PRODUCT DEVELOPMENT OF BENCHALOKAWICHIAN AND MANGOSTEEN FOR SKIN INFECTION AND CANCER TREATMENT

BY

MISS THANA JUCKMETA

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
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FACULTY OF MEDICINE
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was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy in Medical Sciences

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ABSTRACT

Benchalokawichian (BLW) remedy as a Thai Traditional medicine preparation, consist of five plant roots as follows: *Ficus racemosa* Linn., *Capparis micracantha* DC., *Clerodendrum petasites* S. Moore., *Harrisonia perforata* Merr., *Tiliacora triandra* Diels.. Water extract and ethanolic extracts of individual plants and Benchalokawichain remedy were tested for *in vitro* cytotoxic activity against three lung cancers (A549, COR-L23, H226), oropharynx and larynx cancer cell lines (KB, Hep2). All of the water extracts showed no cytotoxic activity (IC_{50}>50 µg/mL). Four of ethanolic extracts which were FR, CM, CP, HP also showed the same result. The ethanolic extract of *Tiliacora triandra* (TTE) inhibited the growth of all types of respiratory system (three lung cancer, oropharynx and larynx) cancer cell lines with IC_{50} values in range of 19.5-45.2 µg/mL. In the same way, the 95%EtOH extract of Benchalokawichien (BLWE) showed *in vitro* cytotoxic activity against A549, COR-L23, H226, KB, Hep2 cell lines with IC_{50} values in the range of 10.1-33.7 µg/mL. Both of them showed specific cytotoxicity against H226 compared with other cell lines. In addition, BLWE showed higher cytotoxicity against the skin cancer (C32) cell line than TTE with IC_{50} values of 29.1 and 37.9 µg/mL, respectively.

The results of this research suggest that the *Tiliacora triandra* ethanolic extract (TTE) would be a good marker for cytotoxic activity of BLW remedy, so it was

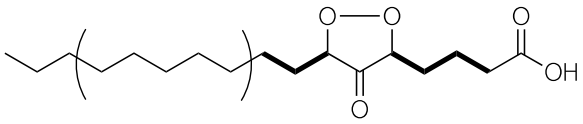
investigated using a bioassay-guided fractionation technique. Following VLC method, five fractions (TTF1-TTF5) were collected, a percentage of yield was calculated. TTF4 was the most abundant compound (48.4%) followed by TTF5, TTF2, TTF3, TTF1 (24.1%, 4.6%, 3.82%, 0.1%). The result showed that TTF4 like TTE exhibited *in vitro* cytotoxic activity against all cancer cell lines. Surprisingly, TTF5 showed specific against H226 with an IC_{50} value of 33.02 µg/mL. TTE1 was isolated from the TTF4 ethanolic extract of *Tiliacora triandra*. This is first report of tiliacoric acid [4-((3*S*,5*R*)-5-decyl-4-oxo-1,2-dioxolan-3-yl) butanoic acid] a new compound whose structure was elucidated by spectroscopy (NMR, MS, IR, UV). CPE1 compound which was isolated from the *Clerodendrum petisites* ethanolic extract was identified as oleanolic acid acetate. The main compound of *Harrisonia perforata* was HPE1, identified as perforatic acid. Compound BLWE1-3 was isolated from the chloroform fraction of crude BLW ethanolic extract. By comparison of ^{1}H and ^{13}C NMR data with previous studies, BLWE1-BLWE3 were identified as **β**-sitosterol, pectolinarigenin and perforatin A or *O*-methylalloptaeroxylin, respectively.

Cytotoxic activity of isolated compounds against two lung cancer cell lines, melanoma, and a normal cell line were investigated. The TTE1 inhibited the growth of two lung cancer cell lines, A549 and H226, with IC_{50} values of 6.49 and 13.98 µg/mL, which was better than TTE (IC_{50}=33.65 and 19.48 µg/mL). Notably, TTE1 showed no toxicity in a normal cell line (HaCaT). CPE1 also showed strong cytotoxic activity against A549 and H226 with IC_{50} values of 1.24 and 1.95 µg/mL whereas CPE wasn't active (>50 µg/mL). Although HPE1 was the main compound from *Harrisonia perforata* and Benchalokawichian remedy, it showed no cytotoxic activity in SRB assay (>50 µg/mL). In contrast, BLWE2 was cytotoxic to two lung cancer cell lines (A549, H226) and melanoma cancer cell line (C32) with IC_{50} values of 7.76, 6.25, and 6.78 µg/mL respectively. However, BLWE2 also showed toxicity in a normal cell line.

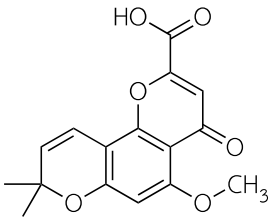
From literature reviews, pectolinarigenin and **α**-mangostin were used as the markers of mixed extract (Benchalokawichian or BLW and *Garcinia mangostana* or GM) in our research. Comparison of GM yield in various solvent extractions, the ethanolic extract showed the highest yield 21.38%, following soxhlet extraction with methanol, chloroform and hexane were 20.82%, 4.35%, 0.92%, respectively. The results showed

that BLWE inhibited *S. aureus* MRSA and *S. aureus* at a concentration 5 mg/mL, but it wasn't killing them at the same dose (MBC >5mg/ml). In similarly, BLWE exhibited antimicrobial activity against *S. pyogenes* at a concentration 400 µg/mL (MBC >400µg/mL). In an investigation of the antimicrobial activities against *S. aureus* MRSA, GMC showed the highest activity follow by GMH, GME, MIX 1:2 and MIX 1:1 (MIC values 0.78, 1.56, 3.13, 6.25, 12.5 µg/mL, respectively). Alpha-mangostin exhibited higher antibacterial activity against MRSA than vancomycin (MIC=0.19, 0.78 µg/mL, MBC=0.39, 0.78 µg/mL, respectively) while gentamicin and clindamycin were not active (MIC > 100 µg/mL). The GMH extract showed highest anti-inflammatory activity, followed by GMC (IC_{50} values 6.24 , 7.84 µg/mL). Nevertheless, they also showed toxicity at a concentration of 30 µg/mL. Both MIX1:1 and GMM showed moderate anti-inflammatiory activity (IC_{50} = 34.70, 37.84 µg/mL), and they showed no toxicity at a concentration of 50 µg/mL. It is possible that BLWE can reduce toxicity of GME.

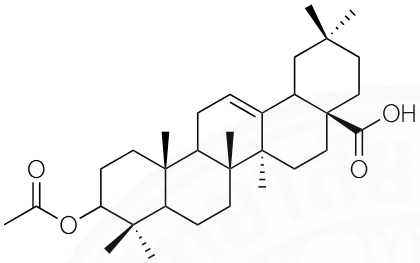
The biological activities and chemical fingerprint of the mixed extract was investigated in an attempt to improve their efficacy over long-term periods. Samples were placed in a stability incubator at 40 celsius degree, with RH 75% for 6 months. The HPLC chemical analysis of the extracts showed that the alpha-mangostin was decreasing over time while pectolinarigenin was increasing. However, they showed a barely different but not significant change in relative concentration (p-value < 0.05). Inhibitory effect on the release of nitric oxide induced by lipopolysaccharide (LPS) and toxicity of mix extract in various days (D0-D180) were not different. The effect of antimicrobial activity against *S. aureus* MRSA were influenced by oxidation and acid hydrolysis in preformulation study. We found that the Rx4 formulation which provided moisturizer and soothing skin might be useful to develop a product. As the result of antimicrobial activity, 0.3% of mixed extract was added in the cream formulation. The triplicate of formulated creams extracted by methanol, yields were average in range 4.51-5.88%. Alpha-mangostin which represented the major chemical compound in the mixed cream was analyzed using HPLC, but the compound concentration various days showed no significant difference. The research showed that the cream preparation was stable and can be kept for periods up to two years.



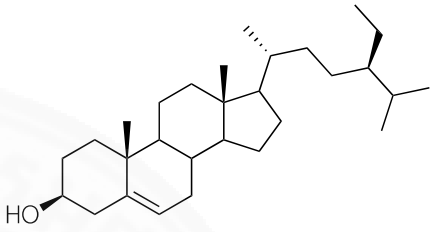
TTE1



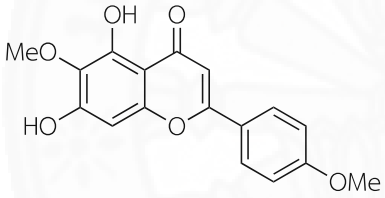
HPE1



CPE1



BLWE1



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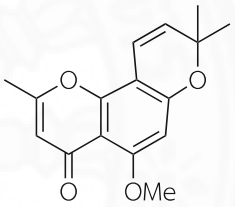

BLWE3

Keywords: product development, Benchalokawichian, mangosteen, isolation, cytotoxic, compounds

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Miss Thana Juckmeta

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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
3D	Three dimensions
ASA	Acetylsalicylic acid
BHI	Brain Heart Infusion
BLW	Benchalokawichian
CAT	Catalase
CC	Column chromatography
CCl_4	Carbon tetrachloride
$CDCl_3$	Deuterated chloroform
CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
cm^{-1}	centimeters
CO_2	Carbondioxide
COSY	correlation spectroscopy
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DPPH	α, α-diphenyl-β-picrylhydrazyl
EC_{50}	Half maximal effective concentration
ESIMS	Electrospray Ionization Mass Spectroscopy
FBS	Fetal Bovine Serum
GM	*Garcinia mangostana*
gm	Gram
GSH	Glutathione
HMBC	Heteronuclear Multiple Bond Correlation

Symbols/Abbreviations	Terms
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High-performance liquid chromatography
IC_{50}	Half maximal inhibitory concentration
IR	Infrared spectroscopy
LOD	Limit of Detection
LOQ	Limit of Quantification
LPS	Lipopolysaccharide
m/z	Mass-to-charge ratio
MBC	Minimum Bactericidal Concentration
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
Me_2CO	Acetone
MeOD	Deuterated methanol
MeOH	Methanol
mg	Milligram
MIC	Minimum Inhibitory Concentration
mm	Millimeters
MS	Mass Spectroscopy
nm	Nanometer
NMR	Nuclear Magnetic Resonance Spectroscopy
NO	Nitric oxide
NT	Not test
PBS	Phosphate Buffer Saline
rpm	Round per minute
Rx	Recipe
SAR	Structure-Activity Relationships
SD	Standard Deviation
SEM	Standard Error of Mean

Symbols/Abbreviations	Terms
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamate-Pyruvate Transaminase
SOD	Superoxide Dismutase
SRB	Sulforhodamine B
TCA	Trichloroacetic acid
TLC	Thin Layer chromatography
TPC	Total Phenolic Content
UV	Ultraviolet spectroscopy
VLC	Vacuum liquid chromatography
w/w	Weight by weight
^{o}C	Degree celsius
%	Percent
>	More than
<	Less than
=	Equal
/	Per
α	Alpha
β	Beta
γ	Gamma
&	And
µg	Microgram
µg/ml	Microgram per milliliter
µl	Microliter
µM	Micromolar

CHAPTER 1
INTRODUCTION

Ministry of public health of Thailand reported the statistic of number and rates of out-patients from the health service units per 1000 persons in 2009-2014 that showed the infectious and parasitic diseases had high rated as more than one-hundred in each year. In addition, the incidence rates of patients who diagnosed as the neoplasms and tumors were increasing. The first cause of mortality in Thailand is cancer, the incidence of cancer in 2005 showed 98,852 new patients (48,596 males and 50,256 females). In 2012 mortality of cancer patients in Thailand are 61,082 patients and trend to increasingly. National Cancer Institute of Thailand reported that cancer in respiratory systems (bronchus and lung cancer) was top three in both men and women (NCI, 2013). Additionally, Thai society of clinical oncology concerned about 300-400 new patients of skin cancer was annually reported and trend to increased (TSCO, 2013). About 2 in 3 of causes inducing cancer relative with smoking, foods, behavior, environment, lack of exercises and infections. As bacterial infection wasn't the major cause of cancer but their mechanisms can lead to provide the cancer (Parsonnet, 1995). Traditional methods for cancer treatment are including chemotherapy, radiotherapy, surgery, and immunotherapy, however, many anticancer drugs also showed various side effects such as fatigue, weight loss, nausea, vomiting, bleeding etc. Not only the mechanism of infection and cancer but their drugs for treatment were also relavance. Many anticancer drugs exhibited antimicrobial activity are classified as an "antitumor antibiotic" for examples, the anthracycline group such as doxorubicin and quinone group such as mitomycin. In the same way, macrolide antibiotic as clarithromycin which is traditionally used for bacterial infections is repurposing to be a cancer drug (Van Nuffel *et al.,* 2015)

There are several types of skin infection, the three main causes are bacteria, virus, and fungi. According to the New York State Department of Health, the two most common are *Staphylococcus aureus* and *Streptococcus*. The most symptoms depend on causes of infection for example; redness, rash, cellulitis, swelling etc. *Staphylococcus aureus* (*Staph. aureus* or "*Staph*") is a bacterium that is carried on the skin or nasal lining of up to 30 percent of healthy individuals. When skin is damaged, *Staph* can cause many problems from a mild pimple to severe illness, especially in young children, older adults, and people with a weakened immune system. The first drug of choice for treatment is penicillin. In the 1950s, many infections became resistant to penicillin and methicillin (related drug were developed to treat these germs such as oxacillin, nafcillin, dicloxacillin). More than 70 percent of the bacteria that cause of these infections are resistant to at least one of the antibiotics commonly used to treat them. By 1990, some strains of *Straph* became resistant to methicillin and other similar antibiotics called "MRSA" which can't cure by traditional drugs (Lui *et al.,* 2011). Vancomycin, a new synthesis drug, was generated and recommended for treatment of MRSA infection. As the prevalence of MRSA is high, the document report susceptibility of *S. aureus* to vancomycin was changing in May 1996. Center of Disease Control and Prevention (CDC) definitions for classifying isolates of *S. aureus* with reduced susceptibility to vancomycin are based on the laboratory breakpoints established by the Clinical and Laboratory Standards Institute (CLSI). In 2009, CLSI classify 3 types as follow; vancomycin-susceptible *S. aureus* (VSSA) showed MIC value ≤2 µg/ml, vancomycin-intermediate *S. aureus* (VISA) showed MIC value = 4-8 µg/ml. and vancomycin-resistant *S. aureus* (VRSA) showed MIC value ≥16 µg/ml (Division of Healthcare Quality Promotion Centers for Disease Control and Prevention, 2015). Treatment of skin infection depend on the cause of infection and severity. The good personal hygiene is an important for cure skin diseases, in some cases topical medications was added such as anti-inflammatory and antibiotic drugs. In severe cases, the combination oral antibiotics or possibly intravenous medication may be needed (Krucik, 2014).

The alternative medicines which are meditation, yoga, Chakra, Reiki, massage, acupuncture, homeopathy, herbal medicines commonly used for treatment in several diseases, the most of them are purpose of recovery the body, mind, and spirit. From literature reviews, the many sources of western medicines were developed from natural, especially plants. Anticancer drugs as "vincristine and vinblastine" are alkaloids which were found from *Catharanthus roseus* or Madagascar periwinkle (Tadeusz Aniszewski, 2007). *Garcinia mangostana* is a source of xanthones which well-known as strongly antibacterial and anti-inflammatory properties (Chomnawong *et al.,* 2005; Chen *et al.,* 2007; Al-Massarani *et al.,* 2013; Tewtrakul *et al.,* 2009), the traditional medicine used the pericarp for treatment of diarrhea, dysentery, infected wound, and chronic ulcer (Chaverri et al., 2008).

Benchalokawichian (BLW) or Ha-Rak remedy as a Thai Traditional medicine preparation, consist of five plant roots as follows: *Ficus racemosa* Linn., *Capparis micracantha* DC., *Clerodendrum petasites* S. Moore., *Harrisonia perforata* Merr., *Tiliacora triandra* Diels. It is commonly used to reduce fever (Rehabilitation Foundation for the Promotion of traditional Thai medicine. Ayurvedic College (Cheevakakomalapaj, 2001). Folk doctors appropriately used by adjust the ratios of plants according to the symptoms for the example, *C. petasites* was added high ratio in case of fever with thirsty nausea and vomiting, *C. micracantha* and *H. perforata* were added in case of fever with blister rashes and *F. racemosa* was added in case of fever with diarrhea. There are few reports of this remedy. Many researchers studied based on traditional usage, for example, the powder of roots showed antipyretic efficacy in the animal models (Konsue *et al*., 2008; Jongchanapong *et al*., 2010). The roots have used the components of remedy, thus Singharachai *et al.,* (2008) studied on the morphological and anatomical characteristic or pharmacognostic evaluation. They found that the stems and roots showed similar appearances. Moreover, stem adulteration was commonly founded in commercial BLW capsules (Nutmakul *et al.,* 2013). Preliminary *in vitro* studies, the ethanolic extract showed moderate anti-inflammatory, anti-allergic, antibacterial and cytotoxic activities whereas the water extract showed no activities (Chartsuwan *et al.,* 2009; Suranart *et al.,* 2009; Sangrapee *et al.,* 2009; Tabpueng *et al.,* 2009;). Although, the BLW extract showed moderate

antibacterial activity (Nuaeissara *et al.,* 2011). Interestingly, the combination of BLW remedy and *Garcinia mangostana* which inhibited against *Staphylococcus aureus* MRSA registered the petty patent in Thailand no.10082. The combination of extract inhibited against 3 types MRSA which were isolated from the blood of infected patients with MIC values 6.25-31.25 µg/ml. Recent year, two compounds as pectolinarigenin and *O*-methyllaloptaeroxyrin were isolated from BLW remedy and showed higher anti-allergic activity than the crude of BLW ethanolic extract (Juckmeta *et al.,* 2014). Furthermore, the development and validation of RP-HPLC method to determine anti-allergic compound (pectolinarigenin) in BLW remedy was investigated (Sakpakdeejaroen *et al.,* 2014).

Therefore, the aims of this research were divided into two parts. Initially, the BLW remedy and its components were extracted and studied on cytotoxic activity against 5 types of the respiratory systems cancer cell lines (including A549, H226, COR-L23, KB, and Hep2) and a skin melanoma cell line (C32). The most effective extract was further studied to isolate the pure compound for being a marker of cytotoxic activity. In addition, the principal chemical constituents of BLW also investigated. The isolated compounds were elucidated and identified by using spectroscopy techniques including NMR, MS, IR, UV. Then, they were examined cytotoxicity by SRB assay as same as their crude extract. Another part, the development of a product from Benchalokawichian (BLW) remedy and *Garcinia mangostana* (GM) were studied base on a petty patent of mixed extract for the skin infection. GM was extracted in various organic solvents using soxhlet method and traditional maceration, the GM ethanolic extract was combined with BLW ethanolic extract in 2 ratios to obtain MIX1:1 and MIX1:2. All extracts were studied on antimicrobial activity against 4 gram-positive bacteria, i.e. *Staphylococcus aureus, S. aureus* MRSA, *S. epidermidis* and *Streptococcus pyogenes*. Likewise, the inhibitory effect against nitric oxide production of all extracts were considered an anti-inflammation activity whereas toxicity by MTT assay also studied. According to the review of literature, pectolinarigenin and alpha-mangostin were promoted to be a marker of BLW and GM. Thus, these two compounds were verified their efficiency on the antimicrobial and anti-inflammatory activities. Additionally, development and validation of pectolinarigenin and alpha-mangostin was

studied using RP-HPLC the modified method according to quality control of ICH guidelines. Stability of mixed extract was studied on chemical and biological activities before designed the product development. Following the results of force degradation, we produced the topical cream for skin treatment. An objective of the cream product was formulated to prevent skin infection and reduce skin inflammation. Percentage of mixed extract which added on cream formula was decided by the concentration of antimicrobial activity. The quality control of cream product was examined by HPLC analysis using the alpha-mangostin as a marker of mixed extract, moreover, *in vitro* antimicrobial activity against *S.aureus* MRSA was tested.

1.1 Research Problems

Benchalokawichian or Ha-Rak (BLW) remedy as a Thai Traditional medicine preparation is commonly used to reduce fever and skin treatment. The previous studied on anti-inflammatory, anti-allergy and antimicrobial activities provide information to support their treatment. There are few previous reports on cytotoxic activity of Benchalokawichian remedy. *Garcinia mangostana* (GM) and major compounds as alpha-mangotsin have many reports antimicrobial and antiinflammatory activities, but it will not launched be product for antibiotic drug. Moreover, there has been no report on active compounds for cytotoxic and antimicrobial activities of Benchalokawichian remedy.

1.1.1 Do the Benchalokawichian remedy and its compounds show cytotoxic activity?

1.1.2 Is it possible to develop combination extracts from BLW and GM to be an alternative instead of antibiotic for treatment of drug resistance bacteria strain?

1.2 Objectives

1.2.1 To investigate the cytotoxic activity of water extracts and ethanolic extracts from Benchalokawichian (BLW) remedy and each plant

1.2.2 To isolate the active compounds for cytotoxic activity against cancer cells from Benchalokawichian (BLW) remedy

1.2.3 To investigate antimicrobial activity of formulary from combination Benchalokawichian (BLW) remedy and extracts from *Garcinia mangostana*

1.2.4 To develop the antibacterial product from Benchalokawichian (BLW) remedy combined with *Garcinia mangostana* extract for skin infection treatment

1.2.5 To determine the quality control of Benchalokawichian (BLW) remedy, combination of BLW and GM for cytotoxic and antimicrobial activities by using High Performance Liquid Chromatography (HPLC) technique

1.3 Keywords

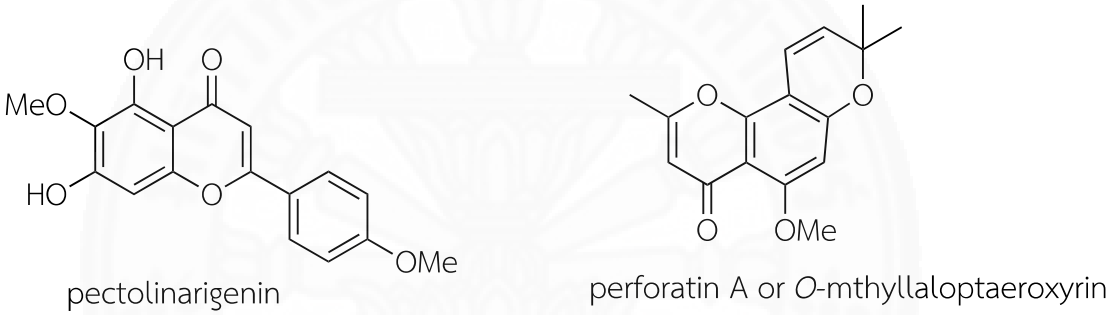
Benchalokawichian (BLW, Harak), *Garcinia mangostana* (GM), cytotoxic activity, antimicrobial activity, anti-inflammatory activity, product development

CHAPTER 2
REVIEW OF LITERATURE

2.1 Benchalokawichian remedy

Benchalokawichian remedy is widely used as an antipyretic and skin treatment by many traditional practitioners in Thailand. Root powder of BLW formula showed the antipyretic efficacy by using a Baker's yeast-induced fever model in rats (Konsue *et al*., 2008). Antipyretic and antinociceptive activity of ethanolic extract, all doses of BLW 25-400mg/kg significantly ($p<0.05$) attenuated the increased rectal temperature produced by LPS injection and were found to be as potent as ASA. BLW (400 mg/kg) produced a significant analgesic response in the hot-plate test, while all doses of BLW, except the lowest dose (25mg/kg), produced significant analgesic responses in the tail-flick test. For acetic acid-induced writhing models in mice, BLW doses of 200 and 400 mg/kg significantly ($p<0.05$) decreased the mean writhing response compared to vehicle controls (Jongchanapong *et al*., 2010). For Identification of adulteration, Singharachai *et al.,* (2008) recommended identification of five roots base on the morphological characters including macroscopic examination of whole plant, microscopic investigation of transverse section and powder of root crude drugs examination. Their result showed that the main distinguishing features of five root species were obtained from the morphological and histological characters as well as 3D-HPLC chromatogram. Another research, they found that the stems and roots of each plant presented similar appearances, especially *C. micracantha* and *F. racemosa*, which were similar in both morphological and anatomical characters. Thin Layer Chromatography Technique used for distinguishes adulterate comparing authentic root and stem. Results of TLC technique, stem adulteration was commonly found in commercial capsule of BLW remedy (Nutmakul *et al.,* 2013). *In vitro* study, its ethanolic extract showed moderate anti-inflammatory, anti-allergic, antibacterial and cytotoxic activity whereas the water extract showed no all activities (Chartsuwan *et al.,* 2009; Suranart *et al.,* 2009; Sangrapee *et al.,* 2009; Tabpueng *et al.,* 2009; Nuaeissara *et al.,* 2011; Juckmeta & Itharat, 2012). Pectolinarigenin and *O*-methylalloptaeroxylin were

founded in this remedy, which showed higher anti-allergic activity (IC_{50}=6.3, 14.2 µg/ml) than chlorpheniramine (IC_{50}=16.2 µg/ml) as a positive control and crude BLW extract (IC_{50}=39.8 µg/ml) (Juckmeta *et al.,* 2014). Studied on development and validation of RP-HPLC method determined anti-allergic compound in BLW remedy. Pectolinarigenin was absorption at wavelength 331 nm for quantification, the spectra of peak was detected at a retention time at 21.49 min. Based on the HPLC analysis, pectolinarigenin was a minor compound with a content of 0.18% w/w (Sakpakdeejaroen *et al.,* 2014)



pectolinarigenin perforatin A or *O*-mthyllaloptaeroxyrin

Figure 2.1 Two compounds found from BLW remedy

2.1.1 *Clerodendrum petasites/ Clerodendrum indicum*

Clerodendrum petasites has two botanical synonyms as *Clerodendrum petasites* and *Clerodendrum indicum* in the family Verbanaceae or Lamiaceae, the local name are Thao yai mom (Central, Chanthaburi); Kasalong, Charto phra thorani, Dok khan (Yala); Thao yai mom pa (Ubon Ratchathani); Ping khom, Ping luang (Northern); Phaya rak diao, Phaya leng chon, Leng chon tai, Phom phi (Udon Thani), Pho-kwo (Karen-Kamphaeng Phet), Phin phi (Loei), Pho phing (Ratchaburi) Mai thao ruesi (Northern, Peninsular), Ya lin chon (Prachuap Khiri Khan) in Thailand (Thai Biodiversity, 2012).

Microscopic study stems had a pericyclic band of sclerenchyma, and lignified parenchyma and sclereids lining around hollow pith, which absented in the root (Nutmakul *et al.,* 2013). Anti HIV-1 Integrase of aqueous aerial extract exhibited activity with IC_{50} value 43.5 µg/ml whereas the ethanolic extract showed no activity, IC_{50} > 100µg/ml (Bunluepuech & Tewtrakul, 2009). The methanol and ethanolic extract showed anti-inflammatory in animal model and inhibit NO effect in RAW264.7 cell line with IC_{50} value 51.5, 46.6 µg/ml (Panthong *et al.,* 2003; Suranart *et al.,* 2009; Juckmeta & Itharat, 2012). The ethanolic and water extracts also showed no antioxidant with DPPH scavenging assay and no cytotoxic activity on COR-L23, MCF-7, MRC5 cell line (Chartsuwan *et al.,* 2009; Juckmeta & Itharat, 2012). For anti-allergy, the ethanolic extract from its roots exhibited effect against release β-hexosaminidase with IC_{50} value 90.0, 57.8 µg/ml (Tabpueng *et al.,* 2009; Juckmeta *et al.,* 2014). The ethanolic extract inhibited all gram negative and positive bacteria except *C. albicans*. The water extract showed no activity against all microbes (Nuaeissara *et al.,* 2011). Moreover, the ethanolic extract was tested to evaluate the spasmolytic activity on isolated guinea-pig tracheal smooth muscle. The crude extract (2.3–9.0 mg/ml) dose-dependently caused relaxation of tracheal smooth muscle which was contracted by exposure to histamine. The active principle was isolated and identified as the flavonoid hispidulin (EC_{50}=3.0×10^{-5}M, 9 µg/ml), which showed more effective than asthma drug; aminophylline as positive control with EC_{50} values 26 µg/ml (Hazekamp *et al.,* 2001).

2.1.1.1 Chemical constituents of *Clerodendrum petasites/ indicum*

The Isolated chemical constituents from *Clerodendrum petasites* or *indicum* which were reported showed in Table 2.1 and Figure 2.2.

Table 2.1 Chemical constituents found in *Clerodendrum indicum*

Part of plant	Chemical constituents	References
aerial	hispidulin **(1)**	Hazekamp *et al.,* 2001
root	3b-hydroxy-D:B-friedo-olean-5-ene **(2)**	Somwong *et al.,* 2015
	oleanolic acid-3-acetate **(3)**	
	taraxerol **(4)**	
	lupeol **(5)**	
	(22E)-stigmasta-4,22,25-trien-3-one **(6)**	
	stigmasta-4,25-dien-3-one	
	stigmasta-4,22-dien-3-one	
	22-dehydroclerosterol	
	clerosterol **(7)**	
	stigmasterol	
	22-dehydroclerosterol-3-O- β -D-glucopyranoside	
	Clerosterol-3-O- β -D-glucopyranoside **(8)**	
	Stigmasterol-3-O- β -D-glucopyranoside	
	pectolinarigenin **(9)**	
	Hispidulin	

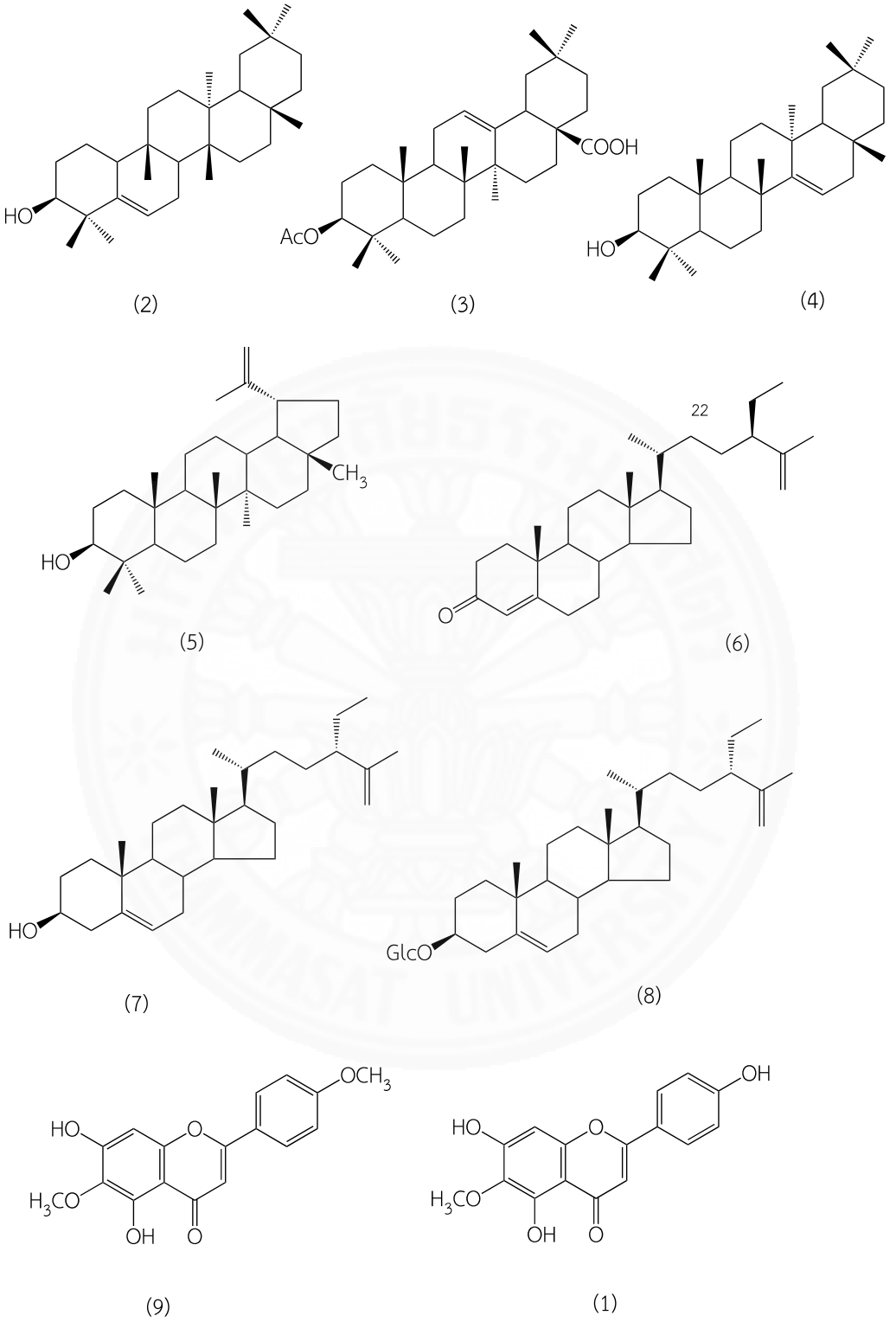


Figure 2.2 Selected chemical constituents of *Clerodendrum indicum*

2.1.2 *Capparis micracantha*

Capparis micracantha DC. (CAPPARACEAE) has two botanical synonyms *Capparis odorata* Blanco (1837), *Capparis myrioneura* Hallier f. (1906). It has vernacular names in various countries which are Indonesia: balung, kledung (Javanese), sanek (Madurese). Malaysia: kaju tuju. Philippines: salimbagat (Tagalog), tarabtab (Iloko), salimomo (Bisaya). Cambodia: kanchoen bai dach. Laos: say sou. Thailand: chingchee (central), kradaat khaao (central), nuat maeo daeng (northern). Vietnam: b[uf]ng ch[ef], c[as]p gai nh[or].

The ethanolic extract showed moderate antioxidant activity by DPPH assay with EC_{50} value 42.91, 61.35 µg/ml (Chartsuwan *et al.,* 2009; Juckmeta & Itharat, 2012), weak anti-inflammatory activity inhibit NO effect with IC_{50} value 95.79, 61.37 µg/ml (Suranart *et al.,* 2009; Juckmeta & Itharat, 2012), antimicrobial activity against general pathogen such as *S. aureus,* MRSA, *B. subtilis* (Nuaeissara *et al.,* 2011), but its ethanolic extract showed no cytotoxic against lung, breast cancer and normal lung cell [COR-L23, MCF-7, MRC5 respectively] (Chartsuwan *et al.,* 2009). The aqueous and ethanolic extracts of CM wood showed no HIV-1 Integrase activity (IC_{50} > 100 µg/ml). The water extract exhibited no anti-inflammatory, anti-allergic, antioxidant, in contrast, it had better inhibited microbes such as *A. buamannei, K. pneumoniae* and *Shigella* species (except *S. sonnei)* than the ethanolic extracts (Nuaeissara *et al.,* 2011). The macro-microscopic study, stems and roots presented similar appearances. *Capparis micracantha* stems had marked lignified sclereids, arranged in pericyclic band underneath rhytidome, groups in cortex and densely packed in secondary phloem. On the contrary, the roots had few groups of sclereids scattered in cortex and secondary phloem (Nutmakul *et al.,* 2013). The chemical constituents of Caper *spp.* were reported, but there is no research which studied on isolation compounds from CM.

2.1.2.1 Chemical constituents of *Caper* species

The Isolated chemical constituents from *Caper* Species which were reported showed in Table 2.2. Most of them are alkaloids and flavonoids. However, there is no report of chemical constituents from *C. micracantha.*

Table 2.2 Chemical constituents found in *Caper sp.*

Resources	Part of plant	Chemical constituents	References
C. aphylla	fresh root	capparilinecapparinecapparinine	Manzoor-i-Khuda & Jeelani, 1968
C. himalayensis	whole plant	capparin Acapparin B	Li *et al.,* 2008
C. decidua	root bark	a spermidine alkaloid, capparisine	Ahmad *et al.,* 1986
C. decidua	root bark	a spermidine alkaloid, isocodonocarpine	Ahmad *et al.,* 1989
C. decidua	root	15-N-acetyl capparisine	Ahmad *et al.,* 1992
C. decidua	root bark	capparasinine	Ahmad *et al.,* 1987
C. decidua	bark, root	capparidisine	Ahmad *et al.,* 1985
C. spinosa	aerial	kaemp-ferol-7-rhamnoside, flavonoid glycosides	Sharaf *et al.,* 1997
C. spinosa	fruit	(6S)-hydroxy-3-oxo-α-ionol glucosides	Calis *et al.,* 1999
C. spinosa	fruit	stachydrine	Afsharypuor *et al.,* 1999
C. spinosa	aerial	3-O-[6-α-L-rhamnosyl-6″-β-D-glycosyl]-β-D-glucoside, flavonoid	Sharaf *et al.,* 2000
C. spinosa	fruit	1H-indole-3-acetonitrile glycosides	Calis *et al.,* 2002
C. spinosa		cadabicine	Khanfar *et al.,* 2003
C. spinosa	young shoot and bud	glucocapperin glucosinolate	Matthaus &Özcan, 2002

Resources	Part of plant	Chemical constituents	References
C. spinosa	root	cadabicine 26-O-β-D-glucoside hydrochloride capparispine	Fu *et al.,* 2008
C. spinosa	fruit	capparisine A Capparisine B Capparisine C 2-(5-Hydroxymethyl-2-formylpyrrol-1-ol) propionic acid lactone N-(3'-Maleimidyl)-5-hydroxymethyl-2-pyrrole formaldehyde	Yang *et al.,* 2010
C. tomentosa	root	14-*N*-acetylisocodonocarpine	Dekker *et al.,* 1987

2.1.3 *Harrisonia perforata*

Harrisonia perforata Merr. (SIMARUBACEAE) has two botanical synonyms *Harrisonia paucijuga* Oliv. (1868) and *Harrisonia bennettii* Benn. (1875). It has vernacular names in various countries which are Indonesia: sesepang (Lampung), garut (Sundanese), ri kengkeng (Javanese). Malaysia: kait-kait (Murut, Sabah). Philippines: asimau, mamikil (Tagalog), muntani (Bisaya). Laos: dok kin ta. Thailand: khonthaa (Central), naam chee (Northern) (Flora of Thailand (various editors), 1970). Vietnam: s[aa]n, da da, h[ar]i s[ow]n (Pételot, 1952-1954).

Characteristic of dried stems and roots presented similar appearances. *H. perforata* stems had tangential bands of lignified fibers arranged in secondary phloem and area of pith whereas the roots had small-scattered groups of lignified fibers in secondary phloem and absented pith area (Nutmakul *et al.,* 2013). HP, which was extracted with organic solvent, showed antimicrobial activity against both gram-positive *(S. aureus,* MRSA*, S. pyrogenes, S. mutans, B. subtilis,)* and negative bacteria (*S. dysenteriae, A. buamannei)* (Limsong *et al.,* 2004; Chea *et al.,* 2007; Nuaeissara *et al.,* 2011). Cytotoxic activity, the ethanolic extract inhibited against cervical cancer cells (Hela), normal lung fibroblast (MRC-5), normal African green monkey kidney (Vero) cell, lung carcinoma cell line (COR-L23) and breast cancer cell line (MCF-7) (Nguyen-Pouplin *et al.,* 2007; Prayong *et al.,* 2008; Chartsuwan *et al.,* 2009). The water extract showed no cytotoxic and anti-inflammatory activity, but it exhibited anti-HIV-1 IN activity (IC_{50} = 2.3 µg/ml) higher than that of suramin, a positive control (IC_{50} = 3.4 µg/ml). Moreover, water extract of HP at the oral doses treated did not cause either acute (at the dose of 5,000 mg/kg) or subchronic toxicities in rats (Sireeratawong *et al.,* 2009). Noticeable, Juckmeta *et al.,* (2014) presented that ethanolic extract exhibited stronger anti-allergy activity than Tabpueng *et al.,* (2009) with IC_{50} = 14.5 and 84.4 µg/ml, respectively.

The chemical constituents of branches found peucenin-7-methyl ether, *O*-methylalloptaeroxylin, perforatic acid, perforamone A, B, C, eugenin, saikochromone A, perforamone D, greveichromenol, most of compounds inactived antiplasmodial activity. Only *O*-methylalloptaeroxylin also showed antiplasmodial with EC_{50} value 10.5 µg/ml and antimicrobial activity with MIC value 100 µg/ml. The MIC value of perforamone B and perforamone D showed highest antimicrobacterial activity (25 µg/ml) following by peucenin-7-methyl ether and greveichromenol (50 µg/ml), compound *O*-methylalloptaeroxylin and eugenin (100 µg/ml), perforamone A and C (200 µg/ml), respectively (Thadaniti *et al.,* 1994; Tuntiwachwuttikul *et al.,* 2006). Its stems and leaves were extracted by organic solvent and were isolated compounds such as perforatinolone, (a tetranortriterpenoid with an A, D-ring seco-limonoid structure, which is closely related to perforatin), gallic acid, sitosterol, 3-O-fl-D-glucopyranosyl sitosterol, haperforine A, haperforine E, 12-Desacetylhaperforine, haperforins C2, F, and G, harrisotone A, harrisotone B, harrisotone C, harrisotone D, harrisotone E, harrisonol A. Three structures (harrisotone A, harrisotone C and harrisonol A) exhibited significant cytotoxic activity against P-388 tumor cell line with IC_{50} values of 1.56, 2.35, and 0.27 µM, respectively. Harrisotone A and harrisonol A also showed moderate activity against lung cancer cell (A-549) with IC_{50} of 24.5 and 26.6 µM, pseudolaric acid B15 as positive control showed IC_{50} values as 0.74 and 1.99 µM against P-388 and A-549, respectively. (Sung *et al.,* 1995; Khuong-Huu *et al.,* 2000; Khuong-Huu *et al.,* 2001; Yin *et al.,* 2009). Isolated compound from its fruits and roots were 5,6-dehydrodesepoxyharperforin C2, harrpernoid B, harrpernoid C, harperforin C2, perforin A, 12b-acetoxyharrisonin, 11b,12b-diacetoxyharrisonin, rutaevine, umtatin, greveichromenol, one triterpene, pachymic acid, one lignan, pinoresinol, gallic acid, methyl gallate. Only harrpernoid B showed very weak cytotoxicity against human lung cancer and human leukemia (A-549 and HL-60) cell lines. Harperfolide exhibited stronger potent anti-inflammatory activity by suppressing nitric oxide (NO) production from activated murine macrophages J774.A1 cells than indomethacin (IC_{50} value of 6.51, 28.42 µM). Harrisolanol A, harperamone, peucenin-7-methyl ester, perforatic acid methyl ester, *O*-methylalloptaeroxylin, obacunone showed antiinflammatory activity at IC_{50} in range 31.04-83.61 µM. (Yan *et al.,* 2011; Choodej *et al.,* 2013).

2.1.4 *Ficus racemosa*

Ficus racemosa Linn. (MORACEAE) has two botanical synonyms *Ficus glomerata* Roxb. and *Ficus vesca*. (Lassak & McCarthy, 1997). It is a moderate to large-sized spreading tree (Anonymous, 1952). It has vernacular names in various countries which are cluster fig, red river fig (En). Indonesia: elo (Javanese), loa (Sundanese), arah (Madurese). Singapore: atteeka. Burma: atti, umbar. Cambodia: lovië. Laos: düa kiengz. Thailand: duea kliang (central, northern), duca nam (peninsular). Vietnam: sung. It is distributed in North-eastern Africa, India to Indo-China, Malaysia to north Western Australia (Jansen *et al.,* 1991), India, Sri Lanka, Thailand, Myanmar, Laos, Vietnam and Cambodia (Vo Van Chi, 1998).

F. racemosa Linn. is a moderate-sized avenue tree found throughout India either wild or cultivated for its fruits eaten by villagers. It is popular in Indigenous System of Medicine like Ayurveda, Siddha, Unani and Homoeopathy. In the Traditional System of Medicine, various plant parts such as bark, root, leaves, fruits and latex are used in dysentery, diarrhoea, diabetes, bilious affections, stomachache, menorrhage, haemoptysis, piles and as carminative and astringent (Paarakh, 2009). The fruits used for laxative, digestive, aphthae, menorrhagia, hemoptysis and gargling for sore throat. The leaves mixed with honey used for bilious affection, which are curing juandice, billious remittants and all varieties of disease generated by an unnatural condition of involving organ. The bark used for diabetes, cooling, gonorrhea, ulcers, skin diseases, scabies, hiccup, vomiting. The roots used for dysentery, infusion with oil used external treatment for excema, leprosy, rheumatism. In India contemporary ethnomedical commonly used decoction fruits for gripping gastralgia, root juice (sap) for stomachic and fresh leaf juice for pneumonia (Lansky *et al.,* 2008). Its was found tannins, kaempferol, rutin, arabinose, bergapten, psoralenes, flavonoids, ficusin, coumarins, phenolic glycosides (Baruah & Gohain, 1992).

In vitro antioxidant properties, extract/fractions from *F. glomerata* were studied for TPC, AOA, RP, DPPH, O^{-2},·OH scavenging activities and LPO. Among all the extract/fractions, ethyl acetate fraction (FEF) has shown potent antioxidant activity and was also found effective in protecting oxidative DNA damage. The *in vivo* evaluation of oxidative stress (LPO) and antioxidant defenses (concentration of GSH, as well as CAT and SOD activities) were measured in CCl_4 induced toxic rats. FEF was found to inhibit the toxicity as seen from the decreased LPO and increased GSH, SOD and CAT levels. FEF has higher phenolic content and showed the presence of gallic, chlorogenic and ellagic acid. Based on these results, it is concluded that *F. glomerata* protects tissues from oxidative stress and these effects are probably related to the antioxidant properties (Verma *et al.,* 2010). Another research, ethanolic extract showed stronger antioxidant activity study in DPPH and OH scavenging assay (Manian *et al.,* 2008; Juckmeta & Itharat, 2012). Petroleum ether extract from leaves (400 mg/kg, p.o.) exhibited a significant reduction in the CCl_4 induced increase in the levels of SGOT, SGPT, alkaline phosphatase and serum bilirubin. However, treatment with Neutrosec (a popular liver tonic) also better reversed the hepatotoxicity than the extract significantly (Mandal *et al.,* 1999). *In vivo,* the extract of FR showed anti-diarrhoeal, anti-inflammatory, antipyretic, antidiabetes, antitussive, antihyperglycemic, chemoprotective and gastroprotective activity (Mukherjee *et al.,* 1998; Mandal *et al.,* 1999; Mandal *et al.,* 2000; Rao *et al.,* 2002; Rao *et al.,* 2002; Rao *et al.,* 2003; Khan & Sultana, 2005; Rao *et al.,* 2008; Ahmed & Urooj, 2008; Chomchuen *et al.,* 2010) Subacute toxicity study of an aqueous extract of FR bark in rats. Indicated that administration of the aqueous extract of herb for 15 to 21 days (incremental as well as fixed, in dose 30 mg/100 gm and 20 mg/ 100 gm up to 320 mg/100 gm of body weight) in subacute toxicity study showed definitive liver damage. Hepatotoxicity is appeared to be reversible. Histopathologicaly renal damage was not marked. Serum creatinine and blood urea were increased significantly (Panwar *et al.,* 2010). The ethanolic extract of *F. racemosa* inhibited *S. typhimurium*, *A. buamannei*, *S. pyrogenes* and *B. subtilis* with inhibition zone 9.0, 8.5, 11.7 and 8.2 mm, respectively. The water extract showed no activity agianst all microbacterial in Nuaeissara *et al.,* (2011).

2.1.5 *Tiliacora triandra*

Tiliacora triandra (Colebr.) Diels, is in the family of Menispermaceae. It is a species of flowering plant native to mainland Southeast Asia, and is widespread in the northeast of Thailand. Yanang is named differently from region to region in Thailand. In the north, it has been called Joy-Nang whilst it is known as Toa-Ya-Nanag, Toa-Wan-Keaw and Toa-Pakinee in central Thailand; in the south, Yanang is known as Wan-Yo (Smitinand & Larsen, 1991).

The characteristic of dry stems and roots presented similar appearances. *T. triandra* stems had broad vascular bundles alternating with narrow medullary rays, contrast to the roots, which had narrow vascular bundles alternating with broad medullary rays (Nutmakul *et al.,* 2013). Nanasombat & Teckchuen (2009) found that methanolic extract of leaves had the antioxidant activity with an EC_{50} of 3,903.9 mg extract/ mg DPPH (AE = 25.6×10^{-5}) and contained the content of phenolic compounds (13.3 mg GAE/mg dry extract). Ethanolic of *T. triandra* roots showed strong antioxidant in DPPH assay with EC_{50} value 23.35, 15.38 µg/ml (Chartsuwan *et al.,* 2009; Juckmeta & Itharat, 2012) nearly the previous study. Tabpueng *et al.,* (2009) presented good anti-allergic activity with IC_{50}=10.3 µg/ml, in contrast, Juckmeta *et al.,* (2014) showed no activity (IC_{50} >100 µg/ml). Root ethanolic extracts inhibited *S. aureus, B. subtilis, E. coli* and *C. albicans* with inhibition zone 11.2, 13.8, 9.5 and 20.5 mm, respectively. The residue extracted inhibited *C. albicans* (9mm) whereas the water extract showed no activity (Sangrapee *et al.,* 2009). In the same way, ethanolic extract showed most effective activity against *E. coli* strains, *Shigella* species, *A. buamannei,* gram-positive bacteria and *C. albican* except *P. aeruginosa* and *K. pneumoniae.* On the other hand, the water extract only inhibited *S. aureus*, *S. aureus* MRSA and *C. albican* with the inhibition zone 6.7, 7.3 and 10.8, respectively (Nuaeissara *et al.,* 2011). However, the water extract does not cause acute or subchronic toxicities in either male or female rats (Sireeratawong *et al.,* 2008).

Tiliacolinine, tiliacorine and nortiliacorinine A, were isolated from root of *T. triandra* which showed antimalarial activity with IC_{50} values 3533 ng/ml, 675 ng/ml and 558 ng/ml, respectively (Mahidol *et al.,* 1994). Antimycobacterial activity of bisbenzylisoquinoline alkaloids from TT against 59 clinical isolates of multidrug-resistant *Mycobacterium tuberculosis* (MDR-MTB) found that tiliacorinine and 2'-nortiliacorinine showed stronger inhibition than tiliacorine (Sureram *et al.,* 2012). Additionally, oxonanolobine which isolated from the methanol extract from leaves possessed moderate activity against lung cancer (NCI-H187) with IC_{50} value of 27.6 ± 4.30 µg/ml whereas showed no cytotoxic activity against oral cavity cancer and breast cancer (KB and MCF-7, IC50 > 50 µg/ml). (Rattana *et al.,* 2016)

2.1.5.1 Chemical constituents of *Tiliacora triandra*

The chemical constituents of roots from *Tiliacora triandra* reported as alkaloids while leaves reported fatty acids showed in Table2.3 and Figure 2.3.

Table 2.3 Chemical constituents found in *Tiliacora triandra*

Part	Chemical constituents	References
root	tiliandrine	Paris *et al.,* 1967
root	tiliacorine **(10)**	Wiriyachitra *et al.,*
	tiliacorinine **(11)**	1981
	nortiliacorinine A	
	tiliacorinine 2'-N-oxide	
root	tlianangine	Pachaly *et al.,*
	1S,1'S-bisbenzylisoquinoline alkaloid yanangine	1986
	1R,1'S-dinklacorine	
root	noryanangine	Pachaly *et al.,*
	norisoyanangine	1988
	tilitriandrine	
root	tiliacolinine	Mahidol *et al.,*
	tiliacorine	1994
	nortiliacorinine A	
root	tiliacorine	Saiin *et al.,* 2003
	tiliacorinine	
root	tiliacorinine	Sureram *et al.,*
	2’-nortiliacorinine	2012
	tiliacorine	
leave	hexadecanoic acid **(12)**	Kaewpiboon *et*
	octadecanoic acid **(13)**	*al.,* 2014
	(Z)-6-octadecenoic acid **(14)**	

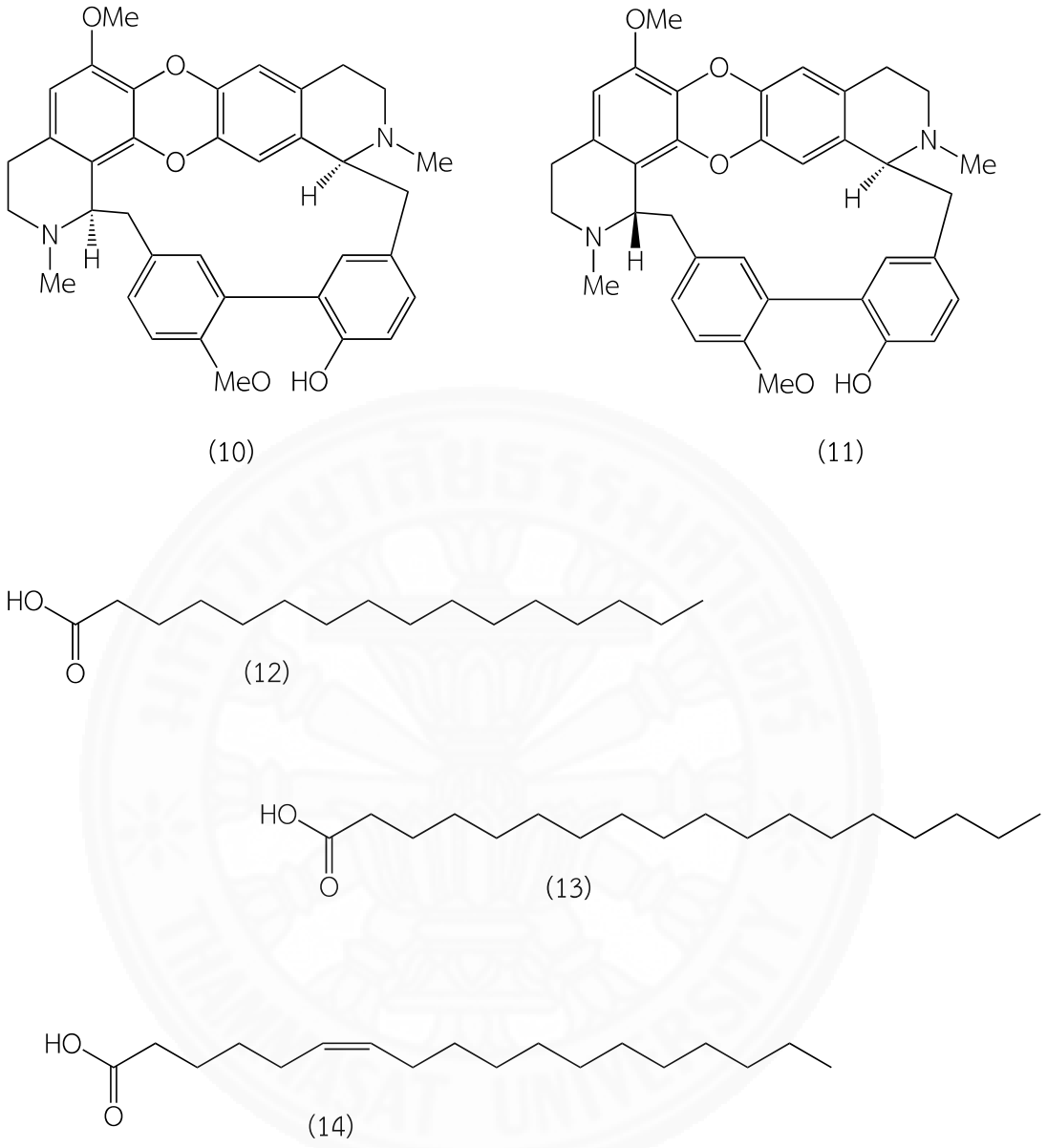


Figure2.3 Selected compounds of *Tiliacora triandra* from root and leave

2.2 *Garcinia mangostana*

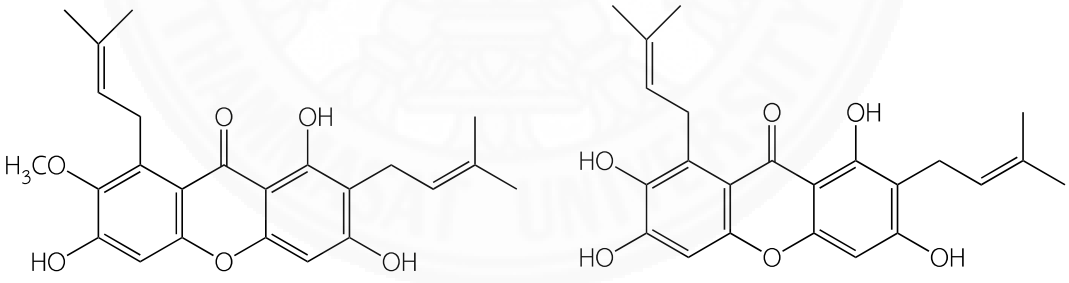
Garcinia mangostana Linn. (GM) belongs to the family of Guttiferae and is named "the queen of fruits". It is cultivated in the tropical rainforest of some Southeast Asian nations like Indonesia, Malaysia, Sri Lanka, Philippines, and Thailand. Traditional medicine has used the pericarp (peel, rind, hull or ripe) of GM for the treatment of abdominal pain, diarrhea, dysentery, infected wound, suppuration, and chronic ulcer (Pedraza-Chaverri *et al.,* 2008). Many researchers investigated antioxidant, antitumoral, anti-allergic, anti-inflammatory, antibacterial, and antiviral activities of the GM extract. Surprisingly, xanthone derivatives are major compounds, a group of oxygen-containing heterocyclic compounds including alpha-mangostin, gamma-mangostin, mangosteen extract which provide remarkable and diverse pharmacological effects (Jindarat *et al.,* 2014).

2.2.1 Anti-allergic, Antioxidant and Anti-inflammatory Activities

All GM extracts (100%, 70%, 40% ethanol and water) potently inhibited A23187-induced prostaglandin E2 synthesis in C6 rat glioma cells while *Rubus suavissimus*, positive control, had no effect. The 40% ethanol of GM extract (100, 300 mg/ml) showed more than 80% inhibition in a concentration-dependent manner of the histamine release. In contrast, the water extract from *Rubus suavissimus* significantly inhibited the histamine release only at the concentration of 300 mg/ml. Major constituents of mangosteen α- and γ-mangostin had no effect on IgE-mediated histamine release (Nakatani *et al.,* 2002). The ethanolic extract of *G. mangostana* (GM) showed the best antioxidant activity using DPPH assay and could inhibit 50% of free radicals at the concentration of 6.13 µg/ml (r^2 =0.9992). *G. mangostana* significantly reduced the ROS production with the highest inhibitory ratio at 77.80±1.28% (Chomnawang *et al.,* 2007). GM ethanolic extract possessed potent inhibitory effect on NO release, LPS-stimulated PGE_2, TNF-α and IL-4 releases with an IC_{50} value of 1.0, 6.0, 10.6 and 19.8 µg/ml. Two xanthones (α-and γ-mangostin) also better possessed inhibitory against NO with IC_{50} 3.1 and 6.0 µM, respectively than indomethacin with IC_{50} value 25 µM (Tewtrakul *et al.,* 2009). Similarly, α-and γ-mangostin, which were isolated

from 70% acetone of fresh hulls, inhibited NO production with IC_{50} value 12.4 and 10.1 μM (Chen *et al.,* 2008). In addition, γ-mangostin had stronger efficacy reduce PGE_2 production than α-mangostin in a dose-dependent manner in the same way with previous reports (Tewtrakul *et al.,* 2009; Chen *et al.,* 2008; Nakatani *et al.,* 2002). Both xanthones at concentration 5.0 μg/ml weakly inhibited iNOS activity in activated RAW264.7 with 4.24% and 28.69%. On the other hand, L-NAME (positive control) significantly inhibited nitrite accumulation by more than 50% at 200 μM (Chen *et al.,* 2008). α-mangostin higher inhibited than γ-mangostin inhibitory effect on LPS-stimulated TNF-α and IL-4 releases with IC_{50} value in range 31.8-64.8 μM, (Tewtrakul *et al.,* 2009). In enzyme assay in *vitro*, γ-mangostin inhibited the activities of both constitutive COX (COX-1) and inducible COX (COX-2) in a concentration-dependent manner, with the IC_{50} values of about 0.8 and 2 μM, respectively (Nakatani *et al.,* 2002).

In animal model, α-mangostin and sulindac treatment (20mg/kg) showed significant acute anti-inflammatory on paw edema inhibition when compared with control group at 3 h and 5 h, respectively whereas γ-mangostin did not. Interestingly, on-set time of α-Mangostin was more quickly than that of sulindac (Chen *et al.,* 2008).



alpha-mangostin gamma-mangostin

Figure 2.4 Xanthones (α-mangostin and γ-mangostin) main constituents of *G. mangostana*

2.2.2 Antimicrobial and antiplasmodial activities

Many reports studied on antimicrobial activities against microorganism of GM differential extracts. MIC values of GM extract were the same (0.039 mg/ml) for both bacterial species and the MBC values were 0.039 and 0.156 mg/ml against *Propionibacterium acnes* and *Staphylococcus epidermidis,* respectively (Chomnawang *et al.,* 2005). The crude CH_2Cl_2 extract (> 0.53 mg/disc) exhibited a favorable activity against *L. monocytogenes*. Furthermore, MIC and MBC of antibacterial against strain were 8.50 and 17.00 mg/ml, which MBC was two-fold higher than the corresponding MIC. The GM extract at conc. 17 mg/ml treated to cells and studied by Transmission Electron Microscopy (TEM). There was some loss and change of the membrane and cytoplasm in cells of the bacterium following exposure to crude extract. This may be due to the yield of **α**-mangostin content about 316.40 µg/mg of dry matter of extract (31.64%) from dried inner mangosteen hull extraction with CH_2Cl_2 (Tangwatcharin *et al.,* 2012). The ethyl acetate extract showed no antiprotozoal activity at all, a pronounced inhibitory effect (IC_{50}) was obtained with the dichloromethane extract against *Plasmodium falciparum* (IC_{50} 2.7 µg/mL) and *Trypanosoma brucei* (IC_{50} 0.5 µg/mL), but only with acceptable selectivity (SI) for *T. brucei* (SI 18.8). **α**-mangostin could be indicated against *B. subtilis* and *S. aureus* (MIC 1.6 and 3.2 µg/mL) and the *Mycobacterium* species (MIC 1.5 µg/mL), selectivity was quite low in view of the observed cytotoxicity on MRC-5 cells (IC_{50} 7.5 µM). Moreover, not at all was found against *C. albicans, E. coli* and *P. aeruginosa* (IC_{50} >200 µg/mL) (Al-Massarani *et al.,* 2013). Reports in same year, the MeOH extract of its bark and pericarp showed the highest inhibition against *Listeria monocytogenes* and *Staphylococcus aureus*, both water and ethanolic extracts showed high effective inhibition in high acidity (pH4) (Palakawong *et al.,* 2013). The 70%ethanolic extract of pericarps, leaf and resin (100mg) showed inhibition against *Staphylococcus aureus* and *Escherichia coli* strains at clear zone 10, 5, 1mm, respectively. The GM leaf extract inhibited cytotoxic activity against mouse melanoma B16 - F10 cells with IC_{50}=0.04 mg/ml (Cunha *et al.,* 2014).

Mangostanaxanthones I, and II displayed promising antioxidant activity with IC_{50} 12.07 and 14.12 µM, respectively using DPPH assay. **α**-mangostin and mangostanaxanthones II had weak to moderate activity against *Staphylococcus aureus* with MICs 0.79, and 1.0 mg/mL, respectively. All compounds were inactive against *Candida albican* (Mohamed *et al.,* 2014). Sakagami *et al.,* (2005) investigated combination of conventional drug with natural substances. **α**-mangostin was found to be active against vancomycin resistant *Enterococci* (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA), with MIC values of 6.25 and 6.25 to 12.5 µg/ml, respectively. **β**-mangostin showed low inhibitory effect against VRE and MRSA with MIC values more than 25 and 100 µg/ml, respectively. Surprisingly, the study showed synergism between **α**-mangostin and gentamicin (GM) against VRE, and**α**-mangostin and vancomycin hydrochloride (VCM) against MRSA might be useful in controlling VRE and MRSA infections. Recently, the 90%ethanol extract of husk was isolated two compounds (**α**, **δ**-mangostin). They found that **α**-mangostin was more active against the resistant *Plasmodium falciparum* chloroquine-resistant (FCR3) strain (IC_{50}= 0.2 µM) than **δ**-mangostin (IC_{50} = 121.2 µM) (Upegui *et al.,* 2015).

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Conceptual framework

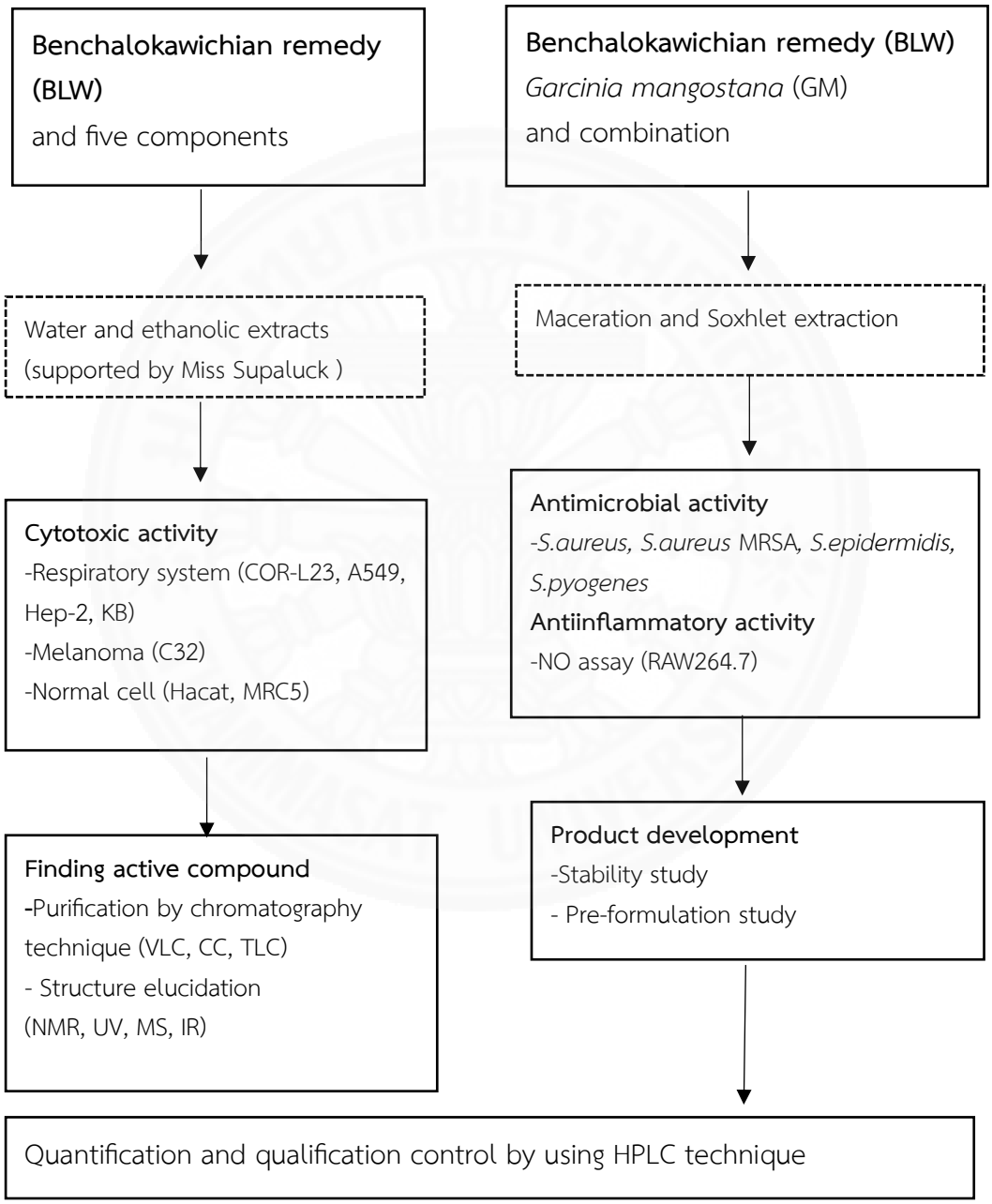


Figure 3.1 Conceptual framework divided into two parts

3.2 Plant materials and Preparation of crude extracts

Five plant roots were collected from Suphanburi in Thailand. They were dried, grounded and homogeneous provided an equal propotion as Benchalokawichian remedy (BLW). *Garcinia mangostana* (GM) were bought from the market at Pathumthani province. The pulps of GM were collected, dried and divided to three portions. Benchalokawichian remedy, GM and combination of BLW: GM (1:1) were macerated with 95% ethanol, then filtered and concentrated by rotary evaporator (under reduced pressure) to obtain the ethanolic extracts. Another portion of GM were continuously extracted with hexane, chloroform and methanol in Soxhlet extractor. For cytotoxic activity, the water and ethanolic extracts of Benchalokawichian remedy and each plant were supported from Miss Supaluck Noaeissara.

3.2.1 Determination of Plant Extract Yield

The yield of dried extracts based on dry weight will be calculated from the following equation:

$$\% \text{ Yield} = (W2 / W1) \times 100$$

W1 was the weight of the plant materials.

W2 was the weight of the extract after the solvent evaporation.

3.3 Determination of Cytotoxic activity

Five types of cancer cell lines in respiratory system included human lung carcinoma COR-L23 (ECACC 92031919), human lung adenocarcinoma A549 (ATCC CCL-185), human lung squamous carcinoma NCI-H226 (ATCC CRL-5826), Hep-2 (ATCC CCL-23), epidermoid carcinoma KB (ATCC CCL-17), human amelanotic melanoma C32 (ATCC CRL-1585), human keratinocyte HaCaT (CLS-300493), and human fibroblast MRC-5 (ATCC CCL-171) cell lines were used to determine cytotoxic activity. The ethanolic extracts were dissolved in sterile dimethylsulfoxide (DMSO), the water extracts were filtered with 0.22 microns membrane and dissolved in water sterile. All of them were prepared to concentration 10 mg/ml. For screening, the concentration 50 µg/ml of sample were tested. The sample which showed more than 50 percent of inhibition were further studied, the various percentage of each concentration were determined the value IC_{50} of cytotoxic activity.

3.3.1 Principle

The anti-proliferative SRB assay is perform to assess growth inhibition by a colorimetric assay. SRB dye bind to protein components of cells that have been fixed to tissue culture by trichloroacetic acid (TCA). The estimates cell number is directly by staining total cellular protein with the dye SRB (Skehan *et al.,* 1990).

3.3.2 Cytotoxic activity using Sulphorhodamine B (SRB) assay

Cytotoxic activity using SRB assay followed by Itharat *et al*., 2014. Cancer cell lines were cultured in appropriately media; RPMI medium or MEM medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, 1%sodium pyruvate. Monolayer of cell culture in 75 cm^3 flask were washed with phosphate buffer saline (PBS) and detached with trypsin-EDTA to make a single cell suspension. The densities of each cancer cell line were provided according to their growth profiles. For A549, COR-L23, HL-226, Hep-2, KB, C32 and Hacat were determined to be 1×10^3, 1×10^3, 1×10^3, 2×10^3, 1.5×10^3, 2×10^3, 8×10^3 cells/well, respectively. 100 µl per well of monolayer culture of each cell line were seeded in 96 well-plate and incubated at 37 ^{o}C in a 5% CO_2 atmosphere with 95% humidity for 24 hours. The sample were prepared at least 4 concentrations (100-1 µg/ml) in medium. 100 µl

medium of cell culture were added for control of media, 2%DMSO were added for control of solvent. Then, 100 µl of sample were added and incubated for the exposure time at 72 hours. After that, the medium in 96 well-plate were removed and washed with calcium free phosphate buffer saline (PBS) 200 µl. Freshly medium with supplement were added 200 µl and incubated 72 hours for recovery period. Finally, cells culture was fixed by 100 µl of ice-cold 40% trichloroacetic acid and incubated at 4 ^{O}C for 1 hour. After plates were washed five times with tap water and drained off to dry in air. SRB solution 50 µl were added to 96 well-plate and left at room temperature for 30 minutes. Excess dye was removed and washed with 1%acetic acid before air drying. 100 µl of 10 mM Tris base [tris (hydroxy methyl) aminomethane, pH 10.5] were added to each well to solubilise the dye. The colors were detected at a wavelength of 492 nm. According to National Cancer Institute (NCI) guidelines (Suffness and Pezzuto, 1990) crude extracts with an IC_{50} values < 30 µg/ml were considered as "active".

$$\text{Calculation: \% inhibition} = \frac{(\text{OD control} - \text{OD sample}) \times 100}{\text{OD control}}$$

3.4 Determination of Antimicrobial activity

In this study, we investigated four stains bacteria which cause of skin infections. *Staphylococcus aureus* (ATCC 25923), *S.aureus* MRSA (DMST 20651), *S. epidermidis* (ATCC 12228) were inoculated on Nutrient Agar (NA), while *Streptococcus pyogenes* (ATCC 19615) were inoculated under anaerobic condition for 18-24 hours at 37 °C. Differential extracts from *Garcinia mangostana*, Benchalokawichian remedy and combination remedy were determined.

3.4.1 Disc diffusion assay

Agar disc diffusion method was used to determine all of extracts, followed by Lorian (1996). The extracts were dissolved in DMSO at concentration 500 mg/ml. 10 µl of solution were applied on 6 mm sterile paper disc. Air-dried disc were placed on inoculated Mueller-Hinton agar (MHA) for *S. aureus, S. aureus MRSA, S. epidermidis* and Brain Heart Infusion agar (BHI) surface for *S. pyogenes*, then incubated at 37°C for 18-24 h. The triplicate inhibition zone (clear zone) were calculated by measuring the diameter (mm). The positive control are gentamicin and ampicillin.

3.4.2 Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The minimal inhibitory concentration (MIC) values were determined using microdilution assay by Sarker *et al.,* (2007). The extracts from maceration and soxhlet extract which were dissolved in Dimethylsulfoxide (DMSO), were prepared at concentration 400 mg/ml. 50 µl of sample solution were added in 96 well-plates and prepared serial two-fold dilution with broth medium. After 18-24 hours culture of *S. aureus, S. aureus* MRSA, *S. epidermidis*, the inoculums were adjusted turbidity equal to 0.5 McFarland standard and diluted with sterile Mueller-Hinton Broth (MHB) at 1:200 to give a final concentration of 5×10^5 CFU/ml. 50 µl of inoculums were added, the 96 well-plates covered with plastic wrap. They were mixed well using a plate shaker and incubator at 37 °C. For *S. pyogenes* was adjusted turbidity equal to 0.5 McFarland standard with sterile Brain Heart Infusion broth (BHI) and were incubated were inoculated under anaerobic condition. After 18-24 hours, 10 µl of 1 mg/ml resazurin solution (blue dye) were added into 96 well-plates and incubated 37 °C for 2-3 hours.

The irreversible reaction of resazurin (blue) to resorufin (pink) is proportional to aerobic respiration. Thus, the last wells that presented blue color were reported as Mininal Inhitibitory Concentration (MIC). The assays were repeated in triplicate. Positive control, negative control and viable control are included. An aliquot of 100 µl from each well which were blue (no bacterial growth) after MIC determination were streaked onto the media agar (MHA, BHI) and incubated at 37 °C for 18-24 hours. The lowest concentration which showed not any colonies growth was considered as the minimal bactericidal concentration (MBC).

3.5 Determination of Anti-inflammatory activity

3.5.1 Principle

Nitric oxide (NO) is representative inflammatory mediators produced by macrophages under inflammatory conditions (Ding *et al*., 1988). Nitric oxide production is a critical step in modulation of NO-mediated diseases (Jung *et al*., 2006). The Griess reagent use to determine the nitrite, which is a stable end product of nitric oxide in cell culture supernatants. The end product evaluated by spectrophotometry (Cho *et al.*, 2000)

3.5.2 Inhibitory effect on NO production and cytotoxicity test in RAW264.7 cells using NO assay and MTT assay (Worawattananutai *et al.,* 2014)

Inhibitory effect on NO production by Mouse Macrophage Leukemia-like RAW264.7 cells were evaluated using a method, as previously reported (Worawattananutai *et al.,* 2014). RAW 264.7 cell line were cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/ml) and streptomycin (100 µg/ml). The cells were seeded in 96-well plate (cell concentration 1×10^5 cells/well) and incubated in CO_2 incubator at 37°C for 24 hours. 100 µl/well of RPMI medium containing 100 µg/ml of LPS were added into control and sample wells, whereas only RPMI medium were added into a blank well. 100µl/well of different sample concentrations (100, 50, 30, 10, 1 µg/ml) were added into sample wells and their corresponding blank sample wells. Then cells were incubated at 37°C for 24 hours. Supernatant (100µl) were added in another 96-well plate and followed by the addition of 100 µl/well of Griess reagent. The color detected at a wavelength of 570 nm.

Cytotoxicity were also determined using the MTT method. After 48 h incubation with the test samples, MTT solution (10 µl, 5 mg/ml in PBS) were added to the wells and incubated at 37°C for 2 hours. The medium was removed, and isopropanol containing 0.04 M HCl were added to dissolve the formazan production in the cells. The formazan solution was measured with a micro-plate reader at 570 nm. The test sample were considered to be cytotoxic when the optical density of the sample-treated group were less than 70-80% of that in the control (vehicle-treated)

group. Indomethacin was used as positive control. %Inhibition and IC_{50} values were calculated from the Prism program.

$$\%\text{Inhibition} = \frac{A - B}{A - C} \times 100$$

Control (A) : LPS (+), Test sample (-)

Sample (B) : LPS (+), Test sample (+)

Blank (C) : LPS (-) , Test sample (-)

3.6 Isolation using Bioassay-guided fractionation

3.6.1 General Experimental Procedures

HPLC was performed on a Waters 1525 HPLC pump system, equipped with a Waters 2487 dual λ absorbance detector using the following column: Phenomenax Luna 5u C_8 column (10 × 250 mm) and CHIRALCEL OD-H column (4.6 × 250 mm). Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 100 (63–200 μm, SiliCycle® Inc.). Waters 10 g Sep-Pak's C18 were used for reversed-phase flash chromatography. Sephadex LH-20 was also used for CC. Precoated plates of silica gel 60 F254 were used for analytical purposes. UV-vis spectra were recorded with a Varian Cary 5000 UV-vis-NIR spectrophotometer. The NMR spectra were recorded using Bruker Avance 600 MHz spectrometers. Chemical shifts are reported in parts per million (δ), and the coupling constants (*J*) are expressed in hertz. HREIMS, and EIMS spectra were obtained on a Bruker-Hewlett-Packard 1100 Esquire-LC system mass spectrometer. The IR spectra were recorded using a Perkin-Elmer FTS FT-IR spectrophotometer.

3.6.2 Isolation of *Tiliacora triandra* exthanolic extract (TTE)

The pure compounds of TTE separated by the following modified method Itharat *et al.,* (2014). An aliquot of TTE were separated by vacuum liquid chromatography (VLC), using five systems of solvent from non-polarity to polarity; Hexane, Hexane: $CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$: MeOH (1:1) and MeOH as TTF1-TTF5. Each fraction was evaporated and the percentage of yield were calculated shown on Figure 3.2.

The fractions (TTF2-TTF5) of extract were preliminary studied for cytotoxic activity, the fraction TTF4 showed cytotoxic activity against all cell lines as KB, Hep2, A549, COR-L23 and H226 with IC_{50} values 27.71, 34.96, 35.72, 28.33, 26.08, respectively. All chromatography techniques including CC, TLC, HPLC were used for isolation and investigation. Then, the pure compounds were elucidated and identified the chemical structures by spectroscopy methods as NMR, MS, IR and UV.

Fraction TTF4 (2g) was subjected to column chromatography (CC) on sephadex using 20%DCM: MeOH isocratic solvent system, 20ml of diluent was collected then five fractions (TTA-TTE) were classified by TLC analysis.

TTB (112.5 mg) was chromatographed using CC on silica gel 60 and eluted by Me_2CO: hexane (1:9) to give three subfractions (B1-B3).

Then, B2 (6.5 mg) was purified by HPLC (column) using isocratic as diluent (flow rate 10 ml/min.) to afford TTE1.

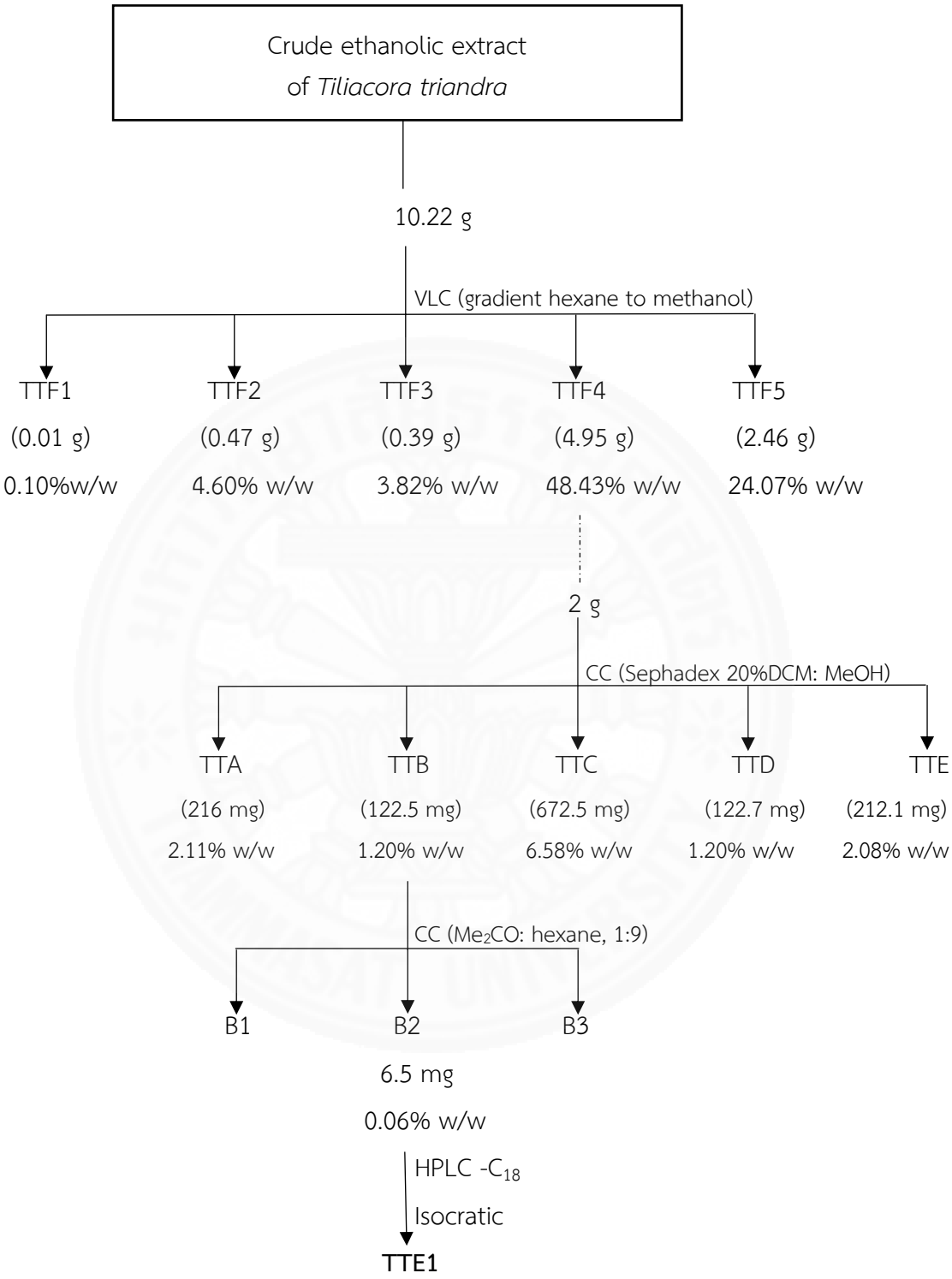


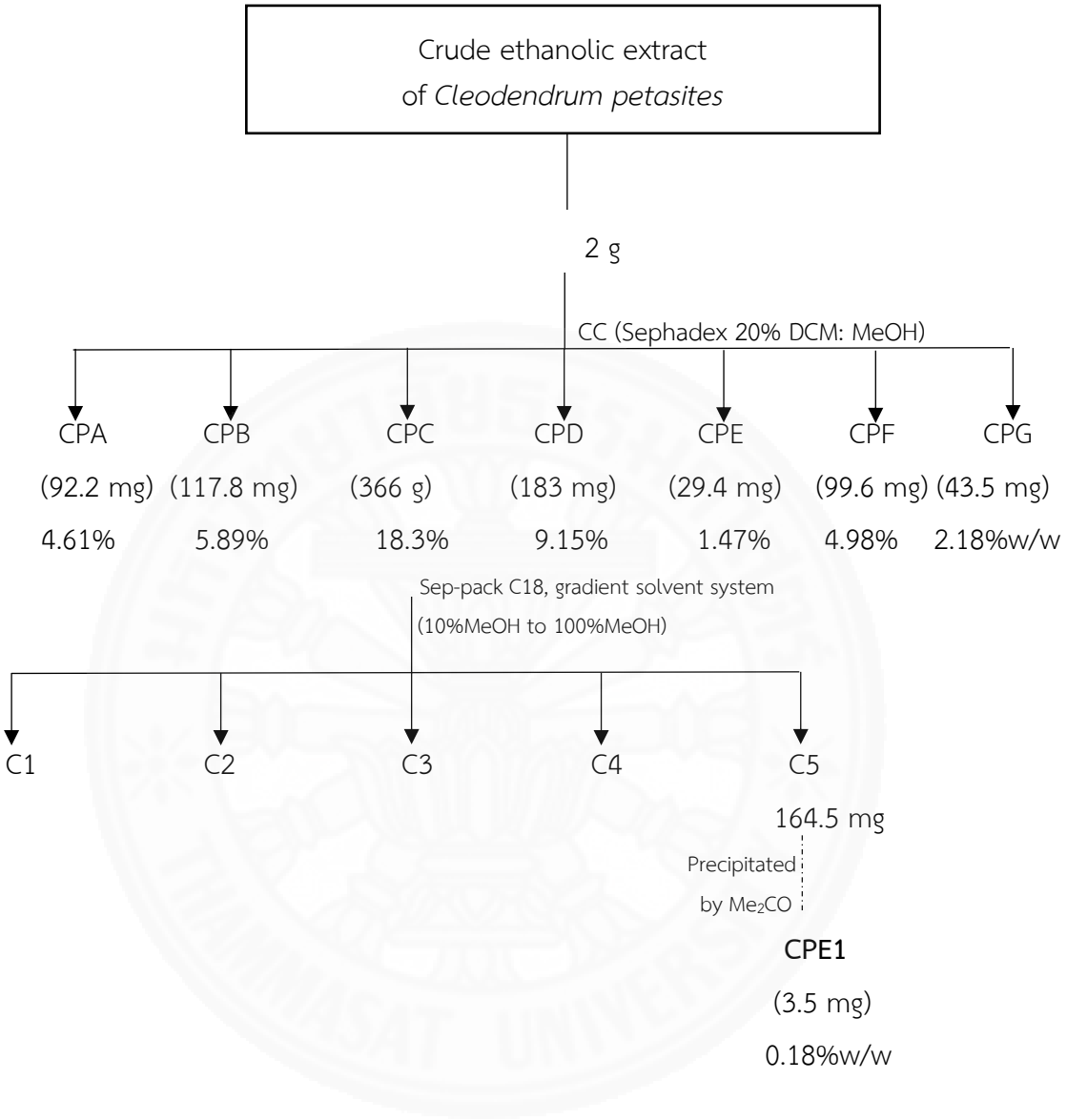
Figure 3.2 Isolation of TTE1 from ethanolic extract of *Tiliacora triandra*

3.6.3 Isolation of *Cleodendrum petasites* extract (CPE)

The ethanolic extract of *Clerodendrum petisites* (2 g) was separated by sephadex LH-20 with dichrolomethane and methanol (ratio 2:8) to give seven fractions (CPA-CPG). Fraction CPC was separated by sep-pack C18 eluting start with 10%methanol and water, then decreasing polarity to 100% methanol afford five subfractions (C1-C5). The C5 (164.5 mg) was washed from methanol which gave a compound as CPE1, the colorless crystal (3.5mg, 0.18%w/w). By comparison of their ^{1}H and ^{13}C NMR data with those previously reported data, CPE1 was identified as oleanolic acid acetate (Hichri *et al.,* 2003; Hwang *et al.,* 2014).

3.6.4 Isolation of *Harrisonia perforata* extract (HPE)

The ethanolic extract of *Harrisonia perforata* was separated by vacuum liquid chromatography (VLC) to give five fractions (A-E), All fractions were investigated by high-performance liquid chromatography. The dominant peak at retention time of 26.4 minutes was collected following the HPLC method, then further purified with increasing polarity of dichloromethane: methanol by CC to give HPE1 as yellow wax, 6.3 mg. The ^{1}H and ^{13}C NMR were indicated as perforatic acid which found from *Harrisonia perforata* as previous study (Thadathiti *et al.,* 1994), and the result of IR and ESIMS were confirmed.



Notes: DCM = Dichloromethane, MeOH = Methanol, Me_2CO = Acetone, and %w/w = %w/w of crude extract

Figure 3.3 Isolation of CPE1 from ethanolic extract of *Cleodendrum petasites*

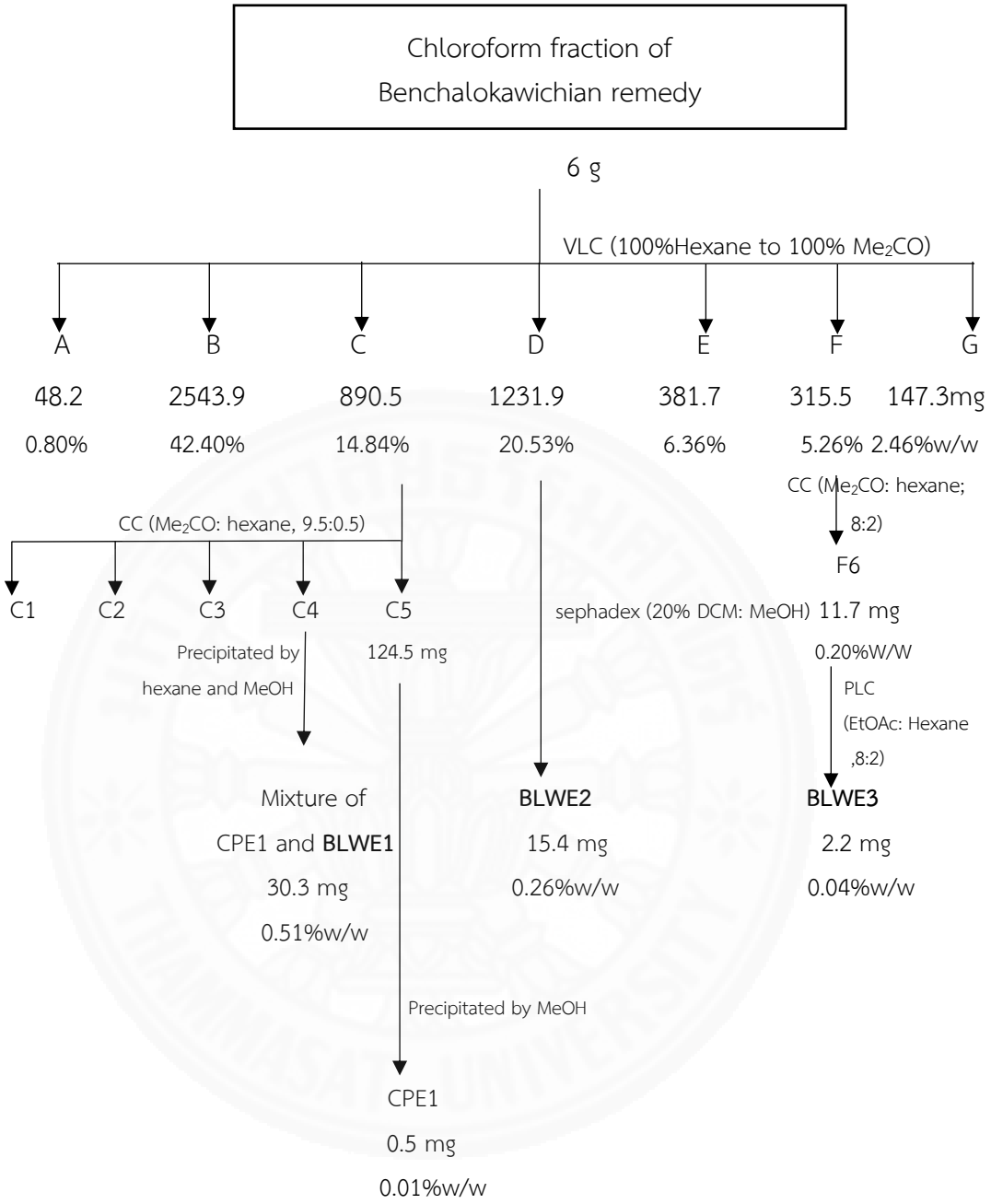
3.6.5 Isolation of Benchalokawichian remedy (BLWE)

The chloroform fraction of crude BLW ethanolic extract was supported from Miss Supaluck Nuaeissara. The F3 ($CHCl_3$ fraction) was separated by VLC elution start with hexane increasing polarity with chloroform and acetone to give seven fractions (A-G). Each fraction was dried and obtained yield as shown in Figure 3.4.

Subfraction C (890.5 mg) was further separated by CC eluting with 5%acetone: hexane to afford 5 subfractions (C1-C5). Recrystallization of C4 using hexane-MeOH afforded CPE1 and BLWE1 as colorless crystals and white powders (30.3mg, 0.51%w/w), the ^{1}H NMR data indicated the mixture of oleanolic acid acetate and β-sitosterol (Hichri *et al.,* 2003; Sayeed *et al.,* 2016). In addition, C5 was washed with MeOH to give colorless crystals as CPE1 (0.5 mg, 0.01%w/w).

Subfraction D (1231.9 mg) was washed with Me_2CO-MeOH to obtain BLWE2 as light-yellow crystals (15.4mg, 0.26%w/w). By comparison of ^{1}H and ^{13}C NMR data with previous study, BLWE2 was identified as pectolinarigenin (Hase *et al.,*1995)

Subfraction F (315.5 mg) was further separated by CC eluting with Me_2CO: hexane (8:2) to obtain 6 fractions (F1-F6). Further PLC separation of F6 with EtOAc: hexane (8:2) to afford BLWE3 as white/yellow wax (2.2mg, 0.04%w/w), data of NMR spectra was confirmed with the previously reported data as perforatin A or *O*-methylalloptaeroxylin (Thadanithi *et al*., 1994).



Notes: DCM = Dichloromethane, MeOH = Methanol, Me_2CO = Acetone, EtOAc = Ethyl acetate

Figure 3.4 Isolation of BLWE1-BLWE3 from chloroform fraction of BLW extract

3.7 Development of product

3.7.1 Stability study of combination extracts

The accelerated stability testing was performed according to guidelines for stability testing of pharmaceutical product, WHO, (1996). The stability testing was carried out in triplicate using transparent vials. Stability studies were undertaken at 40±2ºC with 75±5% RH duration 6 months as accelerated condition. The extracts were stored in vials under conditions for 15, 30, 60, 90, 120, 150, 180 days and control samples (Day 0) were kept at -20°C. After accelerated conditions, the extracts were evaluated chemical using HPLC analysis and biological testing included anti-microbial and anti-inflammatory activities comparison with control samples.

3.7.2 Force degradation study of combination extracts

The extracts were weighted to 50 mg and placed in tube. Heat, humidity, acid hydrolysis, and oxidation were tested the typical tests. The samples were determined for antimicrobial activity assay.

Moisture hydrolysis - Three drops of deionized water of 3 drops were added and heated up at 80 °C for 3 hours was performed.

Temperature forced degradation - The sample were heated it at 80 °C for 3 hours and then placed to cool down to room temperature.

Acid hydrolysis - Three drops of 3N hydrochloric acid were added and heated up at 80 °C for 3 hours was performed.

Alkaline hydrolysis - Three drops of 3N sodium hydroxide were added and heated up at 80 °C for 3 hours was performed.

Oxidation – Three drops of 3N hydrogenperoxide were added and heated up at 80 °C for 3 hours was performed.

3.7.3 Product formulation

Variation of components were applied for finding the best formulation which is appropriate to use for skin topical cream. Individual ingredients were weighed and separated into two phases. Sepigel, sodium EDTA, propylene glycol, glycerine, uniphen and bronidox were dissolved in water phase (A). Liqiud paraffin light, isopropyl myristate, glyceryl sterate, cetyl alcohol, steryl alcohol, cremophor A25, cremophor A6, lanolin anhydrous were melted to be oil phase (B).

Phase A was heated at 80 °C and phase B also heated in water bath at 70 °C. Placed phase A into phase B under stirring and cooling down to get a cream formulation. General appearance including color, odor and pH determination were recorded. The extract was added to the best formula which was considered a good for being a topical cream.

Table 3.1 Ingredients and their functions of cream formula

phase	ingredient	function
A	sepigel	emulsifier, thickener
	sodium EDTA	chelator agent, stabilizer
	propylene glycol	humectant, solubilizer
	glycerine	humectant, solubilizer
	uniphen	preservative
	bronidox	preservative
	water	solubilizer
B	liqiud paraffin light	occlusive
	isopropyl myristate	emollient, emulsifier
	glyceryl sterate, GMS	emulsifier, thickener
	cetyl alcohol	emollient, thickener
	steryl alcohol	emollient, thickener
	cremophor A25 (powder)	emulsifier
	cremophor A6 (hard)	emulsifier
	lanolin anhydrous	moisturizer

3.7.4 Stability testing of product

The accelerated stability testing was also performed according to WHO guidelines, (1996) as same as the extracts. The stability testing was carried out in triplicate using plastic tubes. Stability studies were undertaken at 40±2ºC with 75±5% RH duration 6 months as accelerated condition. Cream tubes were stored under conditions for 15, 30, 60, 90, 120, 150, 180 days and control samples (Day 0) were kept at 4°C before further study. All sample were extracted by 7 ml of MeOH centrifuged 3500 rpm for 5 min., then filtrated the suspension through the glass tubes. The 2 ml of MeOH were added and repeated procedure once, the filtrate was evaporated to obtain cream extracts.

Yield of cream extracts were calculated and also presented by percentage. For HPLC analysis, extract of cream was dissolved in MeOH and prepared in concentration 50 mg/ml. The area under curve of alpha-mangostin was examined for determination of stability preparation.

3.8 Determination of quantification and qualification by using High Performance Liquid Chromatography (HPLC) technique

Studied on validate method of two compounds, pectolinarigenin and alpha-mangostin, from Benchalokawichien (BLW) and *Garcinia mangostana* (GM). Pectolinarigenin, which showed cytotoxic activity in our study as same as previous reports (Bonesi *et al.,* 2008 and Tundis *et al.,* 2005), was used for marker of BLW. Morover, pectolinarigenin also showed anti-inflammatory and anti-allergic activities in the recent report (Juckmeta., 2012 and Juckmeta *et al.,* 2014). Alpha-mangostin is well known for antibacterial activity, and also showed their activity against *S. aureus* MRSA better than some positive control in this research. There is no research study on validation method of this remedy. Thus, determination of quantification and qualification followed by ICH guidelines and modified according previous study This research modified method from Sakpakdeejaroen *et al.,* (2014), Aisha A. *et al.,* (2012) and Yodhnu *et al.,* (2009). Following ICH guidelines, the parameters which are selectivity, linearity, precision and accuracy, limit of detection (LOD) and limit of quantification (LOQ) were examined.

The HPLC instrument (Agilent® LC 1100/1200 system) consists of a quaternary pump (model G1311A), an automatic injector (model G1329A), a photodiode array (PDA) detector (model G1315D). Chromatographic fingerprint was constructed using a reverse-phase C18 column (Phenomenex® Luna, 5 µm, 250 mm x 4.6 mm). The mobile phase composed of 0.1% ortho phosphoric acid(A) and acetonitrile (B). Chemical constituents in sample were gradiently eluted with flow rate of 1.0 ml/min using the following program: 0 – 30 min., 95%A; 30 – 35 min., 5%A; and 35 – 40 min., 95% A. Stock solution of extract was prepared at concentration 10 mg/ml whereas pectolinarigenin and alpha-mangostin at concentration 1, 2 mg/ml were stocked, respectively. Sample injected for 10 µl and the spectra were collected at 331 nm. Data were analyzed by ChemStation® software.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Cytotoxic activity of the water and ethanolic extract of individual plant, Benchalokawichian remedy and its compounds using SRB assay

4.1.1 Cytotoxic activity of crude extracts

For screening of cytotoxic activity using SRB assay against KB, Hep2 and three types of lung cancer ; A549, CORL23, H226 at concentration 50 µg/mL (n=2), the extract which showed >50% cytotoxic were further studied in serial dilution for finding IC_{50}. Following the results, all of water extract showed percent inhibition less than 50 which were classified no cytotoxic activity. Only Benchalokawichien (BLW) and *Tiliacora triandra* (TT) ethanolic extract which showed percentage of toxicity more than 50 (Table 4.1).

IC_{50} of TTE and BLWE were investigated after screening test, the results showed on Table 4.2. The ethanolic extract of *Tiliacora triandra* (TTE) exhibited oropharynx cancer cell line (KB) as well as larynx cancer cell line (Hep2) with IC_{50} 42.1 and 45.2 µg/mL. As the same result, BLWE showed moderate cytotoxic activity against KB and Hep2 with IC_{50} values 32.1 and 32.7 µg/mL. For non-small lung cancer cell, BLW showed cytotoxic activity against epithelial lung adenocarcinoma (A549) quite similar value as TTE with IC_{50} = 32.3, 33.7 µg/mL, respectively. Furthermore, TTE exhibited lung carcinoma (COR-L23) better than BLWE (IC_{50}= 25.7 and 33.7, respectively). Both of them showed specific cytotoxic against squamous lung cancer (H226) better than others cell lines, BLWE showed IC_{50} value 10.1 µg/mL whereas TTE as 19.5 µg/mL. In addition, BLWE showed higher cytotoxic against skin cancer (C32) cell line than TTE with IC_{50} value 29.1 and 37.9 µg/mL, respectively. Nevertheless, their activity was not better than NCI standard (<30 µg/mL). So, the active compound from TT was interesting to investigate.

Two compounds which were isolated from BLW extract in previously report also studied. Pectolinarigenin exhibited cancer cell in respiratory system better than TT and BLW extract with IC_{50} value in range1.91-9.12 µg/mL while *O*-methyllaloptaeroxyrin show no cytotoxic activity in all types of cancer cell lines (>50 µg/mL). Obviously, pectolinarigenin showed cytotoxic against normal cell line (HaCaT) with IC_{50}11.5 µg/mL whereas BLWE and TTE were less toxic (IC_{50} = 64.5, 79.9 µg/mL).

Table 4.1 %Cytotoxic activity of the water and ethanolic extract at concentration 50 µg/mL against five cell lines in respiratory system

Plants	Extract	Code	%Cytotoxic (Mean ± SEM)				
			KB	Hep2	A549	COR-L23	H226
Harrisonia perforata	Ethanol	HPE	0.93 ± 6.00	6.39 ± 0.55	12.35 ± 1.91	53.46 ± 16.67	32.99 ± 9.00
	Water	HPW	-17.90 ± 11.26	0.59 ± 3.33	3.89 ± 2.82	19.42 ± 8.27	-4.48 ± 15.68
Capparis micracantha	Ethanol	CME	3.22 ± 5.31	7.94 ± 0.99	7.23 ± 0.14	17.29 ± 0.99	61.47 ± 4.68
	Water	CMW	-22.60 ± 4.48	1.92 ± 4.22	-1.33 ± 2.08	10.42 ± 8.26	-4.52 ± 24.31
Cleodendrum petesites	Ethanol	CPE	-0.50 ± 4.05	10.02 ± 0.14	4.54 ± 1.26	40.56 ± 1.46	33.43 ± 8.70
	Water	CPW	-12.51 ± 12.28	2.38 ± 4.73	-0.80 ± 1.36	25.54 ± 7.62	10.06 ± 2.40
Ficus racemosa	Ethanol	FRE	3.05 ± 2.53	8.25 ± 0.91	7.97 ± 3.41	17.33 ± 1.16	29.73 ± 5.92
	Water	FRW	-6.33 ± 3.40	12.33 ± 6.78	3.86 ± 0.30	31.18 ± 0.39	-6.53 ± 0.04
Tiliacora triandra	Ethanol	TTE	67.97 ± 5.45	50.90 ± 5.73	89.89 ± 0.84	83.29 ± 11.91	86.28 ± 0.32
	Water	TTW	7.40 ± 7.90	6.14 ± 0.80	4.31 ± 0.38	18.12 ± 1.47	33.43 ± 3.61
Benchalokawichian	Ethanol	BLWE	90.54 ± 1.52	89.76 ± 4.27	94.26 ± 0.66	89.11 ± 2.93	82.57 ± 6.72
	Water	BLWW	-11.22 ± 10.32	10.57 ± 3.85	3.22 ± 1.07	23.16 ± 11.36	-2.81 ± 2.86

Table 4.2 Cytotoxic activity of the water and 95%EtOH extract of five plants from Benchalokawichian, BLW remedy and two compounds against seven cell lines (three type of lung, two types of larynx and skin cancer cell lines, and normal cell lines)

Sample	Cytotoxic activity, IC_{50} (µg/mL) ± SEM						
	KB	Hep2	A549	COR-L23	H226	C32	HaCaT
HPE	>50	>50	>50	>50	>50	>50	NT
HPW	>50	>50	>50	>50	>50	>50	NT
CME	>50	>50	>50	>50	>50	>50	NT
CMW	>50	>50	>50	>50	>50	>50	NT
CPE	>50	>50	>50	>50	>50	>50	NT
CPW	>50	>50	>50	>50	>50	>50	NT
FRE	>50	>50	>50	>50	>50	>50	NT
FRW	>50	>50	>50	>50	>50	>50	NT
TTE	42.09 ± 4.55	45.24± 7.09	33.65 ± 0.58	25.71 ± 7.87	19.48 ± 1.43	37.95 ± 0.36	79.97 ± 4.49
TTW	>50	>50	>50	>50	>50	>50	NT
BLWE	32.05 ± 0.02	32.74 ± 2.06	32.26 ± 0.71	33.65 ± 0.92	10.11 ± 2.85	29.09 ± 4.01	64.35 ± 9.21
BLWW	>50	>50	>50	>50	>50	>50	NT
O-methyllaloptaeroxyrin	>50	>50	>50	>50	>50	NT	NT
Pectolinarigenin	6.19 ± 1.71	1.91 ± 0.56	7.76 ± 0.20	9.12 ± 0.74	NT	NT	11.48±0.95

4.1.2 Cytotoxic activity of *Tiliacora triandra* fractions

The compounds from *Tiliacora triandra* (TTE) was interesting to be a marker for cytotoxic activity of BLW remedy. Isolation of compound was investigated using bioassay guided fractionation technique. Vacuum Liquid Chromatography (VLC), TT extract was weighed 10.22 g homogenous mixing with the silica gel no. 0.7734. The non-polar solvent was used to elude from the column increasing the polarity for 5 fractions (Hexane, Hexane: $CHCl_3$, $CHCl_3$, $CHCl_3$: MeOH, MeOH). The five fractions (TTF1-TTF5) were collected and calculated percentage of yield shown in Table 4.3. The fraction 4, TTF4, showed highest yield with 48.43%.

Table 4.3 Percentage yield of five fractions from the TT ethanolic extract using VLC technique

CODE	Fraction	Weight (g)	%yield
TTF1	Hexane	0.01	0.10%
TTF2	Hexane: $CHCl_3$	0.47	4.60%
TTF3	$CHCl_3$	0.39	3.82%
TTF4	$CHCl_3$: MeOH	4.95	48.43%
TTF5	MeOH	2.46	24.07%

Screening of cytotoxic activity against five cell lines in respiratory system at concentration 50 µg/mL shown on Table 4.4, fraction 2-3 (TTF2-3) showed no cytotoxic activity with percentage lower than 50. The fraction 4 ($CHCl_3$: MeOH, TTF4) showed high percentage of cytotoxic against all cell lines (in range 79.93-95.73%) . The fraction 5 (MeOH, TTF5) showed 70% cytotoxic at concentration 50 µg/mL against H226.

Table 4.4 %Cytotoxic of four fractions at concentration 50 µg/mL against five cell lines in respiratory system

Sample	%cytotoxic (Mean ± SEM, n = 2)				
	KB	Hep2	A549	COR-L23	H226
TTF2	0.10 ± 0.95	5.57 ± 0.39	-1.77 ± 0.72	28.68 ± 8.12	14.17 ± 6.58
TTF3	7.19 ± 0.42	10.50 ± 3.02	0.95 ± 5.59	43.43 ± 2.09	22.15 ± 6.65
TTF4	85.02 ± 6.25	79.93 ± 6.72	80.98 ± 2.61	95.73 ± 0.05	81.73 ± 6.79
TTF5	39.03 ± 8.10	34.97 ± 3.22	27.96 ± 0.44	44.92 ± 7.85	70.51 ± 9.29

The result found that TTF4 also showed the cytotoxic activity as same as ethanolic extract (TTE). The fraction4 or TTF4 also exhibited against KB and Hep2 cell lines better than TTE with IC_{50} value 27.71, 34.96 and 42.09, 45.24 µg/mL, respectively. On the other hand, TTE showed higher cytotoxic activity against three types of lung cancer cell lines than TTF4. Surprisingly, the fraction 5 (TTF5) showed specific against H226 with IC_{50} value 33.02 µg/mL.

Table 4.5 Cytotoxic activity show IC_{50} (µg/mL) of fraction TTF2-5 and *Tiliacora triandra* (Mean ± SEM, n=3)

Sample	Cytotoxic activity, IC_{50} (µg/ml) ± SEM				
	KB	Hep2	A549	COR-L23	H226
TTF2	>50	>50	>50	>50	>50
TTF3	>50	>50	>50	>50	>50
TTF4	27.71 ± 7.25	34.96 ± 3.11	35.72 ± 0.96	28.33 ± 1.05	26.08 ± 4.36
TTF5	>50	>50	>50	>50	33.02 ± 2.10
TTE	42.09 ± 4.55	45.24 ± 7.09	33.65 ± 0.58	25.71 ± 7.87	19.48 ± 1.43

4.1.3 Isolated compound from *Tiliacora triandra*

4.1.3.1 Compound TTE1

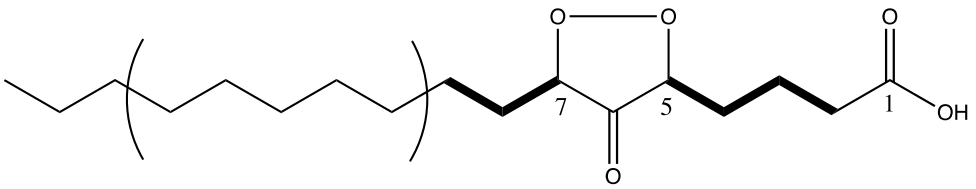


Figure 4.1 Chemical structure of TTE1

The ^{1}H NMR spectrum of TTE1 showed oxymethine protons resonating at δ_H 5.07 (^{1}H, m) and 5.13 (^{1}H, m). A terminal methyl group was observed as triplet at δ_H 0.90 (^{3}H, t). A broad hump between δ_H 1.30–2.80 showed the presence of seven methylene groups. The ^{13}C NMR and DEPT spectra showed eighteen signals comprising one methyl, twelve methylene, two methine, and two quaternary carbons. It showed the presence of a ketone at δ_C 203.59 (s) and a carboxyl at δ_C 179.27 (s), respectively. The two downfield oxymethine carbons at δ_C 89.2 (d) and 91.2 (d), comparing with NMR the data of urticic acid δ_C 91.2 (d) and 89.1 (d) from *Leucas urticifolia* (Fatima *et al.,* 2008). HMBC correlations from δ_H 5.07 (^{1}H, m) and 5.13 (^{1}H, m) to δ_C 203.59 (s) indicated the presence of a five-membered 4-oxo-1,2-dioxolane moiety. The COSY correlations from H7 (δ 5.13, m), to H8 (δ 2.00, m), H8' (δ 2.00, m) to H_2-9 (δ 1.40, m), combined with HMBC correlations from H-17 (δ 0.90, t) to C-16 (δ 22.8) and C-15 (δ 31.5) confirmed the presence of a longchain of methylene carbons. Another fragment which showed COSY correlations from H-2 (δ2.43, t) to H-3 (δ 1.78, m), H-3' (δ 1.78, m) to H-4/H-4' (δ 2.07, m), combined with HMBC correlations from H-2 (δ2.43, t) and H-3 (δ 1.78, m) to C-1, H-2 (δ2.43, t) to C-4 (δ 27.7), H-3 (δ 1.78, m) to C-5 (δ 89.2) confirmed the presence of pentanoic acid. The key COSY correlations from H-4 (δ 2.07, m) to H-5 (δ 5.07, m) and H7 (δ 5.13, m), to H-8/H-8' (δ 2.00, m) combined with HMBC correlations from H-5 (δ 5.07, m) and H7 (δ 5.13, m) to C-6 (δ 203.6) and H-7 (δ 5.13, m) to C-8 (δ 28.5) implied that two fragments were attached

to 4-oxo-1,2-dioxolane moiety (Figure 4.2). Thus, the planar structure of TTE1 was established as 4-((3*S*,5*R*)-5-decyl-4-oxo-1,2-dioxolan-3-yl) butanoic acid.

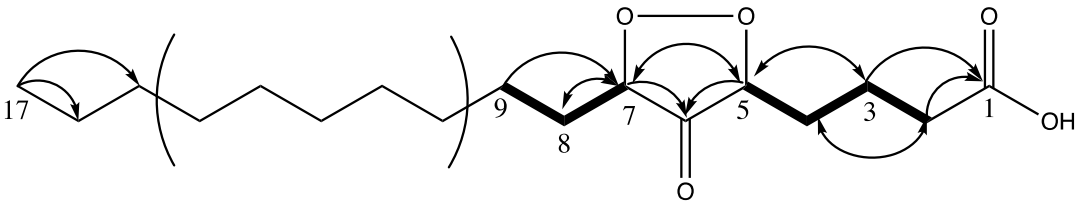


Figure 4.2 COSY and HMBC correlations of TTE1

Table 4.6 The ^{1}H (600MHz), ^{13}C (150 MHz) NMR and HMBC spectral data of TTE1

position	TTE1		
	δ_C	δ_H (*J* in Hz)	HMBC ($\delta_H \rightarrow \delta_C$)
1	179.27		
2	32.8	2.43 (t)	C-1, C-4
3	23.54	1.78 (m)	C-1, C-5
4	27.77	2.07 (m)	C-2
5	89.21	5.07 (m)	C-3, C-6, C-7
6	203.59		
7	91.21	5.13 (m)	C-5, C-6, C-8
8	28.51	2.00 (m)	C-7
9	28.71	1.40 (m)	C-7
10	28.95		
11	28.80		
12	29.06		
13	29.26		
14	29.22		
15	31.50	1.30 (m)	
16	22.80	1.30 (m)	
17	13.71	0.90 (t)	C-15, C-16

4.1.4 Isolated compound from *Cleodendrum petesites*

4.1.4.1 Compound CPE1

The ^{1}H NMR (600 MHz, $CDCl_3$) spectrum revealed seven tertiary methyl peaks as singlets at δ 1.15, 0.96, 0.95, 0.93, 0.89, 0.87, 0.78 that are assigned to Me-27, Me-25, Me-30, Me-23, Me-24, Me-29, Me-26, respectively. An olefinic proton resonance at δ_H 5.30 and a methine proton resonance at δ_H 4.51 were attributed to H-12 and H-3, respectively. A methine double doublet at δ_H 2.84 (J=12 Hz) was assigned to H-18 and a singlet was observed at δ_H 2.07 assigned to OAc. There are 32 carbon signals revealed in the ^{13}C NMR (125 MHz, $CDCl_3$). Carbonyl resonances at presented at δ_C 170.68 and 182.83 were assigned to acetate ester and carboxylic acid functionality, respectively. The olefenic carbon resonances at δ_C 122.17 and 143.20 were attributed to C12-C13. HMBC correlations (Figure 4.3) were observed between the proton resonance at 2.84 (dd) and carbons resonance assigned to C-12 (δ 122.7), C-13 (δ 143.2), C-17 (δ 46.1), C-28 (δ 182.8). The olefenic proton resonance at δ 5.32 showed HMBC correlations with C-9 (δ 47.1), and C-18 (δ 4.1). Moreover, the proton resonance at 4.51 (m) was correlated with carbon resonances assigned to C-23 (δ 27.6), C-4 (δ 37.6), $CH_3\underline{C}OO$ (δ 170.7). Furthermore, the IR spectrum exhibited absorption peaks at 3208, 2944, 2899, 1723, 1252, 1368, 1178, 819 cm^{-1}. Based on all data and comparison with literature data (Hichri *et al.,* 2003). The molecular formula was proposed to be $C_{32}H_{50}O_4$ as determined by ESI mass spectra that gave $[m-H]^-$ =497.3.

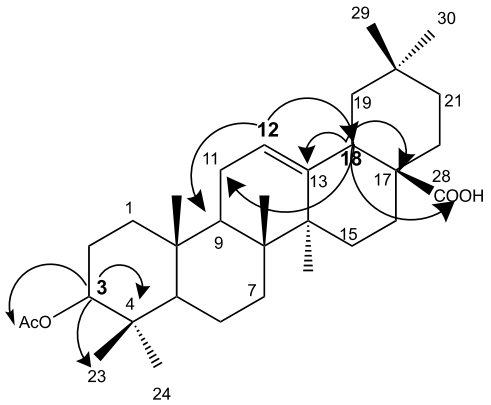


Figure 4.3 Selected HMBC correlations of CPE1

Table 4.7 The ^{1}H (600MHz), ^{13}C (150 MHz) NMR and HMBC spectral data of CPE1

position	CPE1		
	δ_C ($CDCl_3$)	δ_H, *J* in Hz	HMBC ($\delta_H \rightarrow \delta_C$)
1	37.3		
2	25.5		
3	80.5	4.51, 1H, m	C-3a, C-4, C-23
4	37.6		
5	54.8		
6	17.7		
7	32.1		
8	38.8		
9	47.1		
10	36.5		
11	23.0		
12	122.2	5.30, 1H, m	C-9, C-18
13	143.2		
14	45.4		
15	27.2		
16	23.1		
17	46.1		
18	41.1	2.84, dd, 12	C-11, C-12, C-13, C-17, C-28
19	40.5		
20	30.3		
21	33.4		
22	32.7		
23	27.6	0.93, 3H, s	
24	16.2	0.89, 3H, s	
25	15.0	0.96, 3H, s	
26	16.7	0.78, 3H, s	
27	23.2	1.15, 3H, s	

position	CPE1		
	δ_C ($CDCl_3$)	δ_H, *J* in Hz	HMBC ($\delta_H \rightarrow \delta_C$)
28	182.8		
29	32.0	0.87, 3H, s	
30	22.5	0.95, 3H, s	
$\underline{CH_3}COO$	20.9	2.07, 3H, s	
$CH_3\underline{C}OO$	170.7		

Table 4.8 Comparison of ^{13}C and ^{1}H NMR data between CPE1 and oleanolic acid acetate (Hichri *et al.,* 2003 recorded in $CDCl_3$)

	CPE1		Hichri *et al.,* 2003	
position	$\delta_C(CDCl_3)$	δ_H, *J* in Hz	$\delta_C(CDCl_3)$	δ_H, *J* in Hz
1	37.3		38.0	
2	25.5		27.7	
3	80.5	4.51, 1H, m	80.9	4.42, 1H, m
4	37.6		39.0	
5	54.8		55.3	
6	17.7		18.1	
7	32.1		32.8	
8	38.8			
9	47.1		48.0	
10	36.5		37.7	
11	23.0		24.0	
12	122.2	5.30, 1H, m	122.5	5.19, 1H, m
13	143.2		143.6	
14	45.4		41.5	
15	27.2		28.0	
16	23.1		23.4	
17	46.1		46.6	
18	41.1	2.84, dd, 12		2.78, 1H, m
19	40.5			
20	30.3		30.7	
21	33.4		33.8	
22	32.7			
23	27.6	0.93, 3H, s	29.7	0.88, 3H, s
24	16.2	0.89, 3H, s	16.7	0.87, 3H, s
25	15.0	0.96, 3H, s	15.5	1.05, 3H, s
26	16.7	0.78, 3H, s	17..2	0.67, 3H, s

	CPE1		Hichri *et al.,* 2003	
position	$\delta_C(CDCl_3)$	δ_H, *J* in Hz	$\delta_C(CDCl_3)$	δ_H, *J* in Hz
27	23.2	1.15, 3H, s		1.18, 3H, s
28	182.8		178.6	
29	32.0	0.87, 3H, s	33.1	
30	22.5	0.95, 3H, s	23.6	0.79, 3H, s
$\underline{CH_3}COO$	20.9		22.8	1.00, 3H, s
$CH_3\underline{C}OO$	170.7	2.07, 3H, s	171.0	2.06, 3H, s

4.1.5 Isolated compound from *Harrisonia perforata*

4.1.5.1 Compound HPE1

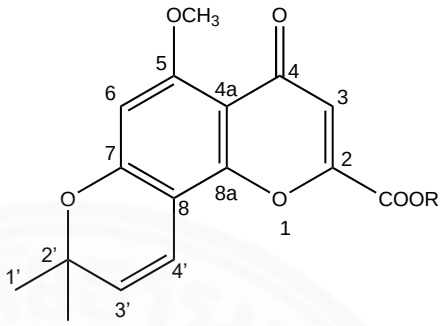


Figure 4.4 Chemical structure of HPE1

Perforatic acid was isolated as a yellow solid. ESIMS showed an $[m+H]^+$ ion at m/z 303.1 which was consistant with a molecular formula of $C_{16}H_{14}O_6$ (MW=302.1). The IR spectrum exhibited absorption peaks at 3377, 2923, 2853, 1618, 1375, 1329, 1203, 1118, 998 cm^{-1}. The ^1H (600 MHz; DMSO-d_6) NMR spectrum contained two methyl resonances at δ_H 2.40 (6H, s), 1.43 (6H, s), methoxy proton at δ_H 3.47 (3H, s), three olefinic proton at δ_H 6.71 (1H, d, *J*=12Hz), 7.74 (1H, d, *J*=12Hz) and 7.48 (1H, s), and aromatic ring proton at δ_H 7.38 (C6-H, s). The comparison of ^1H and ^{13}C NMR spectral data with the previously reported (Thadathiti *et al.,* 1994) showed on Table 4.9.

Table 4.9 Comparison of ^{13}C and ^{1}H NMR data for HPE1 (recorded at 600 MHz in DMSO) and perforatic acid (Thadathiti *et al.,* 1994 in 300MHz recorded in $CDCl_3$+MeOD)

	HPE1		Thadathiti *et al.,* 1994	
position	δ_C(DMSO)	δ_H, *J* in Hz	δ_C($CDCl_3$+MeOD)	δ_H, *J* in Hz
2	161.0		159.8	
3	113.1	7.48, s	113.1	6.89, s
4	178.2		179.5	
4a	103.2		102.5	
5			153.6	
6	97.5	7.38, s	96.4	6.29, s
7	154.5		155.5	
8	109.7		108.2	
8a	158.3		158.4	
1’	28.8	2.40, s	27.4	1.43, s, 6H
2’	78.9		77.9	
3’	115.7	6.71, d, 12	114.6	5.54, d, 10
4’	128.6	7.74, d, 12	126.8	6.88, d, 10
CH_3-2’	28.8		27.4	
OCH_3	57.1	3.47, s	55.4	3.89, s
COOH	162.4		165.5	

4.1.6 Isolated compound from Benchalokawichian

4.1.6.1 Compound BLWE1

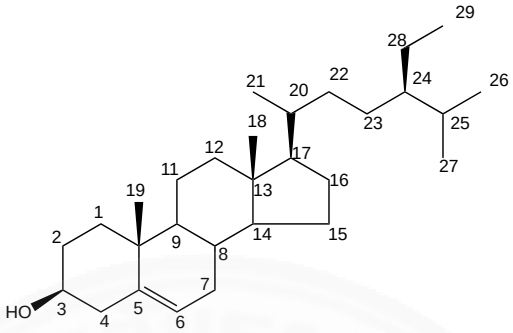


Figure 4.5 Chemical structure of BLWE1

The ^{1}H NMR (600 MHz, $CDCl_3$) spectrum revealed that the sample was a mix structure of BLWE1 and CPE1 as oleanolic acid acetate as showed on Table 4.10. The singlet methyl peaks at δ_H 0.66, 0.82, 0.84, 0.92, 1.04 were assigned to Me-19, Me-27, Me-29, Me-21, Me-18, respectively. However, complex unresolved multiplet resonances were observed at δ_H 0.96-1.02, 1.11-1.31, 1.41-1.53, 1.75-1.83, 2.10-2.24. From these data, compound BLWE1 was tentatived as β-sitosterol (Sayeed *et al.,* 2016).

A B C

Figure 4.6 TLC spots of mixed compound (B, in middle) compared to BLWE1(A, left) and CPE1(C, right) in 30%EtOAc: hexane system

Table 4.10 The ^{1}H NMR data for mixture of CPE1 and BLWE1 (recorded at 600MHz in $CDCl_3$) compared with their references (recorded at 400 MHz in $CDCl_3$)

position	CPE1	Oleanolic acid acetate Hichri *et al.,* 2003	BLWE1	β-sitosterol Sayeed *et al.,* 2016
	δ_H ($CDCl_3$; 600MHz)	δ_H ($CDCl_3$; 400MHz)	δ_H ($CDCl_3$; 600MHz)	δ_H ($CDCl_3$; 400MHz)
1				
2				
3	4.42, 1H, m	4.42, 1H, m	3.45, 1H, m	3.53, 1H, m
4				
5			5.00, 1H, m	5.36, 1H, m
6				
7				
8				
9				
10				
11				
12	5.34, 1H, m	5.19, 1H, m		
13				
14				
15				
16				
17				
18	2.74, 1H, m	2.78, 1H, m	1.04, 3H, s	1.01, 3H,s
19			0.66, 3H, s	0.68, 3H, s
20				
21			0.92, 3H, s	0.93, 3H, s
22				
23	0.86, 3H, s	0.88, 3H, s		
24	0.84, 3H, s	0.87, 3H, s		
25	0.92, 3H, s	1.05, 3H, s		
26	0.77, 3H, s	0.67, 3H, s		0.83, 3H, s
27	1.04, 3H, s	1.18, 3H, s	0.82, 3H, s	0.81, 3H, s

position	CPE1	Oleanolic acid acetate Hichri *et al.,* 2003	BLWE1	β-sitosterol Sayeed *et al.,* 2016
	δ_H ($CDCl_3$; 600MHz)	δ_H ($CDCl_3$; 400MHz)	δ_H ($CDCl_3$; 600MHz)	δ_H ($CDCl_3$; 400MHz)
28				
29	0.78, 3H, s	0.79, 3H, s	0.84, 3H, s	0.84, 3H, s
30	0.93, 3H, s	1.00, 3H, s		
$\underline{CH_3}COO$	1.97, 3H, s	2.06, 3H, s		
$CH_3\underline{C}OO$				

4.1.6.2 Compound BLWE2

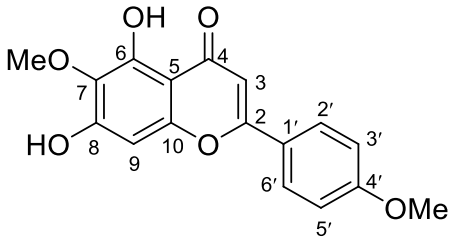


Figure 4.7 Chemical structure of BLWE2

The molecular formula was proposed to be $C_{17}H_{14}O_6$ as deduced from ESI mass spectra [m-H]$^-$ 313.1, ^{1}H NMR (600 MHz, DMSO-d_6) and the ^{13}C NMR (125 MHz, DMSO-d_6) data. The ^{13}C NMR spectrum showed 17 carbon signals observed; 9 of which correspond with 12 protons and 2-OH as observed from the HMQC spectrum (Table 4.11).

The HMBC spectrum showed a pattern of correlations consistent with a flavonone derivative in which a *para*-methoxyphenyl was located at C-2 (δ 163.7). H-2' and H-6' (δ 7.93, dd, J = 6) were correlated with C-2 (δ 163.7) and C-4'(δ 162.2), and one aromatic proton resonance at δ 6.66 (s) was correlated with C-4 (δ 182.5), C-2 (δ 163.7) and C-1' (δ 123.1). The chelated hydroxyl group (δ 13.13, s) at C-5 (δ 152.7) of chomone moiety showed a correlation with with carbonyl group at C-4 (δ 182.5), one methoxyl proton (δ 4.13, s) at C-6 which was confirmed by HMQC and HMBC experiment. Therefore, BLWE2 was assigned as 5,7-dihydroxy-6-methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one or pectolinarigenin (Hase *et al.,*1995).

Table 4.11 Comparison of ^{13}C and ^{1}H NMR data between BLWE2 (recorded at 600Mhz in DMSO) and pectolinarigenin (recorded in $CDCl_3$)

	BLWE2		Hase *et al.,* 1995	
position	δ_C (DMSO)	δ_H, *J* in Hz	δ_C($CDCl_3$)	δ_H, *J* in Hz
2	163.7		163.3	
3	104.0	6.66, s	103.0	6.87, s
4	182.5		182.1	
5	152.7		152.7	
6	129.9		131.4	
7	154.5		157.4	
8	93.2	6.68, s	94.3	6.62, s
9	151.7		152.4	
10	105.3		104.1	
1'	123.1		122.9	
2'	128.1	7.93, d, 6	128.3	8.03, d, 9
3'	114.7	7.10, d, 6	114.5	7.10, d, 9
4'	162.2		162.3	
5'	114.7	7.10, d, 6	114.5	7.10, d, 9
6'	128.1	7.93, d, 6	128.3	8.03, d, 9
6-OH		13.13, s		13.00, s
7-OMe	61.0	4.13, s	59.9	3.86, s
4'-OMe	55.9	3.98, s	55.5	3.75, s

4.1.6.3 Compound BLWE3

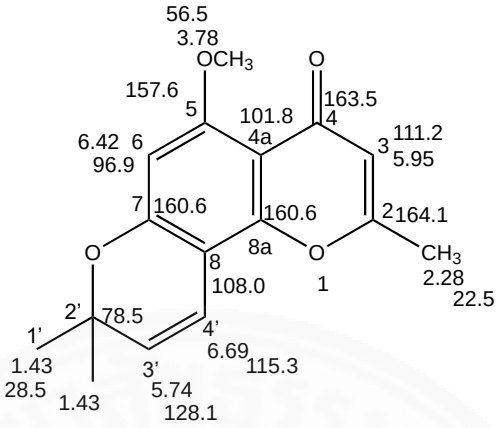


Figure 4.8 Chemical structure of BLWE3

Compound BLWE3 gave a molecular formula $C_{16}H_{16}O_4$ as determined by ESIMS that gave a $[M+H]^+$ ion at *m/z* 273.11. The ^1H (600 MHz; DMSO-d_6) NMR data showed the presence of three methyls at δ_H 2.28 (3H, s), 1.43 (6H, s), methoxy proton at δ_H 3.78 (3H, s), two olefinic protons at δ_H 5.74 (1H, d, *J*=10Hz) and 6.68 (1H, d, *J*=10Hz), 5.95 (C3-H, s), and an aromatic ring proton at δ_H 6.42 (C6-H, s). An aromatic proton resonance at δ 6.42 (s) was correlated to the C-6 resonance of δ 96.9 in the HMQC sprectrum. H-6 showed HMBC correlations with C-5 (δ 157.6), C-8 (δ 108.0), C-7 (δ 160.6) and C-4a (δ 101.8); an aromatic proton resonance at δ 5.95 (s) was correlated with C-4 (δ 163.5) and C-8 (δ 108.0); and the two aromatic proton resonances of the pyran ring at δ 6.69 (d, J=12 Hz, H-4') and δ 5.74 (d, J=12 Hz, H-3') showed the correlations with C-2'(δ 78.5), C-5 (δ 157.6), C-4a (δ 101.8). Thus, the NMR data was consistent with BLWE3 being perforatin A or *O*-methylalloptaeroxylin. The comparison of the ^1H and ^{13}C NMR spectral data with the previously reported data of perforatin A (Thadanithi *et al*., 1994) shown in Table 4.12 confirmed the assignment.

Table 4.12 Comparison of ^{13}C and ^{1}H NMR data between BLWE3 (recorded at 600MHz in DMSO) and perforatin A (recorded at 300MHz in $CDCl_3$+MeOD)

	BLWE3		Thadathiti *et al.,* 1994	
position	δ_C(DMSO)	δ_H, *J* in Hz	δ_C($CDCl_3$+MeOD)	δ_H, *J* in Hz
2	164.1	2.28, s, 3H	163.3	2.29, s, 3H
3	111.2	5.95, s	112.3	6.00, s
4	163.5		178.3	
4a	101.8		103.0	
5	157.6		155.0	
6	96.9	6.42, s	97.0	6.29, s
7	160.6		158.0	
8	108.0		109.0	
8a	160.6		161.0	
1’	28.5	1.43, s, 6H	28.8	1.48, s, 6H
2’	78.5		78.5	
3’	128.1	5.74, d, 12	115.8	5.57, d, 12
4’	115.3	6.69, d, 12	127.9	6.70, d, 12
CH_3-2’	28.3		28.8	
CH_3-2	22.5		20.2	
OCH_3	56.5	3.78, s	57.0	3.92, s

4.1.7 Cytotoxic activity of pure compounds

Three different types of cancer cells, which were epithelial lung cancer (A549), squamous lung cancer cell (H226), and skin melanoma (C32) were selected to investigate the cytotoxic activity of isolated compounds from Benchalokawichian remedy. The toxicity of a normal skin cell line also studied (Table 4.13).

Following the previous result, HPE and CPE showed no cytotoxic activity with IC_{50} > 50 µg/mL. HPE1, which was isolated from *Harrisonia perforata,* gave the same results as their crude extract. On the contrary, CPE1 showed strong cytotoxic activity against two lung cancer cell lines (A549 and H226 with IC_{50} values 1.2 and 1.9 µg/mL) and moderate cytotoxicity against skin melanoma (C32, IC_{50}= 40.1 µg/mL) while CPE wasn't active. The ethanolic extract of *Tiliacora triandra,* TTE inhibited the growth of several types of cancer cell lines (with IC_{50} in range 19.5-37.9 µg/mL) in contrast to the TTE1 compound which showed specific toxicity against lung cancer cell lines (A549 and H226 with IC_{50}=6.5 and 13.9 µg/mL, respectively). However, both TTE and TTE1 showed less cytotoxicity to normal cells (IC_{50}> 50 µg/mL). BLWE showed moderate cytotoxic activity against A549 and C32 but particular activity against H226 although two compounds from BLWE were different. BLWE2 displayed good cytotoxic effects against A549, H226 and C32 with IC_{50} 7.8, 6.3 and 6.8 µg/mL but no toxicity to a normal cell line (HaCaT, IC_{50}>50 µg/mL). Moreover, BLWE3 revealed no cytotoxic activity (IC_{50}>50 µg/mL).

Table 4.13 Cytotoxic activity of isolated compounds compare their crude extracts against two lung cancer, melanoma and normal cell lines with IC_{50} (μg/mL) ± SEM

Sample	Cytotoxic activity, IC_{50} (μg/mL) ± SEM, μM			
	A549	H226	C32	HaCaT
TTE1	6.5 ± 4.8	13.9 ± 2.9	>50	>50
CPE1	1.2 ± 0.0	1.9 ± 0.1	40.1 ± 0.6	48.6 ± 0.3
HPE1	NT	NT	>50	NT
BLWE2	7.8 ± 0.2	6.3 ± 3.2	6.8 ± 1.2	11.5 ± 0.9
BLWE3	>50	>50	NT	NT
TTE	33.7 ± 0.6	19.5 ± 1.4	37.9 ± 0.4	79.9 ± 4.5
CPE	>50	>50	>50	NT
HPE	>50	>50	>50	NT
BLWE	32.4 ± 0.7	10.1 ± 2.9	29.1 ± 4.0	64.4 ± 9.2

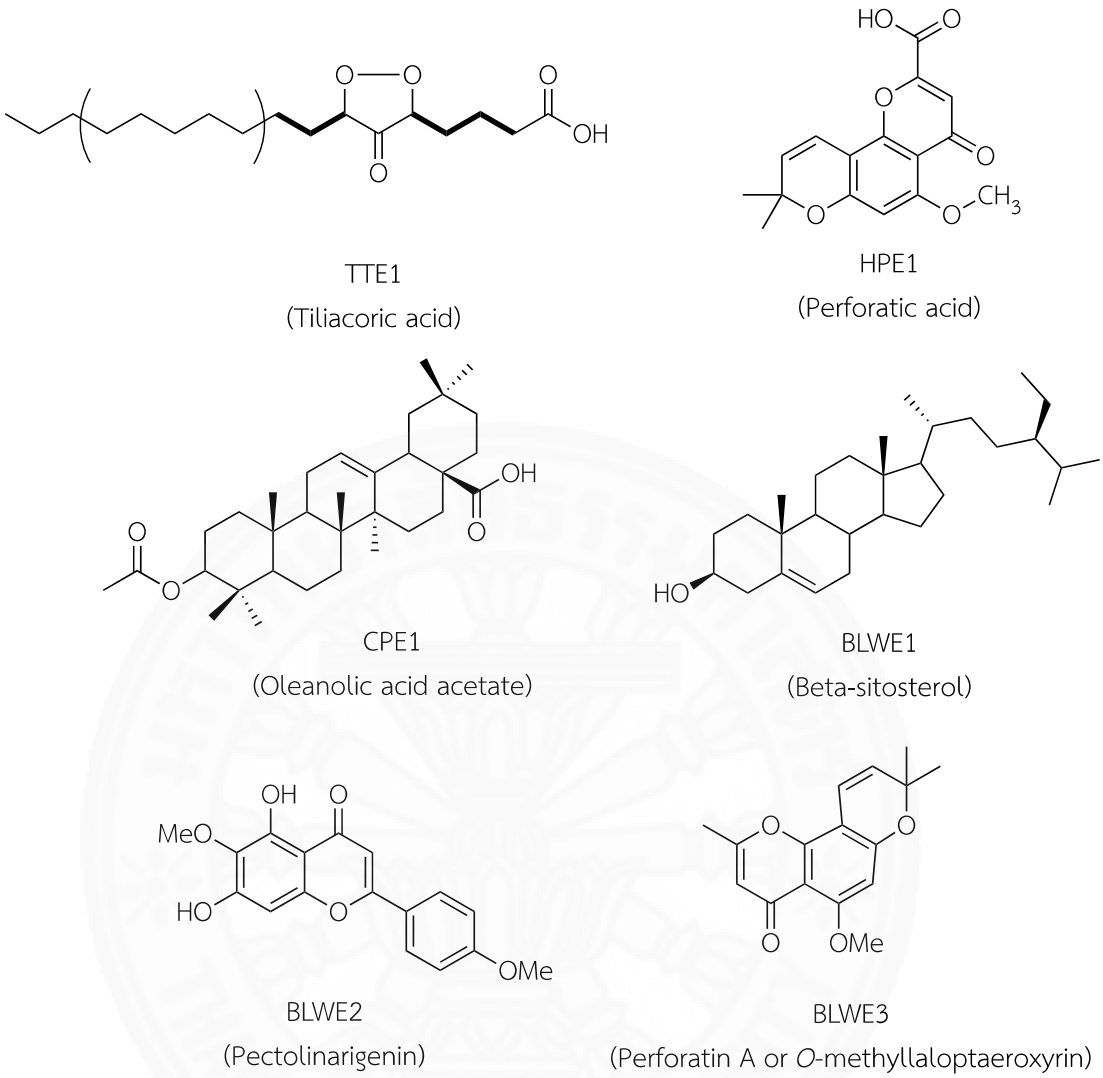
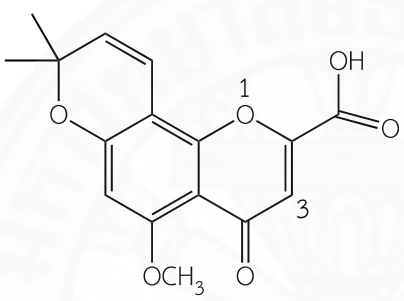


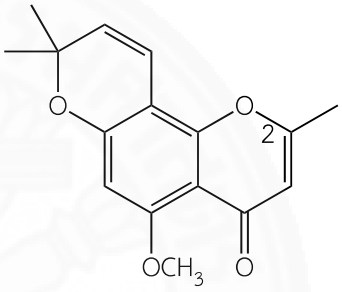
Figure 4.9 The structure of TTE1 from *Tiliacora triandra*, HPE1 from *Harrisonia perforata*, CPE1 from *Cleodendrum petasites,* and BLWE1, BLWE2, BLWE3 from Benchalokawichian remedy

4.1.8 Structure-activity relationships (SAR)

Six isolated compounds isolated from the plants of BLW remedy included the cyclic peroxide (TTE1), chromones (HPE1 and BLWE3), the triterpenoid (CPE1), sterol (BLWE1), and the flavonoid (BLWE2). This is the first report of TTE1, a new compound tiliacoric acid [4-((3*S*,5*R*)-5-decyl-4-oxo-1,2-dioxolan-3-yl) butanoic acid]. It exhibited *in vitro* cytotoxic activity against two types of lung cancer cell lines (A549 and H226) while it showed no toxicity against a skin melanoma cell line and a normal cell line by SRB assay (C32 and Hacat, IC_{50} >50 µg/mL).



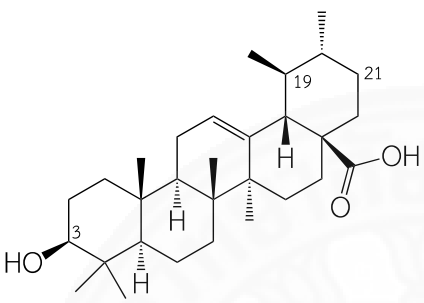
HPE1



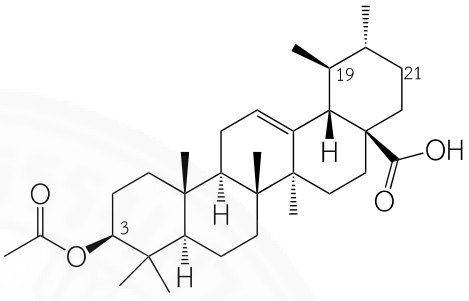
BLWE3

Two chromones, perforatic acid (HPE1) and perforatin A (BLWE3), were found in our study. A literature review showed that BLWE3 has been isolated from several plants, for example, the heartwood of *Cedrelopsis grevei* (Dean & Robinson 1971), the bark of *C. gracilis* (Mulholland *et al.,* 2004), the wood of *Ptaeroxylon obliquum* (Langenhover *et al.,* 1988), the stems of *Cneorum tricoccum* (Gonzales *et al.,* 1984), iranian oil of *Rosmarinus officinalis* (Jalali-Heravi *et al.,* 2011), and branches of *Harrisonia perforata* (Tuntiwachwuttikul *et al.,* 2006). It has been reported to possess antihypertensive, antiplasmodial, antimycobacterial and anti-allergy activity (Langenhover *et al.,* 1988; Tuntiwachwuttikul *et al.,* 2006; Juckmeta *et al.,* 2014). *In vivo* toxicity of BLWE3 has been tested, and it was safe in a rat model with a highest dose of 300 mg/kg (Irwin *et al.,* 1968).

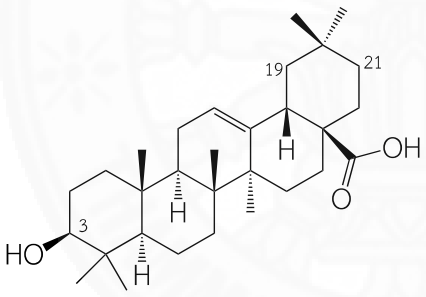
HPE1 was isolated from *H. perforata* a long time ago (Wang *et al.,* 1984; Tanaka *et al.,* 1995) and found to be inactive in antiplasmodial and antimycobacterial assays (Tuntiwachwuttikul *et al.,* 2006). Both chromones HPE1 and BLWE3 have the same core structure, differing only in the substituent at C-2 (-Me and -COOH), which didn't affect the in *vitro* cytotoxic activity.



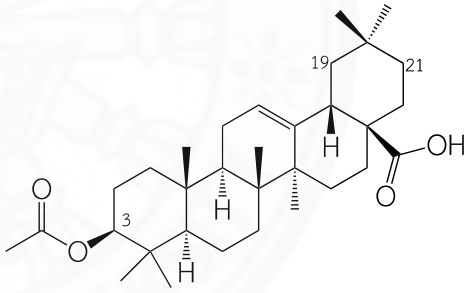
Usolic acid



Ursolic acid acetate



Oleanolic acid

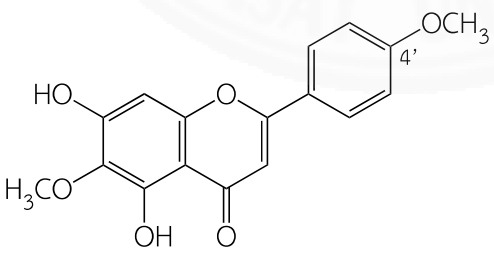


Oleanolic acid acetate

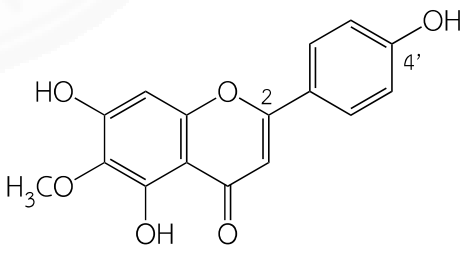
Oleanolic acid or OA has a similar structure to ursolic acid or UA (Jesus *et al.,* 2015; Jamal *et al.,* 2015; Kang *et al.,* 2008). Addition of an acetyl group to the C-3 hydroxyl of the pentacyclic triterpenoid OA gives CPE1 or oleanolic acid acetate (OAA) that improved activity in the type-II collagen-induced arthritis mice model compared with OA (Choi *et al.,* 2016). The cytotoxicity of synthesized oleanolic acid saponins was reported using a panel of human tumor cell lines, including promyelotic leukemia cell line (HL-60), non-small cell lung cancer cell line (A549) and melanoma cancer cell lines (A375), and most of the compounds showed strong cytotoxic activity (Lui *et al.,*

2013). Our results listed in Table4.13 showed the same trend. It is possible that the core structure alone promoted the cytotoxic effect, however, adding C-OOMe at C-3 increased the effect.

Pectolinarigenin, BLWE2, is a flavonoid which has been reported from several plants and in propolis from bees. In previous studies, it was isolated from Scrophulariaceae, Compositae, Bignoniaceae, Fabaceae, Lamiaceae and Verbanaceae (Tundis *et al.,* 2005; Bonesi *et al.,* 2008; Lim *et al.,* 2008; Yoo *et al.,* 2008; Yin *et al.,* 2008; Nugroho *et al.,* 2013; Hase *et al.,* 1995; He *et al.,* 1996; Zaidi *et al.,* 1998; Grayer *et al.,* 2001; Vieira *et al.,* 2003; Pal *et al.,* 1989). Researchers have studied the isolation and identification of the structure of this compound and its analysis by HPLC (He *et al.,* 1996). In addition, they have studied its biological activity. BLWE2 was isolated from *Linaria reflexa* (Scrophulariaceae) which exhibited strong *in vitro* cytotoxic activity against colorectal adenocarcinoma (Caco-2), lung large cell carcinoma (COR-L 23), renal cell adenocarcinoma (ACHN), amelanotic melanoma (C32), lung carcinoma (A459), malignant melanoma (A375) with IC_{50} values of 5.3, 4.1, 15.2, 7.0, 5.6 and 8.2 μM whereas it showed no activity against human hepatocellular carcinoma (Huh-7D12) and human skin fibroblast (142BR, IC_{50} >100 μM) cell lines (Tundis *et al.,* 2005; Bonesi *et al.,* 2008). In the same study, pectolinarigenin exhibited activity against all respiratory system cancer cell lines such as KB, Hep2, COR-L23, A549 with IC_{50} values 6.1, 1.9, 7.8, 9.1 μg/mL, respectively.



BLWE2



Hispidulin

Additionally, pectolinarigenin (BLWE2) found from *Cirsium chanroenicum* (Compositae) was evaluated for anti-inflammatory activity. BLWE2 strongly inhibited

COX-2 mediated PGE2 and 5LOX-mediated LT production at >1 μM, indicating that it is a dual inhibitor of COX-2/5-LOX. An *in vivo* study reported similar inhibitory activities against several animal models of inflammation/allergy: arachidonic acid-induced mouse ear edema, carrageenan-induced mouse paw edema and passive cutaneous anaphylaxis (Lim *et al.,* 2008). BLWE2 which was isolated from Compositae family (*Cirsium setidens*) exhibited hepatoprotective activity mainly *via* an SOD antioxidant mechanism in a rat model of hepatic injury caused by D-galactosamine (GalN) (Yoo *et al.,* 2008).

BLWE2 which was isolated from plants not only exhibited *in vitro* cytotoxic activity against cell lines but also showed effective anti-inflammatory activity *in vitro and vivo.* Hispidulin was reported to have many activities including anti-inflammation, anti-allergy and antitumor activity *in vivo* and *vitro* (Syrov *et al.,* 1985; Liu *et al.,* 1985; *Abdalla et al.,* 1988; *Anulakanapakorn et al.,* 1987; Hazekamp *et al.,* 2001). Following the structures, hispidulin is an analog of BLWE2, differing only in the substituent at C-4' (-OH and -OMe). Thus, the data suggests that core structure directly causes the anti-inflammatory and cytotoxic activities.

4.2 Finding the appropriately ratio of extract for product development treatment skin diseases involve infection and cancer.

Preliminary in previous study, the ethanolic extract of BLW and TT showed cytotoxic activity against all cell lines including five types cell lines in respiratory system and skin cancer cell line. Moreover, pectolinarigenin which was isolated from BLWE showed cytotoxic activity in our study (4.1) and also showed anti-allergic activity in previous study (Juckmeta *et al.,* 2014). In literature reviews, **α**-mangostin is main contents of *Garcinia mangostana* which showed the high ability of antibacterial and anti-inflammatory. Interestingly, it is possibly to develop two extract for skin treatment.

The plants were extracted in the different method and mixing appropriately. All extracts were determined antibacterial, anti-inflammatory and cytotoxic activity involving skin diseases. The stability was tested for approving their efficiency and suitability preparation of product development.

Table 4.14 Yield percentage of the ethanolic extract of Benchalokawichain (BLWE) and *Garcinia mangostana* (GME) and various organic extracts of GM

Sample	Method	Solvent	Code	%Yield
Benchalokawichien or Harak	Maceration	95%EtOH	BLWE	3.18
Garcinia mangostana	Maceration	95%EtOH	GME	21.38
	Soxhlet extraction	Hexane	GMH	0.92
		Chloroform	GMC	4.35
		Methanol	GMM	20.82
BLWE + GME	Mixing	95%EtOH + 95%EtOH	MIX 1:1	-
			MIX 1:2	-

The ethanolic extract of BLW and GM showed yield as 3.18% and 21.38%. Comparison of yield percentage of GM in various solvent extractions, the ethanolic extract showed the highest yield 21.38%, following soxhlet extraction with methanol, chloroform and hexane were 20.82%, 4.35%, 0.92%, respectively.

4.2.1 Antibacterial activity

We focused on four types of bacteria (*S. aureus* ATCC 25923, *S. aureus* MRSA DMST 25923, *S. epidermidis* ATCC 12228, *S. pyogenes* ATCC 19615) which is the most common of skin infection. Disc diffusion were preliminary determined for antibacterial activity, positive control were gentamicin, clindamycin and ampicillin. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) which the lower concentration can inhibit or kill bacteria, were also determined. Gentamicin, clindamycin and vancomycin were positive control.

4.2.1.1 Disc duffusion

The screening antimicrobial activity by disc diffusion against *S. aureus, S. aureus* MRSA, *S. epidermidis, S. pyogenes* showed that MIX1:1 and MIX1:2 exhibited greater antimicrobial activity than GME and BLWE. The extract of GM with methanol (GMM) showed higher antimicrobial activity than ethanol extract (GME) against all microbes in our study. Interestingly, all extract showed inhibition zone against *S. aureus* MRSA while positive control as gentamicin, clindamycin and ampicillin didn't. Three of the top, which showed high inhibition zone against *S. aureus* MRSA, were MIX1:2, following GMC and GME (11.67, 11.00 and 9.67 mm, respectively). However, MIX1:1 also exhibited *S. aureus* MRSA with clear zone 9.0 mm. Overall the results of disc diffusion were shown in Table 4.15.

Table 4.15 The inhibition zone (mm) of disc diffusion against *S. aureus, S. aureus* MRSA, *S. epidermidis, S. pyogenes*

Sample	Disc diffusion, Inhibition zone (mm) Mean ±SD, n=3			
	S. aureus	*S. aureus* MRSA	*S. epidermidis*	*S. pyogenes*
BLWE	7.33 ± 0.58	8.00 ± 1.00	NI	11.00 ± 1.73
GME	11.33 ± 0.58	9.67 ± 0.58	12.00 ± 0.00	11.33 ± 0.58
MIX 1:1	10.67 ± 0.58	9.00 ± 0.00	11.33 ± 0.58	13.00 ± 1.00
MIX 1:2	12.67 ± 3.79	11.67 ± 0.58	12.33 ± 0.58	12.00 ± 0.00
GMH	9.67 ± 1.53	7.67 ± 0.58	10.50 ± 0.71	10.00 ± 0.00
GMC	9.33 ± 0.58	9.67 ± 0.58	11.33 ± 0.58	10.00 ± 0.00
GMM	12.00 ± 0.00	11.00 ± 0.00	13.33 ± 1.15	12.00 ± 0.00
Gentamicin (10µg)	22.67 ± 2.08	NI	27.00 ± 0.00	21.00 ± 0.00
Clindamycin (2µg)	25.33 ± 1.53	NI	30.67 ± 3.21	25.00 ± 0.00
Ampicillin (10µg)	29.00 ± 2.00	NI	16.00 ± 1.73	35.00 ± 0.00

*NI= No inhibition Zone

4.2.1.2 Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

(1) MIC and MBC results against *S. aureus*

The MIC and MBC results of all BLWE and GM in various solvent extracts including Mix extract and their compounds against *S. aureus* displayed on Table 4.16. BLWE showed lowest antimicrobial activity with MIC value 5 mg/mL (MBC >5mg/mL). Pectolinarigenin at concentration 100 µg/mL showed no inhibitory effect. GMC exhibited moderate level of antimicrobial effects with MIC value 3.13 µg/mL, followed by GME which was as well as GMH (MIC values 6.25, 6.25 µg/mL), and GMM (MIC =15.6 µg/mL). The minimum inhibitory concentration of mix1:2 were 15.63 µg/mL whereas mix1:1 was 62.5 µg/mL, as the same result minimal of bactericidal concentration of mix 1:2 was better than mix1:1. Although, the positive control as clindamycin and gentamicin showed high effective against *S. aureus* with MIC values 0.0.9 and 0.19 µg/mL. Nonetheless, alpha-mangostin revealed the stronger antimicrobial activity against *S. aureus* than vancomycin with MIC 0.39 and 0.78 µg/mL, respectively.

(2) MIC and MBC results against *S. aureus* MRSA

The antimicrobial activity of methicillin-resistant reported on Table 4.17. The MIC and MBC results of BLWE and pectolinarigenin against MRSA were same as *S. aureus.* Surprisingly, the most samples showed bactericidal activity against MRSA while gentamicin and clindamycin didn't. The highest inhibitory potentials were GMH and GMC with MIC of 1.56 and 1.56 µg/mL. GME presented good antimicrobial activity, MIC and MBC values were similar (3.13 µg/mL). Likewise, MIC of mix1:2 was better than mix1:2 twice values (6.25 and 12.5 µg/mL). Additionally, alpha-mangostin showed greater antimicrobial activity than vancomycin with MIC values 0.19 and 0.78 µg/mL, respectively.

Table 4.16 The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against *S. aureus*

Sample	Minimal Inhibitory Concentration, MIC (µg/mL)				MBC
	n1	n2	n3	Mean, n=3	Mean, n=3
BLWE	5 mg/mL	5 mg/mL	5 mg/mL	5 mg/mL	>5 mg/mL
GME	6.25	6.25	12.5	6.25	25
MIX 1:1	31.25	62.50	62.50	62.50	125
MIX 1:2	15.63	15.63	15.63	15.63	62.50
GMH	6.25	3.13	6.25	6.25	12.50
GMC	3.13	6.25	3.13	3.13	>50
GMM	15.63	15.63	31.25	15.63	>1 mg/mL
Gentamicin	0.19	0.39	0.19	0.19	1.56
Clindamycin	0.09	0.19	0.09	0.09	0.19
Vancomycin	0.78	0.78	0.78	0.78	1.56
Alpha-mangostin	0.39	0.78	0.39	0.39	0.78
Pectolinarigenin	>100	>100	NT	>100	NT

*NT = not test

Table 4.17 The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against *S. aureus* MRSA

Sample	Minimal Inhibitory Concentration, MIC (µg/mL)				MBC
	n1	n2	n3	Mean, n=3	Mean, n=3
BLWE	5 mg/mL	5 mg/mL	5 mg/mL	5 mg/mL	>5 mg/mL
GME	3.13	3.13	3.13	3.13	3.13
MIX 1:1	12.50	6.25	12.50	12.50	50
MIX 1:2	6.25	3.13	6.25	6.25	>100
GMH	1.56	0.05	1.56	1.56	1.56
GMC	1.56	3.13	1.56	1.56	6.25
GMM	50	6.25	50	50	>50
Gentamicin	>100	NT	NT	>100	NT
Clindamycin	>100	NT	NT	>100	NT
Vancomycin	0.78	0.78	0.78	0.78	0.78
Alpha-mangostin	0.19	0.39	0.19	0.19	0.39
Pectolinarigenin	>100	>100	NT	>100	NT

*NT = not test

(3) MIC and MBC results against *S. epidermidis*

Although, alpha-mangostin showed the highest antimicrobial potentials with MIC 0.39 µg/mL of *S. epidermidis*, clindamycin and gentamicin were better (MIC = 0.04 and 0.09 µg/mL). GMC inhibited *S. epidermidis* as equal as vancomycin with MIC value 0.78 µg/mL however vancomycin exhibited bactericidal activity better than GMC (MBC=1.56 and 3.13 µg/mL, respectively). GMH also showed strong antimicrobial against *S. epidermidis* with MIC of 1.56 µg/mL and MBC of 3.13 µg/mL. Both GMM and GME showed inhibitory activity with MIC value 3.13 µg/mL. In the same manner the MIC of mix1:1 and mix1:2 were 6.25 µg/mL. Notably, BLWE and pectolinarigein revealed no antimicrobial activity agaist *S. epidermidis* (MIC >5mg/mL and > 100 µg/mL, respectively).

(4) MIC and MBC results against *S. pyogenes*

None of them exhibited antimicrobial activity better than positive control which were clindamycin, vancomycin and gentamicin (MIC values 0.09, 0.19, and 0.78 µg/mL, respectively). BLWE at concentration 400 µg/mL showed inhibitory effect against *S. pyogenes* but couldn't kill it. The potent concentration to inhibit and kill *S. pyogenes* of GMH and GMC were 12.5 µg/mL. Both of GMM and GME showed the same MIC value as 25.5 µg/mL while the bactericidal concenteration of them were slightly different. In addition, the results of mix1:1 and mix1:2 were similar with MIC value 50 µg/mL.

Table 4.18 The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against *S. epidermidis*

Sample	Minimal Inhibitory Concentration, MIC (µg/mL)				MBC
	n1	n2	n3	Mean, n=3	Mean, n=3
BLWE	>5 mg/mL	>5 mg/mL	NT	>5 mg/mL	>5 mg/mL
GME	3.13	1.56	3.13	3.13	25
MIX 1:1	6.25	12.50	6.25	6.25	50
MIX 1:2	6.25	3.13	6.25	6.25	25
GMH	1.56	1.56	1.56	1.56	3.13
GMC	0.78	1.56	0.78	0.78	3.13
GMM	3.13	6.25	3.13	3.13	25
Gentamicin	0.09	0.19	0.09	0.09	0.39
Clindamycin	0.04	0.04	0.04	0.04	0.78
Vancomycin	0.78	0.78	0.78	0.78	1.56
Alpha-mangostin	0.39	0.09	0.39	0.39	0.39
Pectolinarigenin	>100	NT	NT	>100	NT

*NT = not test

Table 4.19 The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against *S. pyogenes*

Sample	Minimal Inhibitory Concentration, MIC (µg/mL)				MBC
	n1	n2	n3	Mean, n=3	Mean, n=3
BLWE	400	>400	400	400	>400
GME	25	25	25	25	50
MIX 1:1	50	50	50	50	50
MIX 1:2	50	25	50	50	50
GMH	12.50	3.13	12.50	12.50	12.50
GMC	12.50	6.25	12.50	12.50	12.50
GMM	25	25	25	25	25
Gentamicin	0.78	1.56	0.78	0.78	3.13
Clindamycin	0.09	0.19	0.09	0.09	0.78
Vancomycin	0.19	0.39	0.19	0.19	0.78

*NT = not test

Table 4.20 The results of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against *S. aureus, S. aureus* MRSA, *S. epidermidis, S. pyogenes,* (n=3)

Sample	*S. aureus*		*S. aureus* MRSA		*S. epidermidis*		*S. pyogenes*	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
BLWE	5 mg/mL	>5 mg/mL	5 mg/mL	>5 mg/mL	>5 mg/mL	>5 mg/mL	400	>400
GME	6.25	25	3.13	3.13	3.13	25	25	50
MIX 1:1	62.50	125	12.50	50	6.25	50	50	50
MIX 1:2	15.63	62.50	6.25	>100	6.25	25	50	50
GMH	6.25	12.50	1.56	1.56	1.56	3.13	12.50	12.50
GMC	3.13	>50	1.56	6.25	0.78	3.13	12.50	12.50
GMM	15.63	>1	50	>50	3.13	25	25	25
Gentamicin	0.19	1.56	>100	NT	0.09	0.39	0.78	3.13
Clindamycin	0.09	0.19	>100	NT	0.04	0.78	0.09	0.78
Vancomycin	0.78	1.56	0.78	0.78	0.78	1.56	0.19	0.78
Alpha-mangostin	0.39	0.78	0.19	0.39	0.39	0.39	NT	NT
Pectolinarigenin	NT	NT	>100	>5 mg/mL	>100	>5 mg/mL	>400	NT

*NT = not test

The summary results of antimicrobial (MIC and MBC) against *S. aureus, S. aureus* MRSA, *S. epidermidis, S. pyogenes* showed on Table 4.20. BLWE inhibited *S. aureus* MRSA and *S. aureus* at concentration 5 mg/mL, but in the same dose wasn't killing them (MBC > 5 mg/mL). In similarly, BLWE exhibited against *S. pyogenes* at concentration 400 µg/mL (MBC > 400 µg/mL). In the study on antimicrobial activity against *S. aureus* MRSA, GMC showed highest antibacterial activity follow by GMH, GME, MIX 1:2 and MIX 1:1 (MIC values 0.78, 1.56 ,3.13, 6.25, 12.5 µg/mL), respectively. Alpha-mangostin exhibited higher antibacterial activity by MIC and MBC method than vancomycin (MIC = 0.19, 0.78 µg/mL, MBC = 0.39, 0.78 µg/mL, respectively) while gentamicin and clindamycin were not active (MIC > 100 µg/mL).

4.2.2 Anti-inflammatory activity

The extract which showed highest anti-inflammatory activity were GMH, followed by GMC (IC_{50} values 6.24 , 7.84 µg/mL). Nevertheless, they also showed toxicity at concentration 30 µg/mL. Although, the GME and MIX 1:2 exhibited inhibitory effect of nitric oxide production with IC_{50} values 18.90, 26.67 µg/mL, they also showed toxicity with %survival less than 70% at concentration 50 µg/mL. Both MIX1:1 and GMM showed moderate anti-inflammatiory activity (IC_{50} = 34.70, 37.84 µg/mL), they showed no toxicity at concentration 50 µg/mL. It is possibly that BLWE can reduce toxicity of GME. So, the MIX1:1 is appropriately to be develop the product for reduce inflammatory mechanism.

Table 4.21 Anti-inflammatory activity of the ethanolic extracts, combination extracts in ratio (1:1, 1:2) and different solvent extracts of GM shown in %inhibition, %survival at various concentration and IC_{50} (µg/mL, n=3)**.**

Sample	%Inhibiton and %Survival , Mean ± SEM (n = 3)						IC_{50} (µg/mL)
	100	50	30	10	1	0.1	
BLWE	48.65 ± 10.20	17.43 ± 6.85					>100
	89.37 ± 16.76	98.85 ± 8.86					
GME	97.38 ± 0.65	98.80 ± 1.41	85.47 ± 7.47	19.26 ± 5.10	-0.78 ± 6.32		18.90 ± 0.73
	9.91 ± 2.01	12.53 ± 4.09	65.14 ± 20.60	115.50 ± 6.38	107.97 ± 5.40		
MIX 1:1	99.30 ± 0.59	75.07 ± 6.50	29.47 ± 3.52	-5.42 ± 6.85	-19.77 ± 2.83		34.70 ± 3.51
	10.47 ± 0.67	84.87 ± 9.91	116.62 ± 1.56	108.05 ± 2.76	98.86 ± 6.02		
MIX 1:2	97.70 ± 1.29	96.20 ± 2.43	43.46 ± 2.80	0.62 ± 4.16	-15.24 ± 2.03		26.67 ± 6.94
	9.38 ± 0.87	30.10 ± 2.36	115.47 ± 3.07	107.23 ± 3.61	99.65 ± 5.77		
GMH		99.65 ± 3.84	104.60 ± 5.02	72.69 ± 9.50	-2.24 ± 21.52	-18.67 ± 7.76	6.24 ± 2.52
		11.44 ± 1.88	11.33 ± 1.39	106.84 ± 16.87	100.26 ± 8.28	94.08 ± 5.54	
GMC		95.98 ± 0.29	100.27 ± 0.88	72.72 ± 5.52	-18.45 ± 3.21	-23.20 ± 2.59	7.84 ± 0.49
		10.21 ± 0.95	10.69 ± 1.44	96.40 ± 14.36	99.31 ± 3.22	104.49 ± 1.96	
GMM	85.70 ± 10.56	64.45 ± 12.31	40.87 ± 11.37	12.32 ± 10.59	-0.30 ± 8.72		37.84 ± 9.24
	14.85 ± 0.76	119.71 ± 14.07	117.25 ± 10.24	114.92 ± 3.60	95.24 ± 2.83		

%survival showed more than 70% , it means no toxicity.

4.2.3 Quantitative and Qualitative analysis of MIX remedy using HPLC technique

Studied on validate method of two compounds, pectolinarigenin and alpha-mangostin, from Benchalokawichian (BLW) and *Garcinia mangostana* (GM). Pectolinarigenin, which showed cytotoxic activity in our study as same as previous reports (Bonesi *et al.,* 2008 and Tundis *et al.,* 2005), was used for marker of BLW. Moreover, pectolinarigenin also showed anti-inflammatory and anti-allergic activities in the recent study (Juckmeta *et al.,* 2012 and Juckmeta *et al.,* 2014). Alpha-mangostin is well known for antibacterial activity, and also showed their activity against *S. aureus* MRSA better than some positive control in this research. There is no research study on validation method of this mix remedy. This research modified method from Sakpakdeejaroen *et al.,* (2014), Aisha A. *et al.,* (2012) and Yodhnu *et al.,* (2009), results showed on Table 4.22 and 4.23.

4.2.3.1 Selectivity

The selectivity of this method was confirmed by comparison of mix extract and standard (pectolinarigenin and alpha-mangostin). Spectra of compounds which found from mix extract were identity as same as standard.

Table 4.22 Linear equation, correlation coefficients (R^2), LOD and LOQ of two compounds

parameters	pectolinarigenin	alpha-mangostin
concentration (µg/mL)	10-200	50-1000
linear equation	y= 45.25x + 42.02	y= 18.05x + 236.98
linearity (R^2)	0.9999	0.9999
LOD (µg/mL)	0.4	0.64
LOQ (µg/mL)	0.16	0.49

4.2.3.2 Linearity

Pectolinarigenin was prepared the concentration 10-200 µg/mL, alpha-mangostin was prepared the concentration 50-1000 µg/mL. Standard of two compounds in 5 concentrations were mixed and injected in triplicate. The correlation coefficients (R^2) of them were 0.9999.

4.2.3.3 Precision

The average %RSD of pectolinarigenin and alpha-mangostin in the intra-day data were 0.78% and 2.17% whereas inter-day were 1.4% and 2.7%

4.2.3.4 Accuracy

Two standard compounds which pectolinarigenin (10, 50, 100 µg/mL) and alpha-mangostin (50, 250, 500 µg/mL) were evaluated at 3 concentrations. The percentage of recovery were presented their accuracy.

4.2.3.5 LOD and LOQ

The LOD represent the lowest concentration that can detected whereas the LOQ represents the lowest concentration that can determined with acceptable precision and accuracy by using this instrument and method. Results of LOD and LOQ were revealed on Table 4.23

Table 4.23 Precision and accuracy of pectolinarigenin and alpha-mangostin

pectolinarigenin						
Concentration (μg/mL)	Intra-day (n=3)		Inter-day (n = 9)		Recovery (n=3)	
	Measured Conc. (μg/mL)	%RSD	Measured Conc. (μg/mL)	%RSD	Mean (%)	%CV
100	92.55 ± 1.07	1.16	95.42 ± 4.17	4.37	93.51 ± 0.25	0.28
50	45.13 ± 0.37	0.83	46.55 ± 1.80	3.87	91.87 ± 0.69	0.74
10	9.67 ± 0.03	0.33	9.79 ± 0.21	2.11	100.27 ± 1.62	1.61
alpha-mangostin						
Concentration (μg/mL)	Intra-day (n=3)		Inter-day (n = 9)		Recovery (n=3)	
	Measured Conc. (μg/mL)	%RSD	Measured Conc. (μg/mL)	%RSD	Mean (%)	%CV
500	514.39 ± 2.63	0.51	504.46 ± 17.50	3.46	96.85 ± 2.59	2.67
250	242.77 ± 4.94	2.04	246.41 ± 5.63	2.28	97.43 ± 2.68	2.75
50	65.88 ± 2.61	3.96	60.33 ± 11. 67	19.35	93.83 ± 5.26	5.60

4.2.4 Stability testing of mix extract

The extract was studied on both biological activities and chemical fingerprint for approve their efficacy in long term period. The samples were placed into stability incubator 40 celsius degree, with RH 75% for 6 months. Three samples were randomly collected in each day 0, 15, 30, 60, 90, 120, 150, 180.

4.2.4.1 HPLC technique for quantitative evaluation

The quantitative of two compounds analyze by HPLC technique. The extracts showed that the alpha-mangostin was decreasing on the other hand pectolinarigenin was increasing. They show less different but not significantly (*p*- value < 0.05). However, the biological activity is also necessary.

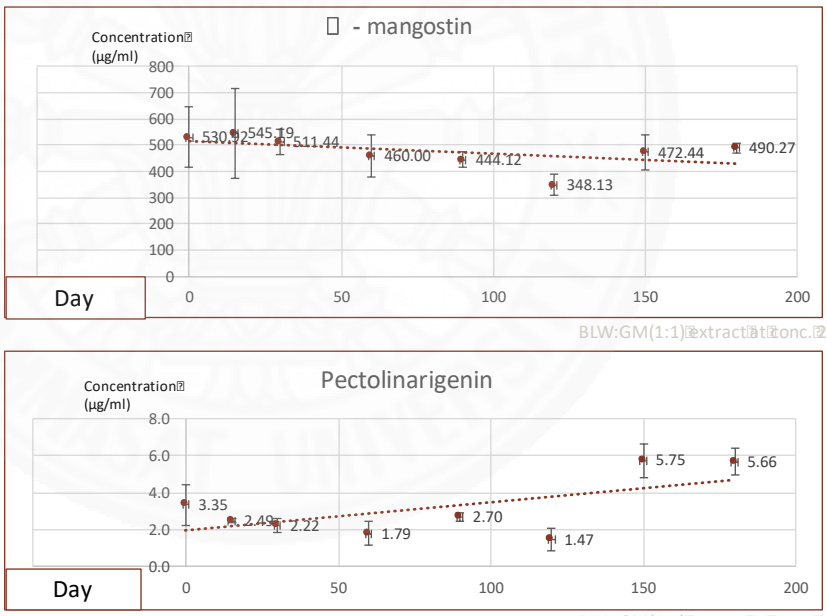


Figure 4.10 Total amounts of alpha-mangostin and pectolinarigenin in stability extract

4.2.4.2 Antimicrobial activity (MIC, MBC)

All extracts also showed not different in *S. aureus* with the MIC in the range of 50-100 µg/mL. The inhibitory effect of mix1:1 extract against *S. aureus* MRSA was decreased after 30 days (MIC from 12.5 to 25 µg/mL) then stable within 90 days before it was decreasing again.

Table 4.24 The results of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of MIX1:1 extract D0-D180 against *S. aureus, S. aureus* MRSA showed in concentration (µg/mL), n=3

MIX1:1 extract	*S. aureus*		*S. aureus* MRSA	
	MIC	MBC	MIC	MBC
D0	50	>100	12.5	100
D15	50	>100	12.5	50
D30	50	>100	25	100
D60	100	>100	25	100
D90	50	>100	25	>100
D120	100	>100	50	>100
D150	50	>100	25	>100
D180	100	>100	50	>100

4.2.4.3 Anti-inflammatory activity using inhibitory release nitric oxide (NO) and MTT assay

Inhibitory effect releasing nitric oxide induced by lipopolysaccharide (LPS) and survival of extract in various days were determined, IC_{50} showed in range 21.0-36.8 µg/ml, Table 4.25. As the result, the samples were not significantly different (*p*-value < 0.05).

Table 4.25 Anti-inflammatory activity of mix extract (D0-D180) with IC_{50} (µg/mL)

MIX1:1 extract	IC_{50} (µg/mL) Mean ± SEM, n=3
D0	21.02 ± 1.13
D15	26.23 ± 13.75
D30	25.02 ± 10.12
D60	32.30 ± 0.64
D90	30.74 ± 1.11
D120	23.74 ± 7.44
D150	36.75 ± 4.71
D180	22.09 ± 2.81

4.2.5 Pre-formulation study

Force degradation of preparation extract were studied before product development. The extracts were weighted to 50 mg and placed in glass tube. Heat, humidity, acid hydrolysis, and oxidation were tested the typical tests. The sample were determined for antimicrobial activity assay.

Antimicrobial activity against *S. aureus* MRSA were influenced by oxidation and acid hydrolysis. In addition, alkaline also showed minor effect on antimicrobial activity. So, the process for product development should be aware and avoid these three factors.

Table 4.26 Force degradation study of mix extract

Sample	*S. aureus* MRSA	
	MIC (µg/mL)	MBC (µg/mL)
Control	6.25	12.5
Oxidation	>100	>100
Acid hydrolysis	>100	>100
Alkaline hydrolysis	25	25
Moisture hydrolysis	6.25	12.5
Temperature forced degradation	6.25	12.5

4.2.6 Product development

The Ingredients were varied and providing five formulations (Rx1-Rx5) showed on Table 4.27. For the topical herbal (mix1:1) cream formulation, we decided to use preservative as less as possible which might not be effect on antimicrobial activity. Rx1-4 were white opaque, smoothness, moisturizer and pH suitable while Rx5 was not homogeneity and texture like balsam.

Table 4.27 Various formulation ingredients of cream (Rx1-Rx5)

Ingredients	Concentration %w/w				
	Rx1	Rx2	Rx3	Rx4	Rx5
Sepigel	1.5	1	1.5	1.5	1.5
Sodium EDTA	0.1	0.1	0.1	0.1	0.1
Propylene glycol	5	5	5	5	5
Glycerine	5.7	5	5	5	5
Uniphen	0.5	-	0.3	-	-
Bronidox	-	0.3	-	-	-
Liqiud paraffin light	1	1.5	1.5	1.5	5
Isopropyl myristate	2	1.5	1.5	1.5	5
GMS	2	2	2	2	2
Cetyl alcohol	2	2	1.5	1.5	2
Steryl alcohol	1	1	0	1	0
Cremophor A25 (powder)	2	2	1.5	2	0
Cremophor A6 (hard)	2	1	1.5	2	0
Lanolin anhydrous	0.5	0.2	0.5	0.5	0.1
Water	q.s.100				

Rx4 formulation which provide moisturizer, soothing skin and film formed after dry is possible to develop product. After the Rx4 showed good stability by using heating-cooling method, 0.3% of extract was added for skin infection product. Cream preparation was filled into tubes, then placed in stability incubator 40 celsius degree, with RH 75% for 6 months.

Table 4.28 Physical properties of the topical cream formulations

Rx	pH	color	Physical appearance	Homogeneity	texture	Phase separation	Immediate skin feels
1	5.5	white	opaque	good	smooth	no	light& dry, not greasy
2	5.5	white	opaque	good	smooth	no	fluid, not greasy
3	5.5	white	opaque	good	smooth	no	viscous, slightly greasy
4	5.5	white	opaque	good	smooth	no	film formed after dry, slightly thicky
5	5.5	white	opaque and transaparent	not good	non-smooth	yes	oily like balsam

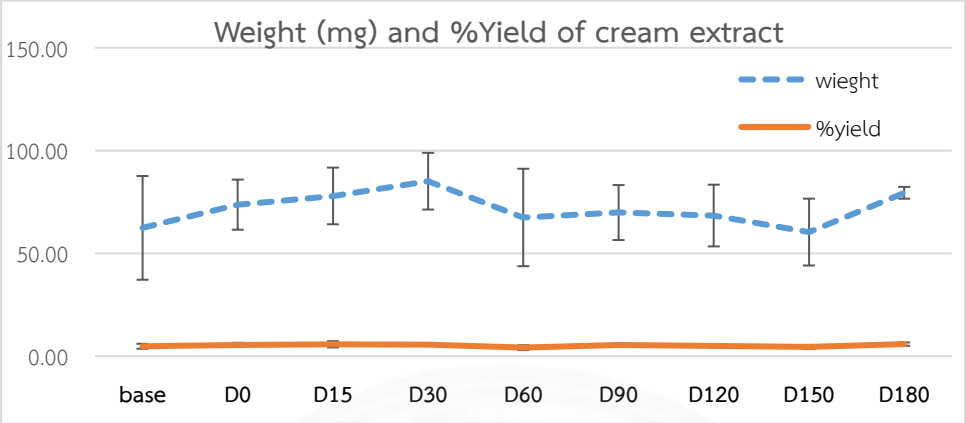


Figure 4.11 Chart illustrated weight and %yield of cream extract

Triplicate samples were weighed into tube, the average weight in range 1200-1600 mg. They were extracted by methanol, centrifuged then filtrated (repeated it twice) to obtain the cream extract. Average of base cream 1248.9 mg provided the percentage of yield as 4.84 (62.4 ± 25.2 mg). Similarly, formulated creams (D0-D180) were extracted and calculated percentage of yield, the results of weight and %yield showed on Table 4.29 and Figure 4.10.

Table 4.29 Weight of stability cream extraction and percentage of yield (Mean±SEM, n=3)

Cream	Weight (mg)	Extract weight (mg)	%Yield
base	1248.97 ± 322.55	62.40 ± 25.23	4.84 ± 121
D0	1382.50 ± 189.86	73.70 ± 12.17	5.51 ± 1.02
D15	1474.23 ± 278.71	77.93 ± 13.78	5.78 ± 1.52
D30	1497.47 ± 123.78	85.10 ± 13.79	5.64 ± 0.57
D60	1587.03 ± 371.72	67.47 ± 23.70	4.20 ± 1.16
D90	1269.83 ± 200.19	69.87 ± 13.36	5.45 ± 0.38
D120	1375.57 ± 185.83	68.40 ± 15.00	5.00 ± 0.82
D150	1351.27 ± 188.79	60.37 ± 16.25	4.51 ± 0.96
D180	1405.90 ± 201.76	79.47 ± 2.87	5.88 ± 0.81

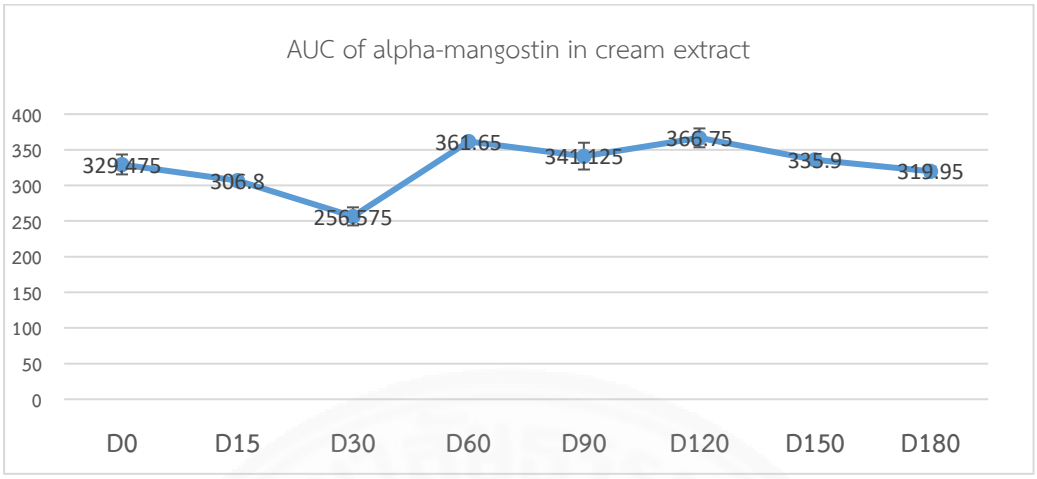


Figure 4.12 Cream stability of analysis using HPLC presented by area under curve of alpha-mangostin.

As the result, area under curve of alpha-mangostin which was used to be an active compound of sample were not difference (p-value < 0.05). It assumed that cream preparation was stability and can kept within two years.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Cytotoxic activity and compounds isolation

Water extract and ethanolic extract of individual plants and Benchalokawichian remedy were tested cytotoxic activity against three lung cancer cells (A549, COR-L23, H226), oropharynx and larynx cancer cell lines (KB, Hep2). All of water extract showed no cytotoxic activity (IC_{50}>50 µg/mL). Four of ethanolic extracts which were FR, CM, CP, HP also showed activity. The ethanolic extract of *Tiliacora triandra* (TTE) exhibited all types of respiratory system (three lung cancer, oropharynx and larynx) cancer cell lines with IC_{50} in range 19.5-45.2 µg/mL. In the same way, the 95%EtOH of Benchalokawichian (BLWE) extract showed cytotoxic activity against A549, COR-L23, H226, KB, Hep2 with IC_{50} in range 10.1-33.7 µg/mL. Both of them showed specific cytotoxic with H226 better than others cell lines. In addition, BLWE showed higher cytotoxic against skin cancer (C32) cell line than TTE with IC_{50} value 29.1 and 37.9 µg/mL, respectively.

Tiliacora triandra ethanolic extract (TTE) was interesting to be a marker for cytotoxic activity of BLW remedy, so it was isolated based on bioassay guided fractionation technique. Following VLC method, five fractions (TTF1-TTF5) were collected. Yield percentage were calculated, TTF4 was the highest yield (48.4%) followed by TTF5, TTF2, TTF3, TTF1 (24.1%, 4.6%, 3.82%, 0.1%). Screening of cytotoxic activity against five cell lines in respiratory system at concentration 50 µg/ml, fraction 2-3 (TTF2-3) showed no cytotoxic activity with percentage lower than 50. The fraction 4 ($CHCl_3$: MeOH, TTF4) showed high percentage of cytotoxic against all cell lines (in range 79.93-95.73%). The fraction 5 (MeOH, TTF5) showed 70% cytotoxic at concentration 50 µg/mL against H226. The result showed that TTF4 exhibited cytotoxic activity as same as TTE. Surprisingly, the fraction 5 (TTF5) showed specific against H226 with IC_{50} value 33.02 µg/ml. Isolation compound from TTF4 of ethanolic extract of *Tiliacora triandra* was TTE1, the ^{1}H and ^{13}C NMR spectrum and COSY, HMBC correlations were proven as 4-((3*S*,5*R*)-5-decyl-4-oxo-1,2-dioxolan-3-yl) butanoic acid.

The ethanolic extract of *Clerodendrum petisites* was separated by sephadex LH-20, and further isolated by sep-pack C18 decreasing polarity to 100% methanol. Compound as CPE1, a colorless crystal was participated by methanol. Base on ^{1}H, ^{13}C NMR (600 and 125 MHz, $CDCl_3$) spectrum all data and comparative their reference, CPE1 was identified as oleanolic acid acetate. Moreover, the main compound was isolated from *Harrisonia perforata* which was HPE1 as the yellow wax. The ^{1}H NMR and ^{13}C were indicated as perforatic acid.

The chloroform fraction of crude BLW ethanolic extract was separated by VLC elution start with hexane increasing polarity with chloroform and acetone to give seven fractions (A-G). Recrystallization of C4 using hexane-MeOH afforded CPE1 and BLWE1 as colorless crystals and white powders, the ^{1}H NMR data indicated the mixture of oleanolic acid acetate and β-sitosterol. BLWE2 was washed with Me_2CO-MeOH to obtain as light-yellow crystals. By comparison of ^{1}H and ^{13}C NMR data with previous study, BLWE2 was identified as pectolinarigenin. BLWE3 as white/yellow wax which was isolated by PLC, data of NMR spectra was confirmed with the previously reported data as perforatin A or *O*-methylalloptaeroxylin.

Cytotoxic activity of isolated compounds against two lung cancer, melanoma and normal cell lines were investigated. The TTE1 exhibited two lung cancer cell lines, A549 and H226, betther than TTE with IC_{50} 6.49, 13.98 and 33.65, 19.48 µg/mL, respectively. Notably, TTE1 showed no toxicity in normal cell line (HaCaT). CPE1 also showed strong cytotoxic activity against A549 and H226 with 1.24 and 1.95 µg/mL whereas CPE weren't. Although HPE1 was the main compound of *Harrisonia perforata* and Benchalokawichian remedy, it showed no cytotoxic activity in SRB assay as same as BLWE3 (>50 µg/mL). In contrast, BLWE2 exhibited against two lung cancers (A549, H226) and malenoma cancer cell lines (C32) with IC_{50} 7.76, 6.25 6.78, µg/mL respectively. Though, BLWE2 also showed toxicity in normal cell line.

5.2 Product development for skin diseases

From review literature, pectolinarigenin and **α**-mangostin which were represented the be a marker of mixed extract. Comparison of yield percentage of GM in various solvent extractions, the ethanolic extract showed the highest yield 21.38%, following soxhlet extraction with methanol, chloroform and hexane were 20.82%, 4.35%, 0.92%, respectively. The results showed that BLWE inhibited *S. aureus* MRSA and *S. aureus* at concentration 5 mg/mL, but in the same dose wasn't killing them (MBC > 5mg/mL). In similarly, BLWE exhibited against *S. pyogenes* at concentration 400 µg/mL (MBC > 400µg/mL). In the study on antimicrobial activity against *S. aureus* MRSA, GMC showed highest antibacterial activity follow by GMH, GME, MIX 1:2 and MIX 1:1 (MIC values 0.78, 1.56 ,3.13, 6.25, 12.5 µg/mL), respectively. Alpha-mangostin exhibited higher antibacterial activity by MIC and MBC method than vancomycin (MIC=0.19, 0.78 µg/mL, MBC=0.39, 0.78 µg/mL, respectively) while gentamicin and clindamycin weren't active (MIC > 100 µg/mL). The GMH extract showed highest anti-inflammatory activity, followed by GMC (IC_{50} values 6.24, 7.84 µg/mL). Nevertheless, they also showed toxicity at concentration 30 µg/mL. Both MIX1:1 and GMM showed moderate anti-inflammatiory activity (IC_{50} = 34.70, 37.84 µg/mL), they showed no toxicity at concentration 50 µg/mL. It is possibly that BLWE can reduce toxicity of GME. Mixed extract was studied on both biological activities and chemical fingerprint for approve their efficacy in long term period.

Validation method of mix extract was developed, pectolinarigenin and alpha-mangostin were investigated based on HPLC analysis, five concentration of compounds provided reliable linearity (R^2= 0.9999). Intra-day and inter-day repeatability were calculated and presented in average %RSD which were refer to the precision. Pectolinarigenin were 0.78% and 2.17% whereas alpha-mangostin were 1.4% and 2.7%, respectively. Both of them revealed an accuracy with %recovery more than 95.

The sample was collected into stability incubator 40 celsius degree, with RH 75% for 6 months. HPLC analysis according to validation method, the extracts showed that the alpha-mangostin was decreasing on the other hand pectolinarigenin

was increasing. However, they show less different but not significantly (p-value < 0.05). Inhibitory effect releasing nitric oxide induced by lipopolysaccharide (LPS) and survival of mix extract in various day were not differented.

The extracts were weighed to 50 mg for pre-formulation before product development. Antimicrobial activity against *S. aureus* MRSA were influenced by oxidation and acid hydrolysis. So, the process for product development should be aware and avoid these three factors. Rx4 formulation which provided moisturizer and soothing skin is possible to develop product. The cream formulations were extracted by methanol in triplicate, the percentage of yields were averaged of 4.51-5.88. Alpha-mangostin which was the chemical compound of mixed cream was analyzed using HPLC analysis, the compound in various days showed not difference. It assumed that cream preparation was stability and can kept within two years. Nevertheless, the cream extract should be confirmed the biological activity which is an antimicrobial activity as same way with their extract.

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APPENDICES

APPENDIX A

Spectrums for Structure Elucidation

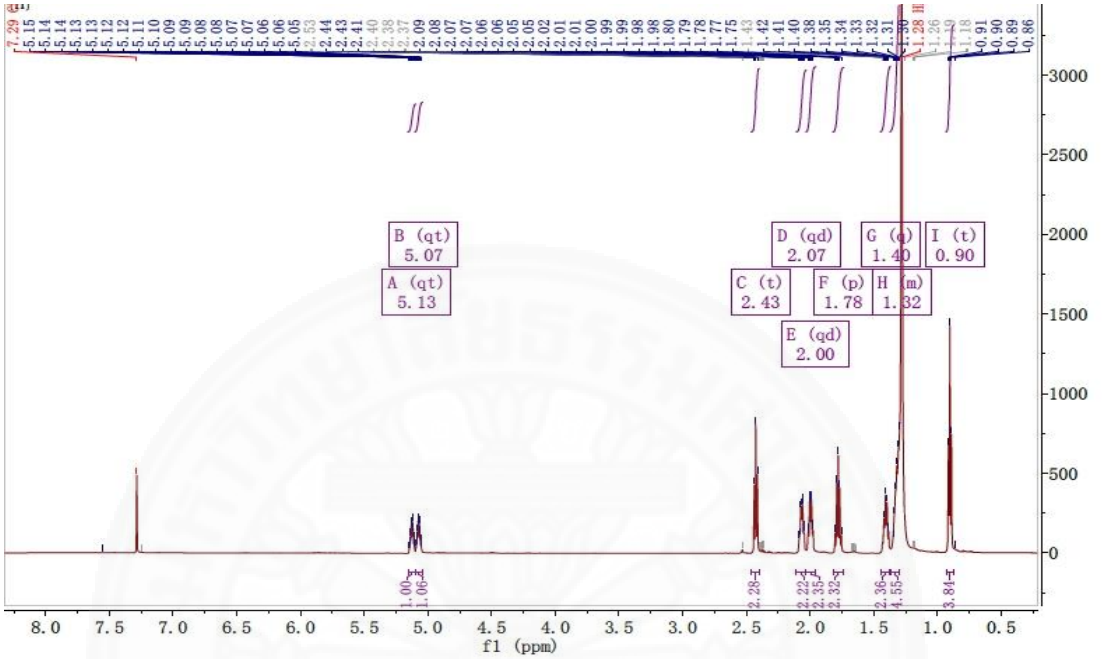


Figure A1 The ^{1}H spectrum of TTE1 recorded at 600 MHz in $CDCl_3$

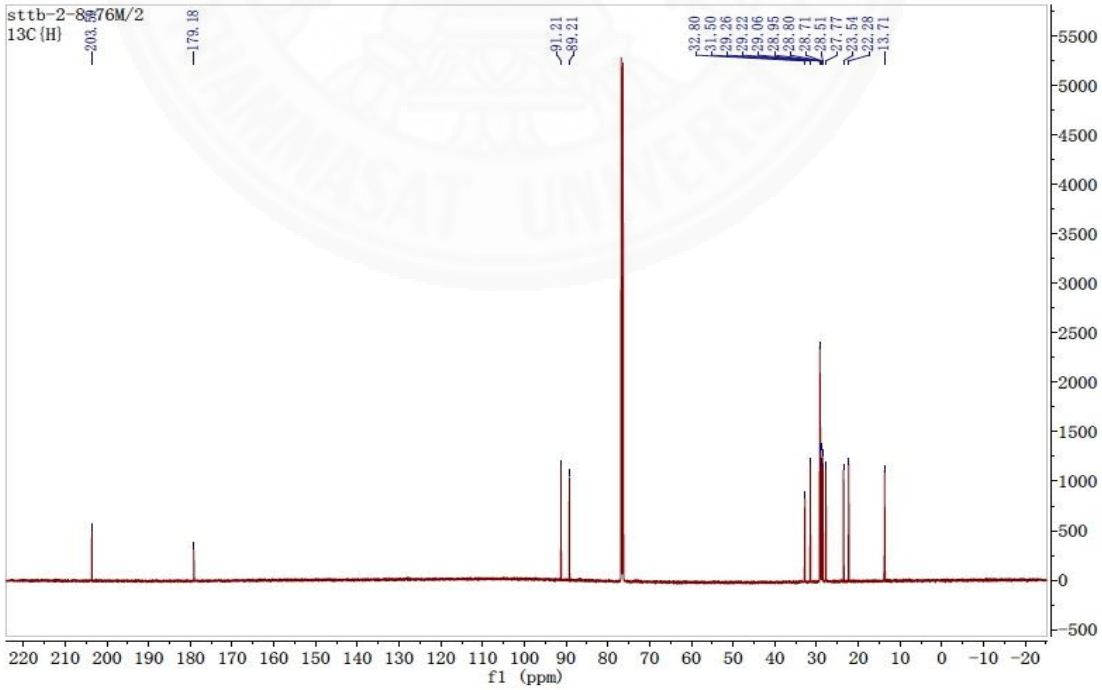


Figure A2 The ^{13}C NMR spectrum of TTE1 recorded at 150 MHz in $CDCl_3$

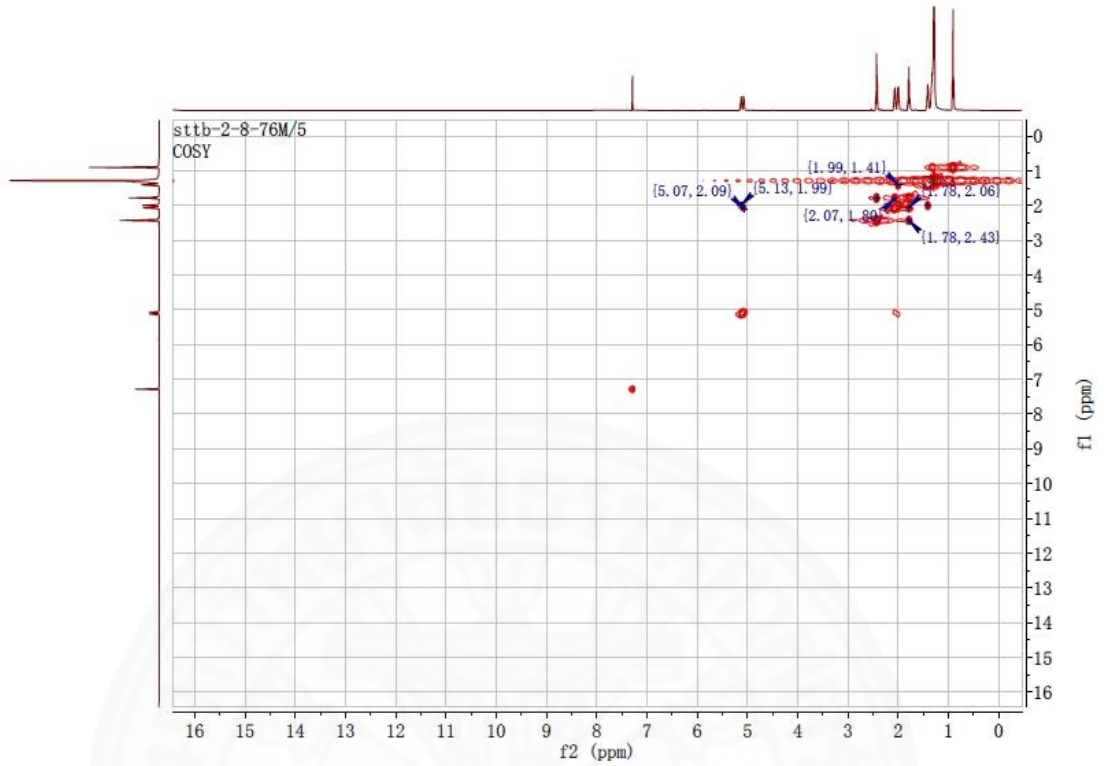


Figure A3 The COSY spectrum of TTE1 in $CDCl_3$

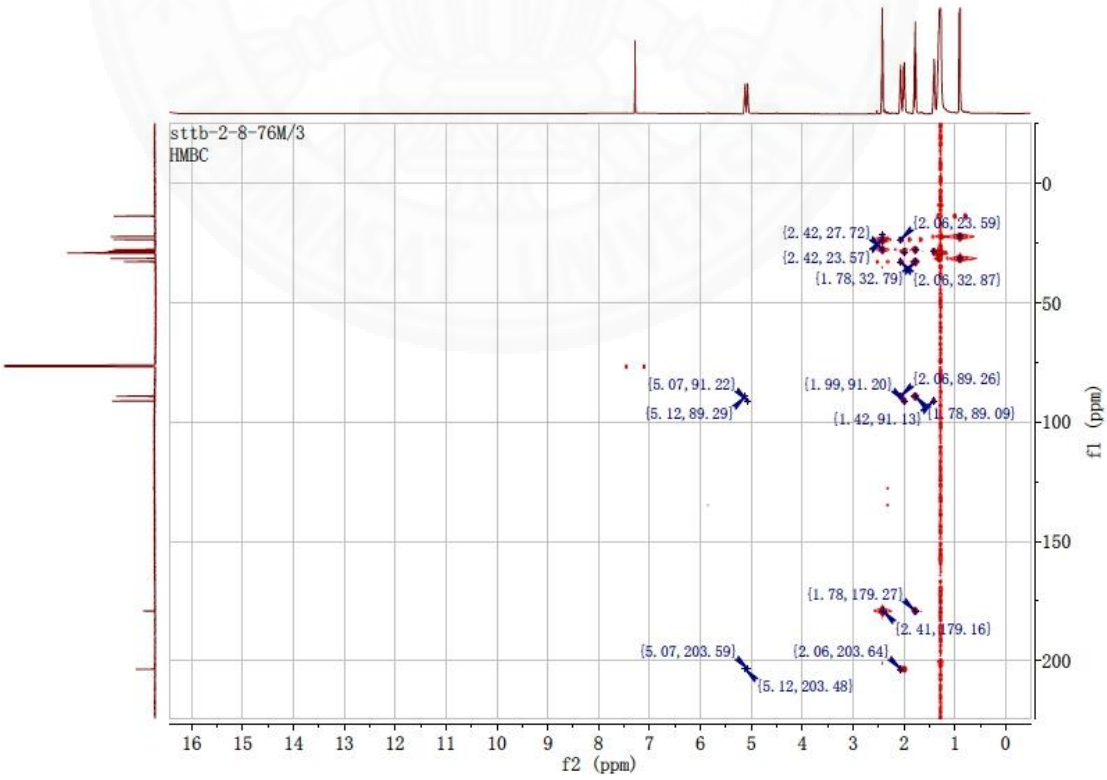


Figure A4 The HMBC spectrum of TTE1 in $CDCl_3$

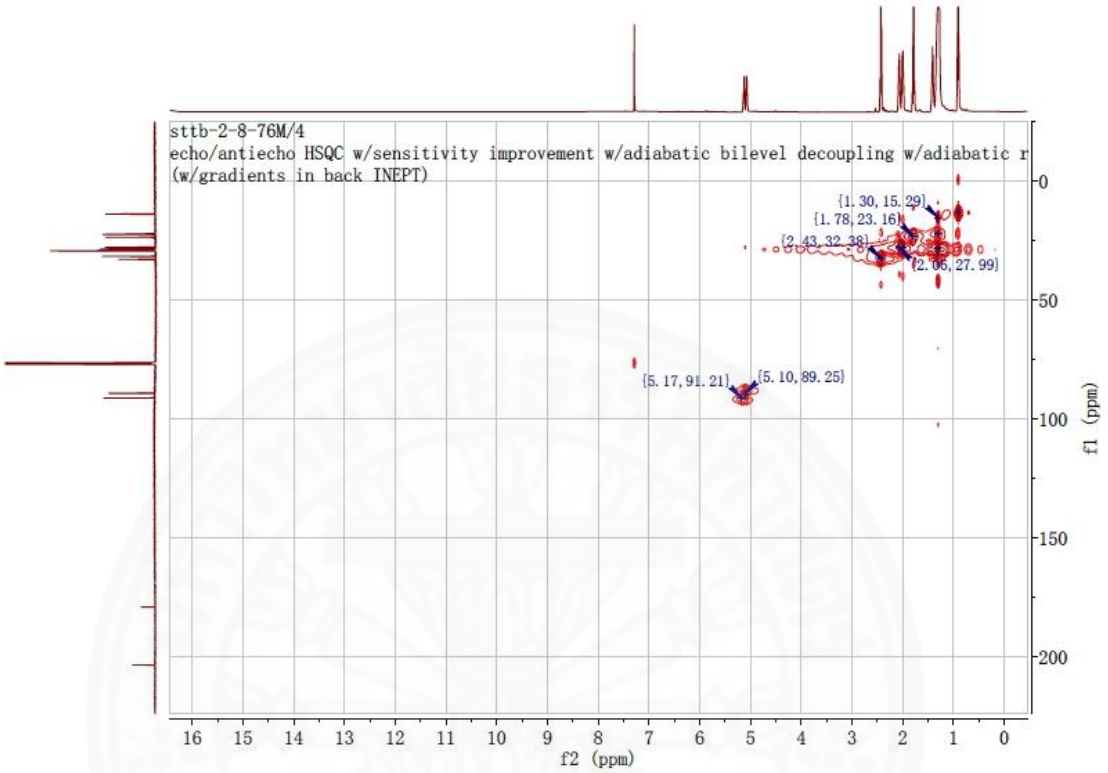


Figure A5 The HSQC spectrum of TTE1 in $CDCl_3$

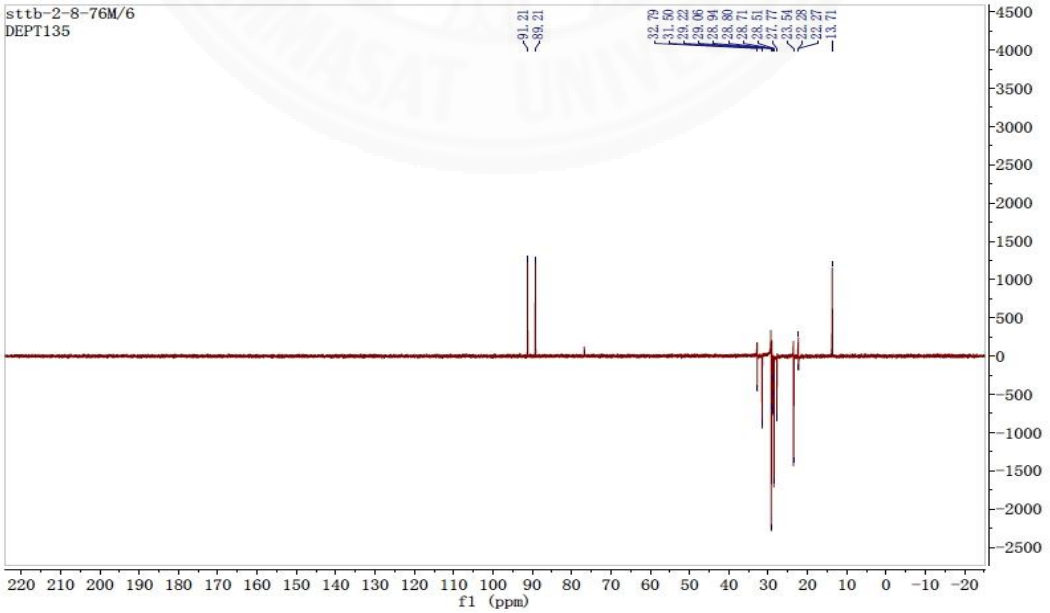
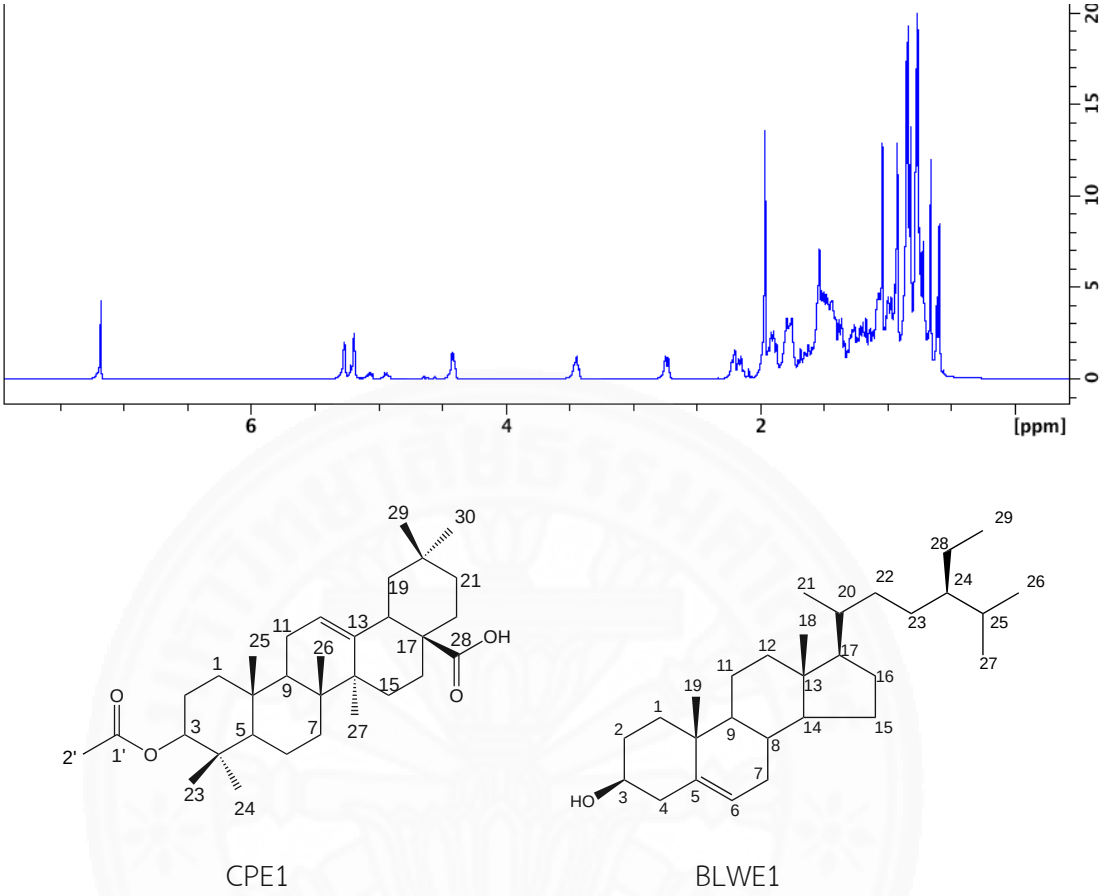


Figure A6 The DEPT135 spectrum of TTE1 in $CDCl_3$



CPE1 BLWE1

Figure A7 The ^{1}H NMR data for mixture of CPE1 and BLWE1 compounds and their structures

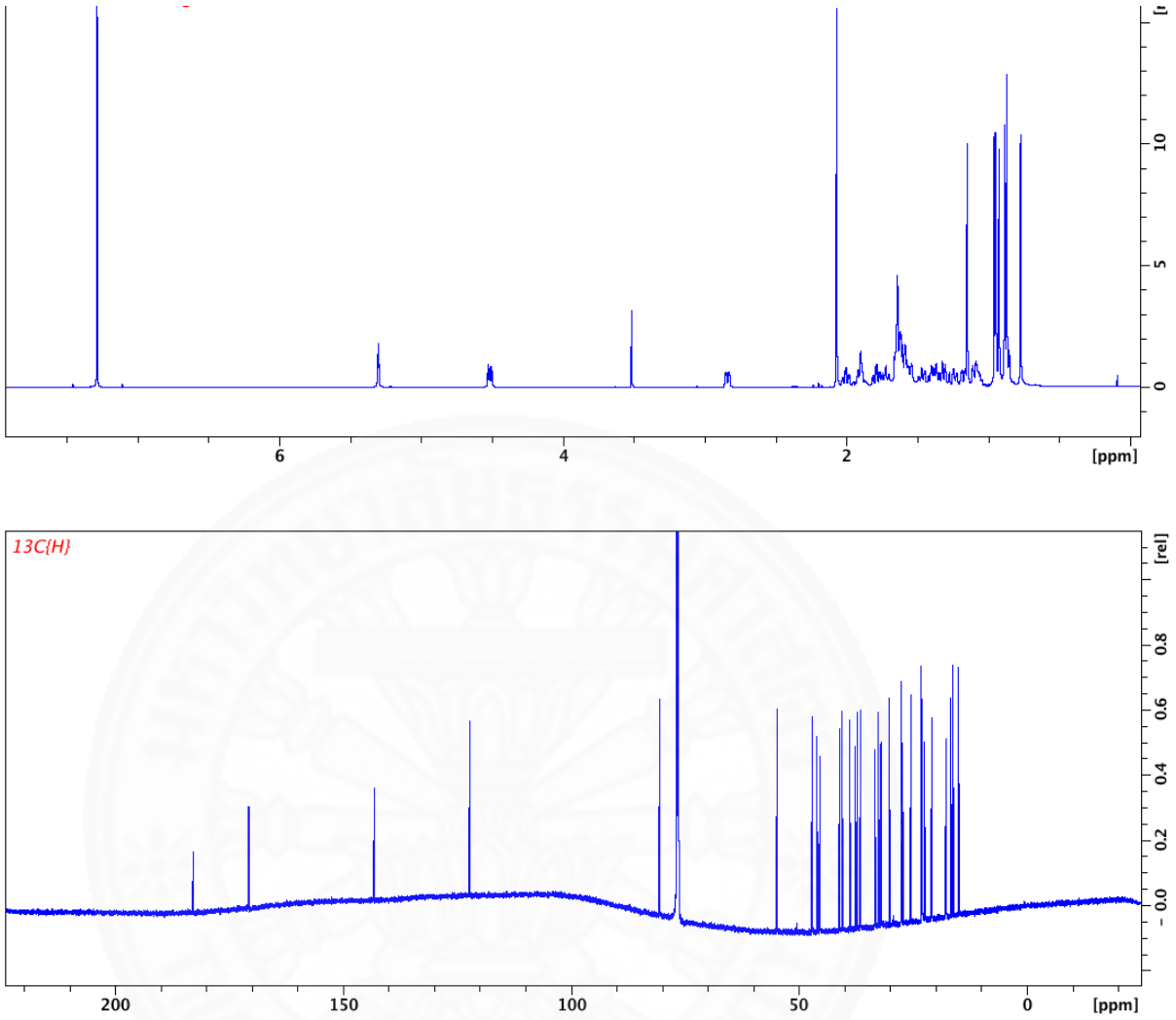


Figure A8 The ^{1}H and ^{13}C NMR spectrum of CPE1 (600 and 150 MHz in $CDCl_3$)

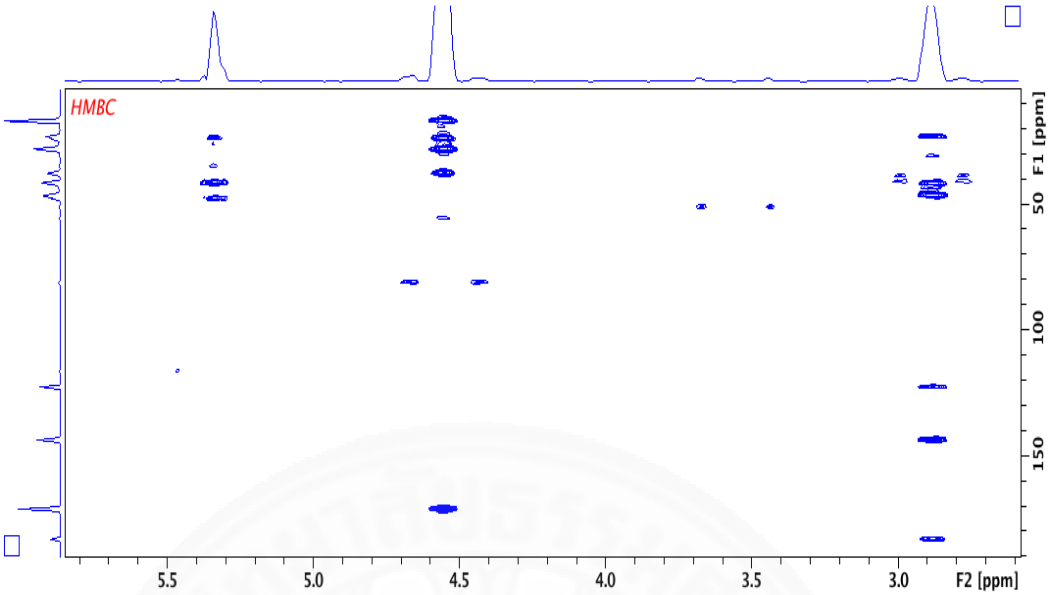


Figure A9 The HMBC spectrum of CPE1 in $CDCl_3$

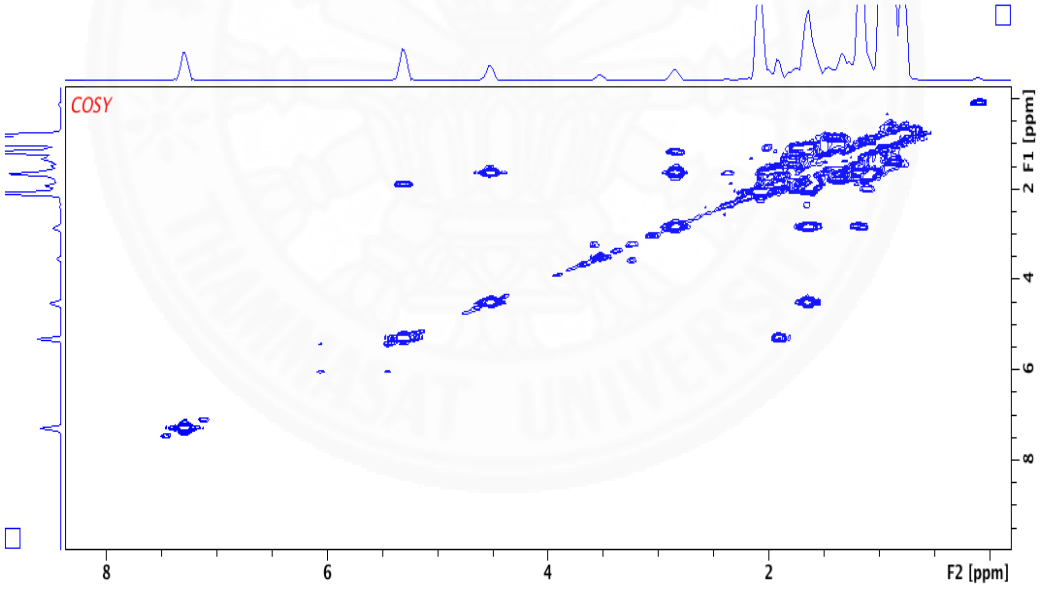


Figure A10 The COSY spectrum of CPE1 in $CDCl_3$

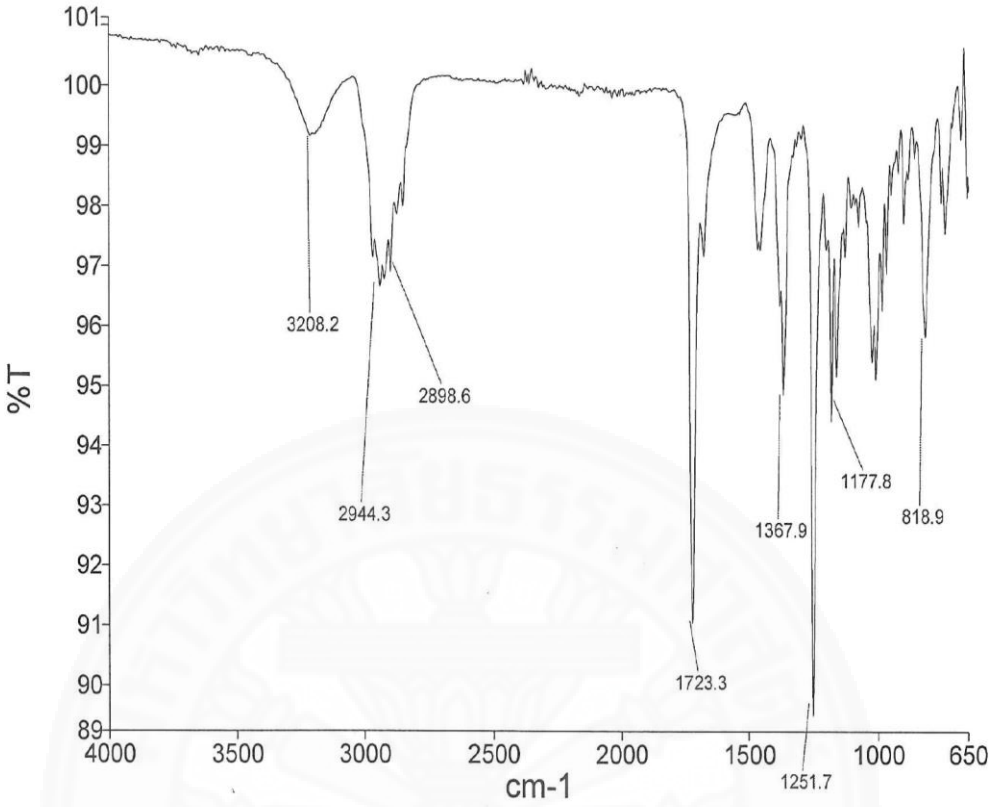


Figure A11 The IR spectrum of CPE1

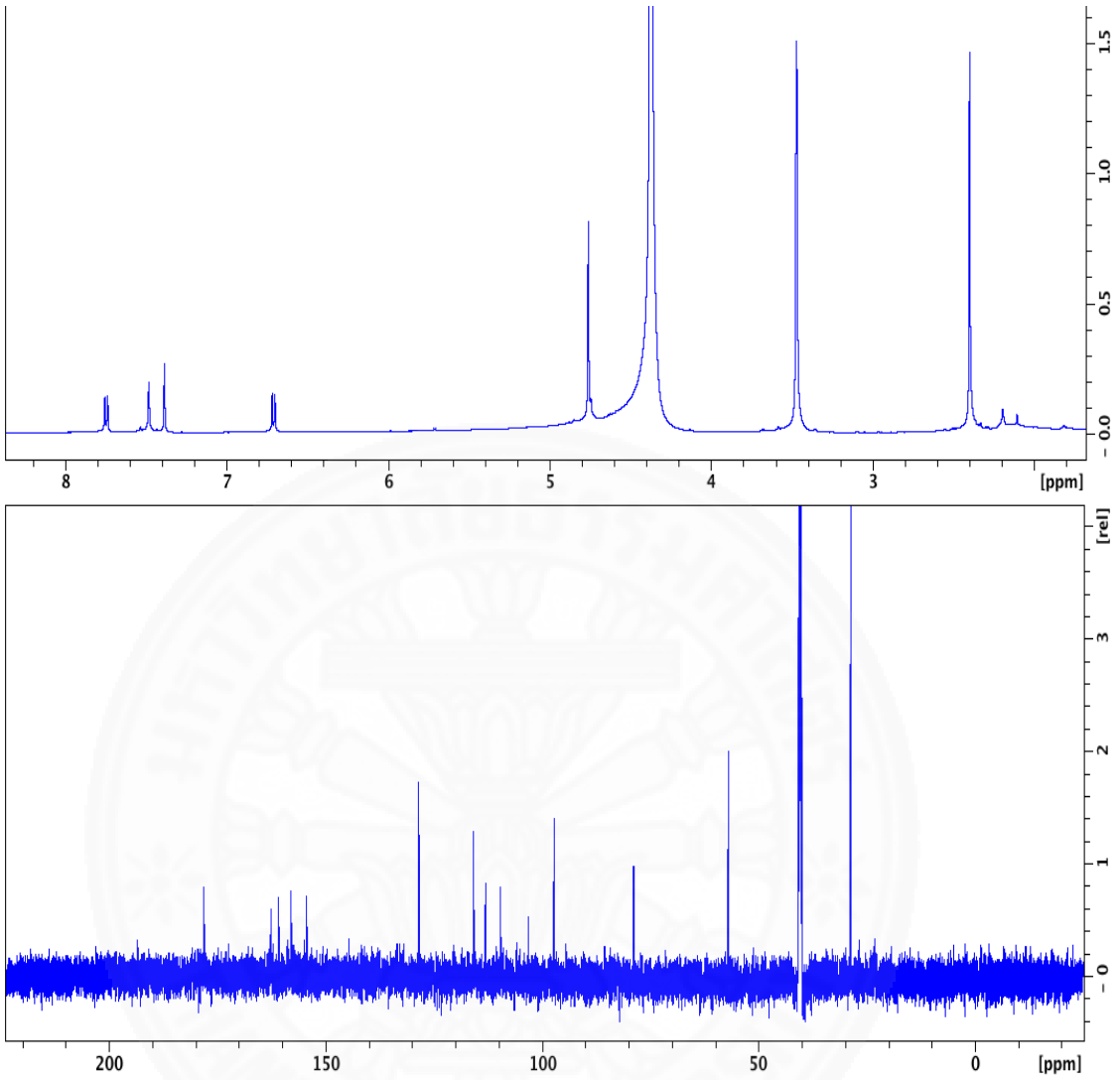


Figure A12 The ^{1}H and ^{13}C NMR spectrum of HPE1 (600 and 150 MHz in DMSO-d_6)

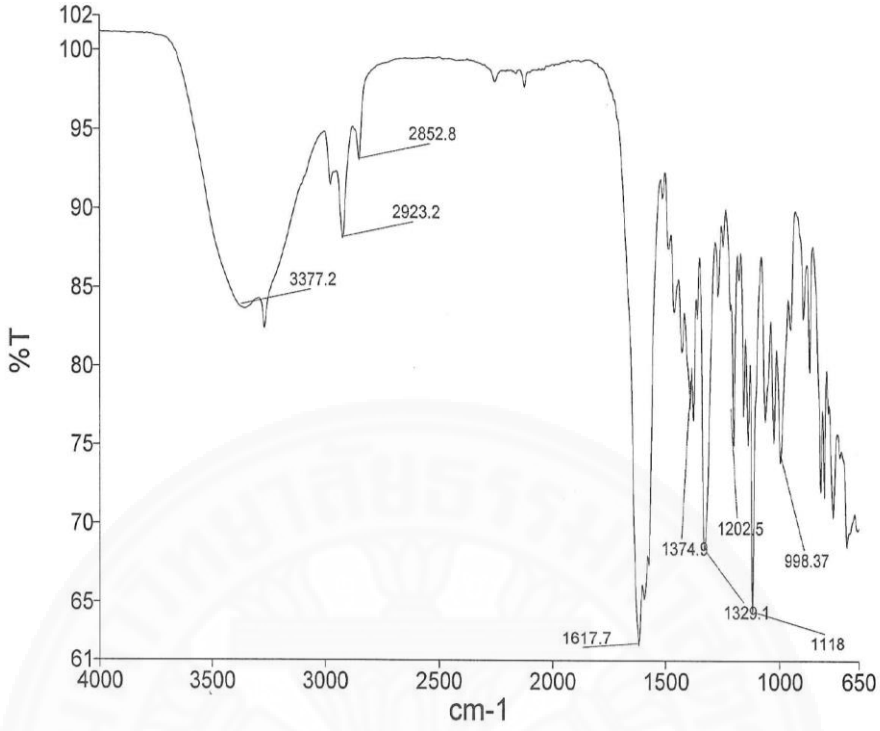


Figure A13 The IR spectrum of HPE1

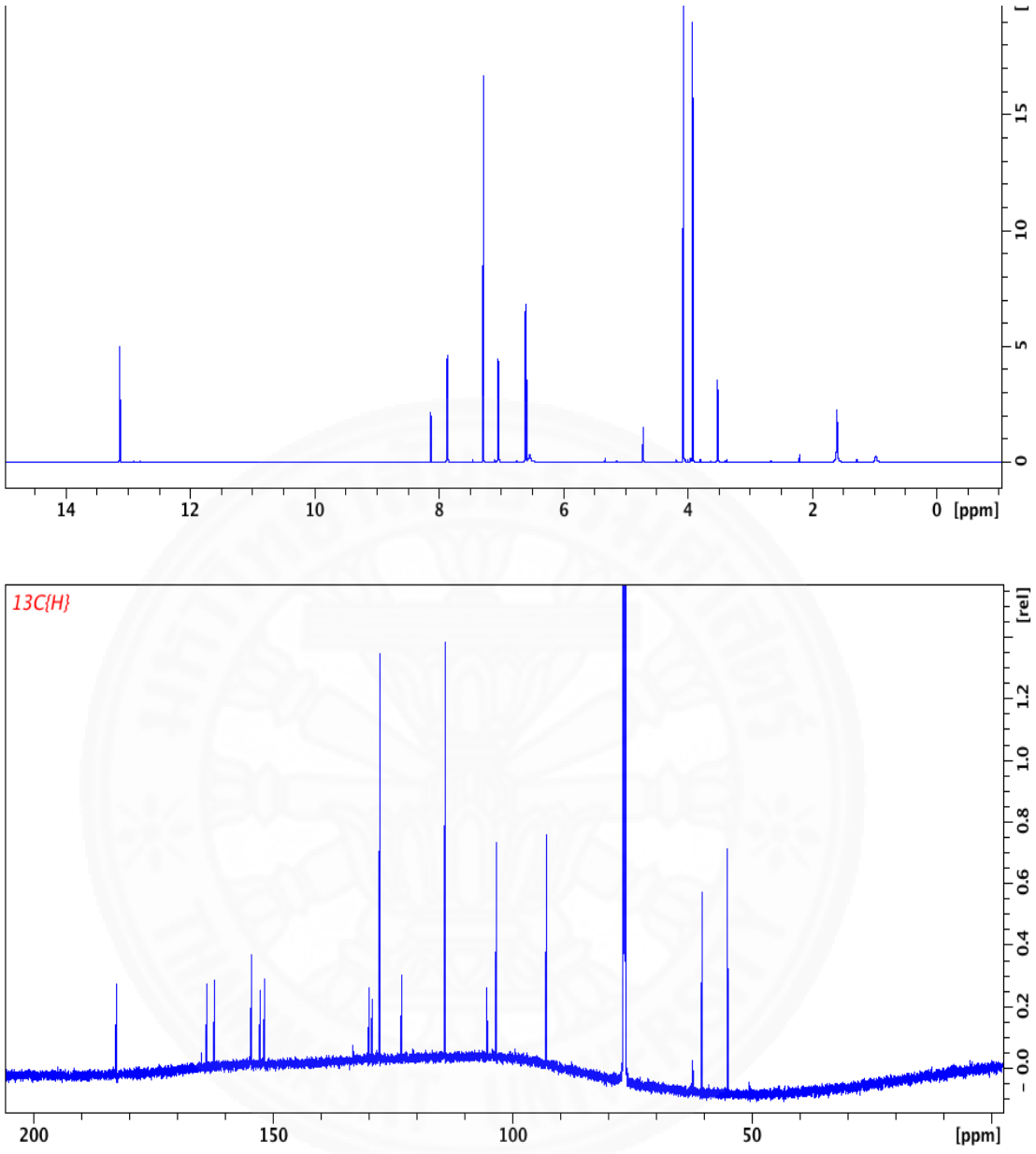


Figure A14 The ^{1}H and ^{13}C NMR spectrum of BLWE2 (600 and 150 MHz in DMSO-d_6)

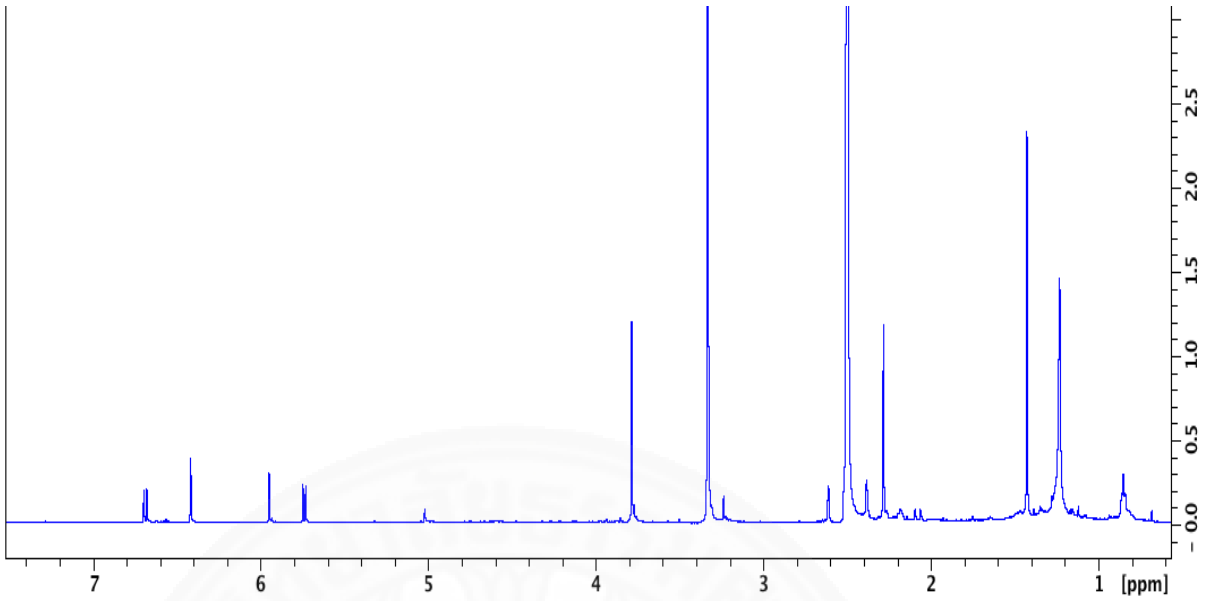


Figure A14 The ^{1}H NMR spectrum of BLWE3 recorded at 600 MHz in DMSO-d_6

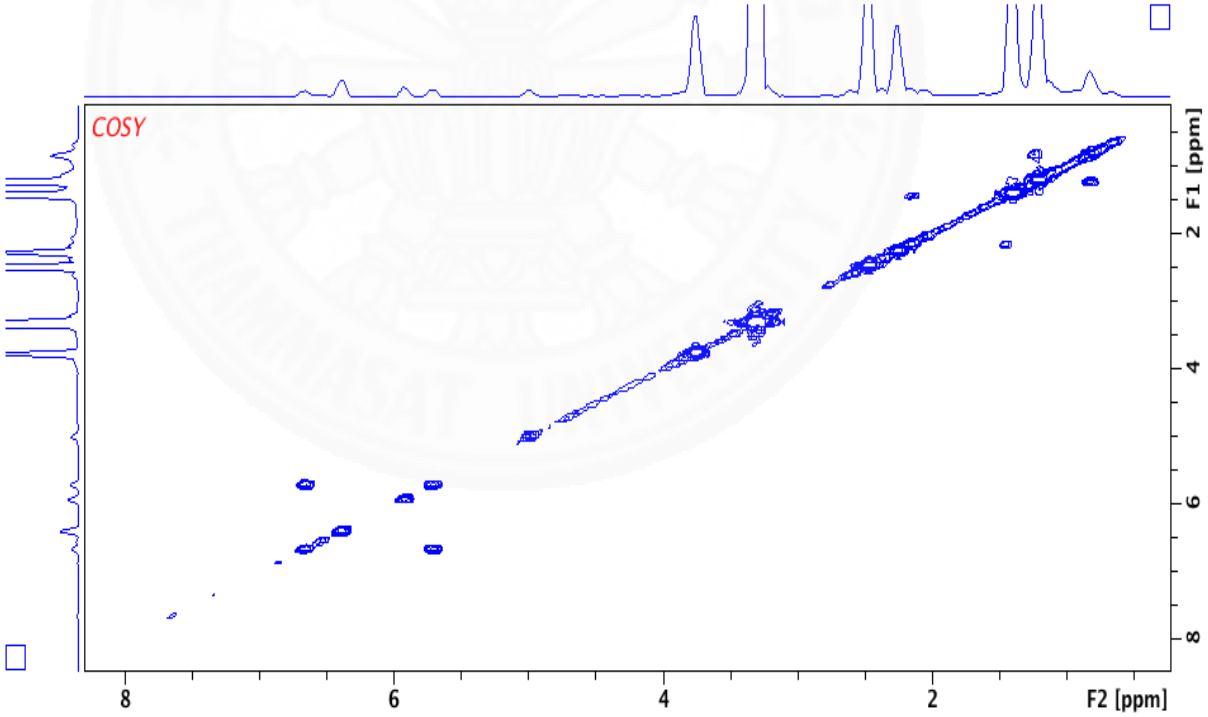


Figure A15 The COSY spectrum of BLWE3 in DMSO-d_6

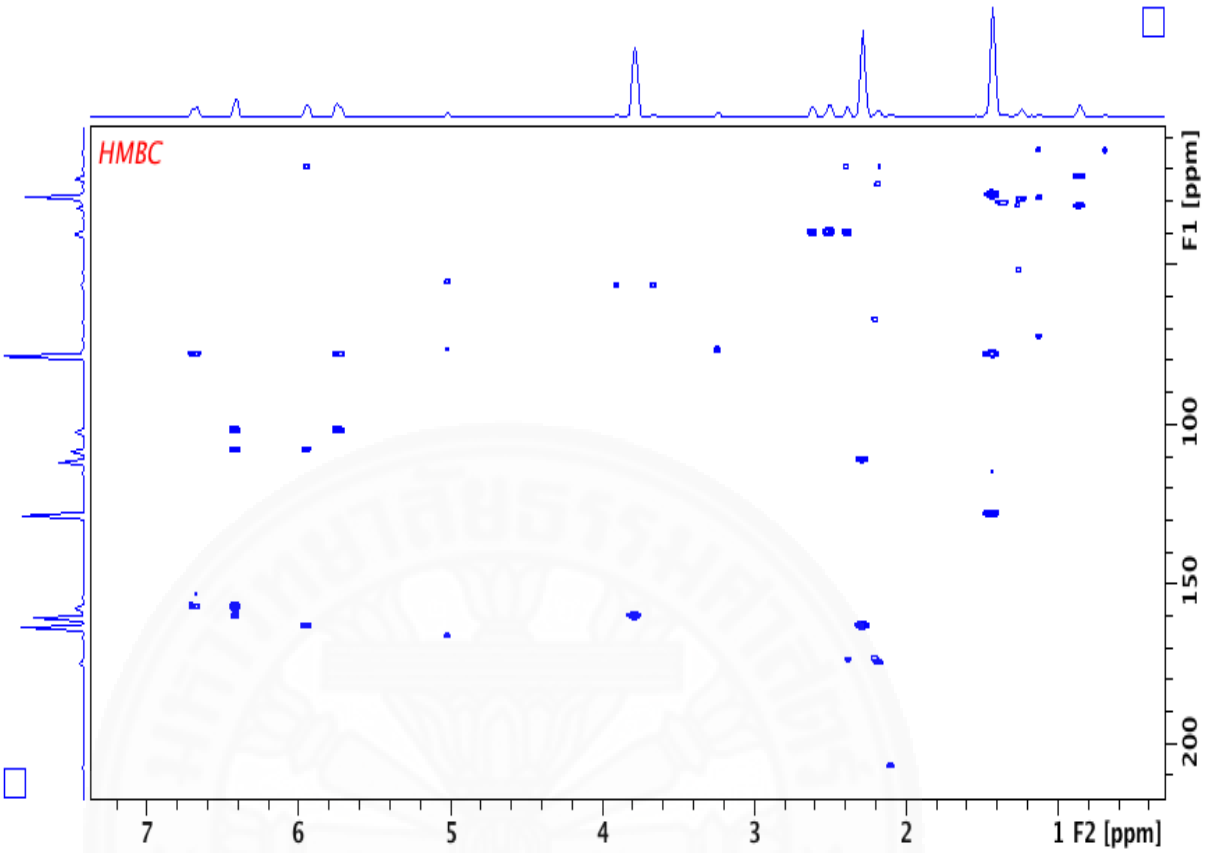


Figure A14 The HMBC spectrum of BLWE3 in DMSO-d_6

APPENDIX B

Chemical Reagents and Preparation

Reagents for cell culture

FBS (inactivated)

- Slowly thaw the frozen FBS, heat inactivate (56 °C, 30 min)

  (Aliquot, stored at -20 °C)

PBS

- PBS 1 tablet
- MQ water 100 ml

  (Autoclave 121 °C, 15 min, stored at 4 °C)

Penicillin-Streptomycin

- Slowly thaw the frozen P/S in water bath at 37 °C till complete thaw

  (Aliquot, stored at -20 °C)

Trypsin-EDTA

- Slowly thaw the frozen trypsin in water bath at 37 °C till complete thaw

  (Aliquot, stored at -20 °C)

Medias preparation

RPMI 1640 (incomplete media)

- RPMI 1640 1X with L-glutamine 1 pack (powder)
- $NaHCO_3$ 2.0 g
- Adjust volume with sterile water to 1,000 ml

  Adjust pH 7.00-7.05 by 1 N NaOH or 1 N HCl

  (Filtered sterile at a pore size of 0.2 µM, stored t 4 °C)

MEM (incomplete media)

- MEM 1X with Earle's salts, L-glutamine 1 pack (powder)
- $NaHCO_3$ 2.2 g
- Adjust volume with sterile water to 1,000 ml

  Adjust pH 7.00-7.05 by 1 N NaOH or 1 N HCl

  (Filtered sterile at a pore size of 0.2 µM, stored at 4 °C

DMEM (incomplete media)

- DMEM 1X with L-glutamine and glucose 1 pack (powder)
- $NaHCO_3$ 2.2 g
- Adjust volume with sterile water to 1,000 ml

  Adjust pH 7.00-7.05 by 1 N NaOH or 1 N HCl

RPMI 1640 (complete media)

- RPMI 1640 (incomplete media) 400 ml
- 10% FBS 40 ml
- 1% Penicillin-Streptomycin 4 ml

  (Stored at 4 °C)

MEM (complete media)

- MEM (incomplete media) 400 ml
- 10% FBS 40 ml
- 1% Penicillin-Streptomycin 4 ml

  (Stored at 4 °C)

DMEM (complete media)

- DMEM (incomplete media) 400 ml
- 10% FBS 40 ml
- 1% Penicillin-Streptomycin 4 ml

  (Stored at 4 °C)

NA (Nutrient Agar)

- NA with beef extract and peptone 8.05 g
- Adjust volume with water to 350 ml
- Mixed well and autocalved (121 °C 15 min.)
- poured into petri dishes and waited until media solidified (Stored at 4 °C)

MHA (Mueller Hinton Agar)

- MHA with beef extract powder, acid digest of casein, starch 13.3 g
- Adjust volume with water to 350 ml
- Mixed well and autocalved (121 °C 15 min.)
- poured into petri dishes and waited until media solidified (Stored at 4 °C)

BHI agar (Brain Heart Infusion agar)

- MHA with calf brain, beef heart, proteose peptone, dextrose 18.2 g sodium chloride, disodium phosphate
- Adjust volume with water to 350 ml
- Mixed well and autocalved (121 °C 15 min.)
- poured into petri dishes and waited until media solidified (Stored at 4 °C)

BHI broth (Brain Heart Infusion)

- MHA with calf brain, beef heart, proteose peptone, dextrose, sodium chloride, disodium phosphate 12.95 g
- Adjust volume with boiled water to 350 ml
- Pipetted to glass tubes with loose screw-caps then autoclaved (121 °C 15 min.)
  (Stored at 4 °C)

MHB (Mueller Hinton Broth)

- MHA with beef extract powder, acid digest of casein, starch 13.3 g
- Adjust volume with boiled water to 350 ml
- Pipetted to glass tubes with loose screw-caps then autocalved (121 °C 15 min.)
  (Stored at 4 °C)

APPENDIX C

Summary of Plants: Literature Reviews

Table C1: Phytochemistry and Biological activity of Benjalokawichian or Ha-Rak remedy

Extraction	Compounds	Activity	Results	References
	-	Antipyretic	The root powder of BLW formula showed the antipyretic efficacy by using a Baker's yeast-induced fever model in rats.	Konsue *et al.,* 2008
-	-	Identification of adulteration by macroscopic	The identification of five authenticated powdered roots were based on the starch granules, fragment of fibers and fragment of pitted vessels. All authenticated powders showed distinctive characteristics which can be established a key to identify the roots of these five species and to detect the adulteration.	Singharachai *et al.,* 2008

Table C1: Phytochemistry and Biological activity of Benjalokawichian or Ha-Rak remedy

Extraction	Compounds	Activity	Results	References
Maceration- 95%ethanol, Decoction and Residue decoction	-	Antioxidant, Cytotoxic	The ethanolic extract showed moderate antioxidant (EC_{50} value 35.3 µg/mL) and also showed cytotoxic activity against lung carcinoma cell line (COR-L23) and breast cancer cell line (MCF-7) with IC_{50} value 29.9, 31.4 µg/mL. Both of water extract showed no activity in this study.	Chartsuwan *et al.,* 2009
Maceration- 95%ethanol, Decoction and Residue decoction	-	Antiinflammatory	The ethanolic extract of BLW showed anti-inflammatory with IC_{50}=91.4 µg/mL whereas the water extract and residue extract showed no activity.	Suranart *et al.,* 2009
Maceration- 95%ethanol, Decoction and Residue decoction	-	Antimicrobial	The ethanolic extract of BLW inhibited *S. aureus, B. subtilis* and *C. albicans* with inhibition zone 7.7, 11.7 and 17.3 mm, respectively.	Sangrapee *et al.,* 2009
Maceration- 95%ethanol, Decoction and Residue decoction	-	Anti-allergic	The ethanolic extract of BLW showed anti-allergic activity with IC_{50}=19.5 µg/mL	Tabpueng *et al.,* 2009

Table C1: Phytochemistry and Biological activity of Benjalokawichian or Ha-Rak remedy

Extraction	Compounds	Activity	Results	References
			whereas the water extract and residue extract showed no activity.	
	-	Antipyretic, Antinociceptive	All doses of BLW 25-400 mg/kg significantly ($p<0.05$) attenuated the increased rectal temperature produced by LPS injection and were found to be as potent as ASA. BLW (400 mg/kg) produced a significant analgesic response in the hot-plate test, while all doses of BLW, except the lowest dose (25mg/kg), produced significant analgesic responses in the tail-flick test. For acetic acid-induced writhing models in mice, BLW doses of 200 and 400 mg/kg significantly ($p < 0.05$) decreased the mean writhing response compared to vehicle controls.	Jongchanapong *et al.,* 2010

Table C1: Phytochemistry and Biological activity of Benjalokawichian or Ha-Rak remedy

Extraction	Compounds	Activity	Results	References
	-	Pharmacognostic evaluations of five roots for diagnosis and distinguishing them from their adulterants.	The samples were collected from wild or non-cultivated from 14 difference places throughout Thailand. The result showed that the main distinguishing features of five root species were obtained from the morphological and histological characters as well as 3D-HPLC chromatogram.	Singharachai *et al.,* 2011
Maceration- 95%ethanol and Decoction	-	Antimicrobial	The water extract of BLW inhibited none of strains whereas the ethanolic extract showed effective against *S. boydii, S. dysenteriae, S. flexneri, A. buamannei, P. aeruginosa, K. pneumoniae, S. aureus* MRSA, *S. pyrogenes, B. Subtilis.*	Nuaeissara *et al.,* 2011
Maceration-95%Ethanol	-	Antioxidant, Antiinflammatory	Ethanolic extract of Benchalokawichian showed moderate antioxidant in DPPH assay and antiinflammatory activity on	Juckmeta&ltharat, 2012

Table C1: Phytochemistry and Biological activity of Benjalokawichian or Ha-Rak remedy

Extraction	Compounds	Activity	Results	References
			RAW264.7 cells (EC_{50} and IC_{50} = 40.93, 40.36 µg/mL)	
-	-	Identification of adulteration by macroscopic, microscopic and TLC Technique	The stems and roots of each plant presented similar appearances, especially *Capparis micracantha* and *Ficus racemosa*, which were similar in both morphological and anatomical characters. Results of TLC technique, stem adultation was common found in commercial capsule of BLW remedy.	Nutmakul *et al.,* 2013
Maceration-95%Ethanol	pectolinarigenin	Development and Validation of RP-HPLC Method	Pectolinarigenin was absorption at wavelength 331 nm for quantification. Mixture of 0.1% ortho phosphoric acid and acetronitrile with gradient elution, flow rate was 1 mL/min, were examined as the mobile phase and optimized to obtaining a good resolution. The spectra of peak found at retention time 21.49	Sakpakdeejaroen *et al.,* 2014

Table C1: Phytochemistry and Biological activity of Benjalokawichian or Ha-Rak remedy

Extraction	Compounds	Activity	Results	References
			min. Based on the HPLC analysis, pectolinarigenin was a minor compound with a content of 0.18% w/w.	
Maceration-95%Ethanol	pectolinarigenin, *O*-methylalloptaeroxylin	Anti-allergic	Pectolinarigenin exhibited stronger anti-allergy activity (IC_{50}=6.3 µg/mL, 20.1 µM) than *O*-methylalloptaeroxylin and chlorpheniramine, positive control (IC_{50} =14.2µg/mL, 51.8 µM and 16.2 µg/mL, 58.8 µM respectively). The ethanolic extract of Benchalokawichian showed moderate inhibitory effect agianst beta-hexosaminidase with IC_{50} value 39.8 µg/mL.	Juckmeta *et al.,* 2014

Table C2: Phytochemistry and Biological activity of *Cleodendrum petasites*

Part used	Extraction	Compounds	Activity	Results	References
Aerials	Maceration-96%ethanol	hispidulin (flavonoids)	Spasmolytic (bronchodilator)	The ethanolic extract was tested to evaluate the spasmolytic activity on isolated guinea-pig tracheal smooth muscle. The crude extract (2.25–9.0 mg/mL) dose-dependently caused relartxation of tracheal smooth muscle which was contracted by exposure to histamine. The active principle was isolated and identified as the flavonoid hispidulin (EC_{50}: 3.0×10^{-5} μM).	Hazekamp *et al.,* 2001
Roots	Maceration-methanol	-	Anti-inflammatory	The methanol extract possessed moderate inhibitory activity on acute phase of inflammation in a dose-related manner as seen in ethyl phenylpropiolate-induced ear edema (ED_{50} = 2.34 mg/ear) as well as carrageenin-induced hind paw edema (ED_{50} = 420.41 mg/kg) in rats.	Panthong *et al.,* 2003
Whole plants	Reflux-water and ethanol	-	HIV-1 Integrase	The aqueous extract of *C. indicum* exhibited anti-HIV-1 IN activity (IC_{50} =43.5 μg/mL) whereas the ethanolic extract showed no HIV-1 IN activity (IC_{50} > 100 μg/mL).	Bunluepuech& Tewtrakul, 2009

Table C2: Phytochemistry and Biological activity of *Cleodendrum petasites*

Part used	Extraction	Compounds	Activity	Results	References
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Antioxidant, Cytotoxic	The ethanolic and water extracts showed no antioxidant activity by DPPH assay with EC_{50} >100 µg/mL and no cytotoxicity on COR-L23, MCF-7, MRC5 cell line.	Chartsuwan *et al.,* 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Anti-inflammatory	The ethanolic extract exhibited anti-inflammatory inhibit NO effect with IC_{50} = 51.46 µg/mL. The water extract showed no activity (> 100 µg/mL).	Suranart *et al.,* 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Anti-allergic	The ethanolic extract showed anti-allergic activity against release β-hexosaminidase with IC_{50} = 90.01 µg/mL whereas the water extract and residue extract showed no activity.	Tabpueng *et al.,* 2009
Roots	Maceration-95%ethanol, Decoction	-	Antimicrobial	The ethanolic extract inhibited all gram negative and positive bacteria except *C. albicans*. The water extract showed no activity agianst all microbacterial in this study.	Nuaeissara *et al.,* 2011

Table C2: Phytochemistry and Biological activity of *Cleodendrum petasites*

Part used	Extraction	Compounds	Activity	Results	References
Roots	Maceration-95%ethanol	-	Antioxidant, Antiinflammatory	Ethanolic extract of *C. petasites* showed weak antioxidant in DPPH assay (EC_{50} >100 µg/mL) and moderate inhibition nitric oxide effect on RAW264.7 cells (IC_{50} value 46.55 µg/mL)	Juckmeta&Itharat, 2012
Stems and Roots	-	-	Identification of adulteration by macroscopic, microscopic and TLC Technique	*Clerodendrum petasites* stems had a pericyclic band of sclerenchyma, and lignified parenchyma and sclereids lining around hollow pith, which absented in the root.	Nutmakul *et al.*, 2013
Roots	Maceration-95%ethanol	-	Anti-allergic	*C. petasites* showed moderate inhibitory effect against beta-hexosaminidase with IC_{50} value 57.8 µg/mL. IC_{50} values of chlorpheniramine, positive control was 16.2 µg/mL, 58.8 µM.	Juckmeta *et al.*, 2014

Table C3: Phytochemistry and Biological activity of *Capparis micracantha*

Part used	Extraction	Compounds	Activity	Results	References
Woods	Reflux-water and ethanol	-	HIV-1 Integrase	The aqueous and ethanolic extracts of *C. micracantha* showed no HIV-1 Integrase activity (IC_{50} > 100 µg/mL).	Bunluepuech &Tewtrakul, 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Antioxidant, Cytotoxic	The ethanolic extract showed moderate antioxidant activity by DPPH assay with EC_{50} = 42.91 µg/mL and no cytotoxicity on COR-L23, MCF-7, MRC5 cell line. The water extract and residue extract showed no activity (>100 µg/mL).	Chartsuwan *et al.,* 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Anti-inflammatory	The ethanolic extract exhibited anti-inflammatory inhibit NO effect with IC_{50} = 95.79 µg/mL. The water extract and residue extract showed no activity (>100 µg/mL).	Suranart *et al.,* 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Anti-allergic	The ethanolic extract of CM showed anti-allergic activity with IC_{50}=9.8 µg/mL whereas the water extract and residue extract showed no activity (>100 µg/mL).	Tabpueng *et al.,* 2009

Table C3: Phytochemistry and Biological activity of *Capparis micracantha*

Part used	Extraction	Compounds	Activity	Results	References
Roots	Maceration-95%ethanol and Decoction	-	Antimicrobial	The water extract inhibited all *E. coli* strains with inhibition zone 9.3-10.5 mm whereas the ethanolic extract inhibited general pathogen *(S. aureus,* MRSA*, B. subtilis)*. The water extracts had better inhibited *A. buamannei, K. pneumoniae* and *Shigella* species (except *S. sonnei)* than the ethanolic extracts. CM and CMW also showed no activity against *P. aeruginosa* and *C. albicans.*	Nuaeissara *et al.,* 2011
Roots	Maceration-95%ethanol	-	Antioxidant, Antiinflammatory	Ethanolic extract of *C. micracantha* showed moderate antioxidant in DPPH assay and antiinflammatory activity on RAW264.7 cells ($EC_{50,}$ IC_{50} =61.35, 61.37 µg/mL).	Juckmeta & Itharat, 2012
Stems and Roots	-	-	Identification of adulteration by macroscopic, microscopic and TLC Technique	The stems and roots presented similar appearances. *C. micracantha* stems had marked lignified sclereids, arranged in pericyclic band underneath rhytidome, groups in cortex and densely packed in secondary phloem. On the contrary, the roots had few groups of sclereids scattered in cortex and secondary phloem.	Nutmakul *et al.,* 2013

Table C3: Phytochemistry and Biological activity of *Capparis micracantha*

Part used	Extraction	Compounds	Activity	Results	References
Roots	Maceration-95%ethanol	-	Anti-allergic	The ethanolic of *C. micracantha* showed no inhibitory effect agianst beta-hexosaminidase (IC_5 >100 µg/mL) whereas chlorpheniramine, positive control exhibited anti-allergy activity with IC_{50} value 16.2 µg/mL (58.8 µM) respectively.	Juckmeta *et al.,* 2014

Table C4: Phytochemistry and Biological activity of *Harrisonia perforata*

Part used	Extraction	Compounds	Activity	Results	References
Stem barks		-	Antimicrobial	The stem bark of 'da da' *(Harrisonia perforata)* extract has high activity against *Shigella shiga*, low activity against *Vibro eltor* and no activity against *Salmonella* and other *Shigella* and *Vibro* species.	Phan *et al.,* 1981 Phan *et al.,* 1983
Branches	Soxhlet-Chloroform	peucenin-7-methyl ether, O-methylalloptaeroxylin, perforatic acid	-	-	Thadaniti *et al.,* 1994
Leaves	Defatted with n-hexane and extracted with Me_2CO and Ethanol	Perforatinolone, (a new tetranortriterpenoid with an A, D-ring seco-limonoid structure, which is closely related to perforatin), gallic acid, sitosterol and 3-O-fl-D-glucopyranosyl sitosterol	-	-	Sung *et al.,* 1995

Table C4: Phytochemistry and Biological activity of *Harrisonia perforata*

Part used	Extraction	Compounds	Activity	Results	References
Woods	n-butanol extracts	perforatin C, perforatin D, perforatin E, perforatin F, perforin G, Heteropeucenin-7-methyl ether, Heteropeucenin-5-methoxy-7-methyl ether, 2-Hydroxymethylalloptaeroxylin-5-methyl ether, Perfiratin A, Perforatic acid, Perforatic acid methyl ester, Scopoletin, Cedrelopsin, Xanthoxyletin, Coniferyl aldehyde	Isolate compounds	Five new chromones, perforatins C-G, together with 10 known compounds were isolated from the wood of *Harrisonia perforata*.	Tanaka *et al.,* 1995
Leaves	J. Polonsky for the isolation of the bitter principles of the Simaroubaceae, with slight modifications.	haperforine A, haperforine E, 12-Desacetylhaperforine	Isolated compound	Acetylationn of 12-Desacetylhaperforine to afford a haperforine A	Khuong-Huu *et al.,* 2000

Table C4: Phytochemistry and Biological activity of *Harrisonia perforata*

Part used	Extraction	Compounds	Activity	Results	References
Leaves	25% aqueous EtOH extract	haperforins C2, haperforins F, and haperforins G	Isolated compound		Khuong-Huu *et al.,* 2001
Twigs	Maceration-50% / 95% ethanol	-	Inhibitory effect on *Streptococcus mutans* (S. mutans) ATCC 25175 and TPF-1 *in vitro*	The inhibitory effect on adherence to glass surface of 0.5%extract was strong inhibiter for *S. mutans* ATCC 25175 and TPF-1.	Limsong *et al.,* 2004
Branches	95% Ethanolic extract - partitioned between water, ethyl acetate, n-buthanol	peucenin-7-methyl ether, *O*-methylalloptaeroxylin, perforatic acid, perforamone A, B, C, eugenin, saikochromone A, perforamone D, greveichromenol	Anti-plasmodial, Antimicrobial	Most of compounds inactived antiplasmodial activity. Only *O*-methylalloptaeroxylin also showed antiplasmodial with EC_{50} value 10.5 µg/mL and antimicrobial activity with MIC value 100 µg/mL. The MIC value of perforamone B and perforamone D showed highest antimicrobacterial activity (25 µg/mL) following by peucenin-7-methyl ether and	Tuntiwachwuttikul *et al.,* 2006

Table C4: Phytochemistry and Biological activity of *Harrisonia perforata*

Part used	Extraction	Compounds	Activity	Results	References
				greveichromenol (50 µg/mL), *O*-methylalloptaeroxylin and eugenin (100 µg/mL), perforamone A and C (200 µg/mL), respectively.	
Leaves	cyclohexane extract, methylene chloride extract, crude extract, methanol extract, methanol extract after tannins removal.	-	Anti-plasmodial, Cytotoxic	The crude, cyclohexane, methanol extract showed antiplasmiodial activity with IC_{50} value 9.4, 6.7 and 5.1 µg/mL. Cytotoxic activity on Hela and MRC-5 showed IC_{50} values following crude (8.7 µg/mL, NT), cyclohexane (3.9, 8.4 µg/mL), methanol (14.3, 56 µg/mL).	Nguyen-Pouplin *et al.,* 2007
Roots and Stems		-	Antimicrobial	*H. perforata* exhibited a bactericidal effect against *S. aureus* (roots, at conc. 500 µg/mL) and *Mycobacterium smegmatis* (roots and stem, at concentration 250 µg/mL)	Chea *et al.,* 2007

Table C4: Phytochemistry and Biological activity of *Harrisonia perforata*

Part used	Extraction	Compounds	Activity	Results	References
Branches	Maceration-50%Ethanol	-	Cytotoxic	The ethanolic extract show exhibited normal African green monkey kidney (Vero) cell with IC_{50} value 276 µg/mL, no cytotoxic with malignant human hepatoma (HepG2).	Prayong *et al.,* 2008
Barks	Decoction	-	Acute and subchronic toxic	The water extract of *H. perforata* at the oral doses treated did not cause either acute (at the dose of 5,000 mg/kg) or subchronic toxicities in rats.	Sireeratawong *et al.,* 2009
Stems and leaves	95% Ethanolic extract-which was suspended in 1.5 L water and then partitioned with ethyl acetate to give ethyl acetate soluble fraction	Harrisotone A, Harrisotone B, Harrisotone C, Harrisotone D, Harrisotone E, Harrisonol A	Cytotoxic	In these tests, pseudolaric acid B15 (with IC_{50} values of 0.74 and 1.99 against P-388 and A-549, respectively) was used as positive control. Harrisotones A, C and harrisonol A exhibited significant cytotoxic activity against P-388 tumor cell line with IC_{50} values of 1.56, 2.35, and 0.27 µM, respectively. Harrisotone A and harrisonol A also showed moderate	Yin *et al.,* 2009

Table C4: Phytochemistry and Biological activity of *Harrisonia perforata*

Part used	Extraction	Compounds	Activity	Results	References
				activity against A-549 tumor cell line with IC_{50} of 24.5 and 26.6 μM, respectively.	
Wood	Reflux-water and ethanol	-	HIV-1 Integrase	The aqueous extract of *Harrisonia perforata* exhibited anti-HIV-1 IN activity (IC_{50} = 2.3 μg/mL) higher than that of suramin, a positive control (IC_{50} = 3.4 μg/mL). Ethanolic extract showed no HIV-1 IN activity (IC_{50} > 100 μg/mL).	Bunluepuech& Tewtrakul, 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Antioxidant, Cytotoxic	The ethanolic extract showed strong antioxidant activity by DPPH assay with EC_{50} =15.98 μg/mL and exhibited cytotoxic on COR-L23, MCF-7 with IC_{50} = 32.07 μg/mL, 27.66μg/mL, respectively and no cytotoxicity on MRC5 cell line. The water extract and residue extract showed no activity (>100 μg/mL).	Chartsuwan *et al.,* 2009

Table C4: Phytochemistry and Biological activity of *Harrisonia perforata*

Part used	Extraction	Compounds	Activity	Results	References
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Antiinflammatory	The ethanolic extract exhibited anti-inflammatory inhibit NO effect with IC_{50} = 41.6 µg/mL. The water extract and residue extract showed no activity (>100 µg/mL).	Suranart *et al.,* 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Antimicrobial	The residue extract of HP inhibited *S. aureus* with inhibition zone 8.3 mm. The water extract showed no activity (>100 µg/mL).	Sangrapee *et al.,* 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Anti-allergic	The ethanolic extract of HP showed anti-allergic activity with IC_{50}= 84.4 µg/mL whereas the water extract and residue extract showed no activity.	Tabpueng *et al.,* 2009

Table C4: Phytochemistry and Biological activity of *Harrisonia perforata*

Part used	Extraction	Compounds	Activity	Results	References
Roots	Maceration-95%ethanol and Decoction	-	Antimicrobial	The ethanolic and water extracts of *H. perforata* showed antimicrobial activity against both gram-positive *(S. pyrogenes, B. subtilis)* and negative bacteria (*S. dysenteriae, A. buamannei).* In addition, the ethanolic was effective against *S. aureus* and *MRSA* with inhibition zone 7.8 and 7.4 mm, respectively. Both extracts showed no activity against *E. coli* strains and *C. albicans.*	Nuaeissara *et al.,* 2011
		5,6-dehydrodesepoxyharperforin C2, Harrpernoid B, Harrpernoid C, harperforin C2, perforin A, 12b-acetoxyharrisonin, 11b,12b-diacetoxyharrisonin, rutaevine, umtatin, gre-veichromenol, one		All isolated limonoids were screened for cytotoxicity *in vitro*, as well as anti-tobacco	

Table C4: Phytochemistry and Biological activity of *Harrisonia perforata*

Part used	Extraction	Compounds	Activity	Results	References
Fruits	Reflux-Methanol	triterpene, pachymic acid, one lignan, pinoresinol, gallic acid, methyl gallate	Cytotoxic	mosaic virus activities only compound 2 showed very weak cytotoxicity to A-549 and HL-60 cell lines.	Yan *et al.,* 2011
Roots	Maceration-95%ethanol	-	Antioxidant, Antiinflammatory	Ethanolic extract of *H. perforata* showed strong antioxidant in DPPH assay (EC_{50} value 16.91 µg/mL) and moderate inhibition nitric oxide effect on RAW264.7 cells (IC_{50} value 53.16 µg/mL)	Juckmeta&Itharat, 2012
Fruits and Roots	Ethyl acetate extract	harperforatin, harperfolide, harperamone, harrisonin, obacunone, Peucenin-7-methyl ester, Perforatic acid methyl ester, *O*-Methylalloptaeroxylin, braylin I , (+)- vouacapenic acid, harrisolanol A	Antiinflammatory	Harperfolide exhibited stronger potent anti-inflammatory activity by suppressing nitric oxide (NO) production from activated murine macrophages J774.A1 cells than indomethacin (IC_{50} value of 6.51, 28.42 µM). Harrisolanol A, harperamone, peucenin-7-methyl ester, perforatic acid methyl ester, *O*-Methylalloptaeroxylin, obacunone showed	Choodej *et al.,* 2013

Table C4: Phytochemistry and Biological activity of *Harrisonia perforata*

Part used	Extraction	Compounds	Activity	Results	References
				antiinflammatory activity at IC_{50} in range 31.04-83.61 µM.	
Stems and Roots	-	-	Identification of adulteration by macroscopic, microscopic and TLC Technique	The stems and roots presented similar appearances. *H. perforata* stems had tangential bands of lignified fibers arranged in secondary phloem and area of pith whereas the roots had small-scattered groups of lignified fibers in secondary phloem and absented pith area.	Nutmakul *et al.,* 2013
Roots	Maceration-95%ethanol	-	Anti-allergic	*H. perforata* extract exhibited stronger anti-allergy activity than chlorpheniramine, positive control (IC_{50} =14.5 and 16.2 µg/mL, 58.8 µM respectively).	Juckmeta *et al.,* 2014

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
		tannins, kaempferol, rutin, arabinose, bergapten, psoralenes, flavonoids, ficusin, coumarins, phenolic glycosides	Chemical composition	-	Baruah&Gohain, 1992
Bark	Soxhlet extraction with ethanol	-	Anti-diarrhoeal	The extracts of *F. racemosa* (400mg/kg) showed significant inhibitory activity against castor oil induced diarrhoea and PGE_2 induced enteropooling in rats and also showed a significant reduction in gastrointestinal motility in charcoal meal tests in rats.	Mukherjee *et al.,* 1998
Leaves	Soxhlet extraction with petroleum ether	-	Hepato-protective	Oral administration of leaf extract (400 mg/kg, p.o.) exhibited a significant reduction in the CCl_4 induced increase in the levels of SGOT, SGPT, alkaline	Mandal *et al.,* 1999

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
				phosphatase and serum bilirubin. However, treatment with Neutrosec (a popular liver tonic) also better reversed the hepatotoxicity than the extract significantly.	
Leaves	Sequentially extracted- petroleum ether, benzene, chloroform, acetone, methanol and water	-	Antibacterial	The leaves extract was tested for antibacterial potential against *Escherichia coli* ATCC 10536, *Basillus pumilis* ATCC 14884, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 25619 and *Staphylococcus aureus* ATCC 29737. The effects produced by the extracts were significant and were compared with chloramphenicol. The petroleum ether extract was the most effective against the tested organisms, following by benzene and chloroform extract.	Mandal *et al.,* 2000

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
Leaves	Soxhlet extraction with petroleum ether	-	Anti-inflammatory	The anti-inflammatory activity of *F. racemosa* extract was evaluated on carrageenin, serotonin, histamine and dextran-induced rat hind paw oedema models. The extract at doses of 200 and 400 mg/kg has been found to possess significant anti-inflammatory activity on the tested experimental models. 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 h with carrageenin, serotonin, histamine, dextran-induced rat paw oedema, respectively. In a chronic test the extract (400 mg/kg) showed 41.5% reduction in granuloma weight. The effect produced by the extract was comparable to that of phenylbutazone, a prototype of a non-steroidal anti-inflammatory agent.	Mandal *et al.,* 2000

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
Stem bark	Methanol extract (MEFR)	-	Antipyretic	A yeast suspension (10 ml/kg body wt.) increased rectal temperature 19 h after subcutaneous injection. The anti-pyretic effect of MEFR was comparable to that of paracetamol (150 mg/kg body wt., p.o.), a standard anti-pyretic agent. The MEFR, at doses of 100, 200 and 300 mg/kg body wt. p.o., showed significant dose-dependent reduction in normal body temperature and yeast-provoked elevated temperature. The effect extended up to 5 h after drug administration.	Rao *et al.,* 2002
Stem bark	Methanol extract (MEFR)	-	Antidiabetic	The glucose-lowering efficacy of a methanol extract of the stem bark of *F. racemosa* Linn. (MEBFR) (Family Moraceae) was evaluated both in normal and alloxan-induced diabetic rats. The MEBFR at the doses examined (200 and 400mg/kg p.o.) exhibited significant hypoglycaemic activity in both experimental animal models when compared with the control group.	Rao *et al.,* 2002

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
Stem bark	Methanol extract (MEFR)	-	Antitussive	The methanol extract of *Ficus racemosa* Linn (Moraceae) (stem bark) (MEFR) was tested for its antitussive potential against a cough induced model by sulphur dioxide gas in mice. The extract demonstrated significant ($p < 0.001$) antitussive activity at all tested dose levels when compared with the control. The antitussive activity of the extract was comparable to that of codeine phosphate (10 mg), a standard antitussive agent. The extract exhibited maximum inhibition of 56.9% at a dose of 200 mg/kg (p.o.) 90 min after administration.	Rao *et al.,* 2003
Bark	Soxhlet extraction with absolute ethanol (EtOH)	-	Anti-inflammatory	Ethanolic extracts inhibited COX-1 with the IC_{50} of 100 µg/mL. The positive control aspirin showed 52% inhibition of COX-1 at 306 µg/mL and indomethacin showed 70% inhibition at 10 µg/mL.	Li *et al.,* 2003

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
Bark	Soxhlet-absolute ethanol (EtOH)	Bergenin, Rasemisic acid	Antiinflammatory, Antioxidant, Cytotoxic	Racemosic acid showed potent inhibitory activity against COX-1 and 5-LOX in vitro with IC_{50} values of 90 and 18 μM, respectively. Its also demonstrated a strong antioxidant activity to scavenge ABTS free radical cations with an IC_{50} value of 19 μM. *F. racemosa* showed no cytotoxicity on the cell lines skin fibroblasts (1BR3), human caucasian hepatocyte carcinoma (Hep G2) and human Caucasian promyelocytic leukaemia (HL-60)	Li *et al.,* 2004
	Reflux increasing polarity petroleum ether, benzene, ethyl acetate, acetone, methanol and double distilled water	-	Chemo-preventive	*F. racemosa* extract is a potent chemopreventive agent and suppresses Fe-NTA-induced renal carcinogenesis and oxidative damage response in Wistar rats.	Khan&Sultana, 2005

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
Leaves	cyclohexane extract, methylene chloride extract, , crude extract, methanol extract, methanol extract after tannins removal.	-	Antiplasmodial, Cytotoxic	The crude, cyclohexane, methylene chloride, methanol extract showed %inhibition less than 50 at concentration 10 µg/mL. None of extract showed cytotoxic activity.	Nguyen-Pouplin *et al.,* 2007
-	-	-	Review ethnopharmacological and Biological	The fruits used for laxative, digestive, aphthae, menorrhagia, hemoptysis and gargling for sore throat. The leaves mixed with honey used for bilious affection, which are curing juandice, billious remittants and all varieties of disease generated by an unnatural condition of involving organ. The bark used for diabetes, cooling, gonorrhea, ulcers, skin diseases, scabies, hiccup, vomiting. The roots used for dysentery, infusion with oil used external treatment for excema, leprosy, rheumatism. In India	Lansky *et al.,* 2008

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
				contemporary ethnomedical commonly used decoction fruits for gripping gastralgia, root juice (sap) for stomachic and freash leaf juice for pneumonia.	
Stem barks	Soxhlet extraction with methanol follow by 70%acetone	-	Antioxidant	The 70% acetone extract of *F. racemosa* contained relatively higher levels of total phenolics than methanol extract. The antioxidant potential of the extracts assessed by employing different in vitro assays such as reducing power assay, DPPH, ABTS and OH radical scavenging capacities, peroxidation inhibiting activity through linoleic acid emulsion system, antihemolytic assay by hydrogen peroxide induced method and metal ion chelating ability. Though all the extracts exhibited dose dependent reducing power activity, methanol extracts of all the samples were found to have more hydrogen donating ability. Similar line of dose dependent activity has been maintained in all the samples in DPPH and OH scavenging systems. All the extracts	Manian *et al.,* 2008

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
				exhibited antioxidant activity against the linoleic acid emulsion system (34–38%).	
Fruits	50% ethanolic extract	gallic acid and ellagic acid	Gastro-protective	FGE showed dose dependent inhibition of ulcer index in pylorus ligation, ethanol and cold restraint stress–induced ulcers. FGE prevents the oxidative damage of gastric mucosa by blocking lipid peroxidation and by significant decrease in superoxide dismutase, H^+K^+ATPase and increase in catalase activity. High performance thin layer chromatography (HPTLC) analysis showed the presence of 0.57% and 0.36% w/w of gallic acid and ellagic acid in FGE. The results show that *F. glomerata* possess significant gastroprotective activity which might be due to gastric defence factors and phenolics might be the main constituents responsible for this activity.	Rao *et al.,* 2008

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
Stem barks	Aqueous extract	-	Antihyper-glycemic	Oral administration of bark powder (FGB) and aqueous extract (FGAE) at 500 mg/kg caused 21% and 52% reduction in fasting blood glucose, respectively and also decreased glycosuria significantly.	Ahmed&Urooj, 2008
		-	A review	*Ficus racemosa* Linn. is a moderate-sized avenue tree found throughout India either wild or cultivated for its fruits eaten by villagers. It is popular in Indigenous System of Medicine like Ayurveda, Siddha, Unani and Homoeopathy. In the Traditional system of medicine, various plant parts such as bark, root, leaves, fruits and latex are used in dysentery, diarrhoea, diabetes, bilious affections, stomachache, menorrhage, haemoptysis, piles and as carminative and astringent. The review is therefore, an effort to give a detailed survey of the literature on its pharmacognosy, phytochemistry, traditional and pharmacological uses.	Paarakh, 2009

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
Barks	Ethanol extract (FRE) and water extract (FRW)	-	Antioxidant, Radioprotective	Ethanol extract (FRE) and water extract (FRW) of *Ficus racemosa* (family: Moraceae) were subjected to free radical scavenging both by steady state and time resolved methods such as nanosecond pulse radiolysis and stopped-flow spectrophotometric analyses. FRE exhibited concentration dependent DPPH, ABTS, hydroxyl radical and superoxide radical scavenging and inhibition of lipid peroxidation with IC_{50} comparable with tested standard compounds. *In vitro* radioprotective potential of FRE was studied using micronucleus assay in irradiated Chinese hamster lung fibroblast cells (V79). Pretreatment with different doses of FRE 1h prior to 2 Gy g-radiation resulted in a significant ($P<0.001$) decrease in the percentage of micronucleated binuclear V79 cells. Maximum radioprotection was observed at 20mg/mL of FRE. The radioprotection was found to be significant ($P<0.01$) when cells were treated with	Veerapur *et al.,* 2009

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
				optimum dose of FRE (20mg/ml) 1h prior to 0.5, 1, 2, 3 and 4 Gyg-irradiation compared to the respective radiation controls. The cytokinesis-block proliferative index indicated that FRE does not alter radiation induced cell cycle delay. Based on all these results we conclude that the ethanol extract of *F. racemosa* acts as a potent antioxidant and a probable radioprotector.	
Woods	Reflux-water and ethanol	-	HIV-1 Integrase	The aqueous and ethanolic extract of *F. glomerata* exhibited anti-HIV-1 IN activity, with IC_{50} =29.5 and 7.8 µg/mL, respectively.	Bunluepuech& Tewtrakul, 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Antioxidant, Cytotoxic	The ethanolic, water extract and residue extract showed no antioxidant (EC_{50} > 100 µg/mL) and cytotoxic activity (IC_{50} > 100 µg/mL).	Chartsuwan *et al.,* 2009

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Anti-inflammatory	The ethanolic extract of FR showed anti-inflammatory activity with IC_{50}=70.2 µg/mL whereas the water extract and residue extract showed no activity.	Suranart *et al.,* 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Anti-allergic	The ethanolic extract of FR showed anti-allergic activity with IC_{50}=15.9 µg/mL whereas the water extract and residue extract showed no activity.	Tabpueng *et al.,* 2009
Green fruits		-	Antioxidant, DNA damage protective	In order to find *in vitro* antioxidant properties, extract/fractions from *F. glomerata* were studied for TPC, AOA, RP, DPPH, O^{-}_{2}, $^{\cdot}OH$ scavenging activities and LPO. Among all the extract/fractions, FEF has shown potent antioxidant activity and was also found effective in protecting oxidative DNA damage. *In vivo* evaluation of oxidative stress (LPO) and antioxidant defenses (concentration of GSH, as well as CAT and SOD activities) were measured in CCl_4 induced toxic rats. FEF was found to inhibit the	

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
				toxicity as seen from the decreased LPO and increased GSH, SOD and CAT levels. FEF has higher phenolic content and showed the presence of gallic, chlorogenic and ellagic acid. Based on these results, it is concluded that *F. glomerata* protects tissues from oxidative stress and these effects are probably related to the antioxidant properties.	Verma *et al.,* 2010
Bark		-	Subacutetoxic	Indicated that administration of the aqueous extract of herb for 15 to 21 days (incremental as well as fixed, in dose 30 mg/100 gm and 20 mg/ 100 gm up to 320 mg/100gm of body weight) in subacute toxicity study showed definitive liver damage. Hepatotoxicity is appeared to be reversible. Histopathologicaly renal damage was not marked. Serum creatinine and blood urea were increased significantly.	Panwar *et al.,* 2010

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
Leaves, Stems, Roots, Fruits	50% ethanolic extract	-	Physicochemical screening	Physicochemical studies reflected that total, water soluble ash content is higher in stem and acid insoluble ash is higher in root. It may be due to the soil components. Extractive value has been found higher in stem water extract, however alcoholic extract has found higher in leaf. Total carbohydrate content has found higher in leaf however, protein, phenol and tannin content found higher in stem portion of *F. racemaosa.*	Arunachalam *et al.,* 2010
Bark	Maceration-ethanol	-	Antipyretic	All doses of FR (ethanolic extract) significantly ($p<0.05$) reduced the pyrexia induced by yeast and FR (ethanolic extract) doses of 200 and 400 mg/kg were equally potent as ASA.	Chomchuen *et al.,* 2010
Roots	Maceration-95%ethanol and Decoction	-	Antimicrobial	The ethanolic extract of *F. racemosa* inhibited *S. typhimurium, A. buamannei, S. pyrogenes* and *B. subtilis* with inhibition zone 9.0, 8.5, 11.7 and 8.2 mm, respectively. The water extract showed no activity agianst all microbacterial in this study.	Nuaeissara *et al.,* 2011

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
-	-	-	A review	The leaf of this plant contains sterols, triterpenoids (Lanosterol) and alkaloids, tannins and flavonoids. Stem-bark gives gluanol acetate, β-sitosterol, leucocyanidin-3-O-β-D-glucopyrancoside, leucopel- argonidin-3-O-β-D-glucopyranoside, leucopelarg- onidin -3-O-α-L-rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate and α-amyrin acetate. From trunk bark, lupenol, β-sistosterol and stigmasterol were isolated. Fruit contains gluanol acetate, glucose, tiglic acid, esters of taraxasterol, lupeol acetate, friedelin, higher hydrocarbons (Hentriacontane) and other phytosterols. A new tetracyclic triterpene glauanol acetate which is characterized as 13α, 14β, 17βH, 20αH-lanosta-8, 22-diene-3β-acetate and racemosic acid were isolated from the leaves. An unusual thermostable aspartic protease was isolated from latex of the plant. The stem bark and fruit showed presence of gluanol acetate.	Shiksharthi & Mittal, 2011

Table C5: Phytochemistry and Biological activity of *Ficus racemosa*

Part used	Extraction	Compounds	Activity	Results	References
Roots	Maceration-95%ethanol	-	Antioxidant, Antiinflammatory	Ethanolic extract of *F. racemosa* showed stronger antioxidant in DPPH assay than positive control, BHT (EC_{50} = 4.87 and 12.75 µg/mL, respectively). It showed moderate inhibition nitric oxide effect on RAW264.7 cells (IC_{50} = 46.55 µg/mL).	Juckmeta & Itharat, 2012
Stems and Roots	-	-	Identification of adulteration by macroscopic, microscopic and TLC Technique	*F. racemosa* stem and root presented similarity in both cell types and arrangement. However, the stems had broad bands of xylem fibers alternating with narrow bands of axial xylem parenchyma in contrast to the roots.	Nutmakul *et al.,* 2013
Roots	Maceration-95%ethanol	-	Anti-allergic	The ethanolic of *F. racemose* exhibited anti-allergy activity (IC_{50} = 27.1 µg/mL) whereas chlorpheniramine, positive control showed inhibitory effect against beta-hexosaminidase with IC_{50} value 16.2 µg/mL (58.8 µM) respectively.	Juckmeta *et al.,* 2014

Table C6: Phytochemistry and Biological activity of *Tiliacora triandra*

Part used	Extraction	Compounds	Activity	Results	References
Roots	-	nortiliacorine A, tiliacorinine 2'-*N*-oxide, tiliacorinine 2-*N*-oxide, tiliacorinine, tiliacorine, dinklacorine, yanangine, yanangcorinine, tilianangine, tiliageine, nortiliacorinine A, tilitriandrine, noryanangine, norisoyanangine	Isolated compound		Thaweephol *et al.,* 1974; Wiriyachitra & Phuriyakorn, 1981;
Roots	-	tiliacolinine, tiliacorine, nortiliacorinine A	Antimararial	Tiliacolinine, tiliacorine and nortiliacorinine A were isolated from *T. triandra* which showed antimararial activity with IC_{50} values 3533 ng/mL, 675 ng/mL and 558 ng/mL, respectively.	Mahidol *et al.,* 1994

Table C6: Phytochemistry and Biological activity of *Tiliacora triandra*

Part used	Extraction	Compounds	Activity	Results	References
Roots	Maceration-chloroform: methanol: ammonia (50:50:1)	tiliacorine, tiliacorinine	Isolated compound	Isolation and purification of the crude extract using column chromatography and crystallization techniques provided two pure alkaloid compounds: tiliacorinine and tiliacorine with 0.0082 and 0.0029 percent yield, respectively.	Saiin & Markmee, 2003
Roots	Maceration-methanol	-	Anti-alzheimer	The methanolic extracts from *T. triandra* (Colebr.) Diel. showed moderate effect of the inhibitory activity on acetylcholinesterase (40-50%)	Ingkaninan *et al.,* 2003
Whole	Decoction	-	Acute and subchronic toxic	The water extract from the *T. triandra* does not cause acute or subchronic toxicities in either male or female rats.	Sireeratawong *et al.,* 2008
Leaves	Maceration-methanol	-	Antioxidant	The methanolic extract had the antioxidant activity with an EC_{50} of 3,903.9 mg extract/ mg DPPH (AE = 25.6×10^{-5}) and contained the content of phenolic compounds (13.3 mg GAE/mg dry extract).	Nanasombat & Teckchuen, 2009

Table C6: Phytochemistry and Biological activity of *Tiliacora triandra*

Part used	Extraction	Compounds	Activity	Results	References
Stems	Reflux-water and ethanol	-	HIV-1 Integrase	The aqueous and ethanolic extracts of *T. triandra* showed no HIV-1 IN activity (IC_{50} > 100 µg/mL).	Bunluepuech & Tewtrakul, 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Antioxidant, Cytotoxic	The ethanolic extract showed antioxidant activity by DPPH assay with EC_{50} = 23.35 µg/mL and high exhibited cytotoxic activity on COR-L23, MCF-7 with IC_{50} = 5.50 µg/mL, 7.88 µg/mL, respectively but no cytotoxicity on MRC5 cell line. The water and residue extracts showed no activity (>100 µg/mL).	Chartsuwan *et al.,* 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Antiinflammatory	The ethanolic extract showed cytotoxic on RAW264.7 cell line which use to test anti-inflammatory activity. The water and residue extracts showed no activity (>100 µg/mL).	Suranart *et al.,* 2009

Table C6: Phytochemistry and Biological activity of *Tiliacora triandra*

Part used	Extraction	Compounds	Activity	Results	References
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Antimicrobial	The ethanolic of TT inhibited *S. aureus, B. subtilis, E. coli* and *C. albicans* with inhibition zone 11.2, 13.8, 9.5 and 20.5 mm, respectively. The residue of extract inhibited *C. albicans* (9mm) whereas the water extract showed no activity (>100 µg/mL).	Sangrapee *et al.,* 2009
Roots	Maceration-95%ethanol, Decoction and Residue decoction	-	Anti-allergic	The ethanolic extract of TT showed anti-allergic activity with IC_{50}=10.3 µg/mL whereas the water extract and the residue extract showed no activity (>100 µg/mL).	Tabpueng *et al.,* 2009

Table C6: Phytochemistry and Biological activity of *Tiliacora triandra*

Part used	Extraction	Compounds	Activity	Results	References
Roots	Maceration-95%ethanol and Decoction	-	Antimicrobial	The ethanolic extract showed most effective activity against *E. coli* strains, *Shigella* species, *A. buamannei,* gram-positive bacteria and *C. albican* except *P. aeruginosa* and *K. pneumoniae*. In contrast, the water extract only inhibited *S. aureus*, *S. aureus* MRSA and *C. albican* with the inhibition zone 6.7, 7.3 and 10.8 mm, respectively.	Nuaeissara *et al.,* 2011
Roots	Maceration-95%ethanol	-	Antioxidant, Antiinflammatory	Ethanolic extract of *T. triandra* showed strong antioxidant in DPPH assay (EC_{50} value 15.38 µg/mL) and moderate inhibition nitric oxide effect on RAW264.7 cells (IC_{50} value 54.65 µg/mL)	Juckmeta & Itharat, 2012

Table C6: Phytochemistry and Biological activity of *Tiliacora triandra*

Part used	Extraction	Compounds	Activity	Results	References
Roots		tiliacorinine, 2'-nortiliacorinine, tiliacorine, 13'-bromo-tiliacorinine	Antimycobacterial	Bisbenzylisoquinoline alkaloids, tiliacorinine, 2'-nortiliacorinine, and tiliacorine, isolated from the edible plant, Tiliacora triandra, as well as a synthetic derivative, 13'-bromo-tiliacorinine, were tested against 59 clinical isolates of multidrug-resistant *Mycobacterium tuberculosis* (MDR-MTB). The alkaloids showed MIC values ranging from 0.7 to 6.2 μg/ml, but they exhibited the MIC value at 3.1 μg/ml against most MDR-MTB isolates.	Sureram *et al.,* 2012
Stems and Roots	-	-	Identification of adulteration by macroscopic, microscopic and TLC Technique	The stems and roots presented similar appearances. *T. triandra* stems had broad vascular bundles alternating with narrow medullary rays, contrast to the roots, which had narrow vascular bundles alternating with broad medullary rays.	Nutmakul *et al.,* 2013

Table C6: Phytochemistry and Biological activity of *Tiliacora triandra*

Part used	Extraction	Compounds	Activity	Results	References
Roots	Maceration-95%ethanol	-	Anti-allergic	The ethanolic of *T. triandra* showed no inhibitory effect agianst beta-hexosaminidase (IC_{50} >100 µg/mL) whereas chlorpheniramine, positive control exhibited anti-allergic activity with IC_{50} value 16.2 µg/mL (58.8 µM) respectively.	Juckmeta *et al.*, 2014

Table C7: Phytochemistry and Biological activity of *Garcinia mangostana*

Part used	Extraction	Compounds	Activity	Results	References
Hulls	-	γ-mangostin	Anti-inflammation	γ-Mangostin had a potent inhibitory activity of prostaglandin E_2 (PGE_2) release induced by A23187, a Ca_2 ionophore. The inhibition was concentration-dependent, with the IC_{50} value of about 5 μM. γ-Mangostin had no inhibitory effect on A23187-induced phosphorylation of p42/p44 extracellular signal regulated kinase/mitogen-activated protein kinase or on the liberation of [^{14}C]-AA from the cells labeled with [^{14}C] AA. However, γ-mangostin concentration-dependently inhibited the conversion of AA to PGE_2 in microsomal preparations, showing its possible inhibition of cyclooxygenase (COX). In enzyme assay in *vitro*, γ-mangostin inhibited the activities of both constitutive COX (COX-1) and inducible COX (COX-2) in a concentration-dependent manner, with the IC_{50} values of about 0.8 and 2 μM, respectively.	Nakatani *et al.,* 2002

Table C7: Phytochemistry and Biological activity of *Garcinia mangostana*

Part used	Extraction	Compounds	Activity	Results	References
Hulls	Maceration-100%, 70%, 40% and water	-	Anti-allergy, Antiinflammation	The 40% ethanol extract inhibited IgE-mediated histamine release from RBL-2H3 cells with greater potency than the water extract of *Rubus suavissimus* that has been used as an anti-allergy crude drug in Japan. All GM extracts potently inhibited A23187-induced prostaglandin E2 synthesis in C6 rat glioma cells, positive control had no effect. The 40% GM extract inhibited the prostaglandin E2 synthesis in a concentration-dependent manner with relatively lower concentrations than the histamine release.	Nakatani *et al.,* 2002
Pericarps	Maceration-95%ethanol	-	Antiinflammation, Antioxidant	*G. mangostana* showed the best antioxidant activity and could inhibit 50% of free radicals at the concentration of 6.13 µg/mL. Ethanolic extract also significantly reduced ROS production using NBT assay with the inhibitory ratio at 77.80%. At concentration 50 µg/mL showed highest inhibitory against TNF-α production in dose-dependent manner.	Chomnawang *et al.,* 2007

Table C7: Phytochemistry and Biological activity of *Garcinia mangostana*

Part used	Extraction	Compounds	Activity	Results	References
(freeze-dried) Fruits	Maceration-methanol	two xanthones, 1,2-dihydro-1,8,10-trihydroxy-2-(2-hydroxypropan-2-yl)-9-(3-methyl but-2-enyl)furo[3,2-a]xanthen-11-one, 6-deoxy-7-demethylmangostanin three known compounds, 1,3,7-trihydroxy-2,8-di-(3-methylbut-2-enyl)xanthone, mangostanin, α-mangostin	Antioxidant	Bioactivity-guided fractionation of a dichloromethane-soluble extract of *Garcinia mangostana* fruits has led to the isolation and identification of five compounds, including two xanthones, along with three known compounds (1,3,7-trihydroxy-2,8-di-(3-methylbut-2-enyl)xanthone, mangostanin, α-mangostin). All isolated compounds were tested in an *in vitro* quinone reductase-induction assay using murine hepatoma cells (Hepa 1c1c7) and an *in vitro* hydroxyl radical antioxidant assay. γ-mangostin exhibited hydroxyl radical-scavenging activity (IC_{50} = 0.20 μg/mL).	Chin *et al.*, 2008

Table C7: Phytochemistry and Biological activity of *Garcinia mangostana*

Part used	Extraction	Compounds	Activity	Results	References
(fresh) Hulls	Homogenization-70%acetone	α-and γ-mangostin	In *vitro* and *vivo* Antiinflammation	∝and γ-mangostin dose-dependently reduced the induction of NO products with IC_{50}=12.4 and 10.1 μM Two xanthones concentration-dependently reduced PGE_2 production at IC_{50} 3–25 μM, and the inhibitive effects of γ-mangostin were also stronger than these of ∝-mangostin. Both of them significantly inhibited the expression of iNOS, but not COX-2. In animal model, ∝-Mangostin and sulindac (positive control) exhibited a potent inhibition on paw edema at 3 h and 5 h, respectively whereas γ-mangostin did not. Interestingly, on-set time of ∝-Mangostin was more quickly than that of sulindac.	Chen *et al.,* 2008
(dried) Hulls	Maceration-dichloromethane	α-and γ-mangostin	Anti-inflammation	GM extract possessed potent NO inhibitory effect with an IC_{50} value of 1.0 μg/mL, α- and γ-mangostin, also possessed inhibitory effect with IC_{50} values of 3.1 and 6.0 μM, respectively. Two compounds similar inhibited PGE_2 production at IC_{50} value 13 μM. α-mangostin higher inhibited than γ-mangostin	Tewtrakul *et al.,* 2009

Table C7: Phytochemistry and Biological activity of *Garcinia mangostana*

Part used	Extraction	Compounds	Activity	Results	References
				inhibitory effect on LPS-stimulated TNF-α and IL-4 releases with IC_{50} value in range 31.8-64.8 μM.	
	-	α-mangostin	Antibacterial	Extracts of GM showing inhibitory effects against the growth of *S. aureus* NIHJ 209p were fractionated according to guidance obtained from bioassay and some of the components with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) were characterized. One active isolate, α-mangostin, presented MIC value of 1.57-12.5 μg/mL.	Linuma *et al.,* 1996
	-	-	Antibacterial	GM extract had the greatest antimicrobial effect. The MIC values were the same (0.039 mg/mL) for both bacterial species and the MBC values were 0.039 and 0.156 mg/ml against *Propionibacterium acnes* and *Staphylococcus epidermidis,* respectively.	Chomnawang *et al.,* 2005

Table C7: Phytochemistry and Biological activity of *Garcinia mangostana*

Part used	Extraction	Compounds	Activity	Results	References
Stem Bark	hexane, methylene chloride and methanol	α, β-mangostin	Antibacterial	α-mangostin was found to be active against vancomycin resistant Enterococci (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA), with MIC values of 6.25 and 6.25 to 12.5 μg/mL, respectively. β-mangostin showed low inhibitory effect against VRE and MRSA with MIC values more than 25 and 100 μg/mL, respectively. The study showed synergism between α-mangostin and gentamicin (GM) against VRE, and α-mangostin and vancomycin hydrochloride (VCM) against MRSA might be useful in controlling VRE and MRSA infections.	Sakagami *et al.,* 2005
Hulls	Maceration-hexane, methylene chloride	α-mangostin	Antibacterial	The crude CH_2Cl_2 extract (>0.53 mg/disc) exhibited a favorable activity against *L. monocytogenes*. Furthermore, MIC and MBC of antibacterial against strain were 8.50 and 17.00 mg/mL, which MBC was two-fold higher than the corresponding MIC. The GM extract at conc. 17 mg/mL treated to cells and studied by Transmission Electron Microscopy (TEM). There was some loss and change of the membrane and cytoplasm in cells of the	

Table C7: Phytochemistry and Biological activity of *Garcinia mangostana*

Part used	Extraction	Compounds	Activity	Results	References
				bacterium following exposure to crude extract. This may be due to the yield of α-mangostin content about 316.40 ± 32.36 µg/mg of dry matter of extract (31.64%) from dried inner mangosteen hull extraction with CH_2Cl_2.	Tangwatcharin *et al.,* 2012
Pericarps	dichloromethane and ethyl acetate extracts	α-mangostin	Antiprotozal, Antimicrobial	The ethyl acetate extract showed no antiprotozoal activity at all, a pronounced inhibitory effect (IC_{50}) was obtained with the dichloromethane extract against *Plasmodium falciparum* (IC_{50} 2.7 µg/mL) and *Trypanosoma brucei* (IC_{50} 0.5 µg/mL), but only with acceptable selectivity (SI) for *T. brucei* (SI = 18.8). Some side activity was also noted against *T. cruzi* and *Leishmania infantum* (IC_{50} 7.6 and 7.5 µg/mL), but with low selectivity. α-mangostin could be indicated against *B. subtilis* and *S. aureus* (MIC 1.6 and 3.2 µg/mL) and the *Mycobacterium* species (MIC 1.5 µg/mL), selectivity was quite low in view of the observed cytotoxicity on MRC-5 cells (IC_{50} = 7.5 µM). No activity at all was found against *C. albicans, E. coli* and *P. aeruginosa* (IC_{50} >200 µg/mL).	Al-Massarani *et al.,* 2013

Table C7: Phytochemistry and Biological activity of *Garcinia mangostana*

Part used	Extraction	Compounds	Activity	Results	References
Bark and Pericarps	80% methanol, water	-	Antibacterial	The MeOH extract of bark and pericarp showed highest inhibition against microorganism, both water and ethanolic extracts showed higher effective inhibition in high acidity (pH4).	Palakawong *et al.,* 2013
Pericarps	-	9-hydroxycalabaxanthone, parvifolixanthone C, α-mangostin, mangostanaxanthones I, and II	Antibacterial	mangostanaxanthones I and II displayed promising antioxidant activity with IC_{50} 12.07 and 14.12 μM, respectively using DPPH assay. α-mangostin and mangostanaxanthones II had weak to moderate activity against *Staphylococcus aureus* with MICs 0.79, 1.0 mg/mL, respectively. All compounds were inactive against *Candida albican.*	Mohamed *et al.,* 2014
Pericarps	95% ethanol, 70% acetone	α-mangostin, total phenolic compounds and tannins	Antibacterial	The 95% ethanol extract contained higher α-mangostin and total phenolic compounds. Tannins of 70% acetone extract were significantly higher than 95% ethanol extract. The 95% ethanol extract exhibited a potent antibacterial activity against oral pathogens with low MIC and MBC values compared to the acetone extract. The morphology of bacteria was significantly changed after treatment with extracts for 24 h. Furthermore, time kill assay revealed that bacterial cells were decreased within 2 hours.	Samprasit *et al.,* 2014

Table C7: Phytochemistry and Biological activity of *Garcinia mangostana*

Part used	Extraction	Compounds	Activity	Results	References
Fruits, Leaves and Resin	Maceration-70%ethanol	-	Antibacterial, Cytotoxic	Pericarps, leaves and resins (100 mg) showed inhibition against *Staphylococcus aureus* and *Escherichia coli* strains at clear zone 10, 5, 1 mm, respectively. The GM leaf extract inhibited cytotoxic activity against mouse melanoma B16 - F10 cells with IC_{50}=0.039 mg/mL.	Cunha *et al.,* 2014
Husks	90% ethanol	α-mangostin, δ-mangostin	Antiplasmodial	α-Mangostin was more active against the resistant *in vitro* antiplasmodial and cytotoxic effects were determined. α-mangostin was more active against the resistant *Plasmodium falciparum* chloroquine-resistant (FCR3) strain (IC_{50} = 0.2μM) than δ-mangostin (IC_{50} = 121.2 μM).	Upegui *et al.,* 2015

BIOGRAPHY

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Publications

Suranart, J., **Juckmeta, T.,** Makchuchit, S., Itharat, A. Study on anti-inflammatory activities of Thai medicinal plants in Pikutbenjalokawichian (Ha-Rak) [Abstract]. *Journal of Thai Traditional & Alternative Medicine* ***2009****, 7,* 120.

Suranart, J., **Juckmeta, T.,** Makchuchit, S., Itharat, A. Study on anti-inflammatory activities of Thai medicinal plants in Pikutbenjalokawichian (Ha-Rak) [Abstract]. *The Journal of Thammasat University Medical School* ***2009,*** 184.

Juckmeta, T., Itharat A. Anti-Inflammatory and Antioxidant Activities of Thai Traditional Remedy Called "Ya-Ha-Rak". *Journal of Health Research* ***2012,*** 26, 205-210.

Sakpakdeejaroen, I., **Juckmeta, T.,** Itharat, A. Development and Validation of RP-HPLC Method to Determine Anti-Allergic Compound in Thai Traditional Remedy Called Benjalokawichien. *Journal of Medicinal Association of Thailand, August* ***2014,*** 97 (Suppl. 8): S76-S80.

Jai-aue, A., Makchuchit, S., **Juckmeta, T.,** Itharat, A. Anti-Allergic, Anti-Inflammatory and Antioxidant Activities of the Different Extracts of Thai Traditional Remedy

Called Prabchompoothaweep for Allergic Rhinitis Treatment. *Journal of Medicinal Association of Thailand, August **2014;*** 97 (Suppl. 8): S140-S148.

Juckmeta, T., Thongdeeying, P., Itharat, A. Inhibitory effect on β-hexosaminidase release from RBL-2H3 cells of extracts and some pure constituents of Benchalokawichian, a Thai herbal Remedy, used for allergic disorders. *Evidence-Based Complementary and Alternative Medicine **2014;*** Article ID 828760, 8 pages, http://dx.doi.org/10.1155/2014/82876

Hansakul, P., Dechayont, B., Phuaklee, P., Pradjuabjinda, O., **Juckmeta, T.,** Itharat, A. Cytotoxic and Antioxidant Activities of Antidesma thwaitesianum MÜll Arg (Euphorbiaceae) Fruit and Fruit Waste Extracts. *Tropical Journal of Pharmaceutical Research, April **2015;*** 14(4), 627-34.

Juckmeta, T., Ruangnoo, S., Itharat, A. Cytotoxic activities against two lung cancer cells of Thai antipyretic drug. *Planta medica. December 2016;* 81(s01): S1-381, DOI: 10.1055/s-0036-1596966.

Chunthorng-Orn, J., Dechayont, B., Phuaklee, P., Prajuabjinda, O., **Juckmeta, T.,** Itharat, A. Cytotoxic, Anti-inflammatory and Antioxidant Activities of *Heliotropium indicum* Extracts. *Journal of Medicinal Association of Thailand, July **2016;*** 99(7):

Dechayont, B., Itharat, A., Phuaklee, P., Chunthorng-Orn, J., **Juckmeta, T.,** Prommee, N., Nuengchamnong, N., Hansakul, P. Antioxidant activities and phytochemical constituents of Antidesma thwaitesianum Müll. Arg. leaf extracts. *Journal of Integrative Medicine, July **2017;*** 15(4): 310-19.

Juckmeta, T., Pipatrattanaseree, W., Jaidee, W., Dechayont, B., Chunthorng-Orn, J., Andersen, J.R., Itharat, A. Cytotoxicity to Five Cancer Cell Lines of the Respiratory Tract System and Anti-inflammatory Activity of Thai Traditional Remedy. Natural Product Communications, ***2018***; *In process*

Presentation

Juckmeta, T., Itharat A. Antioxidant and anti-allergy activities of Thai traditional medicine preparations. 40th Annual Meeting of the Physiological Society of Thailand International Conference, 2-4 May **2011** Pullman Hotel, KhonKaen, Thailand (poster presentation)

Juckmeta, T. Biological activities of the ethanolic extracts from Pikut Benjalokawichian (Ha-Rak) and its isolate compounds. Medicinal Conference of Thammasat university **2012**, Faculty of Medicine, Thammasat University (oral presentation)

Juckmeta, T. Biological activities of the ethanolic extracts from Pikut Benjalokawichian (Ha-Rak) and its isolate compounds. 2nd Applied Thai Traditional Medicine Conference, 5-7 October **2012**, Faculty of Medicine Siriraj Hospital, Mahidol University (oral presentation)

Juckmeta, T., Itharat A. Cytotoxic, Anti-inflammatoryand Antioxidant Activities of *Tiliacora triandra* (Yanang). 1st Joint ACS AGFD-ACS ICSCT Symposium on Agricultural and Food Chemistry, 4-5 March **2014** Montein Riverside Hotel, Bangkok, Thailand (poster presentation)

Juckmeta, T., Itharat A. Cytotoxic Activities of *Tiliacora triandra* "Yanang". 18th World Congress on Clinical Nutrition (WCCN)"Agriculture, Food and Nutrition for Health and Wellness", 1-3 December, **2014,** Ubonratchathani, Thailand (oral presentation)

Juckmeta, T., Itharat A. Biological activities of the ethanolic extracts from Pikut Benjalokawichian (Ha-Rak) and its isolate compounds. The International Conference on Traditional and Indigenous Medicine, 4-5 September **2015** Impact exhibition & Convention center, Nonthaburi, Thailand (oral presentation)

Juckmeta, T., Itharat A. Cytotoxic activities against two lung cancer cells of Thai antipyretic drug. The 9th Joint Natural Products Conference, 24-28 July **2016**, Copenhagen, Denmark (poster presentation)

Award

Winner of oral presentation in Thai Traditional Research Conferences of Faculty of Medicine, **2012,** Thammasat University, Thailand

First runner up in Innovation Award **2015,** "Harberstin" product, Institute of Intellectual Property and Business Incubator Thammasat University, Thailand

Work Experience

2014-present: Member of Center of Excellence on Applied Thai Traditional Medicine Research

2013-2016: Research Manager of Research and Development of Thai Traditional Medicine Holistic Approach to Enhance Health, Prevent and Treat Chronic Diseases from Community to World Class, Thammasat University under the National Research University Project of Thailand (NRU) Fund, Office of Higher Education Commission

2012-present: Research Assistant in Applied Thai Traditional Medicine, Faculty of Medicine