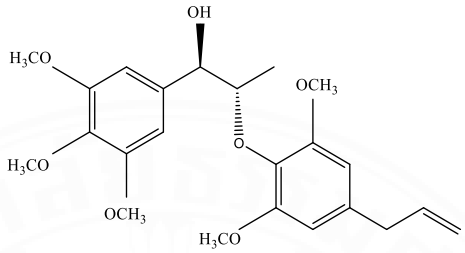



β-sitosterol, fragransin B1

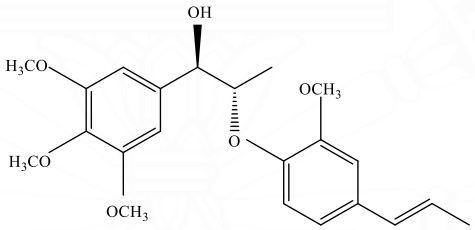
**Figure 2.53** Chemical structure of *M. fragrans* (Ha, 2020; Francis, 2019; Cao, 2015)



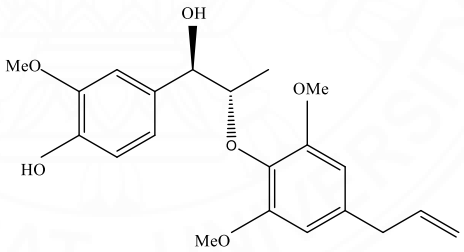

Grandisin



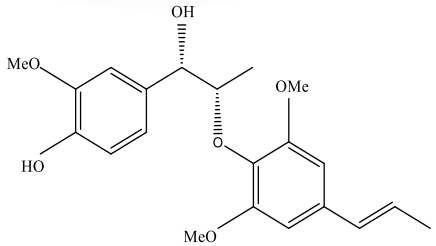

Raphidecursinol B




Surinamensin




Myrislignan




Machilin D

**Figure 2.53** Chemical structure of *M. fragrans* (Cont.) (Ha, 2020; Francis, 2019; Cao, 2015)

### 2.13.13. *Nelumbo nucifera* Gaertn.

#### 2.13.13.1 General data of *N. nucifera*

*N. nucifera* (Nelumbonacae) has different dialects names such as chok, Ubon, Pathum, Sattabongkot, Sattabut (Vudhithammavej, 2009). Description of *N. nucifera* that leaves are single, alternate, peltate, that wide and long 20-50 cm, and entire. Single flowers bloom above water, sepals 2-5 cm that width and length about 1.8 cm. Its many petals are stacked over several layers that are wide 6.5-.7.7 cm and long 7.5– 12.5 cm. Many stamens stacked several layers around the receptacle. The dehiscence is light yellow and long 1.4-1.7 cm **(Figure 2.54, 2.55).** Fruit in the aggregate contains 1 large seed. The seeds have 1-4 dark brown seeds (Picheansoonthon *et al.,* 2004).

**Part used:** Flower

**Traditional use:** Antipyretic, Cardiotonic (Vudhithammavej, 2009)

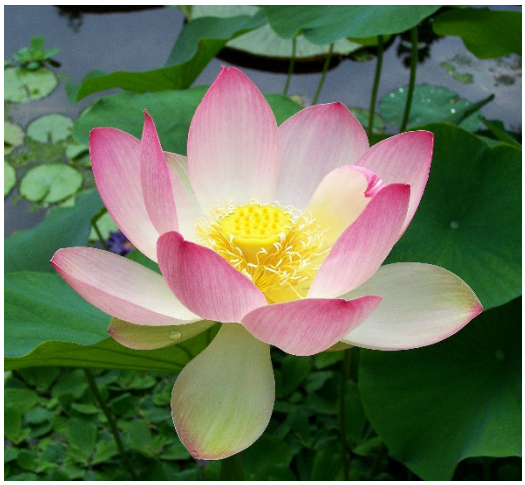


**Figure 2.54** Flower of *N. nucifera*

(Available from http://www.rspg.or.th/plants_data/herbs/herbs_03_5.htm)

**Figure 2.55** Pollen of *N. nucifera*

(Available from http://www.thaicrudedrug.com/main.php?action=viewpage&pid=24)

### 2.13.13.2 Biological activity of *N. nucifera*

**Table 2.15** Biological activity of *N. nucifera*

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *N. nucifera* | Stalks | Antipyretic | The ethanol extract tested the Antipyretic effect on normal body temperature in rats. The result found 200 mg/kg was found to significantly reduce body temperature and 400 mg/ kg reduced body temperature up to 6 hours after administration paracetamol at 150 mg/kg. | Sinha, 2000 |
| *N. nucifera* | Stalks | Antipyretic | The ethanol extract tested for an Antipyretic effect on yeast- induced pyrexia in rats that, after subcutaneous injection 19 hours after injection found the temperature increased. The extract at 200 and 400 mg/kg orally found the temperature could be decrease within 1 hour and the temperature reduction was maintained for 4 hours compared paracetamol at 150 mg/kg. | Sinha, 2000 |

**Table 2.15** Biological activity of *N. nucifera* (Cont.)

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *N. nucifera* | Rhizome | Antipyrrtic | The methanolic extract was studied on normal body temperature and yeast-induced pyrexia in rats. The extract concentration of 200, 300, or 400 mg/ kg significant dose-dependent lowering of normal body temperature and yeast induced pyrexia in rats. The effect produced was comparable with the standard Antipyretic drug, paracetamol (150 mg/kg, i.p.). | Mukherjee, 1996 |
| *N. nucifera* | Leaves | Anti-HIV activity | (+)-1(R)-Coclaurine and (–)-1(S)-norcoclaurine were isolated from the leaves of *N. nucifera*. The compound inhibited HIV viruses activity with $EC_{50}$ values of 0.8 and <0.8 µg/mL, respectively. | Kashiwada, 2005 |
| *N. nucifera* | Seed | Anti-herpes simplex virus type-1 | The ethyl alcohol extract of *N. nucifera* concentration 100 µg/ mL inhibited herpes simplex type 1 (HSV-1) replication by plaque reduction assay with $IC_{50}$ of 50.0 µg/mL | Kuo, 2005 |

**Table 2.15** Biological activity of *N. nucifera* (Cont.)

| Botanical name | Part used | Activities | Results | References |
|---|---|---|---|---|
| *N. nucifera* | Seeds | Anti-inflammatory | NN-B-4 identified by a bioassay-based screening procedure from ethanol extract of *N. nucifera* extracts. NN-B-4 decreases proliferation of primary human peripheral blood mononuclear cells (PMBC) induced by phytohemagglutinin, expression of IL-4, IL-10, and INF and cdk-4 gene. NN-B-4 blocking G1 transition to S phase of PBMC stimulation. | Kuo, 2005 |
| *N. nucifera* | Rhizome | Anti-inflammatory | The betulinic acid isolated from methanol extract of *N. nucifera* rhizome induced rat paw edema by carrageenin and serotonin. 200 and 400 mg/kg at doses of methanol extract and 50 mg/kg and 100 mg/kg p.o. at doses of betulinic acid, showed significant anti-inflammatory activity in both the methods. | Mukherjee, 1997 |

### 2.13.13.3 Chemical constituents of *N. nucifera*

*N. nucifera* has many important chemical components, including quercetin, luteolin, and luteolin glucoside **(Figure 2.56)** (Paudel, 2015).

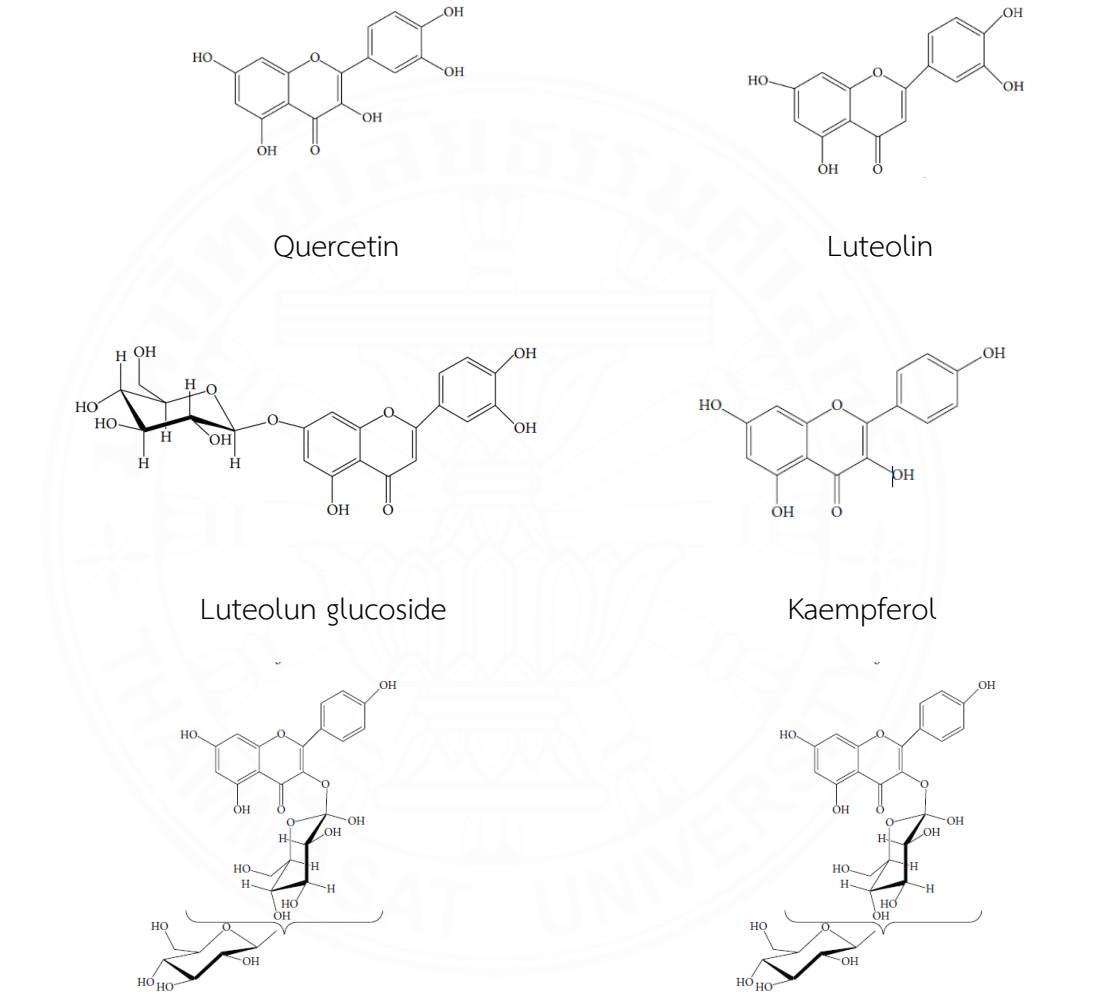



**Figure 2.56** Chemical structure of *N. nucifera* (Paudel, 2015)

# CHAPTER 3
# RESEARCH METHODOLOGY

## 3.1 Chemical reagents

The chemical reagents of this research are shown in Table 3.1.

**Table 3.1** List of chemical reagents

| Name | Source |
|---|---|
| <u>MTT assay</u> | |
| DPBS (1X) Dulbecco's Phosphate Buffered Saline | Gibco, UK |
| Hydrochloric acid | Merck, Germany |
| Mininum Essential Medium (MEM) | Gibco, USA |
| Fetal Bovine Serum (FBS) | Gibco, USA |
| Dimethyl Sulfoxide (DMSO) | RCI LabScan, Thailand |
| Trypan Blue Stain (0.4%) | Gibco, USA |
| Penicillin-Streptomycin (P/S) | Gibco, USA |
| Sodium bicarbonate | BHD, Emgland |
| Trypsin-EDTA (0.5%) | Gibco, USA |
| Sodium Pyruvate | Gibco, USA |
| Thiazolyl blue tetrazolium bromide (MTT) | TCI, Japan |
| Brazilin | Sigma-Aldrich, MO |
| Acyclovir (A1915) | TCI, Japan |
| <u>Antiviral activity assay</u> | |
| DPBS (1X) Dulbecco's Phosphate Buffered Saline | Gibco, USA |
| Hydrochloric acid | Merck, Germany |
| Mininum Essential Medium (MEM) | Gibco, USA |
| Fetal Bovine Serum (FBS) | Biochrom, Germany |

**Table 3.1** List of chemical reagents (Cont.)

| Name | Source |
|---|---|
| Dimethyl Sulfoxide for cell culture (DMSO) | RCI LabScan, Thailand |
| Trypan Blue Stain (0.4%) | Gibco, USA |
| Penicillin-Streptomycin (P/S) | Gibco, USA |
| 37%formaldehyde | RCI Labscan, Thailand |
| Crystal violate | TCI, Japan |
| Sodium bicarbonate | BHD, Emgland |
| Trypsin-EDTA (0.5%) | Gibco, USA |
| Sodium Pyruvate | Gibco, USA |
| Carboxymethylcellulose sodium salt | Sigma, USA |
| Brazilin | Sigma-Aldrich, MO |
| Acyclovir (A1915) | TCI, Japan |
| **Thin layer chromatography (TLC)** | |
| HEXANE (n-Hexane) | ACL Labscan, Thailand |
| ETHYL ACETATE | ACL Labscan, Thailand |
| Acetone | ACL Labscan, Thailand |
| CHLOROFORM 99.8% (1% EtOH) | ACL Labscan, Thailand |
| METHANOL 99.9% | ACL Labscan, Thailand |

### 3.2 Laboratory equipment, plastic wares, and glasswares

Laboratory equipment, plasticware and glassware of this research are shown in table 3.2

**Table 3.2** List of laboratory equipment, plasticware and glassware

| Name | Source |
|---|---|
| **Laboratory equipment** | |
| Autoclave | Hirayama, USA |
| Centrifuge | Boeco, Germany |
| $CO_2$ humidified incubator | Shel lab, USA |
| Freezer (-20°C) | Sanyo, Japan |
| Hot air oven | Memmert, Germany |
| Hotplate | Thermolyne, USA |
| Inverted microscope | Nikon, USA |
| Laminar air flow | Boss tech, Thailand |
| Micropipettes 10,20,200,1000 µL | Gilson, USA |
| Liquid nitrogen tank | Taylor-Wharton, USA |
| Microplate reader | Bio Tek, USA |
| Multichannel pipette | Corning Costar, USA |
| ph meter | WTW inolab, Germany |
| Rotary evaporator | Buchi, Switzerland |
| Sonicator | Elma, Germany |
| Stability incubator | Termarks, Norway |
| Vortex mixer | Scientific industries, USA |
| Water bath | Memmert, Germany |
| Water purification machine | Elga, UK |
| Pipetteboy | Intrgra biosciences, Switzerland |
| TLC plate silica gel 60 $F_{254}$ | MN, Germany |
| TLC Plate Heater | CAMAG, England |

**Table 3.2** List of laboratory equipment, plasticware and glassware (Cont.)

| Name | Source |
|---|---|
| TLC Auto Spotting | Linomat 5, Germany |
| Ultraviolet viewing cabinet for thin-layer chromatography | Uvitec, England |
| **Plasticware** | |
| Centrifuge tube 15 mL | Costar Corning, USA |
| Centrifuge tube 50 mL | Costar Corning, USA |
| Disposable pipette 5, 10, 25 mL | Costar Corning, USA |
| Eppendrof | Corning Costar, USA |
| Filter paper no.1 | Whatman, USA |
| 0.22 microns Membrane filters | Sartorius, Germany |
| Pipette tip | Corning Costar, USA |
| Reagent reservoir (Sterile) | Corning Costar, USA |
| Cryogenic Vial | Corning Incorporated, Mexico |
| 25 $cm^2$ cell culture flask, canted neck | Corning Flask, USA |
| 75 $cm^2$ cell culture flask, canted neck | Corning Flask, USA |
| Syringes | Nipro, Thailand |
| **Glassware** | |
| Glass bottle 50, 250, 500, 1,000 mL | Schott Duran, Germany |
| Glasswares | Schott Duran, Germany |
| Hemotocytometer | Boeco, Germany |
| TLC tank | Anatech, USA |

### 3.3 Plant materials and plant extraction

#### 3.3.1 Plant materials

Plant ingredients of Benchalokawichian and Prasachandaeng were obtained from Mrs. Yupaporn Wichian and Miss Nuntika Prommee, respectively. It is collected from various places in Thailand during June - July 2018. Benchalokawichian and Prasachandaeng were identities and confirmed by comparing the authentic voucher specimens at the Department of national parks, wildlife and plant conservation, ministry of natural resources and environment, Bangkok and southern center of Thai medicinal plants, faculty of pharmaceutical sciences, Prince of Songkla University, Songkla, Thailand, respectively. The voucher specimens number are shown in **Table 3.3**.

**Table 3.3** Summarized data of plant materials

| Scientific name | Place of collection | Voucher specimen number | Thai name | Part of used |
|---|---|---|---|---|
| *Clerodendrum indicum* (L.) Kuntze. | Surin province | BKF no.194783 | Thao-yai-mom | Root |
| *Capparis micracantha* DC. | Prachinburi province | BKF no.194839 | Ching-chi | Root |
| *Ficus racemose* L. | Surin province | BKF no.194784 | Ma-duea-chumphon | Root |
| *Harrisonia perforate* (Blanco) Merr. | Surin province | BKF no.195575 | Khon-tha | Root |
| *Tiliacora triandra* (Colebr.) Diels | Prachinburi province | BKF no.194782 | Ya-nang | Root |
| *Bouea macrophylla* Griff. | Nakhon Pathom Province | SKP 009 02 13 01 | Ma-prang-wan | Root |
| *Caesalpinaia sappan* L. | Nakhon Pathom Province | SKP 072 03 19 01 | Fang | Stem |
| *Citrus aurantifolia* (Christm.) Swing. | Nakhon Pathom Province | SKP 166 03 01 01 | Ma-naw | Root |

**Table 3.3** Summarized data of plant materials (Cont.)

| Scientific name | Place of collection | Voucher specimen number | Thai name | Part of used |
|---|---|---|---|---|
| *Dracaena cochinchinensis (Lour.)* S.C. Chen. | Nakhon Pathom Province | SKP 005 04 12 01 | Chan-daeng | Stem |
| *Jasminum sambac* (L.) Aiton. | Nakhon Pathom Province | SKP 129 10 19 01 | Mali | Flower |
| *Helicia terminalis* (Kurz.) Sleumer. | Nakhon Pathom Province | SKP 157 08 20 01 | Mueng-kon | Root |
| *Kaempferia galangal* L. | Nakhon Pathom Province | SKP 206 11 01 01 | Proh-horm | Rhizome |
| *Ligusticum chuanxiong* Hort. | Nakhon Pathom Province | SKP 199 12 19 01 | Kot-hua-bua | Rhizome |
| *Mammea siamensis* T.Anderson. | Nakhon Pathom Province | SKP 083 13 19 01 | Sa-ra-pee | Flower |
| *Mesua ferrea* L. | Nakhon Pathom Province | SKP 083 13 06 01 | Boon-nag | Flower |
| *Myristica fragrans* Houtt. | Nakhon Pathom Province | SKP 121 13 06 01 | Chan-tet | Stem |
| *Nelumbo nucifera* Gaertn. | Nakhon Pathom Province | SKP 125 14 14 01 | Bua-luang | Flower |

**3.3.2 Preparation of crude extracts**

Benchalokawichian and its plant ingredients extracts were obtained from Mrs. Yupaporn Wichian. Prasachandaengv and its plant ingredients extracts were obtained from Miss. Nuntika Prommee. Plants are extracted according to the process. All Plants were dried at 50°C and ground into powder. After that, plants were weighed and extracted to maceration and decoction. The first part of the crude extracts was macerated with 95% ethanolic (V/V) for three days, filtered and concentrated by a rotary evaporator. The second part of the crude extracts was boiled in water for 15 minutes and dried by a lyophilizer to obtain an aqueous extract. The extracts were stored in the refrigerator at -20 °C until used. The extract was passed the quality control according to the Thai herbal medicine formula for moisture content, total ash, acid insoluble ash, and extractive value (Department of Medical Sciences, 2018).

**3.3.3 Preparation of sample solution**

The ethanolic extracts were dissolved in dimethylsulfoxide (DMSO) to make a concentration of 50 mg/mL. The aqueous extracts were dissolved in sterile water to adjusted a concentration of 10 mg/mL and filtered through 0.22 microns syringe filters. The prepared extract was stored in a refrigerator at -20 °C.

**3.3.4 Acid hydrolysis of Prasachandaeng**

The freeze-dried extract was put in a flask with 0.1 N HCl solution and heated in a water bath at 80 °C for 15 min. Next, the product was extracted with chloroform in a separatory funnel and evaporated in a rotary evaporator. The extracts were stored in containers at - 20 °C until use. The acid hydrolysis extract was dissolved in dimethylsulfoxide (DMSO) to a 50 mg/ml concentration, stored in - 20 °C.

**3.3.5 Pure compound**

Ethyl-*p*-methoxycinnamate that, was isolated from *K. galangal* was obtained from Miss Nichamon Mukkasombut. Brazilin is reported to be a pure compound of Peasachandaeng and *C.sappan* (Prommee *et al.*, 2020). These pure compounds were dissolved in dimethylsulfoxide (DMSO) and adjusted to 50 mg/mL.

### 3.3.6 Positive control

Acyclovir was used as a positive control. It was purchased from Tokyo chemical industry co., ltd, Japan. It was dissolved in DMSO and adjusted to 50 mg/mL.

## 3.4 Cell culture

The Vero cell (African green monkey cell line ATCC CCL-81) was purchased from ATCC. It was maintained in complete Eagle's Minimum Essential Medium (MEM supplement with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% sodium pyruvate). Cells were subcultured every four days and incubated at 37°C with 5% $CO_2$.

## 3.5 Determination of Cytotoxicity activity

### 3.5.1 Principle

The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell viability. The MTT or 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide is used to determine cell viability. Dehydrogenase enzymes reduce MTT in the active cell to generate formazan products. The resulting intracellular purple formazan can be solubilized and measured by spectrophotometric means (American Type Culture Collection, 2011).

### 3.5.2 The method of cytotoxicity activity

For the cytotoxicity of vero cells, an amount of 40,000 cells/well in 100 µL was seeded in a 96-well plate with complete Eagle's Minimum Essential Medium. The plate was incubated at 37 °C, 5%$CO_2$, for 24 hours. After incubation, the supernatant was removed and replaced by 100 µL of 1XMEM (supplemented with 3%fetal bovine serum, 1% penicillin-streptomycin, and 1%sodium pyruvate) and 100 µL of various concentrations of samples. The plate was incubated at 37 °C, 5% $CO_2$ for 2 hours or 3 days. After incubation, supernatant (100 µL/well) was removed, and 10 µL of MTT solution (5mg/mL) was added to each well. The plate was incubated at 37 °C, 5 %$CO_2$, for 2 hours. The supernatant was removed and replaced by 100 µL of

DMSO per well. Then, the optical density was measured at 570 nm by a microplate reader. The percentage of cell viability and cytotoxicity activity was calculated using the equation below. The extract had less than 70 percent survival rate, considered cytotoxic (Standard biological evaluation of medical devices, 2019; Farsalinos *et al.*, 2013*)*.

$$\% \text{ cell survival} = \frac{\text{OD570 of sample}}{\text{OD570 of cell control}} \times 100$$

$$\%\text{cytotoxicity} = 100 - \% \text{ cell survival}$$

**3.6 Antiviral activity of plaque reduction assay**

**3.6.1 Virus propagation**

Herpes simplex virus (HSV-2) ATCC VR734 was propagated in a Vero cell monolayer maintained in 1XMEM (containing 10%fetal bovine serum, 1%penicillin-streptomycin, and 1% sodium pyruvate) in 75 $cm^2$ cell culture flask and incubated 37 ℃ in 5%$CO_2$ for 2-3 days. Cells were harvested when the cytopathic effect (CPEs) were more than 75% of the infected cell. In addition, the supernatant of viruses was contained that collected sterile vial tube and stored at -80℃ (Blaho, Morton, & Yedowitz, 2005).

**3.6.2 Preparation of Vero cell in 12-well plate**

Vero cells were trypsinized and seeded at 350,000 cells/mL in 1XMEM (containing 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% sodium pyruvate) in 12-well plates. The plate was incubated at 37 ℃, 5% $CO_2$ for 2 days.

**3.6.3 Virus plaque titer**

**3.6.3.1 Principle**

Viral plaque titer is used to control research quality and repeat the test to achieve similar results every time. It is possible to calculate the viral load and concentration to be tested with various concentrations of extracts. The calculation

made it possible to test the antiviral activity of the extracts accurately (Puttawattana., 2016; Baer and Kehn., 2014).

**3.6.3.2 Plaque titer process**

HSV-2 was diluted by ten-fold serial dilutions. Eight dilutions ($10^{-1}$ – $10^{-8}$) were added into Vero cells monolayer in a 12-well plate (250 µL/well) and incubated for 1 hour. After that, the supernatant was removed, and 3% carboxymethyl cellulose (CMC) was added and incubated at 37 ℃, 5% $CO_2$ for three days. Then, this plate was fixed with 10% formaldehyde for 2 hours and stained with 0.1% crystal violet for 30 mins. The plaque was counted and calculated in plaque-forming unit per mL using the equation below (Blaho *et al.,* 2005).

$$\text{Pfu/ml} = \frac{\text{Average of plaque}}{\text{D} \times \text{V}}$$

D = dilution V = volume of diluted virus added to the plate

**3.6.4 The method of Plaque reduction assay**

Plaque is a spot that occurs when cells die due to virus infection and fall off, thus appearing as a clear spot shown in **Figure 3.1**.

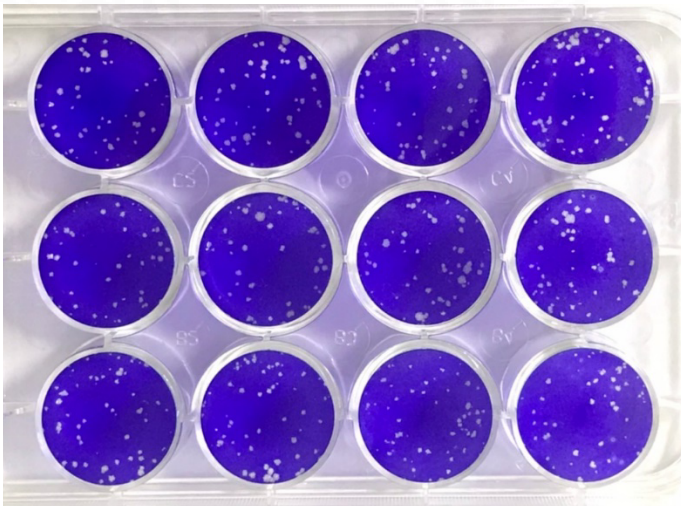


**Figure 3.1** Plaque formed by Herpes simplex virus type-2 in Vero cell

**3.6.4.1 pre-incubation assay**

HSV-2 was incubated with various concentrations of plant extract before migrating to the Vero cell. This method aims to study the extracts effective in killing viruses directly by using the highest concentration of extracts that are non-toxic to Vero cells (%Survival > 70) to test their antiviral activity. Two-fold dilution extract was mixed with 200 PFU of HSV-2. This plate was incubated at 37 °C, 5% $CO_2$ for 1 hour. Then, different concentrations of the extract with the virus were added to Vero cell monolayers in 12- well plate 250 µL/well. This plate was incubated at 37 °C, 5 %$CO_2$ for 2 hours. After that, this plate was removed supernatant and added 1.5 % Carboxymethyl cellulose (CMC). This plate was incubated at 37 °C, 5% $CO_2$, for 3 days. Then, this plate was removed supernatant and was fixed 10% formaldehyde for 2 hours. After that, the plate was stained with 0.1% crystal violet for 30 mins. Finally, the plaque was counted and calculated as percent inhibition (Ang *et al*., 2016).

**3.6.4.2 Pre-treatment assay**

The extract was incubated with cells before HSV-2 infection. The objective of this method was to study extracts that were effective in protecting cells from viral infection by using extracts with the highest concentration of non-toxic to Vero cells (% Survival > 70) to test their antiviral activity. The extract was added to Vero cell monolayers of different concentrations. This plate was incubated at 37 °C 5 %$CO_2$ for 2 hours. Then, the supernatant was removed and infected 100 PFU/mL of HSV-2 (ATCC VR734) to Vero cell monolayers 250 µL/well. This plate was incubated at 37 °C 5 % $CO_2$ for 2 hours. After that, this plate was removed supernatant and added 1.5% carboxymethyl cellulose (CMC). The plate was incubated at 37 °C, 5% $CO_2$ for 3 days. Then, this plate was removed supernatant and fixed 10% formaldehyde for 2 hours. After that, the plate was stained with 0.1% crystal violet for 30 mins. Finally, the plaque was counted and calculated percent inhibition (Ang *et al*., 2016).

**3.6.4.3 Post-treatment assay**

Post-infection was performed by adding extracts after the cells were infected with HSV-2. This method was to determine whether the extract could

inhibit the replication of HSV-2 in Vero cells. The Vero cell monolayers were infected 100 PFU/mL of HSV-2 (ATCC VR734) 250 µL/well. This plate was incubated at 37 °C 5 %$CO_2$ for 2 hours. Then, the extract was added to Vero cell monolayers of different concentrations and incubated at 37 °C 5 %$CO_2$ for 2 hours. . After that, this plate was removed supernatant and added 1.5% carboxymethyl cellulose (CMC). The plate was incubated at 37 °C, 5%$CO_2$ for 3 days. Then, this plate was fixed 10%formaldehyde for 2 hours and stained with 0.1% crystal violet for 30 mins. Finally, the plaque was counted and calculated percent inhibition (Ang *et al*., 2016).

$$\%\text{Inhibition} = \frac{(\text{Average of plaque VC}) - (\text{Average of plaque VC})}{(\text{Average of plaque VC})} \times 100$$

VC = virus control, VT = virus test

The inhibition percentage (% inhibition) at each extract concentration was calculated using the above calculation. The 50% inhibitory concentration ($IC_{50}$) values of the extracts were calculated using GraphPad Prism.

### 3.7 Chemical constituents of its plant components of Prasachandaeng extracts

The pure compound of brazilin and ethyl-*p*- methoxycinnamate was tested for cytotoxicity. In addition, the pure compound was tested for antiviral activity of the pre-incubation assay.

### 3.8 Thin layer chromatography (TLC) fingerprint development

The aqueous and acid- hydrolysis extracts of Prasachandaeng were performed chemical fingerprint by TLC techniques. First, the extract was spotted (10 µL) on TLC silica gel 60 $F_{254}$ plates and developed in three solvent systems, including Hexane: Ethyl acetate (1:1), Hexane: Acetone (7:3), Chloroform: Methanol

(9:1). After that, the TLC plate was examined under UV light at 254 and 365 nm. Then, the TLC was sprayed with anisaldehyde and heated in the hot plate at 105-120 ℃. Finally, the chromatogram results were recorded using the photographic method.

**3.9 Statistical analysis**

All experiments were performed in triplicate. Values of different parameters were expressed as the mean ± standard error of the mean (SEM). Statistical analysis was operated using statistical software.

# CHAPTER 4

# RESULTS AND DISCUSSION

All samples used in this study were obtained from Mrs. Yupaporn Wichian and Miss Nuntika Prommee. The description and code of each sample are shown in **Table 4.1.**

**Table 4.1** Description and codes of samples

| Sample | Extraction | Code |
|---|---|---|
| Benchalokawichian | 95% Ethanol | BLWE |
| | Aqueous | BLWA |
| Prasachandaeng | 95% Ethanol | PJDE |
| | Aqueous | PJDA |
| | Hydrolyze | PJDH |
| *C. indicum* | 95% Ethanol | CIE |
| | Aqueous | CIA |
| *C. micracantha* | 95% Ethanol | CME |
| | Aqueous | CMA |
| *F. racemose* | 95% Ethanol | FRE |
| | Aqueous | FRA |
| *H. perforate* | 95% Ethanol | HPE |
| | Aqueous | HPA |
| *T. triandra* | 95% Ethanol | TTE |
| | Aqueous | TTA |
| *C. sappan* | 95% Ethanol | CSE |
| *D. cochinchinensis* | 95% Ethanol | DCE |
| *K. galangal* | 95% Ethanol | KGE |
| Ethyl-*p*-methoxycinnamate | - | EPMC |
| Acyclovir | - | ACV |

## 4.1 Cytotoxicity activity

The cytotoxicity activity of ethanolic and aqueous extracts of BLW and PJD remedy to Vero cells were performed by MTT assay. All samples were prepared by 2 -fold serial dilution method. The cytotoxicity studies were presented after cell incubation with extract for 2 hours and three days. The percentages of cell viability above 70% are considered as non-cytotoxicity.

### 4.1.1 Cytotoxicity activity of extracts on the Vero cell after 2 hours incubation

The aqueous and ethanolic extracts of Benchalokawichian and Prasachandaeng showed no cytotoxicity on Vero cells at the highest dose (100 µg/mL). The aqueous extracts of plant ingredients of Benchalokawichian also showed no cytotoxicity on Vero cells at the highest dose (survival rate > 70%). In addition, the ethanolic extract of plant ingredients in Benchalokawichian showed no cytotoxicity at any dilution tested except for the ethanolic extract of *H. perforate* (HPE) and *T. triandra* (TTE). HPE and TTE showed cytotoxicity on Vero cells at a 25-100 µg/mL concentration and 100 µg/mL concentration, respectively, as shown in **Table 4.2** and **Figure 4.1-4.2**. Acyclovir was used as a positive control that showed no toxicity at all concentrations, as shown in **Figure 4.3**.

**Table 4.2** Cytotoxicity of extracts on the Vero cell after 2 hours incubation (mean±SEM), (n=3)

| Sample | % cell survival ± SEM / Concentration (µg/mL) | | | | |
|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | 6.25 |
| PJDE | 93.01±3.83 | 90.63±2.94 | 87.64±3.16 | 88.14±2.12 | 87.57±1.92 |
| PJDA | 85.04±1.89 | 86.45±1.17 | 85.56±1.14 | 87.28±1.64 | 87.17±2.16 |
| BLWE | 95.28±1.50 | 93.39±3.90 | 90.42±2.80 | 88.36±1.79 | 85.46±2.32 |
| BLWA | 83.24±2.03 | 84.40±2.22 | 83.79±3.57 | 86.30±1.88 | 91.92±2.25 |
| CIE | 85.90±4.95 | 84.59±3.98 | 84.04±3.58 | 83.84±1.25 | 91.61±2.46 |
| CME | 83.69±1.69 | 82.19±3.08 | 78.70±1.42 | 77.10±2.37 | 81.25±3.79 |
| FRE | 85.34±4.06 | 89.45±2.72 | 89.57±0.74 | 88.75±1.16 | 87.53±4.05 |
| HPE | 5.87±0.37 | 39.71±3.24 | 67.17±1.07 | 72.75±1.98 | 87.68±2.22 |
| TTE | 3.47±0.40 | 92.99±4.65 | 91.89±0.05 | 89.93±2.00 | 88.74±3.81 |
| ACV | 89.03±2.76 | 84.69±3.93 | 80.33±1.92 | 79.38±1.45 | 82.27±0.75 |

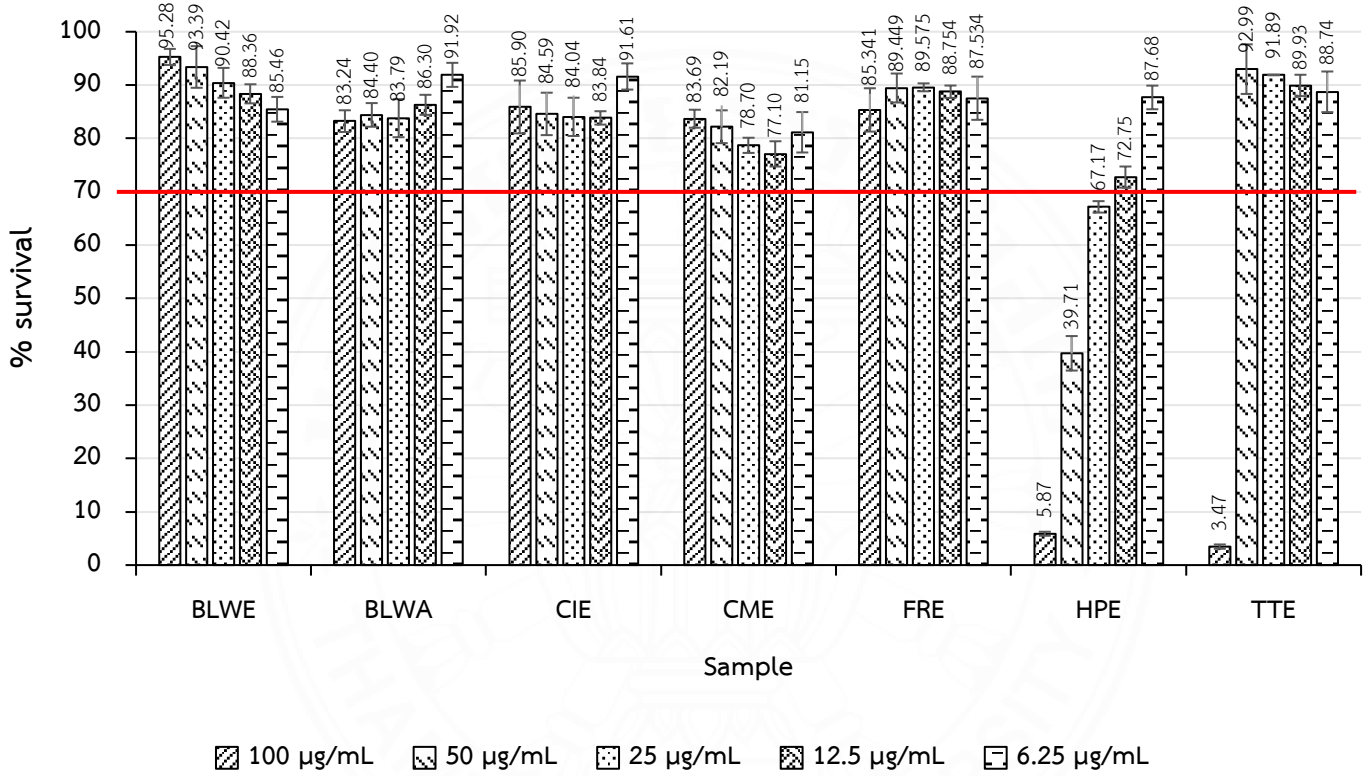



**Figure 4.1** Cytotoxicity of BLWE, BLWA and its plant components extract on the Vero cell after 2 hours incubation

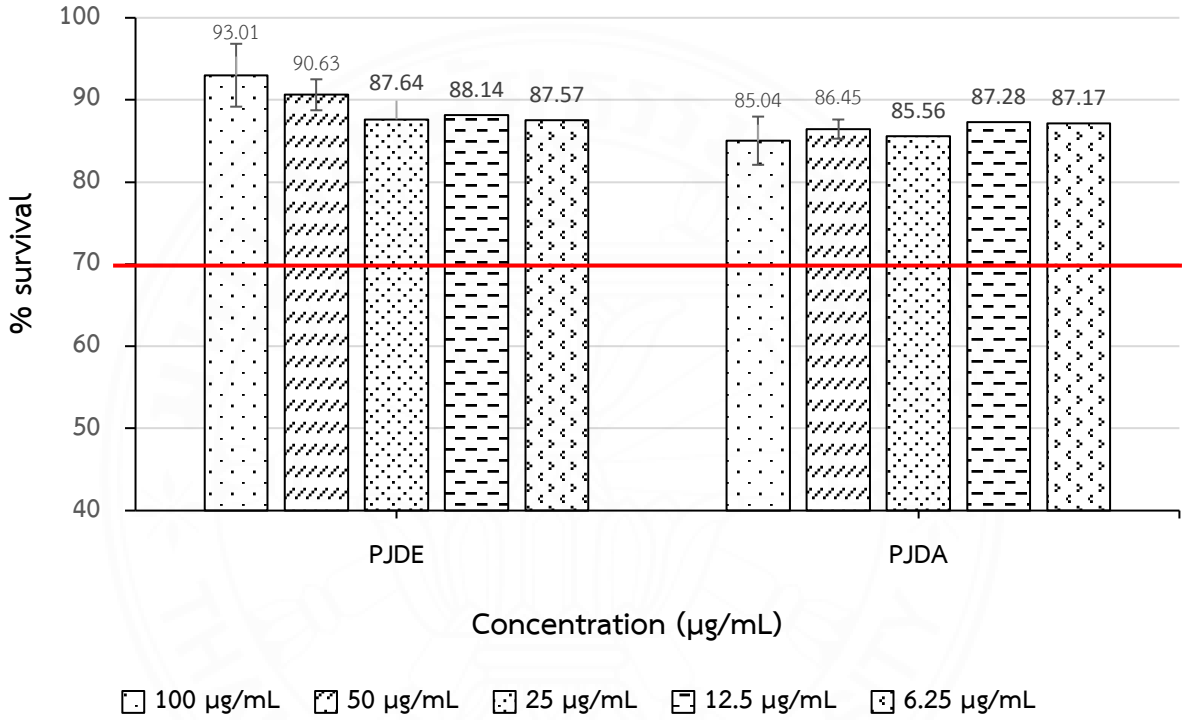



**Figure 4.2** Cytotoxicity of PJDE and PJDA extract on the Vero cell after cell 2 hours incubation

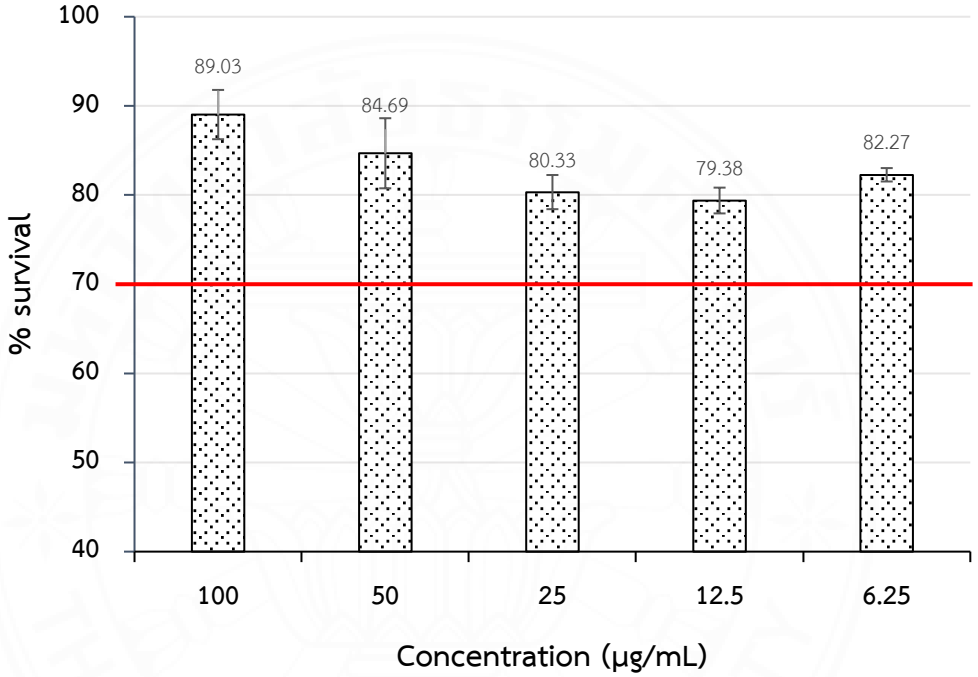



**Figure 4.3** Cytotoxicity of Acyclovir on the Vero cell after 2 hours incubation

**4.1.2 Cytotoxicity activity of extracts on the Vero cell after 3 days incubation**

These extracts were tested for cytotoxicity activity after three days of incubation. The extracts which is continuoly test for virus have to be required more than 70% cell survival, indicating that the extract was non-cytotoxic.

The percent survival of BLWE on Vero cells was below 70% at 50 and 100 µg/mL, while PJDE was non-toxic on Vero cells at 50 µg/mL. The other extracts, including BLWA, PJDA, and acyclovir were non- cytotoxicity at all concentrations, as shown in **Table 4.3** and **Figure 4.4-4.8.** Thus, the non-toxic concentration of each sample was investigated for anti-viral activity against HSV-2 by inhibition of viral replication.

The percentage of cell survival of the extract which was incubated with the Vero cell for 3 days from this study. The extract of BLWE and PJDE were non-cytotoxic to the Vero cell at maximum concentrations of 25 and 50 µg/ mL, respectively. The extract of BLWA and PJDA were non-cytotoxic to Vero cells at maximum concentrations of 100 µg/mL. ACV was non-cytotoxic to Vero cells at the maximum concentration of 100 µg/mL. In previous studies, this drug showed non-cytotoxic to normal cells (Dogan *et al*., 2018).

**Table 4.3** Cytotoxicity of extract on the Vero cell after 3 days incubation (mean± SEM), (n=3)

| Sample | % cell survival ± SEM / Concentration (µg/mL) | | | | |
|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | 6.25 |
| PJDE | 33.77±3.31 | 86.98±5.88 | 90.39±1.68 | 86.72±0.63 | 91.41±2.36 |
| PJDA | 81.48±1.56 | 83.03±4.82 | 88.95±2.20 | 89.27±1.54 | 99.01±1.52 |
| BLWE | 31.22±12.13 | 64.40±5.35 | 86.40±3.32 | 89.10±3.59 | 89.87±6.21 |
| BLWA | 75.20±6.79 | 83.83±2.47 | 83.95±3.61 | 85.44±2.75 | 86.75±3.50 |
| ACV | 89.01±1.98 | 94.76±1.72 | 89.14±1.31 | 83.33±3.10 | 88.37±0.96 |

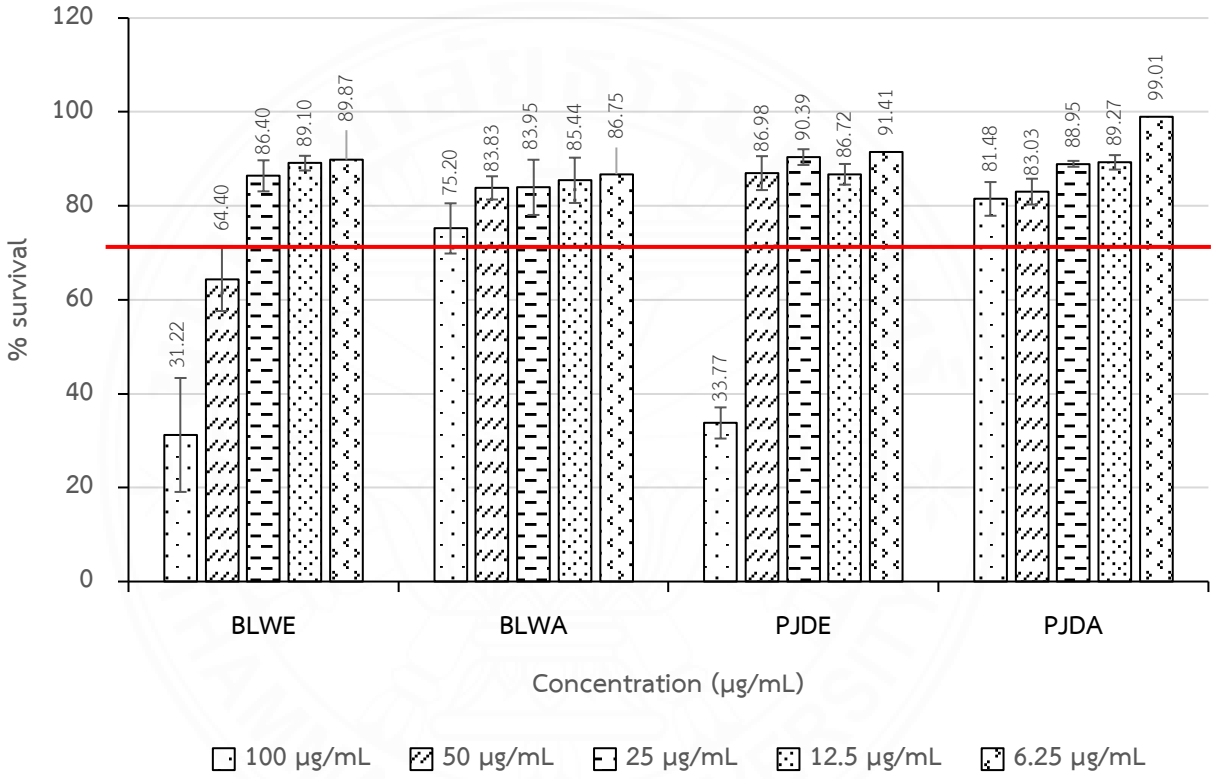



**Figure 4.4** Cytotoxicity of BLWE, BLWA, PJDE and PJDA extract on the Vero cell after 3 days incubation

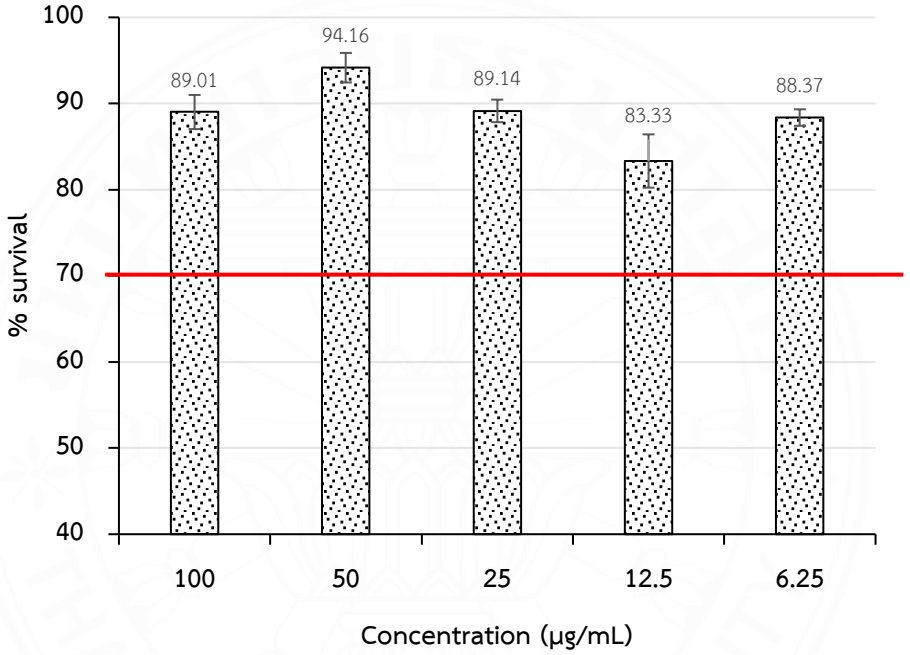



**Figure 4.5** Cytotoxicity of Acyclovir on the Vero cell after 3 days incubation

## 4.2 Antiviral activity

The BLWE, BLWA, PJDE, PJDW, and the extracts of plant components in Benchalokawichian were tested for their antiviral activity against HSV-2 by three mechanisms: pre-incubation assay, pre-treatment, and post-treatment. The plaque reduction technique was used to test the antiviral activity in this study. Acyclovir or ACV is used as a positive control of antiviral activity.

### 4.2.1 Pre-incubation assay

BLWE, BLWA, PJDE, PJDW were incubated with HSV-2 for various concentrations (two hours), then HSV-2 was transferred to Vero cells for infection. After infection, Vero cells were fixed and stained with methylene blue. The number of plaque and percent inhibition was calculated and expressed in **Table 4.4**.

The results showed that PJDE showed potent anti-HSV-2 with an $IC_{50}$ value of 24.46±6.05 µg/mL, while PJDA, BLWE, and BLWA had a low anti-HSV-2 activity with percent inhibition of 43.81±5.48, 28.56±3.89, and 44.25±4.79% at 100 µg/mL. However, acyclovir had the best inhibitory effect against HSV-2 with an $IC_{50}$ value of 12.71±2.94 µg/mL, as shown in **Table 4.4** and **Figure 4.6-4.7**.

Although BLWE had low anti-HSV-2 activity, plant ingredients of BLWE were confirmed anti-HSV-2 effect by pre-incubation assay. The results showed that CIE, CME, FRE, HPE, and TTE displayed a weak anti-viral activity against HSV-2 with a percent inhibition of less than 50 percent at 100 µg/mL, as shown in **Table 4.4** and **Figure 4.8**.

**Table 4.4** Antiviral activity of PJDE, PJDA, BLWE, BLWA, and its plant component of BLWE extracts against HSV-2 in pre-incubation assay (mean± SEM), (n=3)

| Sample | % inhibition / Concentrations (µg/mL) | | | | | | | $IC_{50}$(µg/mL) |
|---|---|---|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.562 | |
| PJDE | 78.07±5.92 | 55.16±7.08 | 49.47± 3.74 | 38.58±7.62 | NT | NT | NT | 24.46±6.05 |
| PJDA | 43.81±5.48 | NT | NT | NT | NT | NT | NT | >100 |
| BLWE | 28.56±3.89 | NT | NT | NT | NT | NT | NT | >100 |
| BLWA | 44.25±4.79 | NT | NT | NT | NT | NT | NT | >100 |
| CIE | 23.10±2.67 | NT | NT | NT | NT | NT | NT | >100 |
| CME | 36.90±0.30 | NT | NT | NT | NT | NT | NT | >100 |
| FRE | 43.65±5.75 | NT | NT | NT | NT | NT | NT | >100 |
| HPE | NT | NT | NT | 32.75±3.51 | NT | NT | NT | >12.5 |
| TTE | NT | 16.82±2.60 | NT | NT | NT | NT | NT | >50 |
| ACV | 98.91±1.09 | 85.85±3.47 | 66.47±4.27 | 56.19±9.35 | 30.94±2.40 | NT | NT | 12.71±2.94 |

*NT = Not tested

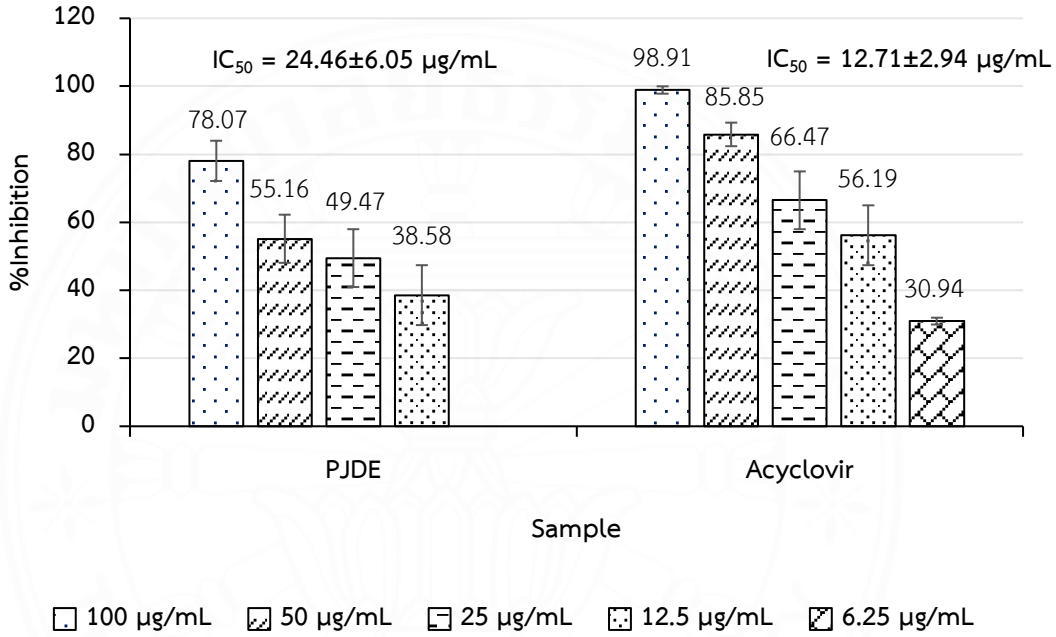



**Figure 4.6** Antiviral activity of PJDE extract and acyclovir against HSV-2 in pre-incubation assay

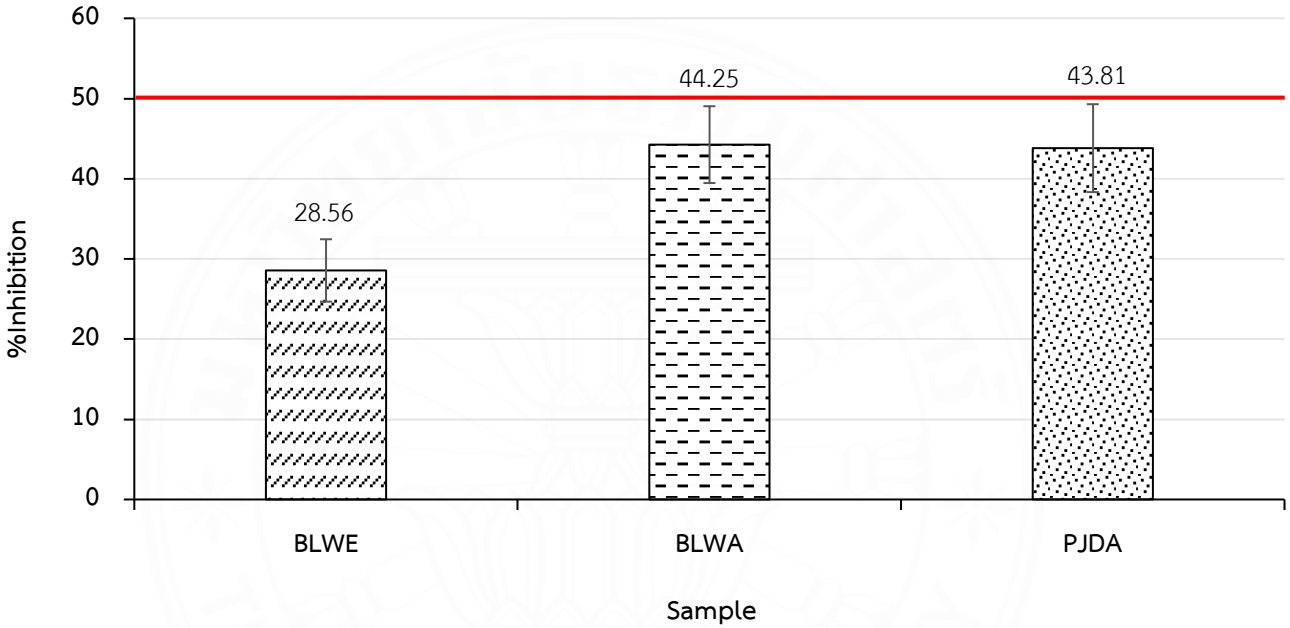



**Figure 4.7** Antiviral activity of BLWE, BLWA and PJDA extracts against HSV-2 at concentration of 100 µg/mL in pre-incubation assay

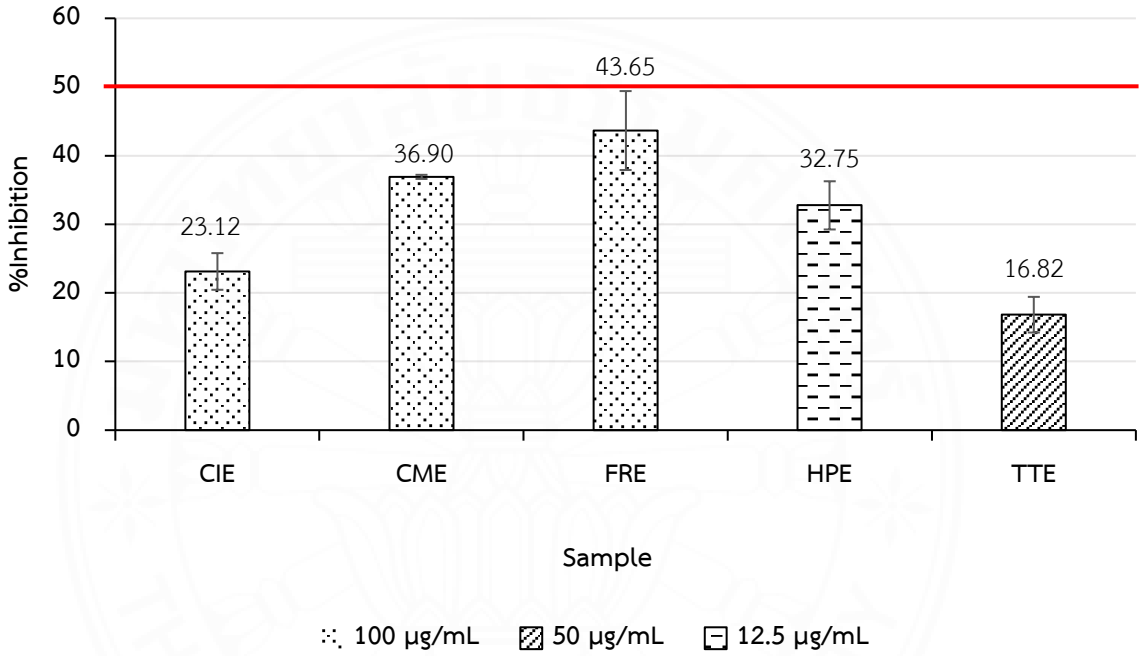



**Figure 4.8** Antiviral activity of plant components of BLWE extracts against HSV-2 in pre-incubation assay

The results showed that the PJDE extract was able to inactivate the virus in only one process of the pre-incubation assay. Therefore, the main ingredient of Prasachandang remedy (DCE) and the ingredients that were reported on antiviral activity, including CSE and KGE, were investigated for anti-HSV-2 activity. First, we studied the cytotoxicity of DCE, CSE, and KGE on Vero cells, as shown in **Table 4.5** and **Figure 4.9**.

**Table 4.5** Cytotoxicity of the CSE, DCE, and KGE extracts on the Vero cell after 2 hours incubation (mean±SEM), (n=3)

| Sample | % cell survival ± SEM / Concentration (µg/mL) | | | | |
|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | 6.25 |
| CSE | 41.05±4.20 | 53.49±2.61 | 87.30±1.29 | 87.88±1.74 | 91.88±1.09 |
| DCE | 84.14±3.85 | 89.13±2.30 | 89.50±1.58 | 85.85±2.73 | 91.28±2.98 |
| KGE | 81.85±2.00 | 84.89±1.96 | 86.66±1.48 | 88.38±1.22 | 87.69±0.52 |

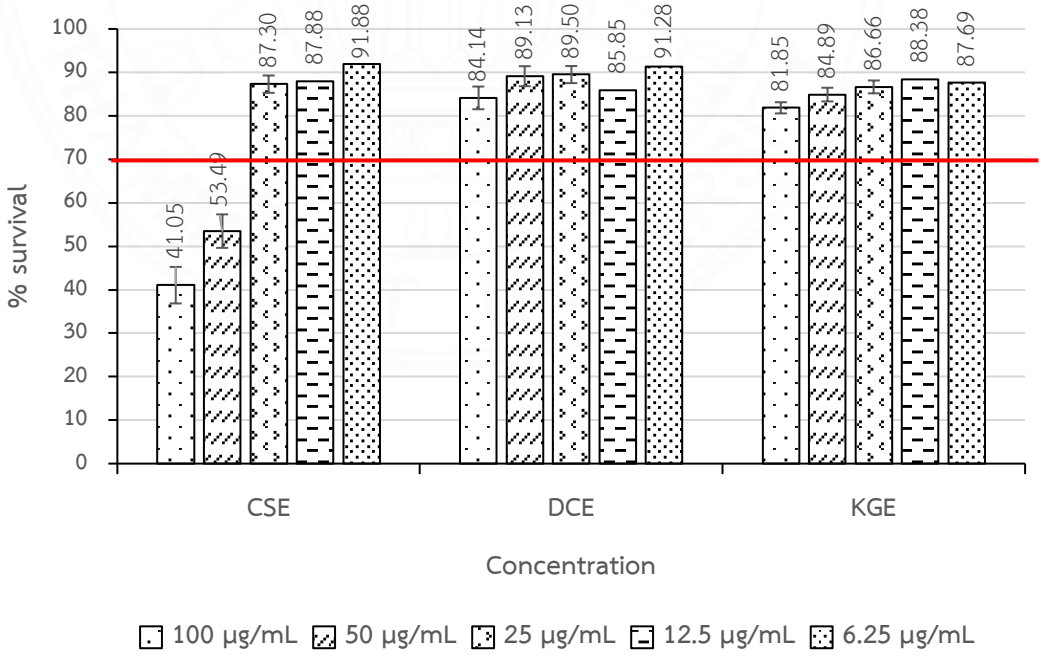



**Figure 4.9** Cytotoxicity of ethanolic extracts of CSE, DCE, and KGE extracts on the Vero cell after 2 hours incubation

The CSE, DCE, and KGE were non-toxicity on Vero cells at 25, 100, and 100 µg/mL concentrations, respectively. The non-toxic concentration of extracts was tested for antiviral activity in the pre-incubation assay.

The results found that DCE and CSE effectively inhibited the HSV-2, while KGE showed low antiviral activity. Surprisingly, CSE showed higher antiviral activity against HSV-2 than PJDE and acyclovir. CSE was the most potent in the pre-incubation assay with an $IC_{50}$ value of 1.91±0.13 µg/mL. DCE had the anti-HSV-2 activity with an $IC_{50}$ value of 37.23±4.15 µg/mL, as shown in **Table 4.6** and **Figure 4.10.**

Previous studies showed that resveratrol, the pure compound of DCE, showed the antiviral activity against HSV-1 and HSV-2 replication, including inhibition of virus attachment and inhibition of reactivation of virus from latently-infected neurons, and reducing the amount of ICP-4 (Docherty *et al*., 1999; Annunziata *et al.*, 1999). Moreover, CSE consists of flavonoids that showed inhibitory effects on HSV-1 and 2 by CPE assay (Lyu *et al.,* 2005). Thus, the herpes virus inhibition of DCE and CSE extracts may cause a group of active ingredients that have been studied for herpes simplex virus inhibition.

**Table 4.6** Antiviral activity of CSE, DCE, and KGE extracts against HSV-2 in pre-incubation assay (mean± SEM), (n=3)

| Sample | % inhibition / Concentrations (µg/mL) | | | | | | | $IC_{50}$(µg/mL) |
|---|---|---|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.562 | |
| PJDE | 78.07±5.92 | 55.16±7.08 | 49.47± 3.74 | 38.58±7.62 | NT | NT | NT | 24.46±6.05 |
| DCE | 92.48±2.44 | 62.83±5.30 | 38.98±2.62 | 28.39±4.03 | 25.84±1.16 | NT | NT | 37.23±4.15 |
| CSE | NT | NT | 98.28±0.20 | 94.01±1.07 | 81.92±7.70 | 67.42±6.04 | 45.25±2.09 | 1.91±0.13 |
| KGE | 3.03±0.92 | NT | NT | NT | NT | NT | NT | >100 |
| ACV | 98.91±1.09 | 85.85±3.47 | 66.47±4.27 | 56.19±9.35 | 30.94±2.40 | NT | NT | 12.71±2.94 |

*NT = Not tested

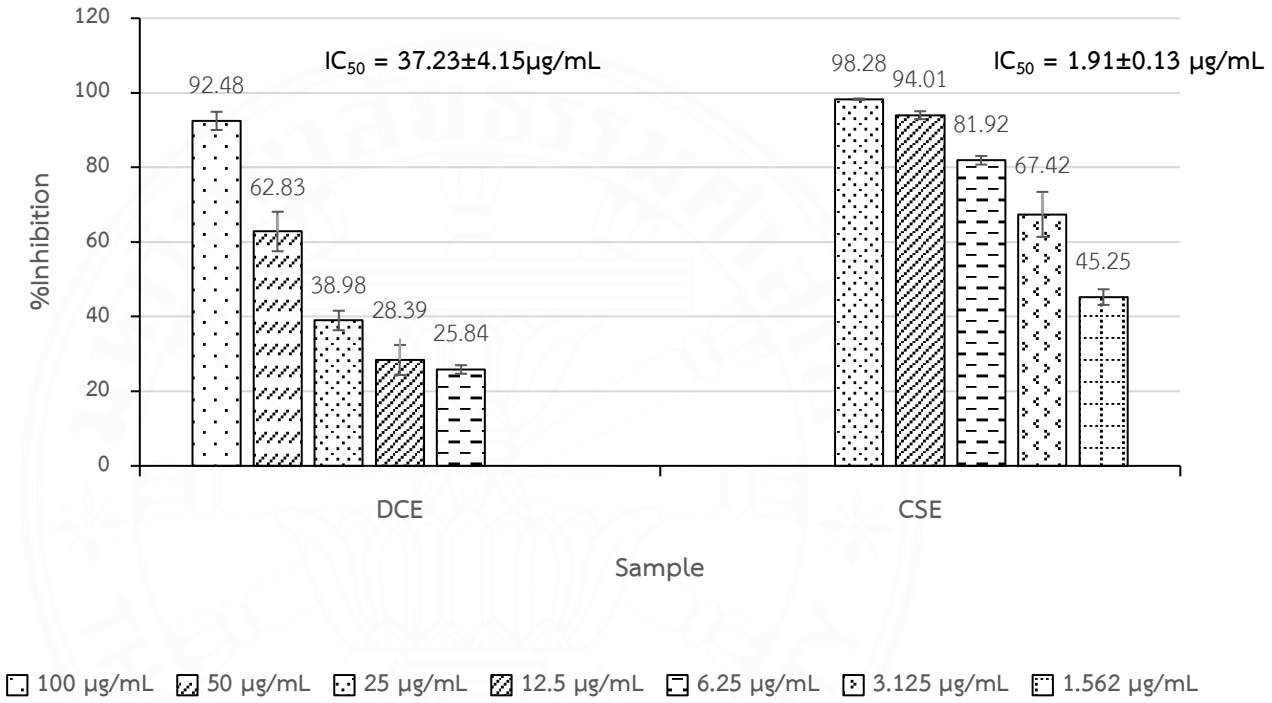



**Figure 4.10** Antiviral activity of DCE and CSE extracts against HSV-2 in pre-incubation assay

**4.2.2 Pre-treatment assay**

The BLWE, BLWA, PJDE, and PJDA were investigated for screening the anti-HSV-2 activity by pre-treatment at 100 µg/mL. The results showed that BLWE, BLWA, PJDE, and PJDA showed low anti-viral activity against HSV-2 with percent inhibition of 14.67±1.97, 11.48±1.17, 4.30±0.82, and 14.04±0.08% at 100 µg/mL. However, the inhibition effect of ACV against HSV-2 also showed a low effect with a percent inhibition of 23.16±4.32% at 100 µg/mL. All samples have a percent inhibition value of less than 50 percent. Therefore, the extract could not prevent the HSV-2 entry to the Vero cells. These data are shown in **Table 4.7** and **Figure 4.11.**

**Table 4.7** Antiviral activity of BLWE, BLWA, PJDE, PJDA extracts and ACV against HSV-2 in pre-treatment assay (Mean ± SEM), (n=3)

| Sample | % inhibition at concentration 100 µg/mL | $IC_{50}$(µg/mL) |
|---|---|---|
| BLWE | 14.67±1.97 | >100 |
| BLWA | 11.48±1.17 | >100 |
| PJDE | 4.30±0.82 | >100 |
| PJDA | 14.04±0.08 | >100 |
| ACV | 23.16±4.32 | >100 |

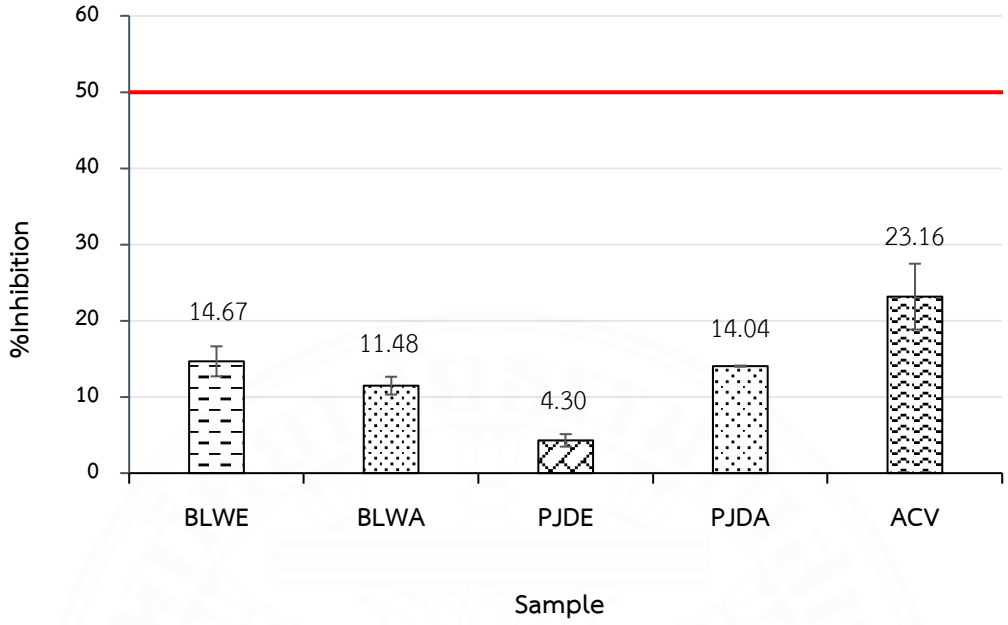



**Figure 4.11** Antiviral activity of BLWE, BLWA, PJDE, PJDA extracts and ACV against HSV-2 at concentration of 100 µg/mL in pre-treatment assay

**4.2.3 Post-treatment assay**

The antiviral activity of the extract was assessed by post-treatment after three-day incubation. The non-toxic concentrations of the extracts were tested for antiviral activity. The BLWE and PJDE inhibit HSV-2 with an inhibition value of 25.97±5.12 and 9.57±2.90% at 25 µg/mL. The BLWA and PJDA showed low inhibition effects against HSV-2 with an inhibition value of 25.16±5.59 and 20.68±3.89% at 100 µg/mL. The results can conclude that all samples had no antiviral activity against HSV-2 because they had less than 50 percent inhibition value. However, acyclovir had an $IC_{50}$ of 0.27±0.04 µg/mL. Acyclovir is activated by viral thymidine kinase and inhibits the DNA polymerase of the Herpes simplex virus (Shiraki., 2018). Thus, acyclovir showed the best antiviral effect against Herpes simplex in this mechanism. The data is shown in **Table 4.8** and **Figure 4.12-4.13.**

**Table 4.8** Antiviral activity of BLWE, BLWA, PJDE, PJDA extracts and ACV against HSV-2 in post-treatment assay (Mean ± SEM), (n=3)

| Sample | % inhibition / Concentration (µg/mL) | | | | | | | | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|---|---|---|
| | 100 | 50 | 25 | 3.125 | 1.562 | 0.781 | 0.390 | 0.195 | |
| BLWE | NT | NT | 25.97±5.12 | NT | NT | NT | NT | NT | >100 |
| BLWA | 25.16±5.59 | NT | NT | NT | NT | NT | NT | NT | >100 |
| PJDE | NT | NT | 9.57±2.90 | NT | NT | NT | NT | NT | >100 |
| PJDA | 20.68±3.89 | NT | NT | NT | NT | NT | NT | NT | >100 |
| ACV | NT | -NT | NT | 99.85±0.15 | 99.04±0.74 | 88.12±3.70 | 68.90±8.04 | 39.40±7.04 | 0.27±0.04 |

*NT = Not tested

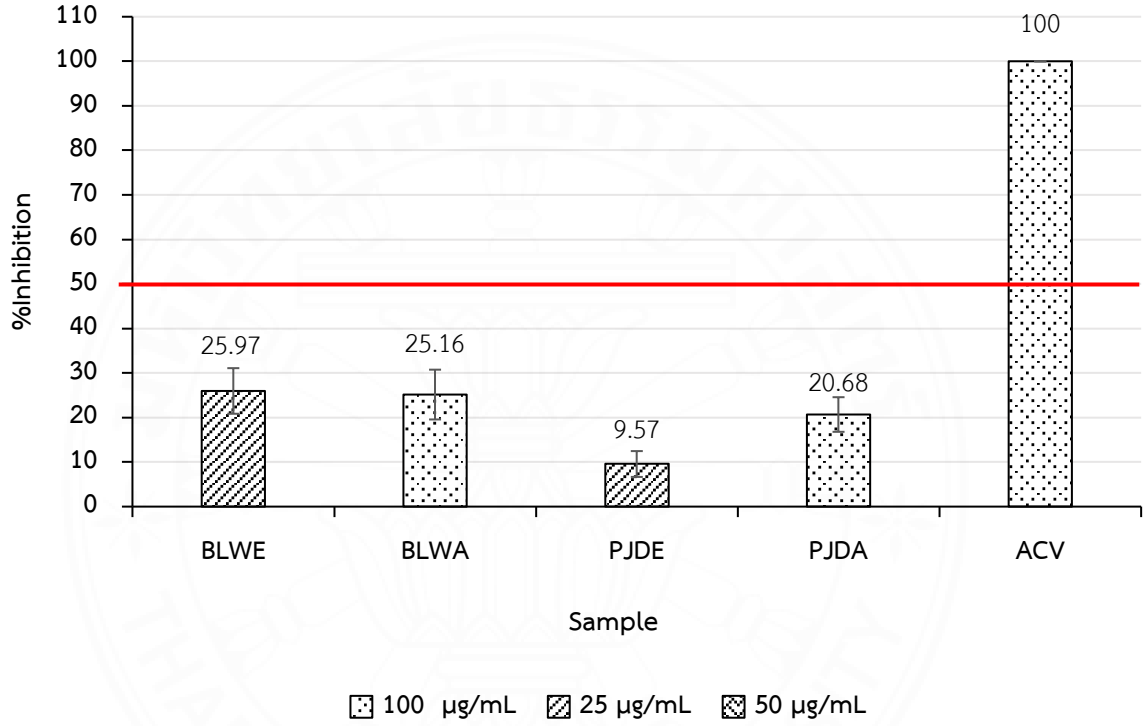



**Figure 4.12** Antiviral activity of BLWE, BLWA, PJDE, PJDA extracts and ACV against HSV-2 in post-treatment assay

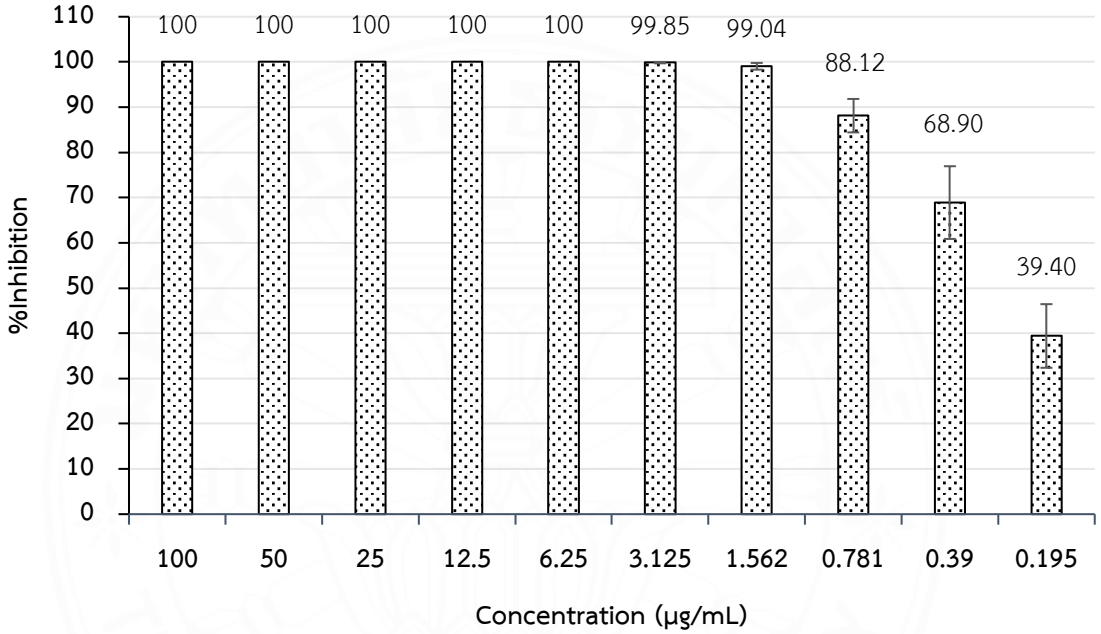



**Figure 4.13** Antiviral activity of ACV against HSV-2 in post-treatment assay

### 4.2.4 Antiviral activity of pure compounds from PJDE

The results showed that PJDE could inactivate the virus in only one process of the pre-incubation assay. The PJDE had the most effective inhibition of HSV-2 with an $IC_{50}$ value of 24.46±6.05μg/mL, while CSE, plant component of the PJD, was the most potent inhibitor of HSV-2 with an $IC_{50}$ value of 1.91±0.13 μg/mL. The previous study found that brazilin is the main pure compound of PJDE and CSE (Prommee *et al*., 2020). In addition, EPMC is a pure compound of KGE (Umar *et al*., 2012) and there has never been a study on the virus. Therefore, a pre-incubation assay tested pure compounds such as brazilin and EPMC for anti-HSV-2 activity. First, brazilin and EPMC was investigated for cytotoxicity to Vero cell. Brazilin was non-cytotoxic to the Vero cell at maximum concentrations of 12.5 μg/mL, which the percentages of cell survival were 86.42±1.21%. In addition, EPMC were non-cytotoxic to the Vero cell at maximum concentrations of 100 μg/mL, which the percentage of cell survival was 89.77±1.27%. The cytotoxic effect of brazilin and EPMC on Vero cells after 2 hours incubation are shown in **Table 4.9** and **Figure 4.14.**

**Table 4.9** Cytotoxicity of the pure compound on the Vero cell after 2 hours incubation (mean±SEM), (n=3)

| Sample | % cell survival ± SEM / Concentration (μg/mL) | | | | |
|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | 6.25 |
| Brazilin | 10.73±1.02 | 34.04±0.95 | 40.52±0.52 | 86.42±1.21 | 91.86±1.37 |
| EPMC | 89.77±1.27 | 94.67±1.37 | 94.27±1.80 | 94.83±0.38 | 94.67±0.41 |

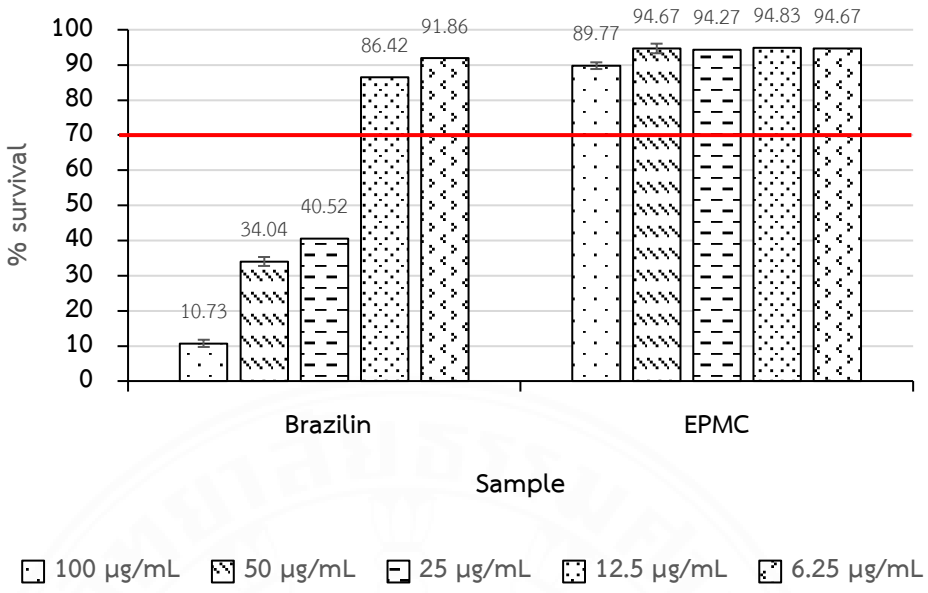



**Figure 4.14** Cytotoxicity of Brazilin and the EPMC on the Vero cell after 2 hours incubation

After that, brazilin and EPMC were tested for Herpes simplex virus activity in a pre-incubation assay by plaque reduction assay. The data are shown in **Table 4.10** and **Figure 4.15.**

The results showed that brazilin effectively inhibited Herpes simplex virus type-2 in Pre-incubation assay with an $IC_{50}$ value of 4.28±1.64 µg/mL, which is the highest anti-viral effect compared to the ethanolic of PJDE and acyclovir. Previous studies have not yet been investigated the antiviral activity of brazilin. However, flavonoid compounds have been reported on antiviral activity against the HSV-1, HSV-2 including influenza A virus and coronavirus (Lyu *et al.*, 2005; Dong *et al.*, 2014; Russo *et al.*, 2020). On the other hand, EPMC is a pure compound of KGE. Unfortunately, it has a low inhibitory effect against HSV-2 with less than 50 percent of herpes virus inhibition.

**Table 4.10** Antiviral activity of pure compound against HSV-2 in pre-incubation assay (Mean ± SEM) (n=3)

| Sample | % inhibition / Concentrations (µg/mL) | | | | | | | | $IC_{50}$(µg/mL) |
|---|---|---|---|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.562 | 0.781 | |
| Brazilin | NT | NT | NT | 71.94±7.84 | 53.95±3.73 | 44.79±3.94 | 30.64±5.07 | 20.32±0.32 | 4.28±1.64 |
| EPMC | 3.76±1.89 | NT | NT | NT | NT | NT | NT | NT | >100 |
| ACV | 98.91±1.09 | 85.85±3.47 | 66.47±4.27 | 56.19±9.35 | 30.94±2.40 | NT | NT | NT | 12.71±2.94 |

*NT = Not tested

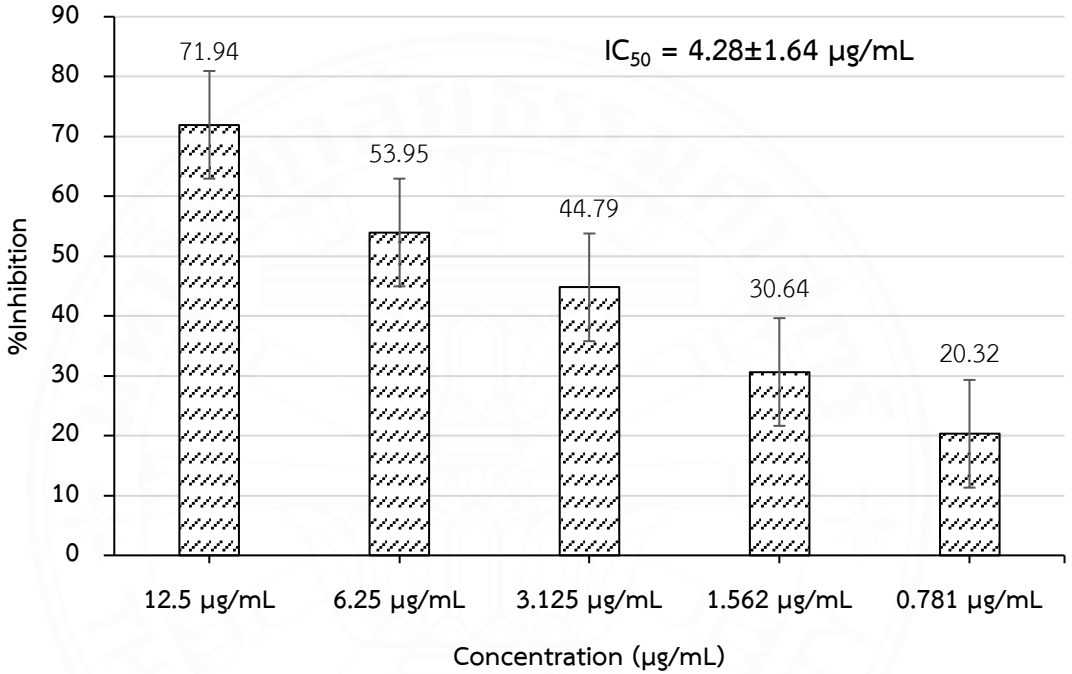



**Figure 4.15** Antiviral activity of Brazilin against HSV-2 in pre-incubation assay

The experimental results found that PJDE, CSE, DCE and brazilin have antiviral effects in the Pre-incubation assay or direct virus killing. The previous study found that the herpes simplex virus (HSV) transmission in human tissues is cell-to-cell spread. The virus spreads from the primary infected cell to the adjacent cells in the mucous tissue (Arvin *et al.*,2007). Another study found that the development and progression of HSV-1 lesions were delayed by the water extract of *C.sappan* (Kurokawa *et al.*,1993). The results showed that our extract directly inhibited the virus and may reduce the spread of the herpes virus from cell to cell and the severity of herpes. However, the results found that the ethanolic and the aqueous extract of Benchalokawichian and its plant component did not inhibit all three mechanisms of herpes simplex virus type-2. The previous studies have shown that Benchalokawichian extract has anti-inflammatory, antipyretic, anti-allergic, and anti-nociceptive effects (Juckmeta *et al.*, 2014; Booranasubkajorn *et al.,* 2017; Jongchannapong *et al.*, 2010). Therefore, Benchalokawichian extract is more suitable for treating anti-inflammatory and anti-pyretic of HSV patients.

**4.2.5 Antiviral activity of PJDH**

PJDE has an inhibitory effect on the HSV-2 in the pre-incubation assay, while PJDA could not inhibit the HSV-2 virus in any mechanism. However, PJDA may be changed the phytochemical structure after digestion in the stomach. Thus, the hydrolysis of PJDA was performed to confirm the antiviral activity of aqueous extract of Prasachandang remedy. PJDA extract hydrolysis (PJDH) was tested for cytotoxicity to Vero cells at two hours and three days. The PJDH showed non-cytotoxic to Vero cells with a cell survival rate of 99.03±1.41% at 100 µg/mL after two hours of incubation. In comparison, it showed non-cytotoxic to Vero cells with a cell survival of 81.04±1.29% at 50 µg/mL after three days of incubation. The data is shown in **Table 4.11 and Figure 4.16**

**Table 4.11** Cytotoxicity of the PJDH extract on the Vero cell after 2 hours and 3 days incubation (mean±SEM), (n=3)

| Sample | incubate with Vero cell | % cell survival ± SEM / Concentration (µg/mL) | | | | |
|---|---|---|---|---|---|---|
| | | 100 | 50 | 25 | 12.5 | 6.25 |
| PJDH | 2 hours | 99.03±1.41 | 96.50±0.73 | 98.26±0.59 | 98.09±0.30 | 101.50±1.41 |
| PJDH | 3 days | 67.37±1.88 | 81.04±1.29 | 79.19±0.71 | 84.62±0.90 | 87.94±1.59 |

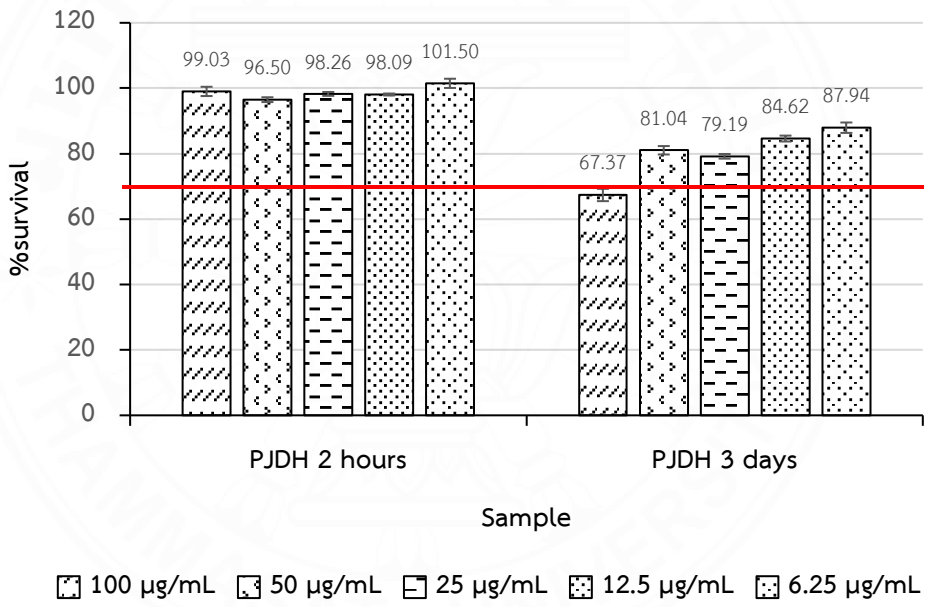



**Figure 4.16** Cytotoxicity of PJDH on the Vero cell after 2 hours and 3 days incubation

The non-toxicity concentrations of PJDH were tested for antiviral activity by plaque reduction assay for pre-incubation assay, pre-treatment assay, and post-treatment assay. The antiviral activity results are shown in **Table 4.12.**

The PJDH extract has the best effect of inhibiting the HSV-2 in the pre-incubation assay with an $IC_{50}$ value of 28.03±6.44 µg/mL. In contrast, the PJDA extract had no antiviral activity in this process. In the pre-treatment assay and the

post-treatment assay, the maximum non-toxic concentration of PJDH showed a low inhibition effect against HSV-2 with an inhibition value of 23.78±0.75 and 12.11±3.35%, respectively.

Hydrolysis can cause a chemical change in the extraction formula, The previous study, hydrolyzed acid extraction cade the substances in the seed cake yields more than the solvent extraction and hydrolyzed acid concentration can enhance the extract quality (Usman *et al.,* 2003). A previous study found that hydrolysis process can transform the chlorogenic acid isomer to its derivative compounds. (Dawidowicz *et al.,* 2017). Another study found the biological activity and chemical content of *Hibiscus sabdariffa* extract were increased after acid hydrolysis, including acidic gastric conditions in the stomach may increase the anti-inflammatory and antioxidant effects of *H. sabdariffa* extract (Ariyabukalakorn *et al.*, 2019). The previous report found that the final biological activity of the drug depends on the final change after digestion (Kumar *et al.,* 2011). These results revealed that hydrolysis of PJDA may change the chemical structure of PJDA and affect on anti-viral activity after digestion.

**Table 4.12** Antiviral activity of PJDH extract against HSV-2 (Mean ± SEM) (n=3)

| Sample | % inhibition / Concentrations (µg/mL) | | | | | $IC_{50}$ |
|---|---|---|---|---|---|---|
| | **100** | **50** | **25** | **12.5** | **6.25** | **(µg/mL)** |
| **Pre-incubation assay** | | | | | | |
| PJDA | 43.81±5.48 | NT | NT | NT | NT | >100 |
| PJDH | 90.78±5.04 | 83.23±2.60 | 37.35±7.83 | 17.41±0.95 | NT | 28.03±6.44 |
| ACV | 98.91±1.09 | 85.85±3.47 | 66.47±4.27 | 56.19±9.35 | 30.94±2.40 | 12.71±2.94 |
| **Pre-treatment assay** | | | | | | |
| PJDA | 14.04±0.08 | NT | NT | NT | NT | >100 |
| PJDH | 23.78±0.75 | NT | NT | NT | NT | >100 |
| ACV | 98.91±1.09 | 85.85±3.47 | 66.47±4.27 | 56.19±9.35 | 30.94±2.40 | 12.71±2.94 |
| **Post-treatment assay** | | | | | | |
| PJDA | 20.68±3.89 | NT | NT | NT | NT | >100 |
| PJDH | NT | 12.11±3.35 | NT | NT | NT | >50 |
| | **3.125** | **1.562** | **0.781** | **0.390** | **0.195** | |
| ACV | 99.85±0.15 | 99.04±0.74 | 88.12±3.70 | 68.90±8.04 | 39.40±7.04 | 0.27±0.04 |

*NT = Not tested

The studies have shown the extracts of PJDE, DCE, CSE, PJDH, and Brazilin have an inhibitory effect on the herpes simplex virus in pre-incubation assay with $IC_{50}$ value of 24.46±6.05, 37.23±4.15, 1.91±0.13, 28.03±6.44 and 4.28±1.64 µg/mL, respectively. While ACV inhibited HSV-2 with $IC_{50}$ value of 12.71±2.94 µg/mL. PJDA, BLWE, and BLWA have a low inhibitory effect against HSV-2 with less than 50 percent of herpes virus inhibition, as showed in **Table 4.13**. Even though PJDH had a similar anti-viral activity to PJDE against Herpes simplex virus, the yield of PJDH was lower than the yield of PJDE. Thus, PJDE is more suitable for the product development for treating Herpes simplex than PJDH.

CSE extract was the highest anti-viral substance against Herpes simplex virus when compared with the other extracts, brazilin and acyclovir. The dry powder extract of *C. sappan* has been investigated the sub-acute toxicity in rats. The results showed that it had no hepatotoxicity and nephrotoxic after oral administration to rats at concentrations of 250, 500, and 1,000 mg/ml for 30 days when compared to the control group (Sireeratawong *et al.*, 2010). Thus, CSE is high potent anti-viral substance against Herpes simplex virus and suitable for the development of oral or topical anti-viral products.

The difference anti-viral activity of each sample may be caused by chemical constituent in extracts, so the chemical fingerprint analysis of each extracts were performed by using Thin layer chromatography (TLC) .

**Table 4.13** Summary of antiviral activity of the extracts against HSV-2 in the pre-incubation assay (mean±SEM), (n=3)

| Sample | % Yield (W/W) | % inhibition / Concentrations (µg/mL) | | | | | | | | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.562 | 0.781 | |
| PJDE | 13.62 | 78.07±5.92 | 55.16±7.08 | 49.47± 3.74 | 38.58±7.62 | NT | NT | NT | NT | 24.46±6.05 |
| PJDA | 2.24 | 43.81±5.48 | NT | NT | NT | NT | NT | NT | NT | >100 |
| PJDH | 7.05 | 90.78±5.04 | 83.23±2.60 | 37.35±7.83 | 17.41±0.95 | NT | NT | NT | NT | 28.03±6.44 |
| DCE | 10.45 | 92.48±2.44 | 62.83±5.30 | 38.98±2.62 | 28.39±4.03 | 25.84±1.16 | NT | NT | NT | 37.23±4.15 |
| CSE | 8.17 | NT | NT | 98.28±0.20 | 94.01±1.07 | 81.92±7.70 | 67.42±6.04 | 45.25±2.09 | NT | 1.91±0.13 |
| BLWE | 3.80 | 28.56±3.89 | NT | NT | NT | NT | NT | NT | NT | >100 |
| BLWA | 8.83 | 44.25±4.79 | NT | NT | NT | NT | NT | NT | NT | >100 |
| Brazilin | - | NT | NT | NT | 71.94±7.84 | 53.95±3.73 | 44.79±3.94 | 30.64±5.07 | 20.32±0.32 | 4.28±1.64 |
| ACV | - | 98.91±1.09 | 85.85±3.47 | 66.47±4.27 | 56.19±9.35 | 30.94±2.40 | NT | NT | NT | 12.71±2.94 |

*NT = Not tested

**4.2.5.1 Thin layer chromatography fingerprint development**

The PJDA, PJDH, PJDE, CSE, DCE, and Brazilin were performed chemical fingerprints by Thin layer chromatography (TLC). The extract was developed in three solvent systems of hexane: ethyl acetate (7:3), hexane: acetone (1:1), chloroform: methanol (9:1) and record the chromatogram results using the photographic method. The results of the study are shown in **Figure 4.17-4.19.**

CSE exhibited a blue color spot under UV 365 nm and color change to an orange spot after spraying with anisaldehyde reagent (spot number 1) that was similar to brazilin's spot, as shown in **Figure 4.18-4.19**. The previous report showed that brazilin is the main chemical compound of CSE (Prommee *et al.,* 2020). Thus, spot number 1 is possible to be brazilin. Furthermore, spot number 1 of CSE should be changed to orange color after confirmation by ferric chloride reaction (Soonthornchareonnon *et al.*, 2008). Surprisingly, CSE is a higher potent anti-viral activity than brazilin. The chemical fingerprint of CSE showed that CSE contained other chemical substances. In the previous study, *C. sappan* was found quercetin and flavonoids that showed anti-herpes virus activity (Gravina *et al*., 2011; Lyu *et al.,* 2005). One possibility is the synergistic effect of substances in CSE may cause the high anti-viral activity of CSE.

Spot number 2 was found in PJDH, PJDE and DCE. It was blue color under UV 365 nm and the color changed to purple after spraying with anisaldehyde. The terpenoids group display a similar result to spot number 2, so spot number 2 is expected to be a terpenoids (Soonthornchareonnon *et al.,* 2008). Terpenoids were found in plant ingredients in Prasachandang such as *J. sambac, K. galangal*, and *M. siamensis* (Esmail, 2018; Umar, 2011; Pootaeng-on., 2005). Spot number 3 and spot number 4 showed similar result to spot number 1. They were blue color under UV 254 and 365 nm and color change to orange after spraying with anisaldehyde. However, spot number 3 and 4 did not match with the Rf of brazilin, so they is expected to be the other flavonoid compounds. Previous studies found that DCE, the main component of Prasachandaeng remedy, contain other flavonoids substance such as cochinchinenenes A-D, cochinchinenenes E-H, cochinchinenin,

cinnabarone, dracaenin A, and biflavocochins A-G (Thu *et al.*, 2020) which the ability to inhibit the herpes virus may be caused by these substances.

The chemical fingerprint of PJDA was difference from PJDH, as showed in **Figure 4.17-4.19**. The chemical fingerprints proved that acid hydrolysis can change the chemical structure of aqueous extract. PJDA after hydrolysis contains similar chemical constituents to PJDE. Moreover, they showed almost the same anti-viral activity. Even though PJDA showed no anti-viral activity against the Herpes simplex virus, the acid-hydrolysis process in the stomach can change the chemical structure of PJDA and increase anti-viral activity. Thus, the decoction of Prasachandaeng which is Thai traditional usage can be used to prepare Prasachandaeng remedy for the treatment of HSV patients. The ethanolic extract of Prasachandaeng remedy that showed high yield and anti-viral activity should be developed topical product for the treatment of HSV in the future.

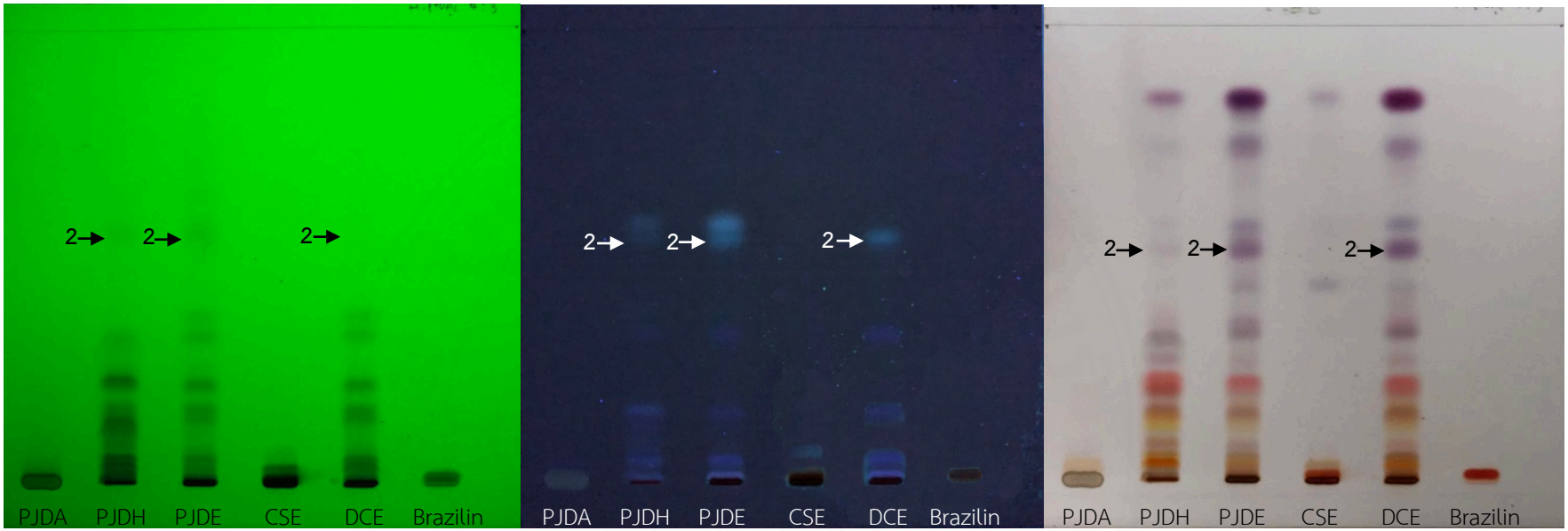



**Figure 4.17** TLC fingerprint of extract at hexane: ethyl acetate (7:3)

A. UV 254 nm B. UV 365 nm C. sprayed anisaldehyde and heated at 105-120 °C

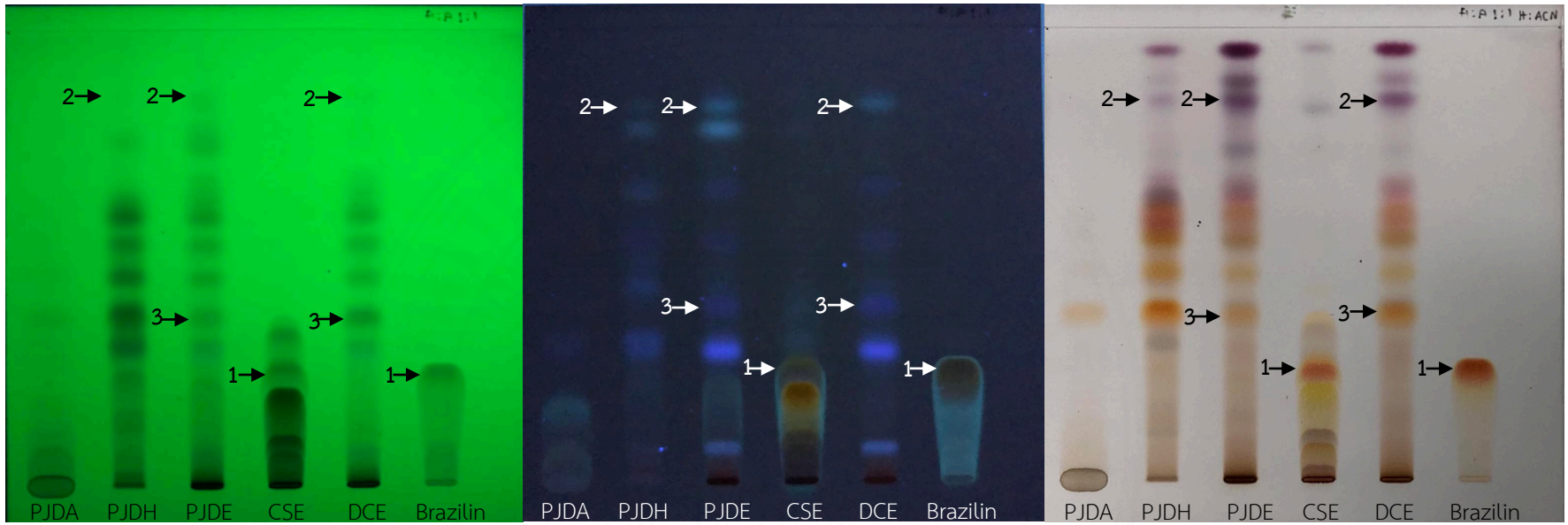



**Figure 4.18** TLC fingerprint of extract at hexane: acetone (1:1)

A. UV 254 nm B. UV 365 nm C. sprayed anisaldehyde and heated at 105-120 °C

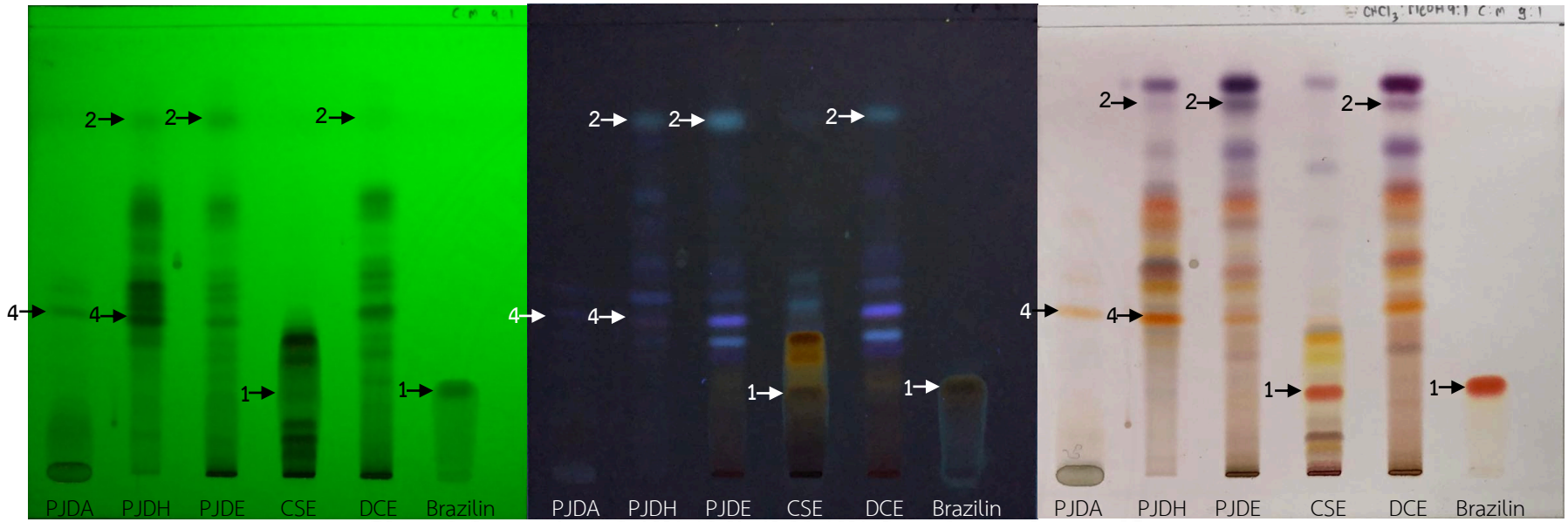



**Figure 4.19** TLC fingerprint of extract at chloroform: methanol (9:1)

A. UV 254 nm B. UV 365 nm C. sprayed anisaldehyde and heated at 105-120 °C

# CHAPTER 5

## CONCLUSIONS AND RECOMMENDATIONS

In the past, herbal medicines were used to treat many infections, including herpes infections. Acyclovir is used to treat herpes. However, they are imported and expensive. In addition, long-term use of the drug can lead to drug resistance. Therefore, medicinal plants are an alternative treatment. BLW remedy is a medicinal formula consisting of 5 roots plants. In Takasila scriptures that mention the properties of Khireimnakhang and Khireimnakhaw. PJD remedy consists of 12 herbs that are indicated for the treatment of fever, Kai-Pid, and Kai-Karn. Folk healers use it to treat fever, pain, burning, reduce heat, and it is also used to treat skin conditions such as Herpes or allergic dermatitis. Traditional healers use their formula to treat fever, reduce inflammation and reduce heat.

The previous study of both recipes have anti-inflammatory, antipyretic, anti-allergic, anti-nociceptive effects, and inhibited some viruses. In addition, both recipes have never been studied to inhibit the Herpes virus. In this study, we investigated the antiviral activity of BLW extract, PJD extract, their plant components extracts, and active markers against HSV-2 and also investigate the chemical fingerprints of antiviral extracts by using thin-layer chromatography (TLC).

The extracts were tested for toxicity on Vero cells by using MTT assay to determine the percentage of viability cells. The extracts were incubated for 2 hours and 3 days to correlate with the antiviral assay method. The results of BLW and PJD extract which were incubated with cells for 2 hourswere found that the ethanolic and aqueous extracts of both extracts had non-cytotoxic to Vero cells at all concentrations. PJDH showed non-cytotoxic to Vero cell at concentrations of 100 µg/mL. CSE, DCE, and KGE showed non-cytotoxic at concentrations of 25, 100, and 100 µg/mL, respectively. HPE and TTE showed non-cytotoxic to Vero cell at concentrations of 12.5 and 50 µg/mL, respectively. The results of BLW and PJD extracts which were incubated with cells for 3 days were found that the PJDE and PJDA were non-cytotoxic to Vero cells at concentrations of 50 and 100 µg/mL, respectively. PJDH was non-

cytotoxic to Vero cells at a concentration of 50 µg/mL. The BLWE and BLWA extracts were non-cytotoxic on Vero cells at a concentration of 25 and 100 µg/mL, respectively. ACV is the standard drug for the treatment of Herpes simplex. The cytotoxicity of ACV was tested at a concentration of 100 µg/mL and also showed non-cytotoxic against Vero cells when incubated with Vero cell at 2 hours and 3 days. The pure compound of PDJ such as Brazilin and EPMC were tested toxicity on Vero cells by using MTT assay. Brazilin and EPMC also showed non-cytotoxic to Vero cell at concentrations of 12.5 and 100 µg/mL, respectively.

The extract were tested activity against HSV-2 by plaque reduction assay. Antiviral activity was tested for 3 mechanisms: pre-incubation assay, pre-treatment assay, and post-treatment assay. In the pre-incubation assay, PJDE and PJDH showed inhibitory activity against HSV-2 with $IC_{50}$ values of 24.46±6.05 and 28.03±6.44 µg/mL, respectively. While CSE showed the best inhibitory activity against HSV-2 with an $IC_{50}$ value of 1.91±0.13 µg/mL. DCE showed inhibitory activity with $IC_{50}$ values of 37.23±4.15 µg/mL. In contrast, BLWE, BLWA, PJDA, its plant components of BLWE and KGE had no antiviral effect against HSV-2. In addition, brazilin, the pure compound of CSE, was tested to kill viruses directly and had an $IC_{50}$ value of 4.28±1.64 µg/mL, which brazilin is the most potent compound which inhibited of the HSV-2. Furthermore, EPMC is the isolated compound of KGE, which could not inhibit the HSV-2. However, the pre-treatment assay and post-treatment assay, the BLWE, BLWA, PJDE, PJDA, and PJDH extracts had no antiviral effect against HSV-2.

From this study, the PJDH extract inhibited the virus in the pre-incubation assay, while the PJDA extract did not have antiviral activity. Therefore, both extracts were performed chemical fingerprint using a Thin layer chromatography (TLC). The PJDH and PJDA extracts showed a different pattern in all solvent systems. Acid hydrolysis causes chemical changes in the aqueous extract to contain active substances that it can inhibit the virus.

The results of the antiviral activity of the PJDE, DCE, PJDH, CSE, and brazilin showed that the extract had an inhibitory effect on the HSV-2 in the pre-incubation assay. However, BLW is not effective against antiviral activity in all mechanisms. The

previous studies have shown that BLW extract has anti-inflammatory, antipyretic, anti-allergic, and anti-nociceptive effects. Therefore, BLW extract is more suitable to be developed as a treatment for herpes symptoms than an antiviral drug.

This study support that the Prasachandaeng, *D. cochinchinensis*, and *C. sappan* have antiviral effects. Therefore, they should be developed as a topical drug for the treatment of Herpes simplex viruses such as cream or gel. However, the pre-formulation study of PJDE under stress condition such as acid, base, high temperature and oxidation conditions should be investigated before product development process.

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# APPENDIX

## Chemical Reagent for Laboratory Experiment

**Reagent for cytotoxic and antiviral activity**

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

**1.1 Stock solution (1X)**

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

**1.2 Complete media (10%FBS in MEM)**

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

**1.3 Maintain media (3%FBS in MEM)**

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

**1.4 Stock solution (2X)**

| | | |
|---|---|---|
| Sterile deionized water | 500 | mL |
| MEM powder | 9.5 | g |
| 5%$NaHCO_3$ | 2 | g |
| 10% hydrochloric acid | 0.5-0.7 | µL |

**1.5 Maintain media (6%FBS in MEM)**

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

**2. Phosphate buffer saline (PBS)**

| | | |
|---|---|---|
| PBS | 1 | Tablet |
| Distilled water | 500 | mL |

Sterilize by autoclave before use

**3. 10% Formaldehyde**

| | | |
|---|---|---|
| PBS | 145 | mL |
| Formaldehyde 37% | 54 | mL |

**4. 0.1% Crystal violet**

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

**5. 3% Carboxymethylcellulose**

| | | |
|---|---|---|
| Distilled water | 500 | mL |
| Carboxy Methyl Cellulose | 15 | mg |

Sterilize by autoclave before use

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| | |
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