Thai traditional medicine as a source for cancer prevention: from local concepts to the discovery of potential chemopreventive extracts

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Declaration

This PhD thesis describes research conducted in the School of Pharmacy, University College London between October 2013 and June 2017 under the supervision of Professor Michael Heinrich and Dr. Jose Prieto-Garcia. I, Natchagorn Lumlerdkij confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature …… Date ……..

(Natchagorn Lumlerdkij)

This thesis is dedicated to

My mom, dad, and grandmothers

And the memory of HM King Bhumibol Adulyadej (1927 – 2016)

and his tireless efforts to maintain the benefits and happiness of Thai people

Abstract

Cancer chemoprevention aims to prevent, delay, or reverse carcinogenesis. Thai Traditional Medicine (TTM) could be a source for cancer chemopreventive agents and – more broadly – could play a role in cancer prevention. Using an ethnopharmacology approach this thesis aims to understand the pharmacological basis of some of these botanical drugs and to discover new extracts which could be useful in cancer prevention.

Interviews with 33 TTM practitioners revealed the five characteristics of cancer in TTM (*mareng*), which is described as an accumulation of waste, chronic inflammation, chronic illnesses (*krasai*), bad condition of body fluids (‘*luead*’ and ‘*namlueang*’), and imbalance of *dhātu si*. Further analysis of preventive methods led to the five strategies for preventing *mareng*. To link TTM actions to pharmacology, we proposed that three strategies, removal of waste, liver protection, and prevention from *krasai*, can be linked to the antioxidant system.

After screening of fifty-two extracts, fifteen exhibited protective effect in a liver cancer cell line. Among them, ethanol extracts of *Thunbergia laurifolia* leaves (TLe) and *Senegalia rugata* leaves (SR1e) exhibited the most potent activities in the induction of NQO1 enzyme and glutathione. Upregulation of antioxidant genes and radical scavenging were among their protective mechanisms. While TLe induced NQO1 expression, SR1e upregulated the expression of Nrf2. Both extracts did not induce CYP1A1 expression nor reduce cell viability of primary rat hepatocytes which provided preliminary safety profile. Using HPLC-HRMS-SPE-*tt*NMR, we could identify some active constituents in the extracts.

This is the first report analysing how cancer is perceived in TTM, what prevention strategies are used, linking this to pharmacological models, and on chemopreventive properties of TLe and SR1e and some of their constituents. The evidence supports the potential use of these medicinal plants in cancer prevention. Future work should be performed with more TTM practitioners and use *in vivo* models.

Impact Statement

This thesis presents an analysis of knowledge in Thai traditional medicine (TTM) and links it to biomedical concepts and practical lab-based experiment, as well as provides *in vitro* pharmacological properties and phytochemical profiles of selected medicinal plants. This is the first time that characteristics of cancer in TTM (or *mareng*) were systematically analysed and grouped. The findings have direct impacts on providing scientific evidence for medicinal plants used in TTM and characteristics of cancer in TTM through a combination of ethnopharmacological and biomedical research. The proposed concept is also beneficial for future research on cancer in TTM or TTM drugs for cancer treatment and prevention. Moreover, it is fruitful for TTM education, most importantly at university level. Our results can be used as preliminary data for future research grants on TTM or cancer drug discovery, as well as another PhD project.

For non-academia impacts, our data supports TTM practice and the integration of TTM to the main health care system. It could help local business, such as farmers and herbal medicine manufacturers, which are usually small and medium-sized enterprises (SMEs). In addition, it is also helpful in the promotion and conservation of TTM.

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List of abbreviations

2-AAF	2-acetyl aminofluorene
5-FU	Fluorouracil
5-LOX	5-lipoxygenase
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
ACF	aberrant crypt foci
ADCC	antibody dependent cellular cytotoxicity
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ARE	antioxidant responsive elements
AST	aspartate aminotransferase
B(a)P	Benzo(a)pyrene
BSA	albumin from bovine serum
BUN	blood urea nitrogen
C/EBP	CCAAT/enhancer binding protein
CAT	catalase
CATTM	Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University
CCA	cholangiocarcinoma
cDNA	complementary DNA
ConA	concanavalin A
COX-1	cyclooxygenase-1
COX-2	cyclooxygenase-2
CRE	creatinine
CuSOD	copper superoxide dismutase
CYPs	cytochrome P450s
DEN	diethylnitrosamine
DMBA	7,12-dimethylbenz[a]anthracene
DMH	1,2-dimethyl hydrazine
DPPH	2,2-diphenyl-1-picrylhydrazyl

DR3	Death receptor 3
EGCG	epigallocatechin gallate
EMT	epithelial mesenchymal transition
EPIC	European prospective investigation into cancer and nutrition
FADD	Fas-associated protein with death domain
FC	frequency of citation
Fe-NTA	ferric nitrilotriacetic acid
FRAP	ferric reducing antioxidant power
G6PDH	glucose-6-phosphate dehydrogenase
γ-GCS	gamma-glutamylcysteine synthetase
γ-GT	gamma-glutamyl transpeptidase
GM-CSF	granulocyte monocyte-colony stimulating factors
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	reduced glutathione
GSSG	oxidized glutathione
GST	glutathione-S-transferase
HMOX-1	heme oxygenase-1
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
HSP70	heat shock 70-kDa protein
HUVEC	Human umbilical vein endothelial cells
IFNγ	interferon gamma
IGF1R	insulin-like growth factor 1 receptor kinase
IL	interleukin
iNOS	inducible nitric oxide synthase
IPA	Ingenuity Pathways Analysis
Keap-1	Kelch-like ECH-associating protein 1
LDH	lactate dehydrogenase
LPO	lipid peroxidation

LPS	Lipopolysaccharide
MDA	malondialdehyde
MeOD	Methanol-d_4
MMP	matrix metalloproteinase
MnSOD	manganese superoxide dismutase
MNTC	maximum non-toxic concentration
MQ	menadione
mtDNA	Mitochondrial DNA
mTOR	mammalian target of rapamycin
NCDs	non-communicable disease
NDMA	*N*-nitrosodimethylamine
NF-kB	nuclear factor kappa B
NK cells	natural killer cells
NMU	*N*-nitroso-*N*-methylurea
NO	nitric oxide
NQO1	NADPH:quinone oxidoreductase
Nrf2	Nuclear factor-E2-related factor 2
ODC	ornithine decarboxylase
OGD-R	oxygen-glucose deprivation followed by reoxygenation
PAHs	polycyclic aromatic hydrocarbons
PARP	poly(adenosine diphosphate-ribose) polymerase
PBMC	peripheral blood mononuclear cell
PCO	protein carbonyls
PCR	Polymerase chain reaction
PDGF-BB	platelet-derived growth factor
PGE_2	prostaglandin E_2
PMA	phorbol 12-myristate 13-acetate
PMSF	phenylmethylsulfonyl fluoride
PPAR	peroxisome proliferator-activated receptor
PVDF	polyvinylidene difluoride

qPCR	quantitative polymerase chain reaction
QR	quinone reductase
RCHY1	ring finger and CHY zinc finger domain containing 1
RFC	relative frequency of citation
RIN^e	RNA integrity number equivalent
ROS	reactive oxygen species
RT	retention time
SD	standard deviation
SDS-PAGE	polyacrylamide gel in the presence of sodium dodecyl sulphate
SGOT	serum glutamate oxaloacetate transaminase
SGPT	serum glutamate pyruvate transaminase
SOD	superoxide dismutase
SPE	solid-phase extraction
SR1e	ethanol extract of the leaf of *Senegalia rugata*
STAT3	signal transducer and activator of transcription 3
TBARS	thiobarbituric acid reactive substances
t-BHP	*tert*-butylhydroperoxide
TBST	Tris-buffered saline with 0.1% Tween 20
TCM	Traditional Chinese Medicine
TEAC	Trolox equivalent antioxidant capacity
Th2	T helper 2
TLe	ethanol extract of the leaf of *Thunbergia laurifolia*
TNF-α	tumour necrosis factor alpha
TPA	12-O-tetradecanoylphorbol-13-acetate
TTM	Thai Traditional Medicine
*tt*NMR	automated tube transfer nuclear magnetic resonance spectroscopy
VEGF	vascular endothelial growth factor
WHO	World Health Organization
XO	xanthine oxidase
ZnSOD	zinc superoxide dismutase

Chapter 1 General introduction

1.1. Cancer- a global health problem

According to the World Health Organization (WHO), non-communicable diseases (NCDs), especially cardiovascular diseases, cancer, and chronic respiratory diseases, caused more deaths than all other causes combined in 2012. Globally, cancer was the second leading cause of death, ranked behind cardiovascular diseases (World Health Organization, 2014). In 2012, there were 14.1 million new cancer cases and 8.2 million deaths from cancer worldwide (Ferlay et al., 2015). It was predicted that the number of new cancer cases would be over 20 million per year by 2025 (Bray, 2014). According to Cancer Research UK, more than 1 in 3 people in the UK will develop some form of cancer during their lifetime (Cancer Research UK, 2014).

Although there have been successes in the treatment of some cancers, such as certain types of leukaemia and lymphoma, the management of cancer is still challenging, with the overall mortality of cancer patients[1] still above 50%, especially in developing countries (Figure 1-1). The concept that cancers are an outcome of over-proliferating cells has led to the extensive research of cytotoxic drugs. Unfortunately, these drugs are also toxic to normal cells; such as those in bone marrow, heart, lungs, gastrointestinal tract, kidney, and brain. This in turn results in the failure of these organs and often leads to cancer patient mortality. Therefore, there is an increased interest in more targeted therapies. However, it is still difficult to cure advanced cancer patients because of the genetic heterogeneity and the tumour burden characteristic (Sporn and Suh, 2000).

[1] The sources of the WHO mortality database are from annual reports submitted by its Member States from their civil registration systems, which the cause-of-death data is coded according to the ICD-9 and ICD-10 system (source: WHO. Available: http://www.who.int/healthinfo/mortality_data/en/ [Accessed 30 October 2017]).

Figure 1-1 Incidence and mortality from all cancers except non-melanoma cancer based on WHO, 2012 (estimated age-standardised rate per 100,000) (Ervik et al., 2016).

1.2. Carcinogenesis

Cancer develops after a long period of multiple exposures to carcinogens as it can be seen that cancer incidence increases with increasing age. Carcinogenesis (also called oncogenesis and tumourigenesis) is a multistep process where normal cells become cancerous. It is caused by both genetic and epigenetic alterations that result in an imbalance between cell proliferation and cell death. An epigenetic alteration is a change in gene expression without changes to the DNA sequence. More than 600 cancer-related genes are known to carry epigenetic changes (Landis-Piwowar and Iyer, 2014, Weston and Harris, 2003). Processes that promote carcinogenesis include genomic instability, abnormal cell proliferation, reprogramming of the stromal environment, and aberrant differentiation between epithelial and mesenchymal states (EMT/MET). Carcinogenesis is generally divided into three main steps - cancer initiation, promotion, and progression (Elinav et al., 2013, Weinberg, 2014).

Carcinogens play an important role in tumour initiation. These agents often form an adduct with DNA, and then this carcinogen-DNA adduct (Figure 1-2) leads to mutation during DNA synthesis, proto-oncogene activation, and tumour-suppressor gene inactivation. Tumour promotion is the step where the numbers of initiated DNA damaged cells are increased by tumour promoters. Tumour promoters are not directly carcinogenic. They promote clonal expansion of initiated cells, increase cell proliferation of mutated cells, and also enable the effect of low concentration tumour initiators to be carcinogenic. In the progression step, precancerous cells/benign tumours transform into malignancy by a sequence of mutations and epigenetic alterations of DNA. This event affects the genes that control cell proliferation, survival, and other characteristics associated with the cancer cell phenotype. In the early phase of progression, cancer cells multiply in or near the original site and result in a primary

tumour mass. A variety of human tumours later undergo invasion-metastasis cascade and travel throughout the body. Cancer metastasis is responsible for approximately 90% of cancer deaths (Weinberg, 2014, Knowles and Selby, 2005). Figure 1-3 illustrates tumour development in various sites.

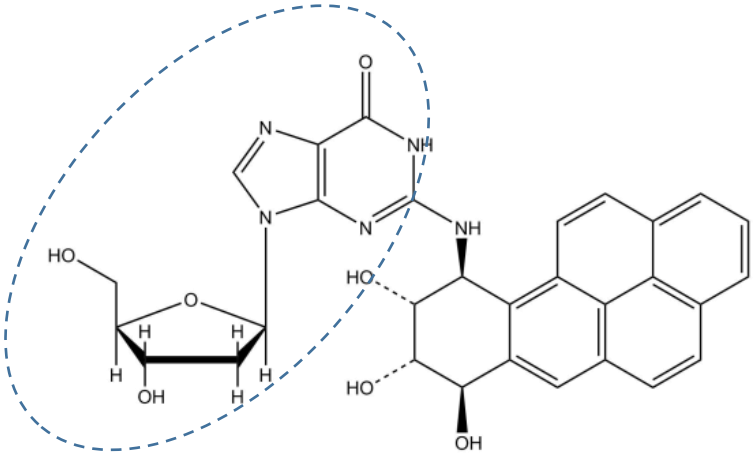

Figure 1-2 Benzo(a)pyrene-DNA adduct. The well-known carcinogen forms a covalent bond with deoxyguanosine in DNA (in the blue circle) (Poirier, 2012).

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Figure 1-3 Multi-step tumourigenesis in different sites. CIN = cervical intraepithelial neoplasia, CIS = carcinoma in situ, DCIS = ductal carcinoma in situ, PIN = prostatic intraepithelial neoplasia (Weinberg, 2014).

1.3. Cancer prevention and chemopreventive agents

The most important strategy for primary prevention of cancer is to avoid or minimize exposures to risk factors. A complementary approach is to make the body become more resistant to mutagens and carcinogens, as well as to suppress progression of the disease (De Flora and Ferguson, 2005). Some recognised risk factors, including lifestyles and diet, are presented in

Figure 1-4. Multiple lines of evidence show that more than 30% of common cancer could be prevented by modifying the diet, weight, and physical activity. Strong evidence suggests that non-starchy vegetables, fruits, physical activity, dietary fibre, and garlic reduce the risks of cancer (World Cancer Research Fund International, 2016a).

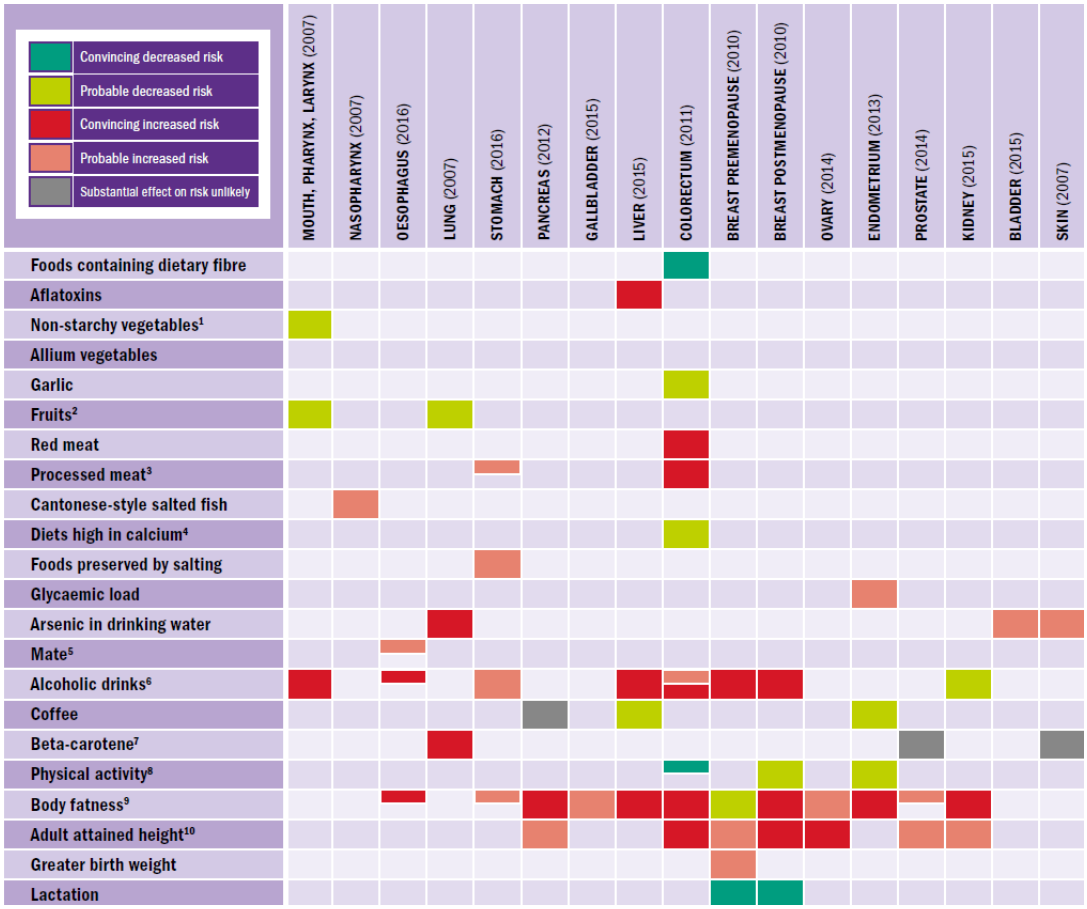

Figure 1-4 Summary of factors that increase or decrease risk of cancer. [1]Includes evidence on foods containing carotenoids for mouth, pharynx, larynx, [2]Includes evidence on foods containing carotenoids for mouth, pharynx, larynx, lung, [3]For stomach, probable increased risk of non-cardia cancer only, [4]For colorectum, evidence is from milk and studies using supplements, [5]Probable increased risk for oesophageal squamous cell carcinoma only, [6]For oesophagus, convincing increased risk for oesophageal squamous cell carcinoma only. For kidney, based on evidence for alcohol consumption up to 30 grams/day, [7]For lung, evidence is from studies using high-dose supplement in smokers, [8]Convincing decreased risk of colon not rectum, [9]For oesophagus, convincing increased risk for adenocarcinoma only. For stomach, probable increased risk of cardiac cancer only. For prostate, probable increased risk of advanced prostate cancer only, [10]Adult attained height in unlikely to directly influence the risk of cancer. It is a marker for genetic, environmental, hormonal and nutritional factors affecting growth during the period from preconception to completion of linear growth (World Cancer Research Fund International, 2016b).

1.3.1. Cancer chemopreventive agents

Cancer chemoprevention is the use of natural, synthetic, or biological substances to prevent, reverse, suppress, or retard carcinogenesis (Gong et al., 2011). Since the development of cancer is a multi-step process and takes time, it is possible to prevent initiated cells to become cancerous. For instance, Vitamin A analogues are able to cause regression of the lesions of leucoplakia and reduce the development of squamous cancer by the ability to modulate the

differentiation of epithelial cells (Tobias and Hochhauser, 2015). The success of chemoprevention using tamoxifen also provides relevant evidence. Tamoxifen is the first FDA approved chemopreventive agent to prevent breast cancer and was found to reduce its incidence by 50% in women at high risk (Anand et al., 2008).

Clinically, chemopreventive agents are used by healthy individuals potentially with premalignant lesions or increased risk, such as an inherited cancer syndrome or a family history of cancer, as well as to prevent cancer recurrence after cancer therapy. At molecular level, chemopreventive agents can be divided into blocking agents and suppressing agents (Figure 1-5) (Landis-Piwowar and Iyer, 2014).

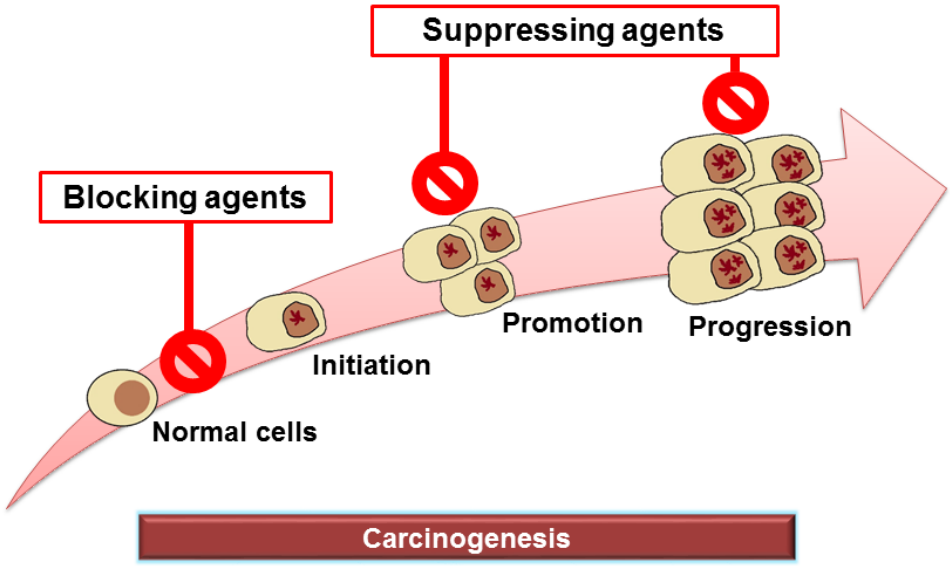

Figure 1-5 Major categories of cancer chemopreventive agents based on molecular mechanisms.

1.3.1.1. Blocking agents mechanisms

Blocking agents are agents that inhibit cancer initiation. The first approach is to block the uptake of carcinogens/mutagens by modification of transmembrane transport to inhibit their penetration or stimulate the pumping out. The second way is a modulation of phase I or phase II enzyme. Many of phase I enzymes, especially some isoforms of CYPs, are known to activate procarcinogens into carcinogens. Phase II enzymes are responsible for detoxification of carcinogens and reactive metabolites, as well as protection from reactive oxygen species (ROS) and electrophiles. Thus, the general idea is to inhibit phase I and induce phase II enzymes. Once mutated DNA has already formed, it is important to inhibit the replication of cells carrying mutated DNA and activate DNA repair mechanisms. Some epigenetic changes also lead to cancer initiation. Therefore, correction of hypomethylation or inhibition of histone deacetylation are also among blocking mechanisms. Moreover, telomerases and oncogenes have also been considered to be primary prevention (Landis-Piwowar and Iyer, 2014, Steward and Brown, 2013, De Flora and Ferguson, 2005).

1.3.1.2. Suppressing agents mechanisms

Suppressing agents reduce or delay cancer promotion and progression by several mechanisms. Firstly, it is important to inhibit cancer cell proliferation. Many agents have been investigated for the activity to down-regulate signal transduction pathways, such as NF-κB, mTOR, and STAT3. Another approach is to inhibit some isoforms of CYPs that are involved in the modulation of signal transduction to hormone responsive element. For instance, aromatase inhibitors block the activity of aromatase (CYP19) resulting in the down-regulation of the survival signal by oestrogen which lead to the decrease in breast cancer metastasis. A proposed method to delay or reduce metastasis is to induce apoptosis and inhibit angiogenesis, EMT, invasion, and dissemination (Landis-Piwowar and Iyer, 2014). In addition, inhibition of COX-2 expression is also a target because it results in the inhibition of PGE_2 production. PGE_2 plays important roles in promoting cancer angiogenesis and cell proliferation, as well as suppression of cancer cell apoptosis (Thun, 2012). Inhibition of tumour promotion could also be performed by prevention of ROS and free radicals formation and modulation of the immune system (De Flora and Ferguson, 2005).

1.3.2. Natural sources: providers of cancer chemopreventive agents

Consumption of vegetables, fruits, and dietary fibre has been recommended in cancer prevention (World Cancer Research Fund International, 2016a). Multiple lines of evidence showed that phytochemicals have potential to prevent or treat cancer. For example, a European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study involving about 11 years of follow-up of 477,312 men and women from 10 European countries showed an inverse association between consumption of flavonols and lignans and risk of bladder cancer (Zamora Ros et al., 2014). Another EPIC study suggested that the intake of lignans before breast cancer diagnosis may help to improve the survival among postmenopausal women, but not in premenopausal women (Kyrø et al., 2015).

As natural sources, especially plants, have proved to be a good source for chemopreventive agents, many research groups have extensively studied phytochemicals for this purpose. Phytochemicals that are well-known for their anti-cancer or chemopreventive properties include genistein from soy, resveratrol from red grapes, peanuts, and berries, epigallocatechin gallate from green tea, lycopene from tomatoes, lupeol from mangoes, curcumin from turmeric, indole-3-carbinol from cruciferous vegetables, and diallyl sulfide from garlic and related taxa. These compounds have shown pharmacological activities as blocking or suppressing agents, such as protection against oxidative stress, induction of apoptosis, modulation of phase II enzymes, and regulation of immune system (Singh et al., 2012).

However, regardless their excellent *in vitro* and *in vivo* activities, the development of phytochemicals into approved cancer chemopreventive agents are still challenging due to several important reasons. Firstly, it is difficult to design intervention studies to evaluate the effectiveness of such preventive intervention. For example, clinical studies investigating the effect of green tea on liver diseases; including liver cancer, suffered from several major

limitations, such as improper comparison between control and treatment groups, no randomisation, and selection bias (Darvesh and Bishayee, 2013). Secondly, the effectiveness of phytochemicals to reduce cancer risk vary among population, such as in the case of soy-rich diets that showed inverse association with breast cancer only in Asian women, but could not be confirmed in European women (Spagnuolo et al., 2015). Thirdly, the development of preparations that overcome/enhance their bioavailability also needs to be considered. Moreover, the question regarding the different effects between a single compound and whole extracts or products is debatable, as well as the safety of isolated compounds.

1.4. Ethnopharmacology and the development of chemopreventive agents

Ethnopharmacology is 'a multidisciplinary area of research concerned with the observation, description, and experimental investigation of indigenous drugs and their biological activity' (Rivier and Bruhn, 1979). It also focuses on the understanding of the benefits and risks of traditional medicine with the aim to provide better and safer uses (Heinrich, 2015).

One of the classic example of drug discovery using ethnopharmacology approach is the discovery of curare. Curare is a plant extract used to make poison-tipped hunting arrows by certain wild tribes in South America. The French researcher, Claude Bernard (1813 – 1878), was the first one who studied curare. He noticed that the animals did not show sign of pain or nervousness. Later, we understood that it kills animals by paralyzing their muscle, including muscles of respiration, and the death occurs as a result of suffocation (Heinrich, 2015). The very first attempts of using curare for therapeutic purposes were for treatment of spastic paralysis, for moderating the convulsions in the shock-therapy of certain psychoses, and as an adjunct to anaesthesia in surgery. However, major problems of the drug during that time regarding the origin, composition, and quality of the extract have limited its uses. Its active component, d-tubocurarine, was isolated from the crude extract by H. King in 1935 (Wintersteiner and Dutcher, 1943) and has led to the successful use as medicine. After that, d-tubocurarine has been used as muscle relaxant in abdominal operations and adjunct to anaesthesia in surgery (Prescott et al., 1946).

The curare case study exemplifies that new drugs can be discovered through ethnopharmacology research. Nowadays the approach is commonly used for research on traditional Chinese medicine (TCM) in Asia, as well as research on medicinal plants in Brazil, Mexico, and South Africa. To date, a fourth of ethnopharmacological research is about anti-cancer effects of traditionally used plants (Heinrich, 2015). This is not a surprise as the previous development of anti-cancer agents has greatly relied on natural sources. More than 60% of the available cancer drugs are developed from natural products. Well-known examples include paclitaxel from *Taxus brevifolia* Nutt. (Taxaceae), vincristine and vinblastine from *Catharanthus roseus* (L.) G.Don (Apocynaceae), and etoposide (a podophyllotoxin derivative) from *Podophyllum peltatum* L. (Berberidaceae) (Gordaliza, 2007).

Herbal medicine is the use of plants or plant mixture to take care of health problems. It has been the main treatment method in most traditional medicines, such as TCM, Ayurvedic

medicine, and Thai traditional medicine. Therefore, it is noteworthy to study herbal medicine for the discovery of new leads.

1.5. Thai traditional medicine

The Kingdom of Thailand has its own system of traditional medicine called “Thai Traditional Medicine” (TTM). TTM is considered a holistic medicine. In the past decades, it has gained more importance especially in chronic diseases, including cancer. The system is in harmony with Thai culture and associated with Buddhism. Various forms of practices, namely herbal medicine, massage, midwifery, maternal and child health care, Buddhist rites, and other rituals related to belief in supernatural power, are practiced in the system (Chokevivat and Chuthaputti, 2005). In the old days, the knowledge (both oral and textual) was transmitted through a process of apprenticeship. Ancient texts were passed on only through this process and mostly only among family members. In recent decades this has resulted in a loss of knowledge when no one in the family wanted to carry on the practice. To preserve the knowledge passed from the ancestors, King Rama III (reign: 1824 – 1851) and Rama V (reign: 1868 – 1910) led the collection and publication of available knowledge and old scriptures and made them available to all Thai people. Since the reign of King Rama V, the most recognized and probably the most important sources of TTM knowledge have been the inscriptions at *Wat Pho* (a Buddhist temple in Bangkok, Thailand) and two old text books (*khamphi*); *Phaetthayasat Songkhro*[2] and *Wetchasuksa*[3] (Mulholland, 1979). Nowadays a degree in TTM can be acquired from a four-year curriculum available from 25 institutes/ universities/ colleges (updated in 2017). Basic knowledge of TTM can also be studied in many schools across the country.

In TTM, *dhātu* (the elements), *utu* (the seasons), *ayu* (age), and *kala (time)* are believed to be the main *samutthan* (causal factors) of diseases. For instance, the fire element is dominant in people aged between 16 and 30 years. Therefore, the fire element is likely to be the cause of illnesses in this group. The body comprises four elements (*dhātu si*) namely earth (*dhātu din*), water (*dhātu nam*), wind (*dhātu lom*), and fire (*dhātu fai*). The number of components of each element in the body is 20 for earth, 12 for water, 6 for wind, and 4 for fire (Figure 1-6). Wind and fire are untouchable, while earth and water form 32 parts of the body. Health problems are caused by the imbalance of the four elements (Foundation for the promotion of Thai traditional medicine and Ayurved Thamrong School Center of applied Thai traditional medicine, 2007b).

[2] A collection of ancient *khamphi* which was published the first time in 1961. It is used nowadays as a manual for people studying Thai traditional medicine and pharmacy.

[3] A manual for students of Thai traditional medicine originally edited and published in 1909.

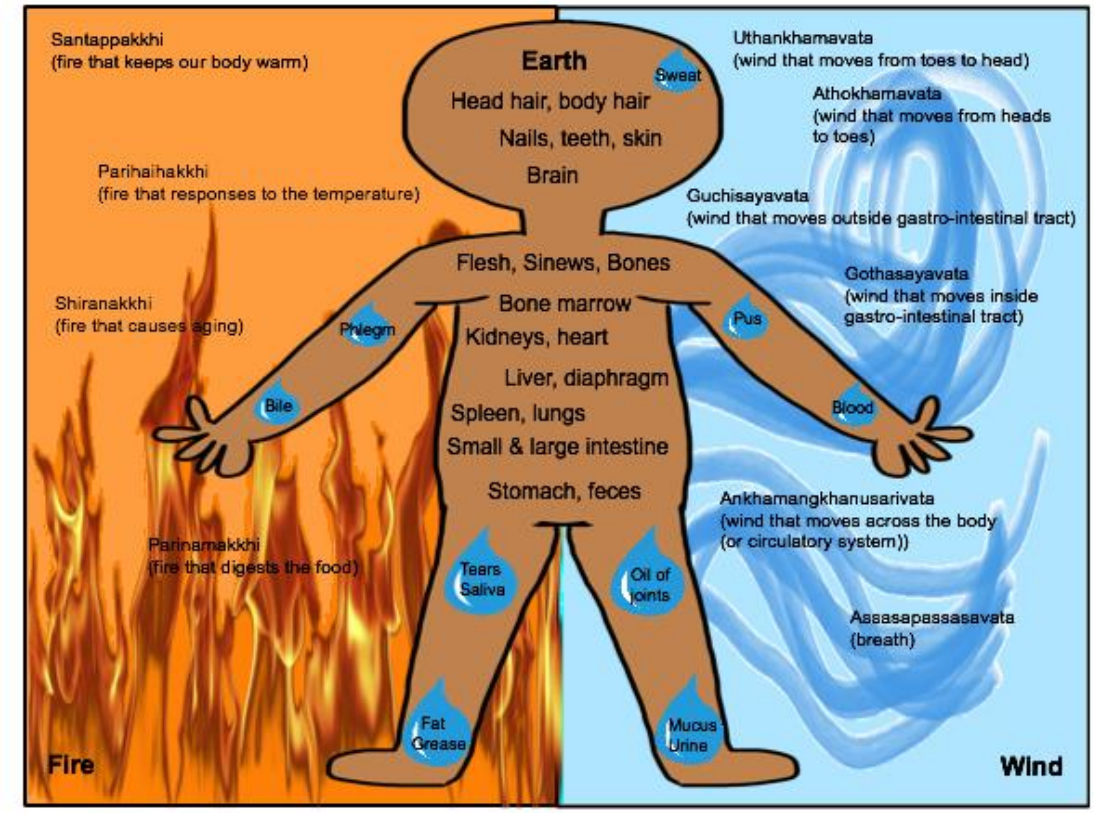

Figure 1-6 The components of each element in the body within the context of Thai Traditional Medicine (Lumlerdkij 2017)

In the past decades, TTM has gained importance especially in chronic diseases, including chronic pain and cancer. Although scientific evidence to support the use of Thai traditional drugs in cancer is lacking, medical experience and anecdotal accounts of the benefits of TTM against the disease are well known throughout Thailand. However, little systematic information is available about the knowledge and practice of Thai healers (officially called Thai traditional practitioners in the country) regarding 'cancer'.

1.6. Potential of Thai medicinal plants as cancer chemoprevention

The main textbook describing uses of herbal medicine in TTM; *Khamphi Sapphakhunya* (Foundation for the promotion of Thai traditional medicine and Ayurved Thamrong School Center of applied Thai traditional medicine, 2007a), was reviewed and ten well-studied and important plants were selected. Then a literature search was performed using mainly Pubmed and Web of Science databases, as well as manual search in Google Scholar to find scientific and biomedical evidence for the use of these plants for cancer prevention. Keywords included plant names, compound names, cancer, chemoprevention, antioxidant, and protection. The main focus was on blocking and supressing mechanisms *(see section 1.3.1),* as well as tumour inhibition *in vivo*. We excluded studies that reported positive activities at unphysiologically high concentrations of test samples e.g. 1000 mg/kg *in vivo* or 250 μg/ml in

cell cultures. Antioxidant assays without suitable positive controls to allow comparisons were also excluded. The search was limited to the period of 1990 – 2017.

Garlic is well-known for its medicinal properties and remarkable biological activities; including cancer chemoprevention. In TTM, the bulb was used as carminative, for treatment of diseases in the chest, indigestion, constipation, fever caused by *Semha* (water element) abnormality, ringworm, and for hair nourishment. The leaf has been used as an expectorant, a diaphoretic, for treatment of bruises, for stomach pain and to improve blood circulation (Foundation for the promotion of Thai traditional medicine and Ayurved Thamrong School Center of applied Thai traditional medicine, 2007a). Globally garlic (*Allium sativum* L.) and other allium vegetables; including garlic, onions, shallots, leeks and chives, are known as 'healthy diet' and often associated with the reduction in cancer risk. Several meta-analyses of population-based studies have been performed and reported differently for the association of allium vegetable consumption and risk of cancer, especially for colorectal and gastric cancer. Kodali and Eslick (2015), who performed meta-analysis of a total of 8,621 cases and 14,889 controls, concluded that garlic intake was associated with reduced risk of gastric cancer (Kodali and Eslick, 2015). This is in agreement with Zhou et al. (2011) who also found that increased garlic and garlic stalks consumption was associated with a reduced gastric cancer risk (Zhou et al., 2011). However, there was no correlation found between garlic intake and reduced risk of colorectal cancer (Hu et al., 2014).

Although the effectiveness of garlic to reduce cancer risk still needs information from more population-based study, the use in cancer prevention has been backed by a lot of mechanistic studies. Their chemopreventive properties have been reviewed extensively elsewhere (Nicastro et al., 2015, Guercio et al., 2014, Schäfer and Kaschula, 2014, Mikaili et al., 2013, Melino et al., 2011). Briefly, *in vitro* and *in vivo* studies reported that garlic, onion, and their allyl sulfur compounds interfered with cancer initiation and cancer promotion. The blocking and suppression mechanisms include suppression of bioactivation of dietary carcinogens (e.g. nitrosamine and heterocyclic amines), induction of NQO1 and heme oxygenase 1 (HMOX-1) enzymes and their regulator Nrf2, cancer cell cycle arrest, induction of apoptosis via several pathways; including JNK, ERK1/2, and p38; p53, NFκB, Bcl-2/Bax and caspase. Their crude extracts and various organosulfur compounds including alliin, allicin, DADS exhibited antioxidant properties, such as inhibition of lipid peroxidation and radical scavenging activity (Nicastro et al., 2015). Water and ethanol extracts of garlic and some compounds also showed immunomodulatory activity by stimulation of lymphocyte proliferation, macrophage phagocytosis, macrophage, and the release of cytokines such as IL2, IL12, TNF-α and IFNγ, and promotion of NK cell number and activity (Schäfer and Kaschula, 2014).

Table 1-1 presents traditional uses and pharmacological activities related to cancer chemoprevention of nine selected plants used in TTM. Most of them or their isolated compounds/fractions exhibited antioxidant properties or anti-cancer effects; such as inducing apoptosis and suppressing tumour growth. This shows that herbal medicine in TTM is a

potential source for cancer chemopreventive agents. However, as most studies were performed in specific types of tumour or cell lines, such as liver cancer, skin cancer, and prostate cancer, the information would be useful only for the prevention in high risk population or secondary prevention.

Since it is impractical and difficult to prevent cancer in all sites, we should have a strategy that is beneficial for cancer prevention in general. Based on the philosophy of holistic medicine like TTM, we believe that it is possible to develop such strategy. This question has led to the aims and development of the study plan of this thesis.

Table 1-1 Selected medicinal plants used in Thai traditional medicine: traditional uses and related pharmacological activities regarding chemoprevention

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
Aegle marmelos (L.) Corrêa (Thai name: matum)	Young fruit: antidiarrheal, antidysenteric, treatment of fever and fix wind element Mature fruit: nourish fire element Ripe fruit: promotes digestion, carminative, nourish fire element, relieve thirst Root: treatment of cough, asthma, phlegm, fever, abnormal vaginal discharge	Methanol extract of the whole plant	*In vivo*, 25 and 50 mg/kg BW orally, normal saline (con)	Pre-treatment of *A. marmelos* for 7 days suppressed lipid peroxidation, xanthine oxidase and release of serum toxicity marker enzymes namely SGOT, LDH, and SGPT in a dose-dependent manner in male Wistar rats. (Khan and Sultana, 2009). It also decreased the incidence of liver carcinogenesis initiated by diethylnitrosamine and promoted by 2-acetyl aminofluorene (2-AAF) in male Wistar rats, together with the ability to restore the level of glutathione, GST, glutathione peroxidase (GPx), inhibited MDA formation, early markers of tumour promotion; ODC activity and hepatic DNA synthesis (Khan and Sultana, 2011).
		Methanol extract of the leaf	*In vitro,* 0 – 110 µg/ml, ascorbic acid (pos)	The extract exhibited DPPH scavenging property with IC_{50} value of 46.7 µg/ml, whereas IC_{50} value of ascorbic acid was 56.2 µg/ml (Nahar et al., 2009).
		Petroleum ether fractions of ethanol extract from stem bark	Various cell lines, 0-500 µg/ml, ethanol extract from the fruit of *Emblica officinalis* (pos)	The fraction showed a strong inhibitory activity against cell proliferation of K562 leukemia cells (IC_{50} = 25 µg/ml), B-lymphoid Raji (IC_{50} = 5 µg/ml), but showed weak to moderate effects in T-lymphoid Jurkat, erythroleukemic HEL, melanoma Colo38, breast cancer MCF7 and MDAMB-231 cell lines (IC_{50} values were between 50 - 500 µg/ml) (Lampronti et al., 2003).
		Hydro-alcohol extract of the fruit	*In vivo*, 50 mg/kg BW/ day orally, water (neg)	Treatment of the extract, before and after DMBA application, significantly reduced the incidence of DMBA-induced skin papillomas to 70% of the control, as well as the size and weight of the papillomas in Swiss albino mice. It also prolonged the average latency period (time lag between the application of the promoter and the appearance of 50% of tumours) from 7.88 in control group to 9.3 – 10.3 weeks in treated groups (Agrawal et al., 2010).
		Ethanol extract of the leaf	*In vivo*, 400 mg/kg BW, normal saline (neg) *In vivo*, 50 and 100 mg/kg BW orally, water (neg), butylated hydroxyanisole (pos)	Administration of the extract once daily for six days increased the survival of tumour bearing mice up to 43 days after the tumour cell inoculation. No survivors in the saline control group (Jagetia et al., 2005). The extract enhanced the basal levels of antioxidant enzymes, such as GST, superoxide dismutase, catalase, GPx, and glutathione reductase in lung or liver of Swiss albino mice. It also elevated the levels of phase I enzymes, such as cytochrome P450 (Singh et al., 2000).

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
		β-caryophyllene and caryophyllene oxide fractions	Cell cultures, 25 – 300 µg/ml, DMSO (con)	The fraction down-regulated anti-apoptotic genes (Bcl-2, mdm2, COX-2 and cmyb) and up-regulated pro-apoptotic genes (Bax, Bak1, Caspase-8, Caspase-9 and ATM) in Jurkat cells (50 µg/ml) and IMR-32 cells (84.5 µg/ml) (Sain et al., 2014).
		Lupeol	Cell cultures, 20 – 80 µM, DMSO (neg) *In vivo*, 1 mg/animal i.p., corn oil (neg)	Lupeol exhibited cytotoxicity in non-metastatic melanoma cells WM35 (IC_{50} = 34 µM) and metastatic melanoma cells 451Lu (IC_{50} = 38 µM), induced apoptosis, PARP cleavage, caspase-3 activation, G1 cell cycle arrest, and caused a shift of Bax/Bcl-2 ratio toward apoptosis in 451Lu cells. In 451Lu xenograft nude mice, it significantly reduced and delayed tumour growth, suppressed cell proliferation, protein expression of cyclinD1, D2, Cdk2, Bcl-2, and induced p21 and PARP cleavage (Saleem et al., 2008).
			Cell culture, 1 - 20 µM, N/A	It increased the formation of dendrites in B16 2F2 melanoma cells and reduced the migration of human G361 melanoma and NB-1 neuroblastoma (Hata et al., 2005).
			Cell culture, 30 – 50 µM, DMSO:alcohol (con)	It inhibited cell proliferation of human pancreatic adenocarcinoma cells AsPC-1 and induced apoptosis, together with the reduction of the expression of Ras and NF-kB activation, and modulation of the protein expression of signalling molecules involved in PKCα/ODC, PI3K/Akt and MAPKs pathways (Saleem et al., 2005a).
			Cell culture, 10 – 150 µM, DMSO (con)	The compound inhibited A431 human epidermoid cancer cell proliferation with IC_{50} value of 75 µM, induced apoptosis, caused cell cycle arrest at sub G1, inhibited Akt/PKB signaling pathway and NF-κB activation (Prasad et al., 2009).
			Cell culture, 6.25 – 200 µM, DMSO (con)	It inhibited cell proliferation, induced apoptosis and expression of Caspase3 and FADD, while suppressed the expression of Death receptor 3 (DR3) in hepatocellular carcinoma SMMC7721 cell (Zhang et al., 2009).
			Cell culture, 1 – 30 µM, DMSO:alcohol (neg) *In vivo*, 1 mg/animal i.p., corn oil (neg)	Lupeol inhibited cell proliferation of human prostate cancer cells LNCaP and CWR22Rv1 with IC_{50} values of 21 and 18.5 µM, respectively. It also induced PARP cleavage, expression of FADD, Fas receptor–mediated apoptotic pathway. It also reduced tumour growth in nude mice implanted with CWR22Rv1 cells (Saleem et al., 2005b).
		Marmelin (1-hydroxy-5,7-dimethoxy-2-naphthalene-carboxaldehyde)	*In vivo*, 200 µg/kg BW i.p. 21 days, N/A Cell cultures, 0–500 µM, N/A	Marmelin reduced the volume, size, weight, angiogenesis, microvessel density, and CD31 (an endothelial cell specific surface marker) of colon cancer cell induced xenograft tumours in nude mice compared to control group. *In vitro*, the compound at 50 µM significant inhibited the cell proliferation of HCT-116 colon and HEp-2 cells, while it did not show the effect in normal mouse embryo fibroblasts up to 500 µM. At 10 -25 µM, marmelin induced apoptosis, caspase 3 protein

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
				expression, sub-G1/G0 cell cycle arrest, expressions of TNF-α, TNFR-1, TRADD and caspase-8 in Hep-2 cells. At 125 nM, it suppressed Akt and Erk phosphorylation, NF-kB activation and IL-8 mediated by TNF-α in HCT-116 cells (Subramaniam et al., 2008).
Atractylodes lancea (Thunb.) DC. (Thai name: kodkhamao)	Dried rhizome: treatment of diseases in oral cavity and neck, asthma, fix Uthankhamavata (see Figure 1-6) that causes shaking and aching	Ethanol extract	Cell culture, 1.95 – 250 µg/ml, 5-FU (pos) *In vitro*, N/A, N/A	The extract showed promising cytotoxicity against CL-6 cholangiocarcinoma and Hep-2 human laryngeal carcinoma with IC_{50} values of 24 and 29 µg/ml, respectively, while the effect was moderate in HepG2 human hepatocarcinoma cells (IC_{50} = 77 µg/ml) and weak in normal human epithelial cell HRE (IC_{50} = 208 µg/ml) (Mahavorasirikul et al., 2010). The extract inhibited LPS-induced nitric oxide production in RAW 264.7 cells with IC_{50} values of 9.7 µg/ml (Kakatum et al., 2012).
		Essential oil and hinesol	Cell culture, 2.5 – 50 µg/ml and 5 – 100 µM, N/A	Essential oil and hinesol, inhibited the proliferation of HL-60 cell with IC_{50} values of 9.6 µg/ml and 4.9 µg/ml (= 22.1 µM), respectively. Hinesol induced apoptosis, activated JNK and ERK (at 100 µM), but not p38 (Masuda et al., 2015).
		Prenylated dihydrobenzofuran derivative (s)	Cell culture, N/A, 10-Hydroxycamptothecin (pos)	Trans-2-Hydroxyisoxypropyl-3-hydroxy-7-isopentene-2,3-dihydrobenzofuran-5-carboxylic acid showed cytotoxicity against HCT-116 and MKN-45 cancer cells with IC_{50} values of 0.4 and 0.5 µM, respectively (Duan et al., 2008).
		Phenol derivative (s)	*In vitro*, N/A, NDGA (pos; 5-LOX) and indomethacin (pos; COX-1)	2-[(2'E)-3',7'-Dimethyl-2',6'-octadienyl]-4-methoxy-6-methyl-phenol showed potent inhibitory activity against 5-LOX and COX-1 with IC_{50} values of 0.1 and 1.8 µM, respectively (Resch et al., 2001).
		β-eudesmol	Cell culture, 2.5 – 50 µg/ml, N/A Cell culture, 10 – 100 µM, 0.1%DMSO (con) *In vivo*, 100 and 200 mg/kg BW, 5-FU (pos)	It inhibited the proliferation of HL-60 cell with IC_{50} value of 10.4 µg/ml (= 46.8 µM) (Masuda et al., 2015). It inhibited the proliferation of HL-60 cell with IC_{50} value of 35.1 µM, induced apoptosis via the mitochondrial apoptosis pathway, activated cleavage of caspase-3, caspase-9, and PARP, JNK signalling, downregulated of Bcl-2 expression; caused a release of cytochrome c, and suppressed MMP (Li et al., 2013b). Treatment with 100 mg/kg BW of β-eudesmol for 30 days reduced tumour growth to less than 10% compared to the control group in CCA-xenografted mice. It also prolonged survival time

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
				from 43 days in control group to 71 days, while 5-FU prolonged survival time to 54 days. In addition, it decreased metastasis from 90% to less than 5% of total lung mass (Plengsuriyakarn et al., 2015).
			Cell culture, 10 – 100 µM, 0.1%DMSO (con) *In vivo*, upto 300 µM, 0.3%DMSO (con), thalidomide (pos)	It inhibited the proliferation of porcine brain microvascular endothelial cells and HUVEC, HUVEC migration, the phosphorylation of ERK 1/2. In mice, the compound inhibited angiogenesis in subcutaneously implanted Matrigel plugs in C57BL/6J mice and in adjuvant-induced granuloma in ddY mice (Tsuneki et al., 2005).
			Cell culture, 10 – 180 µM, nifuroxazide (pos)	The compound showed weak cytotoxicity against (IC_{50} = 167 µM). At 180 µM, it suppressed 2D cell migration, HMOX-1, P-STAT-1 and NF-kB protein expression in time-dependent manner (Mathema et al., 2017).
			Cell culture, 10 – 100 µM, 0.1%DMSO (con), cisplatin (pos) *In vivo*, 2.5 – 5 mg/kg BW, 0.5% CMC-Na containing 0.5% ethanol (con), thalidomide (pos)	The treatment inhibited HUVEC proliferation and CREB phosphorylation stimulated by VEGF and/ or basic fibroblast growth factor (bFGF), proliferation of cancer cell lines HeLa, SGC-7901, and BEL-7402 cells. In mice implanted with H_{22} and S_{180} mice tumour cells, the treatment suppressed the tumour growth, and angiogenesis (Ma et al., 2008).
		n-Hexane extract and its isolated compounds	*In vitro*, N/A, ethanol (con), indomethacin and nordihydroguaiaretic acid (pos)	The crude extract and its isolated compound; atractylochromene and quinone, exhibited potent inhibitory activities against 5-LOX with IC_{50} values of 2.9 µg/ml, 0.6 µM, and 0.2 µM, respectively. Atractylon and osthol also showed moderate activity against 5-LOX with IC_{50} values of 25 µM and 36 µM, respectively. Atractylochromene also showed strong effect against COX-1 (IC_{50} = 3.3 µM), while the crude extract and quinone showed less potent activity with IC_{50} values of 30 µg/ml and 64 µM, respectively. Other isolated compounds; atractylon, osthol, atractylenolide I – III, showed weak effect with IC_{50} values more than 200 µM (Resch et al., 1998). This shows selective activity against 5-LOX of atractylon and osthol.
Coccinia grandis (L.) Voigt (Thai name: tam lueng)	Root and stem: treatment of fever and *fi* (abscess)	Water extract of the leaf and stem	*In vivo*, 50 – 200 mg/kg BW, saline (con) and diclofenac (pos)	All doses of both extracts reduced carrageenan-induced paw oedema in Wistar rats (Deshpande et al., 2011).

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
Ferula assa-foetida L. (Thai name: mahahing)	Resin from root: treatment of constipation, stomach pain, flatulence, *achinarok* (conditions caused by eating prohibited food), aid digestion, fix abnormal elements, cleanse *semha* and *lom*, nourish fire element, swelling	Ethanol extract (unknown part)	*In vivo*, 6.25 and 12.5 mg/kg BW, 1 mM EDTA (con)	All doses of extract reduced the effect of 1,2-dimethyl hydrazine (DMH) by increasing the level of GSH, FRAP, and GST and reducing level of CYPs, β-catenin, and ACF formation in DMH-induced colon carcinogenesis in Wistar rats (Torabi et al., 2015).
		Chloroform fraction of methanol extract of resin and conferol	Cell culture, N/A, doxorubicin (pos)	The chloroform fraction exhibited promising cytoxicity in all cell lines tested with IC_{50} values less than 20 µg/ml. Conferol; a sesquiterpene coumarin, which was isolated from the fraction, showed potent cytotoxicity with IC_{50} values of 0.51, 2.6, and 3.4 µg/ml in HepG2, Hep3B, and MCF-7 cells, respectively (Lee et al., 2009).
		Farnesiferol C	*In vivo*, 0.1 and 1 mg/kg BW daily i.p. for 2 days, PBS (con) *In vitro*, 10 – 40 µM, N/A	In Lewis lung cancer cells (LLC)-inoculated C57BL/6 mice, the treatment reduced the tumour weight, microvessel density, and Ki67 index (proliferation index). *In vitro*, the compound countered the effect of VEGF-A in HUVECs cells by suppression of the induced proliferation (IC_{50} ~15 µM), induced tube formation (IC_{50} = 20 µM), induced 2D-migration (IC_{50} ~ 20 µM), induced invasion in Boyden chamber assay (IC_{50} < 20 µM), secretion of pro–MMP-2 (IC_{50} ~ 20 µM), and induced vessel sprouting (IC_{50} ~ 10 µM). Its mechanisms on the VEGF-VEGFR signalling pathway involved with the inhibition of VEGF binding, VEGFR1 autophosphorylation, ERK1/2, p38, MAPK, and JNK (Lee et al., 2010).
		Umbelliprenin	Cell culture, 25 – 100 µM, 0.5% DMSO (con), staurosporine (pos) *In vivo,* 0.0025% in drinking water, curcumin (pos) Cell culture, 31 µM, 0.15%DMSO (con)	The compound induced apoptosis in Jurkat and Raji leukemia cell lines, but not in PBMC. This effect was not inhibited by IL-4 (Ziai et al., 2012). Pre-treatment with water supplemented with umbelliprenin delayed the formation of papilloma and reduced the number of tumours in TPA-induced papilloma in SENCAR mice. The latter effect was similar to that of curcumin (Iranshahi et al., 2009). Using proteomics approach, treatment with umbelliprenin resulted in differential expression of 45 proteins in QU-DB cells and 70 proteins in A549 cells. In QU-DB cells, downregulatory effect on some tumourigenic proteins, such as heat shock protein, and upregulatory effect on some anti-tumour proteins, such as Nipsnap1, suggested that the compound is a promising anti-cancer agent. However, the downregulation of the tumourigenic proteins; cyclophilin and tumour suppressor MST, in A549 cells contradicted the results in QU-DB cells (Khaghanzadeh et al., 2016).

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
		Galbanic acid	Cell culture, 3.1 – 100 µM, cisplatin (pos)	The compound inhibited the proliferation of ZIPneo and Hras-F cells with IC_{50} values of 58 and 16 µM, respectively. In enzyme-based assay, it inhibited FTase protein (the protein that catalyses posttranslational modifications of Ras protein) with IC_{50} value of 2.5 µM (Cha et al., 2011).
Ligusticum striatum DC. (synonym *L. chuanxiong*) (Thai name: kodhuabua)	Dried rhizome: treatment of problems of wind element and distribute *lom*	Essential oil extracted from the rhizome	*In vitro*, 0.32 – 250 µg/ml, N/A	The essential oil exhibited DPPH and ABTS scavenging activities with IC_{50} values ~ 8 and 1.6 µg/ml, respectively. At 250 µg/ml, the extract protected against UVB-induced damage in mouse skin fibroblast cells NIH 3T3 by reduction of DNA migration, H2A.X phosphorylation, p21 protein expression, and restoration of cyclin D expression (Jeong et al., 2009).
		Supercritical fluid CO_2 extract	*In vivo,* 50, 100 mg/kg/day orally, saline (con), vitamin E (pos) *In vitro*, 25 – 400 µg/ml, vitamin E (pos)	The treatment countered the damages caused by D-galactose injection by reduction of elevated serum ALT, AST, BUN, CRE, hepatic and renal MDA level, increasing activity and gene expression of various antioxidant enzymes; Cu, Zn-SOD, CAT, GPx in liver and kidney, reversal of liver and kidney injury, and reduction of COX-2 and iNOS protein expressions in liver and kidney. However, the extract showed weak DPPH, ABTS, and oxide anion radicals scavenging activity with IC_{50} values of 353, 201, and 707 µg/ml, respectively, as well as weak reducing power with IC_{50} values of 895 µg/ml in *in vitro* (Mo et al., 2017).
		Butylidenephthalide derivatives (BPDs)	Cell culture, 1 – 100 µg/ml, N/A	Seven out of eight BPDs isolated from 95% ethanol extracts showed potent to moderate cytotoxicity with IC_{50} values ranging from 3 to 87 µg/ml in human large-cell lung cancer H460, human liver cancer SMMC7721, and human gastric cancer BGC823 cell lines. In SMMC7721 cells, compound 5 (1.23 µg/ml) and 6 (0.4 µg/ml) reduced cell adhesion, migration, invasion, MMP-2 and MMP-9 expression (Hu et al., 2015).
		Levistolide A	Cell culture, 0 – 200 µM, DMSO (con), 5-FU (pos)	The compound inhibited the cell proliferation and induced apoptosis of both colon cancer HCT116 wild type and HCT116 p53-/- cells. At 50 and 100 µM, it induced caspase-3, PARP cleavage, ROS generation, the expression of ER stress-related proteins in HCT116 wild type. Therefore, anti-cancer effect in colon cancer cells of levistolide A involved with ROS-mediated ER stress pathway (Yang et al., 2017).
Phyllanthus emblica L.	Young fruit: nourish muscle and voice, treatment of constipation, worm in *semha*	Fruit powder	*In vivo*, 20 mg/kg BW orally, N/A	In Dalton's lymphoma ascites (DLA)-tumour bearing BALB/c mice, the treatment increased splenic NK cells activity, antibody dependent cellular cytotoxicity (ADCC), and mean survival time from 20 to 27 days after the tumour cells inoculation (Suresh and Vasudevan, 1994).

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
(Thai name: makhampom)	Mature fruit: treatment of fever	Whole extract of the fruit	*In vivo*, 100 mg/kg BW orally, 10% sucrose (con) Cell culture, 10 – 1000 µg/ml, water (con)	*In vitro,* the extract (300 µg/ml) induced autophagy by upregulation of the expression of autophagic proteins- beclin1 and LC3B-II in OVCAR3 and SW626 cells. It also downregulated angiogenesis-related genes in OVCAR cells; COL4A3, CXCL6, ECGF1, EFNB2, FGF2, IL-1β, PDGFB, TNFRSF12A, and HIF-1α, to less than 40% of the control. Addition of cisplatin to the extract treatment presented synergistic effects in autophagy induction and angiogenesis inhibition. *In vivo,* the treatment reduced the tumour growth and mass, and angiogenesis, and induced autophagy in OVCAR3-induced tumour in nude mice (De et al., 2013).
		Water extract of the fruit	Cell culture, 10 – 300 µg/ml, N/A	The extract inhibited AP-1 DNA binding activity by downregulating the protein expressions of AP-1 family members; c-Jun, JunB, JunD and c-Fos, cell proliferation, and induced apoptosis in two cervical cancer cell lines SiHa (HPV-16 positive) and HeLa (HPV-18 positive). Inhibition of AP-1 has been associated with reduced HPV transcription (Mahata et al., 2013).
			Cell culture, 6.25 – 400 µg/ml, taxol (pos) *In vivo*, 1 – 4 mg/ 0.1 ml acetone tropically, N/A	The extract showed moderate cytotoxicity in various human cancer cell lines; HepG2 (liver), HeLa (cervical), SW620 (colorectal), MDA-MB-231 (breast), and SKOV3 (ovarian) with IC_{50} values between 46 – 106 µg/ml, but no cytotoxicity in normal lung fibroblast with IC_{50} values > 400 µg/ml. At 50 - 200 µg/ml, the extract induced apoptosis in HeLa through extrinsic caspase-8-dependent pathway, while it acted by activating the death receptor Fas/caspase-8-dependent apoptosis pathway in A549, HeLa and SKOV3 cells. In two-stage mouse skin tumourigenesis model using DMBA (initiator) and TPA (promoter), the treatment reduced more than 50% of tumour numbers and volumes over a 20-week period. At 25 – 50 µg/ml, the extract suppressed MDA-MB-231 cells invasion through Matrigel (Ngamkitidechakul et al., 2010).
			In vivo, 100 mg/kg BW orally, water (con) *In vitro*, 1 – 100 µg/ml, vitamin C (pos)	*In vitro*, the extract, at 1 and 25 µg/ml, showed stronger inhibition of $O_2^{-\bullet}$, H_2O_2, NO activity than 100 µM vitamin C. In DEN-treated rats, the extract reduced liver ROS level, bile $O_2^{-\bullet}$, H_2O_2, NO production, plasma ALT, AST, and γ-GT levels (intragastric), infiltrated leukocytes in liver, oxidative stress. It also reversed the effect of DEN treatment by restoring MnSOD and catalase, and suppressing iNOS and CYP2E1 protein expressions in the liver. Moreover, the treatment of the extract after DEN induction suppressed Bax/Bcl-2 ratio, PARP, and Beclin-1 expression which confirmed the ability of the extract in the inhibition of apoptosis and autophagy induced by DEN (Chen et al., 2011).
			In vitro, 5 – 100 µg/ml, N/A	At 50 and 100 µg/ml, the extract decreased the level of ROS and lipid hydroperoxides, while induced GSH, SOD, catalase, GPx, GR, GST and total antioxidant capacity (the ability of antioxidants in the cell lysate to inhibit $ABTS^+$ formation) in HepG2 cells (Shivananjappa and Joshi, 2012).

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
			In vitro, 12.5–75 µg/ml, silymarin/ quercetin (pos)	The extract protected from t-BHP-induced cell death, with the concentration that gave 50% protection of 32.4 µg/ml, and inhibited AAPH-induced fluorescence in HepG2 cells. It exhibited ABTS and DPPH radical scavenging activities with IC_{50} values of 5 and 12 µg/ml, respectively (Hiraganahalli et al., 2012).
			In vitro, 1 – 50 µg/ml, N/A	The extract inhibited cell proliferation of HT1080 human fibrosarcoma cells at 24, 48, and 72 with IC_{50} values of 48, 3.5, and 3 µg/ml, respectively. At 2 and 3 µg/ml, it down-regulated MMP2 and MMP9 expression in the cells after 5 days-incubation. It also showed anti-migration and anti-invasion activity at high dose; IC_{50} values of 640 and 750 µg/ml, respectively, after 4 hour-incubation (Yahayo et al., 2013).
			In vivo, 100 mg/kg BW orally, water (con)	Administration of the extract decreased tumour incidence, yield, burden and cumulative number of papilloma in DMBA-induced papilloma in Swiss albino mice (Sancheti et al., 2005).
			In vivo, 500 mg/kg BW orally, water (con)	The extract reversed arsenic-induced oxidative stress by reduction of serum SGOT, serum SGPT, liver lipid peroxidation, enhancing serum alkaline phosphatase, liver SOD, liver catalase, and liver GST in Swiss albino mice. It also reduced pathological alterations after sodium arsenite treatment (Sharma et al., 2009).
		Methanol extract of the fruit	*In vivo*, 50 – 200 mg/kg BW by gastric intubation, mineral oil (con)	In DMBA-induced oral squamous carcinogenesis in hamsters, DMBA treatment caused a decrease in SOD, CAT and TBARS levels and increase in GSH, Vit E, and GPx in buccal pouch. In plasma, DMBA reduced SOD, CAT, GPx, Vit C, Vit E, GSH and elevated TBARS level. The effects were reversed after the extract treatment (Krishnaveni and Mirunalini, 2012).
		Methanol extract of the fruit (defatted)	*In vivo*, 100 and 200 mg/kg BW orally, saline (con)	In hepatic carcinogenesis model in Wistar rats, the extract recovered the pathological manifestation, γ-GT-positive foci, elevated hepatic ODC activity, elevated GST, elevated γ-GT, and GSH level which were results from DEN initiation and 2-AAF promotion with partial hepatectomy (PH) (Sultana et al., 2008).
		Methanol extract of the fruit (defatted)	*In vivo*, 100 and 200 mg/kg BW orally, saline (con)	Pre-treatment with the extract decreased thioacetamide-induced oxidative stress in Wistar rats by lowered elevated SGOT, SGPT and γ-GT in serum, elevated GST, GR, G6PD in liver, MDA formation, and recovered hepatic GSH and GPx, as well as inhibition of ornithine decarboxylase (ODC) (Sultana et al., 2004).
		Ethyl acetate fraction of 95% ethanol extract	*In vivo,* 500 mg/kg BW orally, 2% gum acacia (con)	Co-treatment with arsenic and the extract reduced the toxicity of arsenic by increasing thymus weight and cellularity, levels of GSH, activity of SOD, catalase, and cytochrome c oxidase, mitochondrial membrane depolarization, while decreasing lipid peroxidation, ROS generation, number of cells in sub G1 phase, and number of necrotic and apoptotic cells in thymus as

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
			In vitro, 1 – 40 µg/ml, N/A	compared to those treated with arsenic alone. *In vitro*, the extract inhibited DPPH radicals with IC_{50} value of 8.32 µg/ml (Singh et al., 2013).
		Polyphenol fraction	*In vivo*, 60 mg/kg BW orally, N/A *In vitro,* 0 – 200 µg/ml, N/A	The treatment reduced NDEA-induced liver tumour development, elevated levels of serum γ-GT, ALP, GPT, bilirubin, hepatic GST, and GSH in rats. *In vitro*, the fraction induced apoptosis in Dalton's Lymphoma Ascites and CeHa cell lines. In addition, it scavenged superoxide and hydroxyl radicals and inhibit lipid peroxidation (Rajeshkumar et al., 2003).
Piper nigrum L. (Thai name: phrikthai)	Fruit (peppercorn), leaf, root: nourish fire element, treatment of *semha* and wind element	Black pepper	*In vivo*, 0.5 – 2% in diet, N/A	Diet containing 1 and 2% black pepper increased GST, -SH, cyt b5, and CYPs level, while inhibited MDA in Swiss albino mice (Singh and Rao, 1993).
		Dichloromethane extract of the fruit	*In vivo*, 100 – 400 mg/kg BW orally, tween 80 (con) *In vitro*, 7.45 µg/ml, N/A	All doses of extract reduced tumour volume, up-regulated p53, down-regulated estrogen receptor, E-cadherin, MMP-9, MMP-2, c-Myc, and VEGF levels in N-nitroso-N-methylurea (NMU)-induced mammary tumourigenesis rats. In human breast cancer cell lines MCF-7, the extract induced protein expression of E-cad, VEGF and c-Myc, but not p53 (Deng et al., 2016).
		Diethy ether fraction of dichloromethane extract of the fruit (piperine-free)	*In vivo*, 100 – 200 mg/kg BW orally , tween 80 (con) *In vitro*, N/A, N/A	Administration of the extract reduced tumour incidence (20%) and weight in NMU-induced mammary tumorigenesis in Sprague-Dawley rats. *In vitro*, the extract exhibited promising cytotoxicity in breast cancer cell lines; MCF-7, MDA-MB-468, and ZR-75-1, with IC_{50} value of 7.5, 18, and 14 µg/ml, respectively, but weaker effect in other cell lines tested (IC_{50} > 20 µg/ml); including normal breast cells, neuroblastoma, colorectal carcinoma, lung carcinoma, and mouse fibrosarcoma. It induced apoptosis by upregulation of p53 and cyt C, while down-regulation of topo II in the MCF-7 cells (Sriwiriyajan et al., 2016).
		Water extracts of the fruit	Cell culture, 10 – 100 µg/ml, vehicle, ConA, LPS (con)	In splenocytes isolated from mice, the extract induced cell proliferation and IFNγ release, but suppressed the release of Th2 cytokines; IL-4 and IL-10, in the presence of ConA. It also promoted the cytotoxic activity of NK cells. In peritoneal macrophages, it increased the release of pro-inflammatory cytokines; IL-6 and TNFα, and NO production in the presence of IFNγ (Majdalawieh and Carr, 2010).
		Piperine	Cell culture, 10–150 µM, DMSO or medium (con)	The compound showed moderate and selective cytotoxicity to colon cancer cells; HT-29 (IC_{50} = 53 µM), Caco-2 (IC_{50} = 54 µM), SW480 (IC_{50} = 126 µM), HCT-116 ($p53^{+/+}$) (IC_{50} = 109 µM), and HCT-116 ($p53^{-/-}$) (IC_{50} = 118 µM). The cytotoxic effect in HT-29 cells are associated with cell cycle arrest at G1 phase, suppression of cyclins D1 and D3 expression, cyclin-dependent

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
				kinases 4 and 6, phosphorylation of the retinoblastoma protein, and promotion of p21/WAF1 and p27/KIP1 expression. It induced apoptosis by induction of ROS generation, loss of mitochondrial membrane integrity, PARP cleavage, caspase activation, and the expression of endoplasmic reticulum stress-associated proteins, c-Jun N-terminal kinase and p38 mitogen-activated protein kinase, as well as inhibition of the phosphorylation of Akt and surviving expression. It also suppressed colony formation and the growth of HT-29 spheroids (Yaffe et al., 2015).
			In vivo, 2.5 – 5 mg/kg BW, N/A Cell culture, 35 – 280 μM, N/A	Injection of piperine into 4T1 tumour, which was inoculated into BALB/c mice, suppressed the tumour growth and lung metastasis. Piperine inhibited cell growth and migration, induced apoptosis, activated caspase 3, caused cell cycle arrest at G2/M phase with a reduction of cyclin B1 expression, and suppressed MMP-9 and MMP-13 expression in mouse mammary carcinoma 4T1 cells (Lai et al., 2012).
			Cell culture, 10 – 200 μM, vehicle (con)	Piperine inhibited cell proliferation, induced apoptosis in HER2-overexpressing breast cancer cells by activation of caspase-3 and PARP cleavage, downregulation of HER2 expression, suppression of cell migration by inhibition of MMP-9 expression, AP-1 and NF-kB activation, and interruption of ERK1/2, p38 MAPK, and Akt signalling pathways (Do et al., 2013).
			Cell culture, 25 – 200 μM, N/A	The compound inhibited proliferation, migration, invasion by suppressing MMP-2 and -9 expression in human osteosarcoma cells HOS and U2OS cells (Zhang et al., 2015).
			In vitro, 3.9 – 250 μM, N/A	At high concentration, piperine inhibited *H.pylori* growth (IC_{50} = 115 μM) and adhesion to gastric epithelial cells (Tharmalingam et al., 2014).
			In vivo, 50 mg/kg BW orally, corn oil (con)	In B(a)P-induced lung carcinogenesis in Swiss albino mice, administration of piperine reversed the effect of tumour by increasing the activities of mitochondrial enzymes; isocitrate dehydrogenase (ICDH), ketoglutarate dehydrogenase (KDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH), glutathione-metabolizing enzymes in lung and liver; GR, GPx, G6PD, and suppressing phase I enzymes in lung and liver; CYPs, cytochrome b5, NADPH-c reductase compared to benzo(a)pyrene-treated group (Selvendiran et al., 2005b). It also increased phase II enzymes; UDP-GT, QR and GST, and reduced DNA-damage in lung cancer-bearing mice (Selvendiran et al., 2005a).
			In vivo, 200 μmol/kg BW i.p., N/A Cell culture, 10 – 100 μg/ml, N/A	Piperine treatment remarkably increased survival rate, reduced number of lung nodules, lung metastatic markers; hydroxyl proline, uronic acid, and hexosaline, serum sialic acid (a marker for metastatic ability) and γGT (a cell proliferation marker) of mice bearing lung metastasis induced by B16-F10 melanoma cells. *In vitro*, the compound inhibited B16-F10 melanoma cell proliferation (Pradeep and Kuttan, 2002).

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
			In vitro and *ex vivo*, 10 – 100 μM, vehicle (con)	At 100 μM, the compound reduced HUVECs migration, tubule formation, collagen-induced angiogenesis, and MDA-MB-231-induced angiogenesis in chick embryo chorioallantoic membrane (CAM) (Doucette et al., 2013).
			In vitro, 5 – 100 μM, 0.1%DMSO (con)	Piperine inhibited 2D-cell migration, MMP-9 expression, PMA-stimulated cell invasion, PMA-stimulated NF-kB and AP-1 expression in fibrosarcoma HT-1080 cells, but not MMP-2 (Hwang et al., 2011).
			In vitro, 1 – 50 μM, verapamil, ketoconazole (pos)	Piperine inhibited digoxin and cyclosporine A transport in Caco-2 cells and CYP3A4 in human liver microsomes (Bhardwaj et al., 2002).
			Cell culture, 2.5 - 10 μg/ml, N/A	It inhibited the cell proliferation, invasion, proinflammatory cytokines; L-1β, IL-6, TNF-α and GM-CSF, and NF-kB in B16F-10 melanoma cells (Pradeep and Kuttan, 2004).
			Cell culture, 10 - 10 μM, N/A	The compound suppressed PMA-induced COX-2 expression by downregulating NF-kB, C/EBP, and AP-1 signaling pathways in RAW 264.7 murine macrophages (Kim et al., 2012).
Saussurea costus (Falc.) Lipsch. (synonym: *Aucklandia lappa* DC., *Saussurea Lappa* Clarke) (Thai name: *kodkraduk*)	Root: treatment of *semha* problems, *lom ammapruek* (causes nausea, colic, loss of hearing, and faint)	Methanol extract	*In vivo*, 300 mg/kg BW orally, water (con)	The extract exhibited hepatoprotective activity by attenuation the effect of deltamethrin (toxic pyrethroid insecticide) by reduction of serum ALT, AST, ALP, and γGT, promotion of serum SH protein, GST, SOD, and CAT in albino rats (Alnahdi et al., 2016).
		Alantolactone	Cell culture, 0 – 15 μM, N/A	The compound suppressed both constitutive and inducible STAT3 activation and translocation in triple-negative breast cancer MDA-MB-231 cells (Chun et al., 2015).
			Cell culture, 20 – 80 μM, N/A	Alantolactone showed promising cytotoxic effect in human lung cancer SKMES-1 cells with IC_{50} value of 40 μM. It induced apoptosis, together with the induction of caspase-8,-9,-3 activation and inhibition of Bcl-2, caused cell cycle arrest at G1/G0 phase, and activated PARP (Zhao et al., 2015).
			Cell culture, 40 μM, 1%DMSO (con)	The IC_{50} value for HepG2 cell proliferation inhibition was 33 μM and induced apoptosis by GSH depletion, inhibition of STAT3 activation, induction of ROS generation, reduction of mitochondrial transmembrane potential, increasing of Bax/Bcl-2 ratio, and caspase-3 activation (Khan et al., 2013).
			Cell culture, 1 – 10 μM, N/A	This compound inhibited iNOS and COX-2 expression, NO, PGE_2, TNF-α, NF-κB activation and MAPKs phophorylation (Chun et al., 2012).

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
			In vivo, 25 – 100 mg/kg BW orally, N/A *In vitro,* 5 – 20 μM, N/A	In C57BL/6 mice, alantolactone inhibited CYP3A4 and CYP2C19. In liver microsomes, the compound exhibited a potent inhibition of CYP3A4 (IC_{50} = 3.6 μM), moderate inhibition of CYP2C19 (IC_{50} = 37 μM), but no inhibition for CYP2A6, CYP2C9, and CYP2D6. It also suppressed gene expression of CYP3A1 and CYP2C39, but not CYP2A4, CYP2D22, and CYP2C37 (Qin et al., 2015).
			Cell culture, 1- 10 μM, N/A	The treatment induced antioxidant enzymes; QR, GST, γ-GCS, GR, and HMOX-1, and Nrf2 nuclear translocation in Hepa1c1c7 mouse hepatoma cells (Seo et al., 2008).
			Cell culture, 1 – 50 μM, N/A	The IC_{50} value for glioblastoma cells U87, U251, U118, and SH-SY5Y proliferation inhibition were 20, 16, 29, and 24 μM, respectively. It also suppressed cell migration, invasion, induced the expression of caspase-3, -9, Bax, PARP cleavage, while decreased Bcl-2 expression of U87 and U251 cells (Wang et al., 2017b).
		Isoalantolactone	Cell culture, 1 – 10 μM, tBHQ (pos)	The treatment induced QR activity to almost two folds in Hepa1c1c7 and its mutant BPRc1 cells. It also increased protein expressions of GR, γ-GCS, GST, and HMOX-1 (β-tubulin was used as a loading control) and Nrf2 nuclear translocation in Hepa1c1c7 and BPRc1 cells (Seo et al., 2009).
Terminalia chebula Retz. (Thai name: *samothai*)	Unripe fruit: treatment of blood problems	Methanol extract of the fruit	*In vitro*, 0.1 – 10 μg/ml, baicalein and curcumin (pos)	The extract protected against OGD-R induced and H_2O_2-induced cell death, reduced lipid peroxidation in thiobarbutic assay in rat pheochromocytoma cells (PC12) cells. It also reduced LPS-induced cell death and NO production in BV2 cells and scavenged DPPH free radical (Gaire et al., 2013).
			In vitro, 0 – 120 μg/ml, *In vivo,* 50 – 200 mg/kg BW orally, saline (con), desirox (pos)	It exhibited considerable iron chelation activity and DNA protection *in vitro*. *In vivo*, it reduced the elevated ALT, AST, alkaline phosphatase, and bilirubin caused by iron-dextran, as well as alleviated the decreased levels of liver SOD, CAT, GST, and non-enzymatic GSH in Swiss albino mice (Sarkar et al., 2012).
		Methanol fraction of the total extract	*In vivo*, 25 and 50 mg/kg BW gavage, saline (con)	The treatment protected from Fe-NTA-induced and DEN-induced renal carcinogenesis and oxidative damage in Wistar rats by restoration of the levels of GSH and cellular protective enzymes and reduction of MDA formation and hydrogen peroxide content. Moreover, it inhibited ornithine decarboxylase activity and DNA synthesis (Prasad et al., 2007).
			In vivo, 25 and 50 mg/kg BW gavage, saline (con)	The pretreatment suppressed $NiCl_2$-induced damage by reduction of the elevated GSH, GST, GR, LPO, H_2O_2 generation, BUN, serum creatinine, DNA synthesis, and ODC activity, as well as, the decreased GPx (Prasad et al., 2006).

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
		Water extract of the fruit	Cell culture, 50 μg/ml, N/A	After 5 hours-treatment in lymphoma Jurkat cells, the extract suppressed protein expressions of β-tubulin, RCHY1, IGF1R, and HSP70, which may negatively affect lymphoma cells. IPA analysis also identified interacting proteins that were associated in the inhibition of NF-kB signalling by the extract (Das et al., 2012). This proteomic study confirmed the result from (Das et al., 2011) that reported NF-kB inhibitory activity of the extract.
			In vitro, 0.5 - 250 μg/ml, N/A	The extract showed strong DPPH radical scavenging activity with IC_{50} value of 1.52 μg/ml and inhibited $CuSO_4$-induced lipid peroxidation. In RAW 264.7 cells, NO production and expressions of iNOS and COX-2 induced by LPS were suppressed by the extract (from 50 μg/ml). In vascular smooth muscle cells, the extract (from 50 μg/ml) inhibited cell proliferation and migration induced by platelet-derived growth factor (PDGF-BB) (Lee et al., 2015b).
			In vivo, 200 mg/kg BW orally, water (con)	The extract reduced mitochondrial lipid peroxidation, lipofuscin (LF), protein carbonyls (PCO), (xantine oxidase) XO in liver and kidney of both young rats and aged rats. In young rats, the treatment enhanced hepatic GST, G6PDH, GSH and renal GST and GSH. In old rats, it promoted MnSOD, GR, GST, G6PDH, GSH, vitamin C, and vitamin E in liver and kidney, while suppressed CAT and GPx in liver and kidney (Mahesh et al., 2009).
		Ethanol extract of the fruit	*In vivo,* 500 mg/kg BW, N/A	Treatment with the extract protected isoproterenol-induced lipid peroxidation in plasma and heart tissue. Isoproterenol caused a reduction in serum GSH, ceruloplasmin, vitamin E, vitamin C, iron, PIBC, heart GSH, and antioxidant enzymes in heart tissues; GPx, GR, GST, SOD, and CAT in Wistar rats. The extract reversed these effects significantly (Suchalatha et al., 2005).
			In vitro, N/A, N/A	IC_{50} values for COX-1 and COX-2 inhibition were 90 and 3.75 μg/ml, respectively (Reddy et al., 2009).
		Chebulic acid	*In vitro*, 0.5 - 250 μg/ml, N/A	It showed strong DPPH radical scavenging activity with IC_{50} value of 1.1 μg/ml and inhibited $CuSO_4$-induced lipid peroxidation (Lee et al., 2015b).
			In vitro, 1 – 100 μg/ml, EGCG (pos)	The compound showed a comparable ferric reducing antioxidant activity to EGCG. It decreased t-BHP-induced oxidative stress by reduction of %cell death, intracellular ROS, MDA formation, and GSSH/total glutathione ratio in primary rat hepatocytes (Lee et al., 2007).
		Chebulagic acid	Cell culture, 0.001 – 100 μM, N/A	In retinoblastoma cells Y79, IC_{50} value for cell proliferation inhibition was 50 μM. At 50 μM, the compound inhibited IkBα, which results in the suppression of NF-kB nuclear translocation (Kumar et al., 2014).

Plants	Traditional uses (from *Khamphi Sappakhunya*)	Extracts/ active ingredients	Model, doses, controls	Chemopreventive effect
			In vitro, 5 – 50 μM, N/A	The compound exhibited promising cytoxicity towards various cancer cell lines; HCT-15 (colon), COLO-205 (colon), MDA-MB-231 (breast), DU-145 (prostate) and K562 (chronic myeloid leukemia) with IC_{50} values of 20, 18, 26, 28, and 30 μM, respectively. It induced apoptosis and PARP cleavage in COLO-205 cells. It also strongly suppressed COX-2 and 5-LOX with IC_{50} values of 0.92 and 2.1 μM, respectively, and weaker inhibited COX-1 (IC_{50} = 15 μM) (Reddy et al., 2009).
			In vitro, 1 – 50 μM, N/A	The compound possessed antiangiogenic property by reduction of sprouting in rat aortic rings, downregulation of CD31 production, E-selection, and VEGF in HUVEC cells (Athira et al., 2013).

Note: con = control or vehicle, pos = positive control

Table 1-1 presents existing data for TTM drugs in the context of their use in cancer prevention. These medicinal plants exhibit promising activities to be developed to cancer prevention and/or chemopreventive agents, with garlic being the best example. The effects of the plants on antioxidant enzymes, apoptotic pathways, PARP (essential for DNA repairs), and NF-kB have been studied extensively. These targets are important for prevention of initiation, promotion, and progression of carcinogenesis.

These botanical drugs could either be used in the traditional way (herbal combinations) or in newly developed preparations (e.g. capsules containing single plant powder). In general, TTM preparations usually contain between 3 to 70 botanical and other drugs. To translate results of a single plant to actual uses, we need to consider its use in a formulation, such as its ratio in the whole recipe and potential synergistic effects with other ingredients. If a single plant preparation were to be developed from a recipe, one should consider safety issues due to the higher amount than its traditional uses. Extracting methods used in the *in vitro* or *in vivo* studies need to be compared to traditional methods, which normally are decoction or ethanol-water maceration, because phytochemical profiles would be different. In addition, some mechanisms have paradoxical effects, such as NF-kB inhibitors often suppress tumour necrosis factor (TNF) which could interfere immune response (Boik, 2001). Thus, it is necessary to see the effects in *in vivo*.

TTM has potential to contribute to cancer prevention, not only because many plants have shown positive results before, but also the opportunities to perform research in the area are increasing. Firstly, TTM is widely accepted among Thai people for treating and preventing diseases. Secondly, herbal medicine used in TTM has a long history of use. Therefore, such usage could provide preliminary data on safety and effectiveness in order to choose high potential medicines for further exploration. Lastly, the Thai government has been keen to develop TTM and integrate it into the main health care system. In 2017, Thailand has had its first master plan for the development of Thai herbal medicine (Ministry of Public Health, 2017). This is a great opportunity to contribute to the sustainable development of Thai healthcare by conducting more research in this field with the support from the government.

1.7. Aims

A basic assumption of this study is that in order to prevent cancer, it is important to prevent cancer development at several mechanisms at the same time, as well as to maintain cell homeostasis. So holistic medicine like TTM which emphasizes maintaining the balance of the body and has been officially promoted in Thailand for health promotion fits well with the finding of natural sources for chemoprevention.

This thesis aims to discover new extracts or compounds which could be useful in the prevention of cancer. An ethnopharmacological approach was used to investigate medicinal plants used in TTM. The project comprises two main parts; fieldwork and *in vitro* experiment. The specific objectives are as follows;

1. To understand cancer prevention strategy from Thai traditional practitioners
2. To document medicinal plants used by Thai traditional practitioners to prevent cancer
3. To investigate chemopreventive properties of selected medicinal plant extracts *in vitro* using an ethnopharmacology approach
4. To study the phytochemistry of selected species in order to identify bioactive metabolites responsible for the chemopreventive effects observed

Chapter 2 Thai traditional medicine concepts of cancer and approaches in cancer prevention: An ethnopharmacological field study

'Culture is at the heart of plant meaning'

Daniel E. Moerman & Ina Vandebroek,

The Anthropology of Ethnopharmacology (Moerman and Vandebroek, 2015)

2.1. Introduction

In the past decades, Thai researchers have been excited about the number of *'cancer therapy'* recorded in ancient texts. According to news published on 3rd of February 2014, Thawatchai Kamoltham, MD, Director General of Department of Thai Traditional and Alternative Medicine at the time announced "In our Thai traditional medicine database containing 12,428 herbal recipes for chronic diseases, there were 3,115 formulations used for treatment of cancer" (ASTV, 2014). This news aids to confirm that herbal medicine is a promising source to find cancer treatments. However, it also provokes the question 'are they really for cancer?'. In global view, the vast majority of medicinal plants that are reported as cancer treatments in indigenous cultures are actually used for infectious conditions of the gastrointestinal tract, respiratory tract, and skin. For example, Heinrich et al. (1992) found that plants used to treat 'cancer' referred by local people in Lowland Mixe, Mexico were actually used for chronic infectious skin conditions (Heinrich and Bremner, 2006). This calls for a systematic assessment of 'what is cancer' in the context of a traditional medical system and how this may be linked to experimental and preclinical research.

Health care systems, both Biomedicine and traditional medicine, are considered cultural systems. Illness is presented as a combination of beliefs about disease causation, the experience of symptoms, specific patterns of illness behaviour, decisions regarding treatment options, actual therapeutic practices, and evaluations of treatment outcomes (Kleinman, 1978). In folk medicine, medical concepts are usually complex and associated with social, cultural, historical, and linguistic aspects (Heinrich and Bremner, 2006). Moreover, they are often different from one society to another. For instance, the concept of benefits of bitter plants is different among cultures. A Dominican community believes that bitter plants 'burn sugar in the blood', therefore they are used for treatment of diabetes. Jamaican culture uses them for skin rashes and other skin problems because they believe that bitter plants could 'cleanse the blood' (Moerman and Vandebroek, 2015). In this project, one of our very first question was '*Is cancer in TTM the same as cancer in biomedicine?*'.

2.1.1. Cross-cultural research and emic & etic concepts

Ethnopharmacological research is cross-cultural as it is a combination of emic perceptions of local people and an etic perception of scientist-researchers and applied science methods.

Cross-cultural research scrutinizes local information and contrasts it with a global view. Therefore, this framework is useful in comparative studies aiming to find general patterns from different cultures and helps us to understand unique cultural characteristics. In addition, it may stand for transdisciplinary approaches that bridge knowledge from different cultural systems together and develop new perspectives based on an equal exchange (Heinrich and Jäger, 2015). One example of cross-cultural studies is from Berger-González et al (2016) who studied Maya healers' conception of cancer from five ethnolinguistic groups in Guatemala and compared with biomedicine. In Guatemala, most Maya people still rely on Maya healers and have poor compliance for Western medicine. This problem is partly caused by cultural disagreements with procedures used in hospitals. From the findings, the authors proposed that primary health care providers should understand the influence of Maya medicine (especially healing settings which normally comprise physical, spiritual, and social methods) on the behaviour of indigenous patients in order to improve compliance which leads to a more efficient and successful treatment, such as spiritual blessing of chemotherapy (Berger-González et al., 2016).

In Thai traditional medicine, as well as other traditional medical systems, diagnosis is based on observation and interpretation of patients' symptoms, past history, causes, effects, and responses, mostly without objective information. On the other hand, in biomedicine, diagnosis relies on objective physical examinations together with patient's history and pathophysiology information obtained from laboratory. This results in cultural gaps between the two systems (Berger-González et al., 2016). To link information from TTM to experimental models in biomedicine, the 'emic-etic concept' has been applied. The terms emic and etic were originally coined in the context of anthropological research by Kenneth Pike in 1954. According to Pike "The etic viewpoint studies behaviour as from outside of a particular system, and as an essential initial approach to an alien system. The emic viewpoint results from studying behaviour as from inside the system." (Pike, 1967).

2.1.2. '*Mareng*' - Cancer in Thai Traditional Medicine?

In modern Thai, '*mareng*' is the commonly used term for cancer. It is believed that the word is derived from '*merengsa*' in Melayu which means untreatable ulcer. However, it is also used to refer to other diseases in TTM text books, which might or might not be cancer in a biomedical sense. In this study, the term '*mareng*' was used when referring to a disease in TTM, which might or might not be the same with cancer. Cancer was used only when referring to biomedical diagnosis.

The word '*mareng*' was mentioned several times in *Phaetthayasat Songkhro*[4]. Each *khamphi* gives symptoms of illnesses, treatment options, and herbal remedy composition and its usage, which depends on the expertise of masters who wrote it (Foundation for the promotion of Thai

[4] A collection of ancient scripture (*khamphi)* which was published the first time in 1961. It is used nowadays as a manual for people studying Thai traditional medicine and pharmacy.

traditional medicine and Ayurved Thamrong School Center of applied Thai traditional medicine, 2007b). Among twenty manuscripts from the first volume, *mareng* was mentioned in five *khamphi*, namely *Khamphi Chanthasat*, *Khamphi Maha Chotarat*, *Khamphi Chawadan*, *Khamphi Mutcha Pakkhantha*, and *Khamphi Takkasila*.

According to *Khamphi Takkasila*[5], there are ten malignant sores (*Fi Kan*) which could occur along with toxic fevers, including *Mareng Tamoi*, *Mareng Pakthum*, and *Mareng Pleo-fai-fa*. It explains the symptoms of each as follows (the original text is shown in appendix I);

- *Mareng Tamoi* – A nodule which has equal size to a thumb or a garden pea. The severe one has a white base and a black head. It causes shivering, watery eyes, or blurred vision. It occurs on the body, arms, or legs. The nodule must be treated as fast as possible, otherwise, a burst could cause death or develop into *mareng*.
- *Mareng Pakthum* (or *Mareng Thum*) – A swollen nodule at the back, one side or both side. It has dark blue colour. If it is not cured, a burst could cause death or develop into *Mareng Pak Mu*.
- *Mareng Pleo-fai-fa* (or *Mareng Pleo-fai*) – A nodule which has equal size to *wong saba*[6]. Its head is green. It causes a burning sensation. A burst causes death.

Another one mentioned in the manuscript is *Mareng Nakkharat* which occurs on finger(s). Its size is varying, from a mung bean to a rock. It causes fever, shivering, watery eyes, blurred vision, headache, and a burning sensation. It also makes hands become black, and stops the heart beating. If hands and legs become black, patients will die. In *Khamphi Maha Chotarat*[7], some postpartum complications[8] are mentioned, such as obstruction of menstrual blood and menstrual blood clots, which might develop into internal *Fi* (nodule), such as *Fi Mareng Suang Krasun*.

From the given examples, it can be seen that a dangerous nodule or later stage of some conditions are common symptoms of *mareng*. And this might be the reason why modern Thai physicians translated tumours “wounds that do not heal (Dvorak, 1986)” as *mareng*. There are also some conditions in the old texts that are similar to the symptoms of some forms of cancer. For instance, the symptoms of liver with impaired earth element include lower position of liver, nodules in liver, blood in the stool, abdominal pain, red eye, coughing, panting, and loss of appetite (Foundation for the promotion of Thai traditional medicine and Ayurved Thamrong School Center of applied Thai traditional medicine, 2007b), which are similar to those of liver cancer (American Cancer Society, 2014). According *to Khamphi Roknithan*, *Ben-*

[5] An ancient Thai medical manuscript about toxic fevers (unknown date).

[6] Fruits of *Entada phaseoloides (*L.) Merr.. Approximate size is 1 x 6 x 0.3 cm.

[7] An ancient Thai medical manuscript about female diseases (unknown date).

[8] After a woman gave birth, toxic blood occurring during the delivery should not be left in the body. If there are some remains, it becomes rotten. Rotten blood can circulate back into the heart and cause several symptoms, such as fever or madness.

cha-am-ma-rit[9] is one of the remedies used for these conditions. Early 2014, this medicine was featured in local newspapers as a medicine that can be used to treat liver cancer (Variety team, 2014), resulting in a lot of liver cancer patients seeking for it. Although it helps in the relief of some symptoms, such as uncomfortable and swelling abdomen, the use of the drug alone caused a huge concern from many academics and health sectors, especially TTM practitioners. According to TTM, this remedy is used to remove waste from the body which is usually the very first step in any disease treatment. Moreover, it must be used together with other remedies to treat diseases and support the body (Temsiririkkul, 2014).

Although there is a lack of scientific evidence to support the use of Thai traditional drugs in cancer, there have been many experiences and anecdotal evidence of their benefits towards cancer. Therefore, we believe that TTM is potentially a good source for cancer treatment and, more importantly, prevention. However, we need to understand its concept of cancer in order to use it properly. Such understanding will also provide a great potential to find novel treatment or prevention for cancer.

2.1.3. Objectives

The first objective of this field work is to understand Thai traditional practitioners' perceptions of cancer in the definition, causes, diagnosis, treatment, and prevention, as no one ever systematically studied it before. The second goal is to document medicinal plants used to treat and prevent cancer in order to find potential plants for further pharmacological investigation. The ultimate aim is to provide a documentation that preserves the knowledge and offers an opportunity for TTM practitioners to discuss and improve it in the future.

2.2. Methods

2.2.1. Ethnopharmacological field survey

Information from TTM practitioners living in different parts of Thailand was collected. The surveys were carried out during December 2013 – April 2014. The project has been approved by the UCL Research Ethics Committee, Project ID: 5068/001, and Siriraj Institutional Review Board (Thailand), Protocol number 779/2556(EC4). All data were collected and stored in accordance with the Data Protection Act 1998.

2.2.1.1. Location of ethnopharmacological surveys

The field work was conducted in five provinces in Thailand, namely Lamphun which is located in the north; Suratthani which is located in the south; Chanthaburi which is located in the east; and Bangkok Metropolitan Region (Bangkok and the five adjacent provinces of Nakhon Pathom, Pathum Thani, Nonthaburi, Samut Prakan, and Samut Sakhon) which is located in the centre (Figure 2-1). We chose the locations from different regions because the healing

[9] Ingredients: mahahing (*Ferula assa-foetida* L.), yadam (dried latex from *Aloe spp.*), rongthong (resin from *Garcinia hanburyi* Hook.f.), tongtaek (*Baliospermum solanifolium* (Burm.) Suresh), di-kluea (epsom salt)

practice may be influenced by indigenous medicine, local culture, and religion, even though some influences from written sources may be of relevance. For instance, Northern Thailand was influenced by Lanna culture, some parts of Eastern Thailand were influenced by Khmer, and most parts of Southern Thailand were influenced by Islam. Each part of the country also has different food cultures.

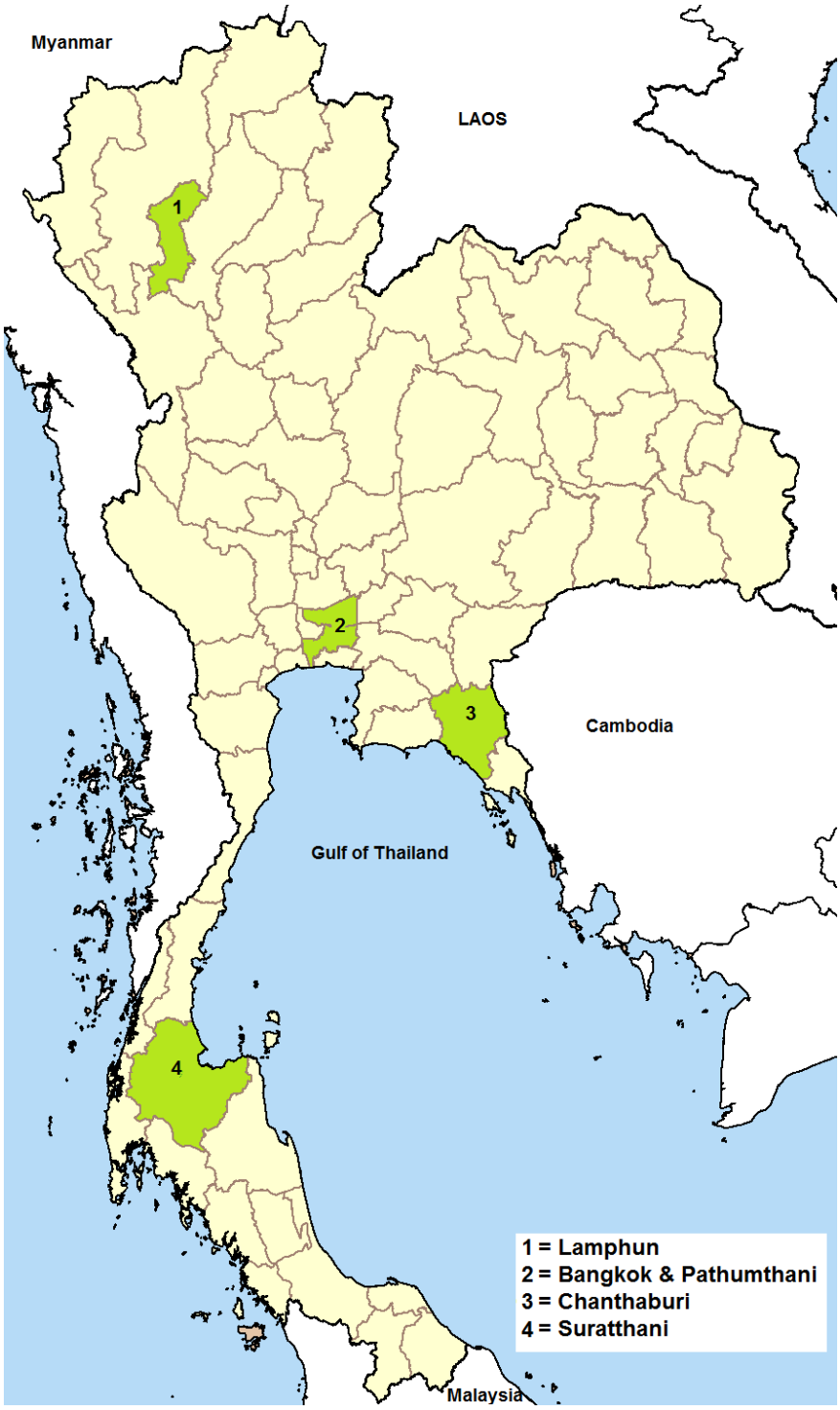

Figure 2-1 Interview locations in green colour. (Map modified from Wikimedia commons)

2.2.1.2. Data collection by interviews

An interview guide (Table 2-1) was prepared in advance in English and Thai versions. The word *'mareng'* was used in the guide (also in the interviews) as it is commonly used to refer to cancer in modern Thai. To validate the Thai version of the interview guide, other Thai students in the UCL School of Pharmacy who did not know about the project helped in the translation back from Thai to English. This English translation was then compared to the original English version.

To recruit participants, we went to locations that are known to be home of the practitioners and visited them. To obtain their addresses, we asked our colleagues who work at Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital (CATTM, Bangkok), Tharongchang Hospital (Suratthani) and Phrapokklao Hospital (Chantaburi), villagers, and local herbal dispensaries.

Semi-structured, open-style interviews with 33 TTM practitioners were performed in an open-style in informal settings, such as at practitioners' houses. All interviews were performed in Thai language. Prior to each interview, the informants were informed about the aims of the project and were advised that they could withdraw at any point. Informed consent and a permission to record the conversation were also obtained.

Figure 2-2 An interview at a practitioner's house. The house is also a TTM clinic and pharmacy.

Table 2-1 Interview questions

1. Personal information:
    1.1. Name
    1.2. Age
    1.3. Gender
    1.4. Address
    1.5. Expertise (eg. spiritual healers, herbalists, bone healers, or masseurs.)
    1.6. How long have you worked as a Thai traditional practitioner? How long have you been treating?
    1.7. What is the average number of patients per week or per month?
2. What is the symptom that you treat most?
3. Please list herbs that most important to you or that you frequently use.
4. Is there anything to eat to protect from diseases?
5. Please describe *'mareng'*:
    5.1. What is '*mareng*'?
    5.2. What is the cause of '*mareng*'?
    5.3. How do you know that somebody has '*mareng*'?
    5.4. How often that you treat patients with '*mareng*'?
    5.5. Please tell me about '*mareng*' that you have experience with.
        5.5.1. Please describe how did you diagnose or identify it?
        5.5.2. What were the main symptoms?
        5.5.3. What plants did you use to treat it?
        5.5.4. What was the dosage regimen?
        5.5.5. How long did the remedy take to make patients feel better?
    5.6. What is the theory or treatment concept of '*mareng*'?
    5.7. Can '*mareng*' be prevented? How?
6. How do you select the plants or medicines for each patient?
7. How have you learned about the use of this medicine/ plants?
8. How do you obtain the plants? Buy, grow, collect?
9. Can you give or show me the plants?

2.2.1.3. Plant sample collections and identifications

After each interview, the informant was asked to show the mentioned plants. Then the living plant samples were photographed and collected, with the informants' permission, into plastic bags with some water inside. All possible parts of the plants were collected, including roots, stems, twigs, leaves, fruits, and flowers. However, in many cases roots, flowers, and fruits were not available. For these cases a description of the flowers and fruits was obtained in order to help with the identification. The voucher specimens were labeled with local name, common name, date of collection, locality, habitat, and other notes, such as height, presence of saps ('rubber'), bark feature, smell, and colour of fresh fruits and flowers. When the living plants were not available, especially for introduced species, dried crude drugs were photographed and collected instead. In addition, some specimens were collected from local botanical gardens, including Chanthaburi botanical garden (Chanthaburi), Siri Ruckkhachati Nature Park (Salaya Campus, Mahidol University), and Suan Chalerm Phrakiat (Faculty of Medicine Siriraj Hospital).

Figure 2-3 Plant voucher specimens collection

Identification of voucher specimens was done by comparison with books, monographs, or authentic specimens from botanical gardens with help from experienced TTM practitioners from CATTM. Imported crude drugs were identified by comparing macroscopic features and thin layer chromatography fingerprints with authentic materials obtained from Sun Ten Pharmaceutical Co., Ltd. (provided by Dr. Anthony Booker). Some common food plants, such as ginger, garlic, shallot, and mung bean were not collected. Plant voucher specimens were deposited at Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

In addition, some informants were re-visited or called to get more information, such as the colour of flowers, in order to confirm or help in identification of the plant species. During the re-visits, we asked them to look at plant samples or pictures from textbooks/monographs and tell us the plant names. We also asked them to prepare some crude drugs for us to buy.

2.2.1.4. Analysis of qualitative data

All interviews were recorded, transcribed, and translated into English with the help from CATTM. The medical terms used in this report were translated to English in two steps; from Thai traditional medical terms to modern Thai, and then from modern Thai to English. Data grouping and association was used for the qualitative analysis of the data. Medicinal plants were ranked according to the frequency of citation (FC) (so called 'number of use-reports') as it is recommended as a tool to select candidates for further bioactivity investigation (Heinrich and Jäger, 2015). FC of specie A is calculated by counting the number of informants who

mentioned that A is useful. Relative Frequency of Citation (RFC) was calculated by dividing FC with the total number of informants participating in the study. The use of RFC is to give the information of relative importance of each species (Tardío and Pardo-de-Santayana, 2008).

2.2.2. Plants selection for pharmacological and phytochemical studies

The selection strategy of species for further bioactivity investigation is shown in Figure 2-4. The first step was to include all the plants mentioned by the informants. The second step was to separate only the plants used in cancer/disease prevention as this is the focus of this thesis. Then some criteria were applied to obtain samples to be investigated.

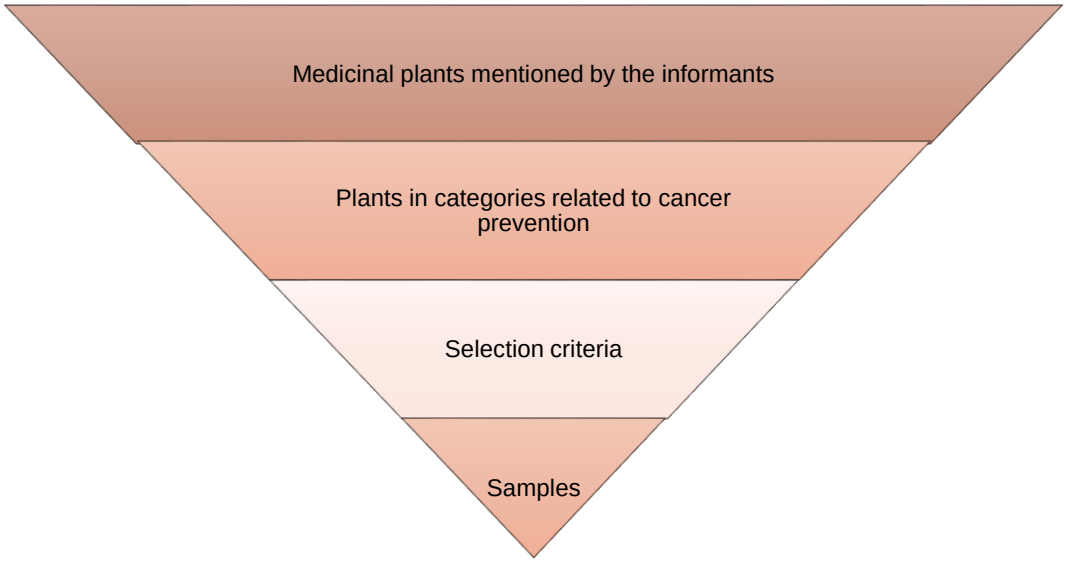

Figure 2-4 Selection strategy of plant samples for further bioactivity investigations. The selection criteria in the third steps were 1) the plants are not endangered species AND 2) the plants can be sustainably supplied by local people AND 3) the plants were mentioned by at least two informants. AND 4) the plants have not been studied extensively with regards to their chemopreventive activities.

2.3. Results and discussion

2.3.1. Informants' characteristics

Thirty-three informants were interviewed independently. Each interview lasted about 1-3 hours. Informants' general characteristics are shown in Table 2-2. Most of them were male (73%). This might be a result of a local belief in some locations that healers should have been in a monkhood before. Thirty informants out of thirty-three treat at home or private clinics and three of them work in public hospitals. Most of them learned their practice from family members. Herbal medicine was the main treatment in general. Physical methods, such as massage, and spiritual approaches, such as ritual blessing, are used together with herbal medicine in some cases.

Table 2-2 Informants' characteristics

Parameters		Results
Gender (N)	**Female**	9
	Male	24
Age (years)	**Min**	31
	Max	96
	Average±SD	64.3 ±14.7
Treatment experience (years)	**1 - 20**	14
	21 – 40	11
	41 – 60	6
	61 - 80	2
Types of expertise (N)	**Herbal medicine**	31
	Physical methods	8
	Spiritual approach	5
Major source of knowledge about therapeutic practice	**Family**	19
	Masters (not from the family)	6
	TTM school	5
	Scriptures/ textbooks	2
	Buddhist temple	1

Figure 2-5 The house of an informant was full of dried herbs, ready to prescribe to patients. Each jar contains one herb, labelled with its local name. Herbs were mainly from the informant's own garden or herbal shops in the area.

Figure 2-6 One informant and his assistant were repelling 'phi' (a ghost or bad spirit) from a patient. The assistant (left, standing) was spitting holy water onto the forehead of the patient.

2.3.2. Informants' cancer experiences

Twenty-nine out of thirty-three informants (87.87%) have had experience in cancer treatment. Three practitioners (9.10%) have been visited by cancer patients but the practitioners refused to give the treatment, instead, they referred those patients to another practitioner in the same area because they are better than them in cancer care. One informant (3.03%) has never been visited by any cancer patients before the interview. In general, cancer patients who have visited the informants were those with cancer of the lung, uterus, liver, breast, colon, neck, bone, cervix, trachea, pancreas, nose, blood, prostate, skin, and lymphoma. The most common types were liver, uterine, lung, and breast cancer.

Cancer patients who have visited the informants commonly come at an advanced stage or because they do not want to have surgery to remove tumours, or have been rejected or discontinued cancer treatments in hospitals due to the treatment guidelines that give priority to palliative care in incurable cases.

Figure 2-7 Medical records. An informant has monthly medical records (A) and medical records for each patient (B) in some cases. In this figure, (B) shows a medical record for a neck cancer patient.

2.3.3. Local concept of cancer and causes

'*Mareng*' is commonly used to refer to cancer in modern Thai. However, it is also used to refer to other diseases in old TTM text books, which might or might not be cancer in a biomedical sense. It is believed that the word is derived from '*merengsa*' in Melayu which means untreatable ulcer. In this study, the term '*mareng*' was used when referring to a disease in TTM, which might or might not be the same with cancer. Cancer was used only when referring to biomedical diagnosis.

As the informants had various views on *mareng*, case studies were found to be the best way to represent their knowledge on causes, diagnosis, treatment, and prevention of *mareng*. The following case studies contain both informants' existing knowledge about *mareng* and some examples of their passed/current cases. A short detailed portrait of each informant was also included. These case studies represent perception of TTM practitioners on causes, diagnosis, treatment, and prevention of *mareng*. Summary of the main concept of *mareng* of each informant was also given.

2.3.3.1. Case studies

Informant A – Mareng is linked with inflammation, blood circulation, chronic conditions, microorganisms, and chemicals

> Informant A studied TTM from a TTM school and graduated with a Bachelor in TTM. Later she also studied Thai traditional massage. She has had experience in TTM for around 10 years. Before running her own clinic, she used to work for a clinic in which she said 'cancer patients were the majority because the place was famous in cancer care'. She takes care of herbal medicine production by herself and she got the formulations from ancient Thai medical textbooks. Her father is also a TTM practitioner.

“In general, *mareng* is caused by internal inflammation (*kan aksep*) and microorganisms. These microorganisms cause wounds or abscesses in the lungs or the liver. When the body cannot fix it or we intake toxins, the wounds will be bigger and distribute to lymph nodes, and then to other organs. In summary, if we have a chronic condition and our body cannot fix it, that condition will become *mareng*.

Mareng in the breast is caused by an obstruction of blood and *lom*. In the early stage, we would find hard lumps, pain in the arms, and headaches. Once there is the obstruction of blood, *phangphued* (fibrosis) would develop and grow bigger if we don’t treat it. If the obstruction continues, waste will accumulate locally. When microorganisms or chemicals get inside, *mareng* will develop. For the treatment, I use herbal remedies which help improving blood circulation, eliminate the *phangphued*, and detoxify the chemicals and microorganisms. The recipe for *phangphued* comprises *thaowanpriang*, *khokhlan*, *phimsen* (borneol camphor), and *karabun* (camphor). The patients will defecate every day to remove the waste. Normally after a month, the lumps will be softer and smaller, and will eventually disappear.

I’ve also treated a case with lung cancer. The patient has received chemotherapy 14 times already before starting TTM therapy with me. I’ve prescribed a detoxifying remedy for the first seven days to wash away chemotherapy. It can be noticed that the patient’s face was brighter and he was less tired, could eat, and had less chest pain. Then I prescribed medicine to treat lung diseases and kill *mareng*.

For *mareng* in the colon, patients will start with detoxifying remedy for three days. Then they will be prescribed *mareng* remedy together with ulcer treatment. I also have to treat other symptoms, such as diarrhoea, indigestion, flatulence, constipation, and bulging stomach which are common co-symptoms of *mareng* in the colon.

In general, *mareng* patients must take medicines that adjust the elements and remove waste, together with *mareng* remedy. Herbs for *mareng* cannot be hot, but it also depends on the type of abnormal elements. I have only one *mareng* remedy for which the main ingredients are *kammathandaeng* (arsenic disulphide), *kammathanlueang* (sulphur), *khaoyennuea*, *khaoyentai*, *khanthongphayabat*, *horathaosunak*, *khanunsampalo*, and *makhampom*. I will add other related remedies, such as liver remedies for patients with *mareng* in the liver. Moreover, I will also add massage to facilitate the blood circulation which benefits the treatment because it pushes the medicine and immunity into tumours. The most important thing is to remove accumulated toxins and waste.

To prevent *mareng*, we have to remove toxins daily because we intake them with food. *Mareng* prevention can also be personalized. For instance, patients with chronic gastric ulcer should take a herbal medicine that heals wounds in the stomach and intestine and detoxifies. Detoxification is the most important step. Also we have to eat properly; like eat five, and avoid food that cause *mareng*, such as internal organs, processed food, eg. sausages, and bamboo shoots.”

Informant B – Mareng is linked to the imbalance of elements and chronic conditions (krasai)

Informant B learned the practice from his aunt. The knowledge has been passed down from generations to generations in his family. He became his aunt's successor in the family's Thai traditional medical clinic. He has helped his aunt since he was a child in their pharmacy. Before he took his aunt's place, he studied the clinical practice beside his aunt for many years. Now he has been treating patients for more than 10 years.

"In TTM, a disease happens because of abnormal *tridosha*. To treat a disease, we have to re-balance *tridosha* and then the earth element will become normal. Every disease is treated like this, including *mareng*.

Mareng is a familial condition that makes a person have an abnormal organ from the beginning. So this kind of disease cannot be prevented because it is already in the blood. However, we could suppress its emergence by preventing *krasai*. A person can develop *krasai* when he/she has a chronic condition. A general method to prevent *krasai* is to eat green mung beans boiled with *rangchued*, *triphala*[10], and sugar. This is a gentle way to wash away poisons to prevent *krasai* with the aim to prevent *mareng*.

An example of *mareng* diagnosis: a patient visited me last week with creamy white vaginal discharge during the menstrual cycle. I performed an examination (touching) and could feel lumps in her cervix. Together with other symptoms, I suspected *mareng* in the uterus. So I asked this patient to check up with the hospital (at the time of the interview we did not know the result yet). The most important symptom which leads to *mareng* diagnosis in this case was the history that the patient has had abnormal vaginal discharge during the menstrual cycle and dysmenorrhea for a long time. I did not tell her that I suspected *mareng* because she would not believe me.

A case where I have suggested *mareng* prevention is a friend of mine who has a familial history of liver diseases. His parent had liver cancer and his brother died because of a liver disease. I knew that he would develop a liver disease in the future. So I recommended him to take care of the liver by having a diet that good for the liver and taking a liver remedy every 2 weeks. I also check the signs of liver diseases, such as tiredness, feeling sleepy, not eating, flatulence, and white tongue. If he has these signs, liver remedy will be prescribed immediately. For the food, he should not eat spicy food and try to eat bitter food more. He also had to take a remedy to wash away poisons in blood every three months to prevent precipitation in blood and *namlueang*. Furthermore, he has to take '*Ya-klom-nang-non*' to nourish the liver for one week every month.

For lung cancer treatment, I start with detoxification of the lung. The medicinal plants have to be those that get into the lungs, such as *yamornoi* and bird nests. When I treat

[10] A mixture of the fruits from *Phyllanthus emblica* L. (Phyllanthaceae), *Terminalia bellirica* (Gaertn.) Roxb. (Combretaceae), and *Terminalia chebula* Retz. (Combretaceae)

cancer patients, I always tell them to use my prescribed herbal medicines together with conventional treatment, but do not tell their doctors otherwise they will be asked to stop herbal remedies. I prefer to use both together because each has its advantages. Herbal medicine only might not be fast enough to kill the tumours. Conventional treatment is very fast. For patients who are taking chemotherapy, I have to prescribe nourishment because their bodies will be very weak."

Informant C – Mareng is linked with inflammation, fire element, abnormal namlueang, and chronic bruises

Informant C has practiced TTM for 28 years. When she was young, she always went with her uncle, who was a TTM practitioner, to treat patients. She decided to become a TTM practitioner when she was 16 years old.

"*Mareng* is caused by a severe inflammation. The fire element is fully activated. There are two conditions that cause *mareng*. One is from abnormal *namlueang* which is caused by over-activated fire element. The second one is from a chronic bruise. A blood clot that has deposited for a long time could develop to *mareng*.

I have experience in treating *mareng* in the breast. The remedy comprises drugs for *namlueang* conditions, including *khaoyen* (means *khaoyennuea* and *khaoyentai*), *haoyang*, *nontaiyak*, *kammathanlueang*, and a charcoal. Patients have to stop using this medicine once they have recovered because this drug affects neurological system. Some patients took three pots and they recovered.

Mareng could be prevented by changing habits; no stress. Fruits and vegetables which have cooling effects (they have 'cold taste' in TTM) can help because *mareng* is related to fire element."

Informant D – Mareng is linked with chemicals and chronic mahok.

Informant D is a Lanna healer. Lanna medicine is practiced widely by TTM practitioners in the North of Thailand. He obtained the knowledge from his ancestors and also from a famous master in the area. He has had his license for TTM practice for more than 20 years.

"*Mareng* is caused by *lom saan* or *lom khaan*. My master said that smoking causes *khaan* to eat liver and lung. *Mareng* in the liver is caused by *khaan* eating liver. The cause of *mareng* is food, such as vegetables contaminated with chemicals. Long term *mahok* could develop into *mareng*. Patients who have *lom khaan* will have pains. There are several *khaan*; some cause pale faces or yellow or thin.

Patients with *mareng* in the liver have yellow skin and vomit after eating. I treat them by asking them to drink *nammon* (holy water) and *rangchued* drink or *phakkhaotong* drink or a decoction of *kasalong*, *oidam*, and *yapakkhwai*.

Patients with *mareng* in the intestine have stomach pain, twisted intestine, gas, and flatulence, which are similar to *mahok* in the intestine. The treatment is drinking holy water and a decoction of *kasalong*, sugar cane, and *yapakkhwai*.

To prevent from a disease, we could drink a decoction from boiled *rangchued* with rice, or *kasalong*-sugar cane-*yapakkhwai*, or have an herbal steam bath."

Table 2-3 Summary of informants' perception of cancer and causes

The questions used were 'what is *mareng*?/ what is the cause of *mareng*?'.

Informant's code	Quotes	Remarks
BK1	1) *Mareng* is caused by internal inflammation and microorganisms*. These microorganisms cause wound or abscess in lung or liver. When the body cannot fix it or we intake toxins, the wounds will be bigger and distribute to lymph nodes, and then to other organs. 2) Chronic conditions. 3) *Mareng* in the breast is caused by an obstruction of blood and wind. 4) Difference in defected elements cause different type of *mareng*. 5) External *mareng* is caused by blood and lymph. 6) Internal *mareng* is caused by the microorganisms that get to lung and liver.	*There is no exact definition of mentioned 'microorganisms'. They could be bacteria or viruses or fungi or worms.
BK2	*Mareng* is a familial condition. We could suppress its emergence by preventing *krasai*. A person can develop *krasai* when he/she has a chronic condition. For instance, a diabetic patient would develop *krasai* in the kidney.	
BK3	*Mareng* is caused by the imbalance of the four elements (*dhātu si phikan*). The imbalance starts with fire or wind or water element and then finally affect the earth element. *Mareng* patients have problems with their *luead/ namlueang* system. According to old texts, such as *Takkasila* and Wat Pho, it might be a type of skin disease.	
CH1	It is caused by food (especially meat), chemicals, and toxicants. *Mareng Fai* – caused by bad blood and lymph or got bitten by ticks *Mareng Rai* – caused by mites that carry *mareng**	*From the explanation, *mareng* is transferable like bacteria or viruses or worms.
CH2	*Mareng medluead* (blood cancer) is caused by the imbalance of blood cells. They eat each other. It's from genetics. Very difficult to prevent.	
CH3	*Mareng* is caused by an obstruction of toxic *luead* or *namlueang* in each part of the body.	
CH4	It is caused by a severe inflammation. The fire element is fully activated. There are two conditions that cause *mareng*. One is from abnormal *namlueang* which is caused by over-activated fire element. The second one is from a chronic bruise. A blood clot that has deposited for a long time could develop to *mareng*.	

Informant's code	Quotes	Remarks
CH5	*Mareng* is caused by chronic bruises. *Mareng* in the colon is caused by food. *Mareng* in the uterus is caused by bruises of the uterus. When the uterus moves for a long term (eg. by jumping), the inflammation occurs and it becomes swollen. Chronic bruises form discharge which then turn into sticky rubber. When eat something which causes non-stop bleeding (eg shallot, garlic), the blood will mix together with the sticky liquid and form a clot. Chronic clots suppress fire element, which results in indigestion of food. Then wind element will ascend. After giving birth to two children, uterus will become very bruising and that when *mareng* develops.	
CH6	*Mareng* is a cell that grows abnormally. It is caused by *namlueang*. We have more than 3,000 lymph nodes. If the lymph nodes are good, we will not get allergy easily. Some symptoms like urticaria, rash, or diarrhea are symptoms of *mareng*. It distributes fast because it eats white blood cells, so patients don't have immunity to fight it.	
CH7	*Tua* developed within the four elements causes an inflammation by an interaction with toxic food that we eat. When there is an imbalance of the elements cancer would develop. It can be treated when it just newly developed.	
CH8	Mareng is caused by eating food contaminated with germs.	
CH9	An abscess that is difficult to cure or chronic shingles can develop to *mareng*. The cause is an accumulation of microorganisms or chemicals.	
LA1	Bruises and shingles are *mareng*. We call it '*ma-**eng**'* which is external *mareng*.	
LA2	*Mareng* is *saan* in Northern Thai dialect. *Saan* is something that cause an abnormality of the body, such as shingles is *mareng* in the skin. Some *saan* cannot be seen, such as *mareng* in the intestine and *mareng* in the uterus. The causes of *saan* are many, such as poisons, abnormal secretion from the body, food, or genetics.	
LA3	*Mareng* is caused by *lom saan* or *lom khaan*. My master said that smoking causes *khaan* to eat liver and lung. *Mareng* in the liver is caused by *khaan* eating liver. The cause of *mareng* is food, such as vegetables contaminated with chemicals. Long term *mahok** could develop to *mareng*.	**Mahok* is a disease which causes different symptoms depending on the affected area of the body, such as *mahok* in the intestine have similar symptom with *mareng* in the intestine.

Informant's code	Quotes	Remarks
LA4	*Mareng* is involved with allergy.	
LA5	We don't know what cause *mareng*. It is developed from wind. This wind distributes throughout the body and causes pain. When it stops, it causes *daan** and *fi*. Nowadays all *fi* are called *mareng*.	**Daan* (ดาน) = a lump
LA6	*Mareng* is caused by sunlight and *phayat**	**Phayat* (พยาธิ) in modern Thai refers to a worm. In TTM, it means illness.
LA7	*Mareng* in the intestine is from *khaan* in the stomach that invade to the intestine.	
LA8	*Mareng* is *saan*. It might be caused by beef and catfishes.	
LA9	*Mareng* is poisonous *lom**. It is caused by genetics and food.	**Lom* is literally translated as wind. But in this context it should mean wind in the body, which could involve with wind element.
SU1	*Merang* is a type of *phayat*. Sometimes they are visible but sometimes not. They are in the environment. *Mareng* in the breast is caused by inflammation of breast.	
SU2	*Mareng* is any disease, which progresses very fast. The cause of the disease is unknown.	
SU3	*Mareng* is from abscess.	
SU5	Chronic wound would develop to *mareng*. When a wound expose with fungi or chemicals, it would become untreatable and distribute.	
SU6	*Mareng* is bruise and inflammation, and bad blood.	

Informant's code	Quotes	Remarks
SU7	*Mareng* is caused by bad immune system. Therefore, wounds or abscess can progress.	
SU8	*Mareng* is caused by something in the spinal cord.	
SU9	*Mareng* is caused by improper eating habit, such as rotten food, black cooking oil, preserved food	

Table 2-4 Summary of cancer diagnosis by Thai traditional practitioners

The questions used were 'how do you know that somebody has *mareng*?/please describe how did you diagnose or identify it./what were the main symptoms?'

Informant's code	Quotes	Remarks
BK1	Beside from looking at the symptoms, I ask about their habits, such as what did they eat, what they smelled, as well as cancer history in the family because the disease passes through genetics.	
BK2	For example, a patient visited me last week with creamy white vaginal discharge during the menstrual cycle. I performed an examination (touching) and could feel lumps in her cervix. Together with other symptoms, I suspected *mareng* in the uterus. So I asked this patient to check up with the hospital (at the time of the interview we did not know the results yet). The most important symptom which leads to the diagnosis of *mareng* in this case was the history that the patient has had abnormal vaginal discharge during the menstrual cycle and dysmenorrhea for a long time. I did not tell her that I suspected *mareng* because she would not believe me.	
BK3	I've never diagnosed *mareng* myself, normally patients came already with the diagnosis. I just use TTM to help them. However, I still examine them with TTM methods to see which elements are defective and correct those elements.	
CH1	The symptoms of some mareng are described below; *Mareng Kam Chang* – open wounds with black edges in oral cavity and painful *Mareng Fai* – it is a skin condition with blackened skin (like burnt wound). Hot and painful. *Mareng Rai* – open wounds which distribute very fast (like in a day), itching, thick edges together with rotten flesh. If not treated, it will eat the bones.	

Informant's code	Quotes	Remarks
	Mareng Pak Moo – chronic wounds (years), 2-3 cm deep, hot and painful, not itching *Mareng Lamsen* – occurs internally, local swollen tendon or ligaments, the whole body aches regardless the affected location Every *mareng* wound is hot and painful, and bloody because the blood was bad.	
CH2	I use a spiritual method called *Pheng Kasin* (เพ่งกสิณ) where the patient's spirit is invited to give information about the illness.	
CH3	Most cancer patients have been diagnosed with cancer already from the hospital.	
CH4	I check symptoms. For instance, patients who have *mareng* in the breast have several small nodules which will be bigger one year later. The nodules do not move. If they move, they are not *mareng*, they are cysts.	
CH5	I check symptoms. However, cancer patients who came here always came in a critical condition.	
CH8	The main symptoms are lumps and bleedings.	
LA1	The symptoms are feeling hot and cannot eat. I will also ask what they ate. If they've eaten prohibited food, they got *mareng*.	
LA2	We could see rashes or lumps in some types of *mareng*, but for *mareng* inside we cannot see. In the latter cases, patients have to be diagnosed with cancer from the hospital.	
LA3	Patients who have *lom khaan* will have pains. There are several *khaan*; some cause pale face or yellow or thin. Patients with *mareng* in the liver have yellow skin and vomit after eating. Patients with *mareng* in the intestine have stomach pain, twisted intestine, gas, and flatulence, which are similar with *mahok* in the intestine.	
LA4	*Mareng* produces a lot of heat. For instance, patients with *mareng* in the skin have nodules with heat which is different from a skin disease. In general, the symptoms of *mareng* are pain, heat, and fever.	
LA5	Stomach ache and have daan.	
LA6	*Mareng* patients have daan and high fever.	

Informant's code	Quotes	Remarks
LA7	We call it *saan*. Pain is the symptom of *saan lom*. Western doctors call it tumour. We could see *saan*. But for *saan* inside the body, we could not see. We would know it after a diagnosis from the hospital.	Lom = wind
LA9	Patients with *mareng* have hot flushes and difficulties in excretion.	
SU1	We can know that someone has *mareng* when they have a condition, which does not respond to any treatment.	
SU2	I'm not an expert in *mareng* treatment. The symptoms of *mareng* patients that I know are that they are thin, have muscle pain, internal abscess, and cough with blood.	
SU3	Stomach pain is an important symptom of patients with *mareng* in the liver. We also have to know the colour of urine and feces.	
SU4	Chronic pain in stomach for more than a year. Chronic abnormal menstruation can develop *mareng*.	
SU5	Check symptoms, eg. patients with *mareng* in the uterus will feel itching and pain when urinate and have seeds in cervix.	
SU6	Yellow skin, thin, inside heat, tired, having difficulties in eating, dizzy, pain around cancer area	
SU7	Pain like being stabbed by needles, but not so often, around cancer area; tired, lack of appetite	
SU8	Common symptoms are tiredness and difficulty in eating and sleeping. I also check the pulse because cancer patients have a very fast pulse.	
SU9	I check the pulse.	

Table 2-5 Summary of cancer treatment

The questions used were 'how often that you treat patients with *mareng*?/ what plants did you use to treat it?/ what was the dosage regimens?/ how long did the remedy take to make patients feel better? What is the theory or treatment concept *of mareng*?'

Informant's code	Quotes	Remarks
BK1	In general, *mareng* patients must take medicines that adjust the elements and remove waste, together with *mareng* remedy. Herbs for *mareng* cannot be hot, but it also depends on which elements that are abnormal. Massage helps to facilitate the blood circulation which benefits the treatment because it pushes the medicine and immunity into tumours. The most important thing is to remove accumulated toxins and waste.	
BK2	We have to re-balance the elements. Start with *tridosha** first and then earth element. Every disease is treated like this. For *mareng*, detoxification is the first step. We have to use herbs that go to the affected organ.	**Tridosha* means *Pitta* (fire), *Vata* (wind), and *Semha* (water).
BK3	The treatment strategy is still the same with other diseases which are to nourish affected organs and relieve symptoms. I will prescribe herbal medicine and patients have to control the diet. Herbs that I use are for treatment of bad *luead* and *namlueang*. In general, I would use *Yaharak*, element adjustment remedy, intoxicated taste remedy, and blood nourishment remedy.	
CH1	- External *mareng* – we must catch *mareng* by putting cotton pads with coconut oil over the affected area and leave for 10-15 minutes. Then burn the pads. - For external *mareng* – make ashes from some herbal materials. Then mix with coconut oil and poultice. - Washing solution – break some herbal materials and soak in water. Wash the wound. Use freshly prepared solution. - Decoction: root of *nontaiyak* (properties: repel *namlueang* and *mareng*), heartwood of *thao-khui-daeng* (property: relief of *mareng*), *khoi* (properties: repel bad lymph, laxative) -*Mareng* patients must not receive massages because massages could cause bruises in that area and would make *mareng* worse. - Some food items are prohibited for cancer patients, such as Chinese kale, eggs, some fish, pineapples, corns, poultry, prawns, shrimps, and crabs. - Main treatment is a decoction. If patients feel better during the first three days, the same remedy will be given continuously for 1-3 courses. If not, patients will be suggested to go to the hospital.	

Informant's code	Quotes	Remarks
CH2	The first step of blood cancer treatment is to enhance the immunity by taking a powdered mixture of young fruits or vines from *marakhinok*, leaves from *khlu*, and camphor until the patients stop feeling tired, mostly within one month. Then I will add a blood tonic which contains *fang*, *wankeeprat*, and *kritsana*. The patients can take these remedies until they are better. The most important things in mareng treatment is to stop the degeneration of the body.	
CH3	I use a decoction as *mareng* remedy.	
CH4	A treatment for *mareng* in breast comprises drugs that are used for lymph conditions, including *khaoyen, haoyang, nontaiyak, kammathanlueang*, and a type of charcoal. Patients have to stop using this medicine once they are recovered because this drug affects neurological system. Some patients took 3 pots and they recovered.	
CH5	There are different herbal remedies for different people because they have different blood group. Before prescribing a treatment, I will check the strength of patients. If they are very weak, I cannot give a very strong medicine. For *mareng* therapy, mix a herbal powder with water. Dosage depends on patients' sizes. We can see that they are better or not in 1-2 days. Chicken and squids are prohibited for cancer patients.	
CH6	When I treat *mareng*, I also give a tonic recipe for that organ and blood tonic (because the patients have less blood). For instance, I give liver tonic recipe together with the treatment for *mareng* in liver. My medicines are in the form of capsules or poultice.	
CH7	I've succeeded in many cases of *mareng* in lymph nodes and breast. It is difficult for *mareng* in stomach, but it is very rare anyway. *Mareng* dies because of intoxicated* drugs. To treat *mareng*, I start with a pot of cleansing remedy to cleanse the body, and then prescribe *ya-lu-thong* (mean laxative). After that I start *fi* treatment and monitor the patients for another 3 days. Then I apply some medication and cast a spell.	*In TTM, drugs are categorized according to their tastes. Intoxicated taste is one of the nine tastes.
CH8	I have treated *mareng* in neck and breast the most often. For internal *mareng*, I use decoction. One pot of a decoction would last for 10 days. For external one, I use *'Soon Poon'* until the lumps are gone.	*Soon poon* = to apply Poon (a product from ground cockle shells mixed with water and turmeric water) on the skin
LA2	Herbal medicine powder with/without topical herbal remedies. Sometimes also with ritual blessings.	

Informant's code	Quotes	Remarks
LA3	*Mareng* treatment composes of the use of herbal medicine, holy water, *pao kra mom**, and avoiding prohibited food items. Example of prohibited food items are preserved food, catfishes, sausages, and some mushrooms.	**Pao kra mom* is a ritual performance where a healer casts spells and blows air onto a patient's forehead. It is a belief that forehead is the centre of the body.
LA4	In general, *mareng* patients will take a detoxification remedy, such as a decoction *rangcheud*. Then they will be prescribed medicine according to their conditions.	
LA5	I use herbal medicine to wash *saan-phit* which is composed of *rangcheud, kasalong, and rangjang*.	
LA6	Herbal medicine used for *mareng* treatment must not be hot. We have to use 'cold medicine'. I also perform ritual blessing. The patients have to avoid drinking alcoholic drinks.	
LA7	Herbal medicine capsules/ decoctions, and also for bathing and external application.	
LA8	I use ritual blessing and spitting herbal medicine on patients. I also use herbal medicine.	
LA9	I have treated the patients with herbal medicine, such as *yanang* or *buabok*, together with their conventional treatment.	
SU1	Herbal medicine and massage. Use an oral remedy to expel *phayat* from inside the body and then use a topical remedy to kill the *phayat*. Massage will be used to treat pain.	
SU2	Use herbs with intoxicated taste, such as *kangplakhao, kangpladaeng, maiyarab*, to treat poisons and abscess inside the body.	
SU3	I use several herbal medicine depends on the type of *mareng*.	The informant showed us notebooks with patients' records and prescriptions.

Informant's code	Quotes	Remarks
SU4	Decoction to dissolve menstruation and make it become normal. Spit herbal medicine on patients and perform ritual blessing. Some food items are prohibited.	
SU5	I use a decoction and ointment from a combination of *sabadeang, wa,* and several mushrooms.	
SU6	Capsules contain *khaoyennuea, khaoyentai, huaroiru, and sabulueat*. Patients who have taken this medicine could live up to 6-7 years. They could take it with conventional treatment, but in a reduced dose of the herbal medicine. They will take this recipe together with a decoction that helps in eating, sleeping, cleansing of bad blood, and excretion.	
SU7	Decoction, according to symptoms, to expel internal wounds. Once the wounds come out, they will be treated with topical remedies. Herbal medicine will be spiritually processed before giving to patients.	
SU8	I use herbal medicine to treat *mareng*	
SU9	I have several herbal remedies	

Table 2-6 Summary of cancer prevention by Thai traditional practitioners

The questions used were 'can *mareng* be prevented?/how can we prevent it?'

Informant's code	Quotes	Remarks
BK1	We have to remove toxins daily because we intake them with food. Prevention of *mareng* can be personalized. For instance, patients with chronic gastric ulcer should take herbal medicine that heals wound inside the stomach and intestine, as well as detoxify. Detoxification in the most important step. Also we have to eat properly, such as eat five and avoid food that cause *mareng* e.g. internal organs, processed food, sausages, and bamboo shoots etc.	
BK2	By preventing *krasai* by using *triphala*. And we could prevent *mareng* personally as well. For instance, I have suggested a man, which his parent and elder brother passed away because of *mareng* in liver, to take care of his liver by taking food and medicine that nourish liver, and remove waste in blood. A blood tonic is also preventive, not only for *mareng* but for any diseases.	

Informant's code	Quotes	Remarks
BK3	Any disease can be prevented, according to TTM, because we can know the signs, so we prevent before it gets worse. By keeping the balance of the four elements, eating normally, sleeping well, having good blood circulation, and having normal excretion, *mareng* could be prevented.	
CH1	By eating a lot of vegetables to help in regular excretion.	
CH2	Green vegetables, such as okra.	
CH3	It is very difficult to prevent cancer but we could prevent bad *luead* or *namlueang* by using a blood tonic recipe.	
CH4	Yes. By changing habits; no stress. Fruits and vegetables which have cooling effects can help because *mareng* is related to fire element.	
CH5	It's very difficult to prevent *mareng*. We have to maintain the balance of the four elements and prevent the development of bruises to *mareng*.	
CH6	We can prevent *mareng* by nourish *namlueang*. Bruises can develop to *mareng*, so if we get one, we must treat it right away. We can also take *benjakool* to maintain the balance of the four elements, at least once a year.	
CH7	We could take *Ya-lu-thong* which is composed by ten drugs.	
CH8	I have herbal tablets and decoction for cancer prevention. We could also drink the water from *fang* and *thonglang* soaked in lime water.	
CH9	No. But we can prevent the development of shingles to cancer. The remedy called '12 roots'.	
LA1	This preventive remedy composes of *katangbai, thaowanprieng, thaowandaeng, and huayang*.	
LA2	Yes, we have to take care of our health by eating and living properly. I don't recommend to take too much medicine to prevent for any diseases.	
LA3	Boil *rangchued* with rice. Drink the decoction as water. It is a universal detoxifier. There is another detoxifier which composes of *kasalong, oidam, and yapakkwai.* Herbal steam bath is also used to detoxify. I also have a herbal remedy that prevent *mahok* to *mareng*.	

Informant's code	Quotes	Remarks
LA4	I think *mareng* could be prevented by our immunity. To prevent it, we have to avoid habits that cause it. For some people who get ill easily, they should take remedies that help them to eat well, sleep well, and excrete well. The main ingredient is *benchakool* combined with herbs which nourish blood or the four elements.	
LA5	We can use an antidote against poisons.	
LA6	There is no preventive remedy for any diseases.	
LA7	I think we could take *saan* remedy. If it could cure the disease, why not prevent.	
LA8	*Yatatrak* should work.	*Rak* = root. *Yatatrak* is the very last remedy for treating *mareng*.
LA9	Food in very important. I suggested eating local vegetables and avoiding meat. We could take a preventive food recipe composes of *pakwanpa, pakwanban, and mon* to prevent from any diseases and keep healthy.	
SU1	I have herbal medicine which can be used to prevent from *mareng*.	
SU2	To prevent any disease, use a decoction composed of *thaowanprieng, kamphaengjedchan, and five types of kod.*	
SU3	*Mareng* cannot be prevented.	
SU5	Lingzhi and Thai medicine used for cleansing stomach, such as roots of *toeilamchiak* and *yakha* mix with other ingredients.	
SU6	*Triphala* can suppress *mareng*. It can be used as prevention as well.	
SU7	Drinking a decoction from fresh ginger and salt daily could prevent *mareng*. One of cancer preventive remedies is a decoction from *khaoyennuea, khaoyentai*, gingers, *sompoi* leaves, and *makham* leaves.	

Informant's code	Quotes	Remarks
SU8	Use a decoction that contains three ingredients which are leaves from *phaiseesuk* (a type of bamboos), turtle shells, and chicken feathers.	
SU9	It is difficult to prevent from any disease. We have to exercise and eat properly.	

Table 2-7 Summary of the comparisons between cancer and mareng from Thai traditional practitioners

Informant's code	Quotes	Remarks
CH2	In old Thai medical textbooks, there are some abscesses which might be cancer, such as *Fi Kanthasut* and *Fi nai pod*. The first condition is an internal abscess in colon similar to haemorrhoid, with pus. The latter one is abscess in lung, nowadays called lung cancer. Skin cancer is a type of skin disease in Thai traditional medicine.	
CH3	There is a type of *Fi* (abscess) which is very similar to cancer. A western doctor often diagnoses this to be cancer. It is a big lump inside stomach. This is the most aggressive *Fi* and can develop into *mareng*.	
CH4	The common symptoms of *mareng* in colon are frequent defecation and bloody, smelly, and black feces. In TTM, this condition is not *mareng* but a kind of haemorrhoid, which the treatment is different from that of colon cancer. While we use intoxicated* drugs for *mareng*, but we use hot-taste and astringent-taste herbs for this condition to increase blood and wind flow, and heal the wounds.	*In TTM, drugs are categorized according to their tastes. Intoxicated taste is one of the nine tastes.
CH7	In TTM, there is only one *mareng*. But in Western medicine, there are many *mareng* according to the location, such as *mareng* in the liver, *mareng* of the skin etc.	
LA4	Skin cancer can be several types of *mareng*, according to the old medical scripture, such as *mareng khai pla, mareng faman*, or *mareng nomyoung.*	

Informant's code	Quotes	Remarks
SU2	Some skin cancers are psoriasis, not *mareng*. We have to see how deep the condition is. If it goes under the skin, it is skin cancer	
SU3	*Mareng* develops from chronic *fi*. However not all *fi* are *mareng*. For example, *fi* in liver is not *mareng* but *krasai linkrabue*. Exploded internal *fi* will distribute throughout the body.	
SU5	Most of the time invasive wounds were diagnosed to be cancer from the hospital. In TTM, they are not *mareng*. We treat those using wound-healing remedies.	
SU9	Sometimes I examined patients who were diagnosed with cancer from the hospital and found that they don't have *mareng*, such as breast cancer. I could not find the cancer pulse.	

2.3.3.2. Disease explanation of cancer and causes in Thai traditional medicine

Explanation of *mareng* and causal factors from each informant are summarized in Table 2-3. Generally, *mareng* is a disease which develops when there is chronic *fi* (abscess), untreated chronic conditions, spoiled *luead* and *namlueang*, serious inflammation, or chronic bruise. Some informants also stated that *mareng* is any fast progressive diseases or body/organ degeneration. This is similar to the definition of cancer from traditional healers in Malaysia which defined cancer as a prolonged sickness or accumulation of abscesses (Al Naggar et al., 2012) and from Maya healers which stated that cancer is a disease that is difficult to treat (Berger-González et al., 2016). Traditional healers in Nigeria also associated cancer with an ulcer that does not heal easily (Nwoga, 1994). This agreement is obviously not relevant to the location or religion. However, it might be because one common characteristic of traditional medical systems is that traditional practitioners have no modern equipment for physical examination. As a result, abscesses and wounds are among the symptoms that can be seen and can be compared to ancient texts (please see the introduction; *mareng*-cancer in Thai traditional medicine?).

How TTM practitioners diagnose *mareng* is shown in Table 2-4. Since the practitioners do not have any tools that help in a diagnosis or confirmation of cancerous cells, the diagnosis relies on patients' symptoms and their history, especially history of cancer in family, jobs, and eating habits. The practitioners recognized that cancer is a disease in Western medicine. Since most patients were already diagnosed with cancer prior to the visits, the practitioners did not have to diagnose them again. However, the informants just check other information related to the treatment consideration i.e. symptoms and *dhātu*. Sometimes they compared the symptoms, mostly nodules and *fi*, with old texts. One informant uses spiritual diagnosis called *Pheng Kasin*, in which he would invite the patient's spirit to come and give the information about the sickness. Two informants check the pulse as they said cancer patients have a very fast pulse with a specific pattern (Figure 2-8) as they also have knowledge in Traditional Chinese medicine (TCM). They use both Chinese herbs and Thai herbs. TCM and traditional Indian healers in Malaysia also use the pulse checking to check for cancer (Al Naggar et al., 2012).

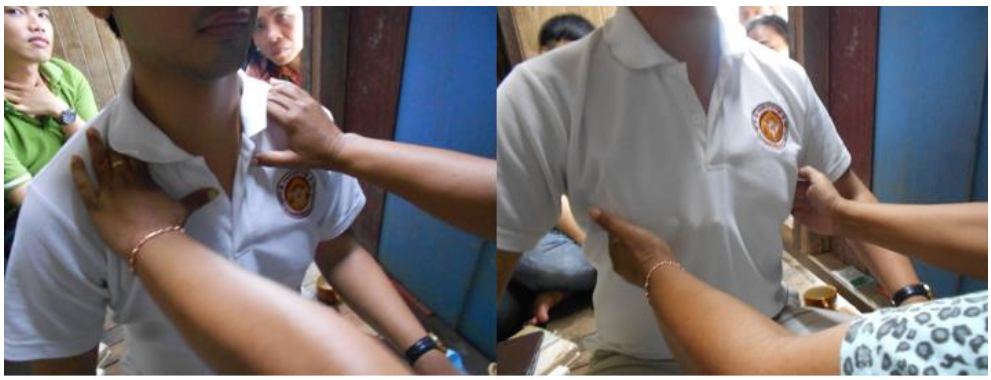

Figure 2-8 Pulse checking demonstration by a Thai traditional practitioner.

Based on the informants' responses, causes of *mareng* can be divided into internal and external factors. External factors are factors that a person is exposed to in the environment which include food, chemicals, *phayat*, *tua*, tobacco, and sunlight. *Tua* is generally used to classify 'living things with legs' and *phayat* means illness or parasites. Thai people use these words to refer to living things, so we could say that some informants view *mareng* as a living entity (Bamber, 1987). Interestingly, this is similar to Maya healers who stated that cancer is moving within the body (Berger-González et al., 2016). In essence these are emic concepts, and some perceived causes are in agreement with current knowledge that smoking, fried food, red meat, sunlight, and pollutants cause cancer (Anand et al., 2008).

Internal factors are now labelled as being linked to modern concepts like genetics and conditions that develop within the body, which include chronic abscesses, serious inflammations, degeneration of body or organs, and an unhealthy immune system.

2.3.3.3. Linking explanatory models of cancer in Thai traditional medicine with biomedical concepts

Understanding characteristics of *mareng* from an etic perspective helps in a better understanding of the actions of *mareng* treatments in TTM. The case studies above were representative of different perceptions of *mareng* from the informants which covered five main characteristics of *mareng* (Figure 2-9). These characteristics; waste accumulation, *krasail*chronic illnesses, *kan aksep* (inflammation), bad *luead* and *namlueang*, and imbalance between elements, were found to be common in patients who suffered from *mareng* and the treatments were based on them. The main characteristics here are our interpretations from the interviews of 33 Thai traditional practitioners (our etic perspective). They were mainly from the description of *mareng*, causes of *mareng*, and the treatment and prevention (see Table 2-3, Table 2-4, Table 2-5, and Table 2-6). In some topics, evidence on species used (this is etic perspective) was given to provide examples for the better understanding (Table 2-8).

Figure 2-9 Main characteristics of cancer from Thai traditional practitioners. The arrows show the relationships between each characteristic. Kan aksep (inflammation) and accumulative waste cause krasai. Bad luead or namlueang causes the imbalance of dhātu si (the four elements). Outer circle shows matching Western medicine concept to each characteristic.

Luead sia (bad blood) and Namlueang sia (bad lymph)

Eight informants mentioned that *luead sia (bad blood) and namlueang sia (bad lymph)* resulted in *mareng* development. The definition of *luead* and *namlueang* in TTM is not clear. *Luead* is recognized as the basic fluid of the body. So blood is accepted to describe *luead*. *Namlueang* is recognized as a specific fluid and is referred to as watery secretions. *Luead* and *namlueang* can be in different states, such as normal or bad. Some reports or books might detail their conditions to be spoiled or rotten or poisonous, but we used 'bad' (especially for *namlueang*) to describe abnormal states, as this needs to be investigated further in order to characterize them. Our findings that bad *luead* and *namlueang* could cause *mareng* correspond to the description of Brun and Schumacher (Brun and Schumacher, 1994) and Maya healers (Berger-González et al., 2016). Similarly, Hausa/Fulani folk healers in Northern Nigeria stated that cancer of male and female gonads and inflammation are caused by excessive production and deposition of fluids and phlegm (Abubakar et al., 2007).

Luead and *namlueang* are also involved with other causes of *mareng* which are the imbalance of the four elements and *fi* (abscesses). According to *Khamphi Maha Chotarat* (a scripture that describes the symptoms and herbal remedies for abnormal menstrual blood and menstruation), rotten or toxic blood could develop to *fi* (Foundation for the promotion of Thai traditional medicine and Ayurved Thamrong School Center of applied Thai traditional medicine, 2007b).

Medicinal plants used for *namlueang* problems are important ingredients in *mareng* therapy. *Khaoyennuea* and *khaoyentai* (Table 2-8) are well-known medicines for cancer treatment in Thailand (Itharat et al., 2004) They are used traditionally for *namlueang* problems. Informant C used them to make a decoction for patients with *mareng* in the breast. Extracts from rhizomes of *Smilax glabra* Roxb. (Smilacaceae), which is a major source for *khaoyentai*, showed significant growth inhibition to cancer cells of colon, gastric, lung, bladder, breast, liver, prostate, cervix, and human leukemia cells *in vitro*, while exhibiting low cytotoxicity to human umbilical vein endothelial cells and normal gastric epithelial cell line GES-1. Mechanistically, this has been linked to cell cycle arrest at S phase, apoptosis induction, and autophagy induction via redox-dependent ERK1/2 pathway (She et al., 2015b).

Nowadays *namlueang* in TTM is often associated with the immune system. Medicinal plants that are good for *namlueang* might be good for immune system and this can be used as a model to test their pharmacological properties. Water extract from *S. glabra* exhibited immunomodulatory activities by down-regulation of over-activated macrophages, up-regulation of the dysfunctional T lymphocytes in adjuvant-induced rats (Jiang et al., 2013), promotion of macrophage phagocytosis, and increasing macrophage-derived biological factors, including nitric oxide (NO), interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) secretion (Wang et al., 2017a). *Derris scandens* (Roxb.) contains flavonoids that exhibited specific cytotoxicity against several breast cancer cells in *in vitro* (Tedasen et al., 2016) and its extract also increased lymphocyte proliferation and NK cells functions (Table 2-8) (Sriwanthana and Chavalittumrong, 2001).

Moreover, informant C stated that abnormal *namlueang* causes inflammation by over-activation of the fire element. Informant A also stated that the obstruction of blood resulted in an accumulation of waste. These show that bad *luead* and *namlueang* are not only directly involved in *mareng* development, but also facilitate other characteristics.

Kan aksep (inflammation)

Five informants mentioned that *kan aksep* (inflammation) was related to *mareng* development. Inflammation can be caused by over-activation of the fire element, or external factors, such as food and microorganisms; or constant impact on an organ. According to informant C, inflammation was a result of an abnormality of *namlueang*. The concept of inflammation and *mareng* was similar to the Ayurvedic concept of cancer, which describes cancer as being of two types; inflammatory and non-inflammatory swelling (Balachandran and Govindarajan, 2005). Thus, this might be due to the influence of Traditional Indian medicine onto TTM

(Chokevivat and Chuthaputti, 2005). It is also in agreement with the current knowledge that chronic inflammation is a risk factor in cancer development (Candido and Hagemann, 2013). Inflammation is a defence mechanism of the body to infections and some conditions, such as trauma and toxins. Chronic inflammation is known to be associated with tumourigenesis and progression. It assists cancer cells to acquire hallmark capabilities, such as sustaining proliferation, limiting cell death, facilitating angiogenesis and metastasis. Nowadays inflammation is accepted as an enabling characteristic of cancer (Hanahan and Weinberg, 2011).

Chronic illnesses/krasai

Every informant said that *mareng* is very difficult to treat. Five informants mentioned specifically that chronic bruises and wounds would develop into *mareng*. According to the informants, chronic conditions can be used to differentiate *mareng* from another disease. For instance, vaginal discharge can result from a problem of the urinary system and dysmenorrhea can result from menstrual blood (*ra-du*) problems. However, informant B suspected *mareng* in the uterus of a patient because this patient had the symptoms for a long time. When a person has an illness for a long time, *krasai* would develop. According to the official Thai dictionary, *krasai* is characterised by emaciation, yellow skin, and cold feet and results in a deterioration of the body (Royal Society of Thailand, 2011). It is also defined as a condition with symptoms generally located in the lower abdominal area, including symptoms recognized in biomedicine as hernia, uro-genital afflictions, ulcers, and possibly gallbladder illness (Bamber, 1987); and a deterioration of the four elements or a decline of the body (Foundation for the promotion of Thai traditional medicine and Ayurved Thamrong School Center of applied Thai traditional medicine, 2007b). Consequently, *krasai* can be described as a degeneration of the body or an organ. As such it is a type of degenerative disease in TTM.

The relationship between degeneration and cancer is generally linked to mitochondrial dysfunction. Mitochondrial DNA (mtDNA) plays a crucial role in maintaining normal functions of organelles. The accumulation of mtDNA mutations or decreased mtDNA copy number have an effect on energy production, cell survival, and increase ROS generation. These have been linked to aging, age-related diseases, and cancer (Hsu et al., 2016b).

Accumulation of khong sia (waste)

One of the key therapeutic strategies to tackle *mareng* is to remove *khong sia* (waste). *Khong sia* is a very broad term, including body waste, such as urine, excrement, and sweat; as well as accumulated blood clots in a womb and unexcreted food in the intestines. According to *Khamphi Maha Chotarat*, blood clots in a womb are very dangerous since they cause pain and death. To treat the clots, one should take medicines that nourish the fire element to re-balance the four elements, followed by a blood tonic (Foundation for the promotion of Thai traditional medicine and Ayurved Thamrong School Center of applied Thai traditional medicine, 2007b). Poonthananiwatkul et al. (2015) reported that medicinal plants used for detoxification have been taken widely by cancer patients in Thailand and they produced some

benefits, such as laxative effect and improved blood test results and breathing in patients (Poonthananiwatkul et al., 2015). Accumulated waste also causes *krasai* (Figure 2-9), so removal of waste also helps to prevent it.

A Thai medicinal plant that is the best known for its detoxifying properties is *rangchued* (*Thunbergia laurifolia* Lindl.) (Table 2-8). Its decoction was mentioned by eight informants to be used in *mareng* treatment and prevention. It also has been reported for anti-cancer, anti-inflammatory, and protective effects (Junsi and Siripongvutikorn, 2016, Jetawattana et al., 2015, Rocejanasaroj et al., 2014, Wonkchalee et al., 2012).

Imbalance of dhātu si (four basic elements); earth, water, wind, and fire

Three informants mentioned that the imbalance *of dhātu si* (the four basic elements) is a cause of *mareng*. The imbalance of body's basic elements causing *mareng* is also an important explanatory model in Ayurvedic medicine. Cancer is seen as a result of a loss of coordination between three main system in the body, which are air, fire, and water elements (Balachandran and Govindarajan, 2005). The balance of elements is the main focus of TTM, as well as in other traditional medical systems. It maintains normal functions of the body and keeps one healthy. Every herbal remedy has to contribute to maintaining or re-balancing health.

To link the balance of the elements to pharmacological models, we propose here that such preparations could be involved in homeostasis. Homeostasis is a state of balance within the body or cells. This state maintains normal function and structure of the body or cells. Losing homeostasis plays an important role in tumourigenesis. For example, the production of cell proliferative signals is carefully controlled in normal tissues to ensure a suitable number of cells and to maintain tissue homeostasis. Cancer cells can avoid this regulation and continue to proliferate, grow, and invade nearby tissues and form tumours (Hanahan and Weinberg, 2011).

Triphala (Table 2-8) is a well-known herbal medicine used widely in the treatment of many diseases and their prevention. It is said to balance the elements, nourish blood, and clean the body. A large body of evidence supports that the medicine is beneficial in cancer and inflammation treatment. It also exhibited immunomodulatory and protective effects (Takauji et al., 2016, Kalaiselvan and Rasool, 2016, Varma et al., 2016, Chandran et al., 2015).

Table 2-8 List of plants/ingredients mentioned in the case studies, their traditional uses, and published relevant pharmacological properties

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
Thaowanprian g	stem	*Derris scandens* (Roxb.) Benth. (Fabaceae)	PBM05189	To fix *en*[11] problems, treatment of cough, cold, and Krasai, diuretic	Anti-cancer activities - Methanol extract/ flavonols exhibited anti-cancer activity against MDA-MB-231 and MCF-7 breast cancer cells and Topoisomerase II poison activity in yeast cells (Tedasen et al., 2016, Sangmalee et al., 2016, Ausawasamrit et al., 2015). Anti-inflammatory activity - *In vitro*, water extract/ ethanol extract/ genistein inhibited inflammatory substances; including myeloperoxide and eicosanoids (Laupattarakasem et al., 2003, Laupattarakasem et al., 2004) - In the rat hind paw edema test, the water extract reduced the edema when given intraperitoneally, but not when given orally (Laupattarakasem et al., 2003). Immunomodulatory activity - The ethanol extract enhanced lymphocyte proliferation and NK cell activity of normal individuals and induced NK cell activity of HIV-infected participants. It also increased IL-2 secretion from normal PBMC, but not IL-4 (Sriwanthana and Chavalittumrong, 2001). Anti-oxidant activities - Its hexane and chloroform extracts showed moderate free radical scavenging activities (Rao et al., 2007).

[11] Tendon or ligament

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
Khokhlan	stem	*Mallotus repandus* (Willd.) Müll. Arg. (Euphorbiaceae)	NL-0021	To fix *en* problems, treatment of *krasai*, renal impairment, diuretic, blood tonic, treatment of internal poisons	Anti-inflammatory activity - Water extract of the stems exhibited anti-inflammatory activity in paw edema induced by carrageenan model (Lin et al., 1992). Anti-oxidant activity - The ethyl acetate fraction of its ethanol extract exhibited strong superoxide radical scavenging activity and the n-hexane fraction showed strong hydroxyl radical scavenging activity (Lin et al., 1995).
Khaoyennuea	root	*Smilax corbularia* Kunth. (Smilacaceae) or mix *Smilax* spp. (Boonyaratanakornkit and Chanthornteptawan, 1993)	NL-0022	Treatment of *pradong*[12], *mareng*, *namlueang* problems, *en* problems, sexually transmitted diseases, thirst, abscesses, ulcers, urination problems, inflammation, kill pus, inhibition of poisons in bones. It is normally used together with Khaoyentai.	Anti-inflammatory activity - Its ethanol extract inhibited TNF-α and NO production. Three flavonols isolated from the ethanol extract; engeletin, astilbin and quercetin, inhibited PGE_2 release (Ruangnoo et al., 2012). Antioxidant activity - The ethanolic extract and its isolated compounds; astilbin and engeletin, exhibited antioxidant activity in DPPH assay and lipid peroxidation assay (Itharat et al., 2007).

[12] A condition in TTM with fever and itching rash

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
Khaoyentai	root	*Smilax glabra* Roxb. (Smilacaceae) or mix *Smilax* spp. (Boonyaratanakornkit and Chanthornteptawan, 1993)	NL-0023	Treatment of *pradong*, *mareng*, 'namlueang' problems, 'En' problems, sexually transmitted diseases, thirst, abscesses, ulcers, urination problems, inflammation, kill pus, inhibition of poisons in bones. It is normally used together with Khaoyennuea.	Anti-cancer activity - Its extracts possessed anti-cancer activity on cancer cell lines of gastric, lung, colon, bladder, breast, liver, prostate, and cervix origins, and human leukemia cells, with little cytotoxicity to HUVECs and normal gastric epithelial cell line GES-1 (She et al., 2015a, She et al., 2015b, Hao et al., 2016b, Li et al., 2015). - *In vivo*, supernatant of the water-soluble extract (SW) reduced the growth of tumour in xenograft mice (She et al., 2015a) and inhibited the metastasis of MDA-MB-231 (She et al., 2015b). Anti-inflammatory activity - *In vitro*, ethanol extract showed 5-LOX inhibition property (Zhao et al., 2016). Its polysaccharide fractions and phenolic-enriched extract inhibited LPS-induced production of TNF-α, IL-6, and NO in RAW 264.7 cells (Wei et al., 2015). Immunomodulatory effect - The water extract/ heteropolysaccharide showed immunomodulatory effects in macrophages and dysfunctional T lymphocytes (Jiang and Xu, 2003, Wang et al., 2017a). Protective effects and anti-oxidant activity - Ethanol extract/ flavonoid-rich fraction exhibited protective effects against CCl_4-induced and lead-induced toxicity and reversed hepatic damage caused by CCl_4 in rats (Xia et al., 2013, Xia et al., 2010).

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
Khanthongphayabat	Root or stem bark	*Suregada multiflora* (A.Juss.) Baill. (Euphorbiaceae)	NL-0024	Treatment of fever, poisoned blood, lung diseases, worms, lymph problems, *mareng*, liver diseases, lung diseases, and skin diseases	Anti-inflammatory activity - The hexane extract and helioscopinolide A inhibited NO production, PGE_2 production, and iNOS and COX-2 mRNA expressions in RAW264.7 cells (Tewtrakul et al., 2011).
Horathaosunak	Dried stem	*Balanophora abbreviata* Blume (Balanophoraceae)	NL-0025	Anti-asthma	Anti-inflammatory activity - Lignans from its extract showed moderate to potent inhibitory activity on LPS-induced iNOS expression in RAW 264.7 cells (Hosokawa et al., 2004).
Khanunsampalo, sake	root	*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg (Moraceae)	PBM05203	Treatment of sexually transmitted diseases and *mareng*	Anti-cancer activity - Diethyl ether extract/ methanol extracts/ partially purified fraction/ geranyl dihydrochalcone showed anti-cancer activity in in T47D breast cancer and DU145 prostate cancer cells, as well as suppressed tumour growth in DU145 xenograft initiation model (Jeon et al., 2015, Arung et al., 2009). Protective effects - The water and ethanol extracts reduced serum AST, ALT and ALP levels in CCl_4-treated rats and restored total protein and albumin to near normal levels. It also suppressed TBARS and increased glutathione level (Sairam et al., 2016). Activities related to blood and lymph - Intravenous administration of the water extract of the leaves caused significant hypotensive and bradycardiac responses (Nwokocha et al., 2012).

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
					- Ethanol extract of the leaves showed ACE-inhibitory activity with an IC_{50} value of 54.08 $\pm$ 0.29 µg/mL (Siddesha et al., 2011).
Makhampom	Root, fruit	*Phyllanthus emblica* L. (Phyllanthaceae)	PBM05199	Young fruit: nourish muscle and voice, treatment of constipation, worm in semha Mature fruit: treatment of fever	Anti-cancer activity - Water extract/ geraniin/ isocorilagin showed cytotoxic effects against MCF-7 cells, HELF cells, and HT1080 and down-regulated MMP2 and MMP9 gene expressions in human fibrosarcoma cells HT1080 (Liu et al., 2012, Yahayo et al., 2013) Anti-inflammatory activity - Ethanol extract of the branch suppressed the expression of LPS-induced pro-inflammatory genes (COX-2, iNOS, TNF-alpha, IL-16 and IL-6) in RAW 264.7 and also suppressed the carrageenan-induced paw edema in rats (Sripanidkulchai and Junlatat, 2014). Protective effects - Administration of the fruits/ methanol extract of the leaves/ compounds isolated from the fruits showed protective effects against cognitive deficits, biochemical abnormalities, apoptosis induced by aluminum chloride, and tau hyperphosphorylation (Thenmozhi et al., 2016), cadmium-induced neurotoxicity in mice (Chatterjee et al., 2016), CCl_4-induced oxidative injuries and tissue damage of lungs of rats (Tahir et al., 2016), H_2O_2-induced injury in PC12 cells (Zhang et al., 2016), and chemical-induced liver injuries in several animal models (Variya et al., 2016). Immunomodulatory activity - The fruit extract reversed the immunosuppressive effect of Cr (VI), enhanced white blood cell count and % lymphocyte distribution in mice, and also activated macrophages (Belapurkar et al., 2014) and the isolated compounds,

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
					geraniin and isocorilagin, stimulated splenocyte proliferation (Liu et al., 2012). Activities related to blood and lymph - Water extract decreased haemolytic activity in red blood cells of human subjects receiving chronic alcohol and cigarette smoking (Lokesh et al., 2016).
Thuakheaw (green mung beans)	dried seed	*Vigna radiata* (L.) R.Wilczek (Fabaceae)	-	Diuretic, tonic, treatment of over-heat, nourish joints	Anti-inflammatory activity - *In vivo*, water extract/ ethanol extract showed potent anti-inflammatory and antinociceptive activities (Ali et al., 2014, Venkateshwarlu et al., 2016). - *In vitro*, water extract/ methanol extract/ ethanol extract inhibited NO, COX-2, and exhibited membrane stabilization activity and protein denaturation activity (Ali et al., 2014, Venkateshwarlu et al., 2016, Zia-Ul-Haq et al., 2013). Immunomodulatory activity - Polysaccharide sub-fractions; MAP-1 and MAP-2, from alkali-extractable polysaccharides from the seeds enhanced the production of NO, TNF-α, and IL-6 in RAW 264.7 cells (Yao et al., 2016). Protective effects - *C. militaris*-fermented mung beans protected DNA damage (Xiao et al., 2015). - Mung bean coat extracts attenuated LPS-induced release of HMGB1 and several chemokines in macrophage cultures. It also rescued mice from lethal sepsis (Zhu et al., 2012).

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
Rangchued	leave	*Thunbergia laurifolia* Lindl. (Acanthaceae)	PBM05178	Universal antidote, treatment of fever, thirst, abnormal menstruation, ear pain, use externally for treatment of swelling, removal of toxin from the liver	Protective effects - In vitro, water extract/ ethanol extract/ acetone extract showed protective effects against oxidative stress in HepG2 cells (Rocejanasaroj et al., 2014), increased QR activity in Hepa1C1C7 cells and showed antimutagenic activity in *Salmonella typhimurium* TA 98 (Oonsivilai et al., 2007), ethanol-induced cell death of primary rat hepatocytes (Pramyothin et al., 2005). - In vivo, leaves/ water extract/ ethanol extract had protective effects against cadmium toxicity in rats (Ruangyuttikarn et al., 2013), inflammation-induced by *O. viverrini* infection or with NDMA administration (Wonkchalee et al., 2012), and $Pb(NO_3)_2$ treated *Oreochromis niloticus* (Palipoch et al., 2011), ethanol-induced liver injury (Pramyothin et al., 2005). Anti-inflammatory activity - Rosmarinic acid isolated from the leaves exhibited antinociceptive activity in the hot-plate test, reduced acetic acid-induced writhing, suppressed formalin-induced pain in the early and late phases, reduced carrageenan-induced and PGE_2-induced paw edema, and inhibited cotton pellet-induced granuloma formation in mice (Boonyarikpunchai et al., 2014). - The powder showed anti-inflammatory properties in *O. viverrini* and NDMA-treated Syrian hamsters by reducing the aggregation of inflammatory cells surrounding the hepatic bile ducts and reversed serum ALT, ALP, BUN and creatinine levels to normal (Wonkchalee et al., 2012).
Triphala	fruits	A mixture of the fruits from *Phyllanthus emblica* L. (Phyllanthaceae), *Terminalia bellirica* (Gaertn.) Roxb.	-	Balance the elements, tonic, nourish water element so this improves blood circulation,	Anti-cancer activity - Methanol extract/ chebulinic inhibited proliferation and induced apoptosis in human colon cancer stem cells (Vadde et al., 2015) and suppressed VEGF- induced angiogenesis (Lu et al., 2012).

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
		(Combretaceae), *Terminalia chebula* Retz. (Combretaceae)		improve digestive system, cleansing especially waste in the intestine	Anti-inflammatory activity - A randomized controlled clinical trial in 90 volunteers with chronic gingivitis showed that the effectiveness of Triphala mouthwash in the treatment of gingivitis was comparable to that of chlorhexidine mouthwash (Pradeep et al., 2016). - Water extract inhibited LPS-induced inflammatory responses in RAW 264.7 cells and adjuvant-induced arthritic rats by inhibition of NF-κB pathway (Kalaiselvan and Rasool, 2016). - Triphala recipe (4 mg/ear) showed an inhibitory effect on the ear edema formation in rats induced by ethyl phenylpropiolate, but not by arachidonic acid. Furthermore, It also decreased carrageenan-induced hind paw edema and showed antinociceptive effect in formalin-treated mice (Sireeratawong et al., 2013). Immunomodulatory effects - Administration of Triphala showed immunomodulatory effects on neutrophil functions, IL-2, IFN-γ levels, CD4+/CD8+lymphocyte phenotype, mitogen-induced T-lymphocyte proliferation, as well as increased phargocytic functions in mice (Belapurkar et al., 2014). - In a clinical trial phase I, volunteers who administered Triphala 3 capsules/ day for 2 weeks showed increase activity of cytotoxic T cells ($CD3^{-}CD8^{+}$) and natural killer cells ($CD16^{+}CD56^{+}$) compared to control group (Phetkate et al., 2012). Protective effects - Water extract/ ethanol extract exhibited protective effects against X-radiation and bleomycin-induced DNA breaks and suppressed ROS generation in HeLa cells (Takauji et al., 2016), H_2O_2-induced damage in fibroblasts or HaCaT cells, and decreased the mRNA levels of tyrosinase in B16F10 cells (Varma et al., 2016).

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
Yamornoi, Yadokkhao	whole plants	*Vernonia cinerea* (L.) Less. (Asteraceae) [Synonym: *Cyathilium cinereum* (L.) Rob	PBM05200	Treatment of fever, pain, hypertension, frequent to urinate, wound, abscess, and herpes zoster, and cleanse the lung	Anti-cancer activity - *In vitro*, dichloromethane fraction of the ethanol extract/ vernolide-A exhibited anti-cancer activity against HeLa, A549, MCF-7, Caco-2 (Beeran et al., 2014) and B16F-10 melanoma cells (Pratheeshkumar and Kuttan, 2011e, Pratheeshkumar and Kuttan, 2011d). - *In vivo*, methanol extract/ vernolide-A reduced lung tumour formation, metastasis, elevated level of serum IL-1β, IL-6, TNF-α, and GM-CSF, and VEGF induced by angiogenesis, and increased life span of C57BL/6 mice (Pratheeshkumar and Kuttan, 2011e, Pratheeshkumar and Kuttan, 2011c). Anti-inflammatory activity - *In vitro*, sesquiterpene lactones isolated from hexane extract of the flower suppressed TNF-α-induced NF-κB activity and NO production in LPS-stimulated RAW 264.7 cells (Youn et al., 2012). - *In vivo*, Intraperitoneal administration of methanol extract inhibited the PMA-induced superoxide generation in mice peritoneal macrophages, increased the levels of catalase, superoxide dismutase, glutathione, glutathione peroxidase and glutathione-S transferase in blood and liver, decreased lipid peroxidation, and inhibited carrageenan-induced inflammation in BALB/c mice (Kumar et al., 2009). Immunomodulatory activity - Methanol extract and vernolide-A stimulated cytotoxic T lymphocyte, NK cell, antibody-dependent cellular cytotoxicity, and antibody-dependent complement-mediated cytotoxicity through enhanced secretion of IL-2 and IFN-γ in *in vivo* and *in vitro* (Pratheeshkumar and Kuttan, 2012). - Vernolide-A increased the production of IL-2 and IFN-γ in metastatic tumour-bearing mice. It downregulated the serum levels of IL-1β, IL-6, TNF-α, and granulocyte–macrophage

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
					colony-stimulating factor (GM-CSF) during metastasis in C57BL/6 mice (Pratheeshkumar and Kuttan, 2011a). Protective effects - Intraperitoneal administration of the methanol extract exhibited protective effects against gamma radiation-induced immunosuppression and oxidative stress in BALB/c mice, while it did not compromise the effectiveness of the radiotherapy (Pratheeshkumar and Kuttan, 2011b).
Nontaiyak	root	*Stemona tuberosa* Lour. (Stemonaceae)	NL-0026	Treatment of skin diseases, itching, bad lymph, cough, phlegm, piles, *mareng* in liver, kill worms, use externally for skin diseases, kill scabies and louse	Anti-inflammatory activity - *In vivo*, water extract/ Tuberostemonine N showed anti-inflammatory activity against cigarette smoke-induced sub-acute lung inflammation in mice (Lim et al., 2015, Lee et al., 2014, Jung et al., 2016). - *In vitro*, water extract/ methanol extract and its eight alkaloids/ methyl ferulate exhibited anti-inflammatory activity in murine BV2 microglial cells and RAW264.7 cells; such as inhibition of LPS-induced NO production, COX-2 expressions, inducible nitric oxide synthase (Lee et al., 2016, Lim et al., 2015, Phuong et al., 2014). Immunomodulatory activity - Tuberostemonine O suppressed IL-2 production and IL-2-induced proliferation in CD4+ T cells (Jang et al., 2014).
Phlukhao, Phak-khaotong	Whole plant	*Houttuynia cordata* Thunb. (Saururaceae)	PBM05204	Treatment of sexually transmitted diseases, bad namlueang, wounds, fever, heat, lung problems, edema, cough,	Anti-cancer activity - Its extracts/ fractions exhibited anti-cancer activity against nasopharyngeal carcinoma cells (Chen et al., 2016), lung cancer A549 cells (Chen et al., 2013), and human leukemic Molt-4 cells (Prommaban et al., 2012). - Fermentation products suppressed the growth of HeLa, HCT116, HT29 cells, human leukemic HL-60 and Molt-4 cell,

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
				haemorrhoids, diuretic, use externally for snake and insect bites, skin diseases, abscess, *mareng khuttharat*	and HepG2 cells (Senawong et al., 2014, Banjerdpongchai and Kongtawelert, 2011). Anti-inflammatory activity - Ethanol extract from *Houttuynia cordata* poultice exhibited anti-bacterial effects and anti-biofilm against methicillin-resistant *Staphylococcus aureus* (MRSA), inhibition effect of *S. aureus* lipoteichoic acid (LTA)-induced IL-8 and CCL20 production in human keratinocyte without any cytotoxicity (Sekita et al., 2016). - Volatile compounds/ polysaccharides/ flavonoids exhibited anti-inflammatory effects in *in vivo* (Chen et al., 2014, Li et al., 2013a, Lee et al., 2015c, Xu et al., 2015, Park et al., 2013). - Ethanol extract/ ethyl acetate fraction/ volatile oil suppressed the production of inflammatory markers/ cytokines in LPS-stimulated RAW 264.7, LPS-stimulated mouse peritoneal macrophages, and HMC‑1 human mast cells (Chun et al., 2014, Li et al., 2013a, Lee et al., 2013, Li et al., 2011). - Hexane extract inhibited prostaglandin synthase in *in vitro*(Bauer et al., 1996). Immunomodulatory activity - Water extract/ ethanol extract/ polysaccharides possessed immunomodulatory effects on the vaginal mucosa (Satthakarn et al., 2015), PBMCs(Cheng et al., 2014), LPS-activated macrophages (Kim et al., 2009), mouse splenic lymphocytes (Lau et al., 2008), Jurkat T cells and HMC-1 human mast cell line (Lee et al., 2008), mast cell-mediated anaphylactic responses (Li et al., 2005). Protective effects - Ethyl acetate extract exhibited hepatoprotective effect in CCl_4-induced hepatotoxicity in mice (Kang and Koppula, 2014) - Water extract showed protective effect against high saturated fat diet-induced obesity and oxidative and inflammatory injury in mice (Lin et al., 2013), prevent lung injury from LPS

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
					stimulation in rats (Cai et al., 2013), cardiac and renal injury and oxidative stress under diabetic condition in mice (Hsu et al., 2016a), and ethanol extract also reduced atherogenic risk and hepatic oxidative damage induced by high saturated fat diet in rats (Kim et al., 2014).
Oidam (sugar cane)	stem	*Saccharum officinarum* L. (Poaceae)	NL-0027	Treatment of gallstones, cough, thirst, fever, wounds, diuretic	Anti-cancer activity - Ethyl acetate extract/ tricin derivatives exhibited cytotoxicity in concentrations in human cancer cell lines; glioma (U521), breast (MCF-7), resistant ovary (NCI/ADR-RES), kidney (786-0), lung (NCI-H460), prostate (PC-3), ovary (OVCAR-3), and colon (HT29), and human keratinocyte HaCat cell lines (Alves et al., 2016, Duarte Almeida et al., 2007). - Its phytocystatin, CaneCPI-4, exhibited a strong inhibitory activity against cathepsins B and L, which are important for cancer cells invasion, in breast cancer cells (Carmona et al., 2008). Anti-inflammatory activity - A mixture of fatty acids from wax oil reduced zymozan-induced β-glucuronidase, cellular infiltration, synovial hyperplasia, and synovitis in mice (Ledon et al., 2007). Protective effects - *In vivo*, sugar cane extracts/ high molecular weight primary acids from sugar cane wax showed protective effects against CCl_4-induced and paracetamol-induced hepatic injury in rats (Mendoza et al., 2003, Noa et al., 2002), LPS-induced liver injury (Motobu et al., 2006), LPS-induced endotoxin shock (Hikosaka et al., 2006), - Water extracts exhibited protective effect against radiation-induced DNA damage in pBR322 plasmid DNA and *Escherishia coli* cultures and also antioxidant properties (Kadam et al., 2008).

Local names	Part used	Scientific names	Voucher number	Traditional uses	Relevant pharmacological properties
					Immunomodulatory effects - Sugar cane extracts and polyphenol-rich fraction stimulated immune responses in chicken by affecting peripheral blood leukocytes, intestinal leukocytes and splenocytes, and delayed-type hypersensitivity responses to human γ globulin in chicken (Hikosaka et al., 2007, Amer et al., 2004).
Kasalong, Peeb	Root, stem bark	*Millingtonia hortensis* L.f. (Bignoniaceae)	PBM05202	Nourishment of lung, treatment of tuberculosis and lung problems	Anti-cancer activity - Water extract and its fraction inhibited cell proliferation and induced apoptosis in RKO colon cancer cells (Tansuwanwong et al., 2009). Antimutagenic activity - Hispidulin and hortensin showed antimutagenic activity against 2–aminoanthracene, aflatoxin BI, and dimethylnitrosamine, while showed no mutagenicity and no cytotoxicity toward *S. typhimurium* strains TA98 and TA100 (Chulasiri et al., 1992). Anti-inflammatory activity - Hispidulin inhibited 5-LOX in *in vitro* (Moongkarndi et al., 1991).

2.3.4. Treatment of *mareng*

The practitioners combine several methods to treat *mareng* patients, such as herbal medicine, or herbal medicines combined with massage, or herbal medicine combined with spiritual methods (such as ritual blessings) (Table 2-5). For herbal medicine, the practitioners received formulation from their ancestors in the form of notebooks or verbal instruction or *khamphi*, well-known *khamphi* (eg. *Phaetthayasat Songkhro and Tamrayaklangbaan*), or from some famous traditional practitioners (Figure 2-10).

Figure 2-10 (A) Samud khoi is very difficult to find and mostly passed on only among family members. (B) A version of Phaetthayasat Songkhro owned by an informant. (C) Tamrayaklangbaan is a very popular textbook published in 1995. It contains more than 500 herbal formulae for many symptoms from many important practitioners, including secret recipes from famous familes.

Prescriptions depend on patients' condition and stages of *mareng*. At the beginning of the treatment, the informants evaluate the suitability of the first therapy in the initial 3-7 days to see the compatibility with the patient's body and good signs for the efficacy. If it does not improve any of the patient's conditions, they will change to the next herbal remedy until they find the right one. The duration of the treatment depends on the disease progress. For instance, an informant recalled a patient with advanced liver cancer who lived up to one year, while in another case with colorectal cancer the person has been on the treatment since four years and still continues the therapy (the interview was performed in April 2013).

Figure 2-11 (A) Herbal recipe to make a decoction for mareng
(B) Ingredients in yatatrak with drug preparation and dosage regimen

Apart from killing *mareng*, there are also other important steps in treating *mareng*. The first one is detoxification as it is believed that accumulated waste in the body could cause *mareng*. Another one is enhancing immunity. Some practitioners also prescribe "*Yatatrak*" (a remedy that kills the 'root' of *mareng*) to prevent the recurrence (Figure 2-11 B). As mentioned previously, some TTM practitioners see *mareng* as a living entity and it has roots. Even though the patients have recovered, *mareng* will grow again if they don't kill the roots.

During and after the treatment, the patients have to avoid some food items which are believed to worsen the condition. Some prohibited food items are catfish, Indo-Pacific mackerel, prawns, shrimps, shellfish, crabs, beef, poultry, sausages, eggs, pineapples, corns, Chinese kale, cucumbers, oyster mushroom (specific to some types of *mareng*), and pickled food. Food restriction is common knowledge in traditional medicine, such as in Mayan medicine (Berger-González et al., 2016). In Thailand, catfish, mackerel, shellfish, shrimps, crabs, chicken meat, beef, and pickled food are general food prohibitions which are not specific to a disease. Patients just have to avoid them after they start to have health problems. In Northern Thailand, eggs are restricted in some diseases, such as *khaan lidsaduan (*lesions of gums/ mouth/ nose/ eyes/ throat/ skin), *bahen* (a group of skin diseases), and *saan* (tumour). Traditional practitioners in Northern Thailand also prohibit some food items which have shapes similar to tumours, such as everything with a head or round fruits or fingers, food that grows by root sprouts, also food with sap because it could make tumours become ripe and pus might develop (Brun and Schumacher, 1994).

2.3.5. Cancer prevention

Twenty-nine informants suggested preventive methods for *mareng* (Table 2-6). Four informants believed that diseases cannot be prevented. They also believed that unnecessary taking of medicine is dangerous. The best way is to seek treatment as early as possible. There are three methods for *mareng* prevention which are herbal medicine, food items, and life style modification. Herbal medicines are multi-herbal preparations or single herbs. In addition, some informants suggested taking herbal products that nourish or adjust the balance of the four elements regularly or at least one course per year. Another preventive method is eating some specific food items which mostly are local fresh vegetables. Lifestyle modifications are mostly dealing with eating habits, such as avoiding some food items. It also involvs avoiding the causal factors specified by western medicine including smoking, fried foods, red meat, alcohol, sunlight exposure, pollutants, infections, stress, obesity, and physical inactivity in order to prevent cancer (Anand et al., 2008). This agreement might be due to the result of the national cancer control program by Ministry of Public Health since 1997 (Ministry of Public Health, 2013) or health information provided by media, especially television program.

In addition, it is suggested that people with an increased risk, such as industrial workers who are exposed to lead or mercury or farmers who use a lot of insecticides, should take herbal medicine that helps in detoxification because chemical exposure leads to toxic blood and could cause *mareng*. This belief coincides with the uses of chemoprevention in high risk

populations, such as the use of raloxifene in prevention of estrogen-receptor-positive invasive breast cancer (Anand et al., 2008). The data suggest that TTM practitioners could have an important role in cancer or other disease prevention in the local community, together with primary health care sectors, because local people still seek help from them, especially in untreatable diseases. However, knowledge sharing and learning from each other (TTM practitioners and health care sector) must be initiated.

Preventive approach from the external causal factors (eg. chemicals, food, smoke) can be achieved by avoiding them or removing them by detoxification. For internal causal factors, the informants suggested some approaches as follows;

- Removal of waste from the body (detoxification)
- Maintaining the balance of the four elements
- Nourishment of *luead* and *namlueang*
- Prevention of *krasai*
- Prevention of liver abnormality because liver is among the most important organs of the body. If the liver becomes abnormal, diseases, including *mareng*, could show up in many organs, such as liver itself, breast, uterus, ovary, or prostate gland.

These approaches help to form our strategy for pharmacological assays in the next steps.

2.3.6. Herbal remedies used in cancer/ *mareng* management

The informants mentioned 113 herbal remedies used in cancer patients and for cancer/*mareng* prevention. The purposes of them are shown in Table 2-9. Our results are in agreement with the findings from a previous study that reported herbal products used by cancer patients in Thailand. Herbal products; single herbs and formulae, were used for directly or indirectly treating of cancer, such as to manage side effects from chemotherapy (eg. aphthous ulcers and fever), detoxification, and enhancement of immunity (Poonthananiwatkul et al., 2015). The routes of administrations are shown in Table 2-10. About half of all remedies are used as a decoction (Table 2-11). Forty-one herbal remedies fall into preventive categories, which are maintain good health, prevention of *mareng*, and prevention of development of cancer/*mareng* from some conditions.

Table 2-9 Summary of purposes of herbal products mentioned by the informants

No	Purposes	Number of remedies
1	Treatment of cancer/*mareng*	65
2	Maintain good health	22
3	Prevention of *mareng*	14
4	Supplement to cancer treatment	4
5	Prevention of development of *mareng* from some conditions	3
6	Treatment of a disease that is sometime diagnosed to be a cancer	3
7	Treatment and prevention of cancer/ *mareng*	2
	Total	**113**

Table 2-10 Directions to use

Directions	**Number of remedies**
Orally consume	100
Cover on affected area	7
Apply to affected area	3
Spit on affected area	2
Wash the affected area	1

Table 2-11 Methods of preparation of all herbal remedies

Methods of preparation	Number of remedies
Decoction	54
Capsules	13
Consume as food	13
Pounding	7
Mixing with oil	6
Powder	6
Alcoholic maceration	4
Freshly press	3
Scratching plants with a stone and water	3
Water/ lime water maceration	3
Pills	1

2.3.7. Plants used in cancer/*mareng* prevention in Thai traditional medicine

There were 346 crude drugs mentioned to be used in cancer/*mareng* management. The crude drugs included 316 plant-derived materials, 15 animal-derived materials, 10 minerals/chemicals, and 4 mushrooms. Thirty-nine plants and three mushrooms could not be collected to verify their correct taxonomic identity (all species listed in Appendix).

Since this project focuses on cancer prevention, only herbal remedies and food plants related to prevention of cancer/*mareng*, including maintaining good health, prevention of *mareng*, and prevention of development of cancer/*mareng* from some conditions, were studied further. A total of 119 plants belonging to 56 families were mentioned by TTM practitioners for their use in cancer/*mareng* prevention (Table 2-12). Five plants could not be verified scientifically. Fabaceae and Zingiberaceae had the highest percentage of medical plant species with 10% and 8%, respectively.

Table 2-12 Medicinal plants used in to maintain good health and prevent from cancer/mareng

No	Local name (other name)	Part used	Scientific name	Family	Frequency (FC)
1	Boraphet (putarwali)	stem	*Tinospora crispa*(L.) Hook. f. & Thomson	Menispermaceae	2
2	Buabok (Asiatic pennyworth, gotu kola)	leaf	*Centella asiatica* (L.) Urb.	Apiaceae	1
3	Buk (elephant yam)	tuber	*Amorphophallus paeoniifolius* (Dennst.) Nicolson	Araceae	1
4	Chaemthet (licorice)	root	*Glycyrrhiza glabra* L.	Fabaceae	2
5	Chan (nutmeg tree)	flower, fruit	*Myristica fragrans* Houtt.	Myristicaceae	2
6	Cha-om (pennata wattle)	root and twig	*Senegalia pennata* (L.) Maslin	Leguminosae	1
7	Chaphlu (wild betel)	leave, whole plant	*Piper sarmentosum* Roxb.	Piperaceae	3
8	Cheng-chu-chai (white mugwort Guizhou group)	aerial part	*Artemisia lactiflora* Wall. ex DC.	Asteraceae	1
9	Chetphangkhi	root	*Cladogynos orientalis* Zipp. ex Span.	Euphorbiaceae	3
10	Chettamunploengdaeng (rose-coloured leadwort)	root	*Plumbaco indica* L.	Plumbaginaceae	3
11	Chingchi, saemathalai	root	*Capparis micracantha* DC.	Capparaceae	2
12	Dipli (long pepper)	fruit	*Piper retrofractum* Vahl	Piperaceae	2
13	Dongdueng (climbing lily)	root	*Gloriosa superba* L.	Colchicaceae	1
14	Fang (sappan tree)	wood	*Caesalpinia sappan* L.	Fabaceae	1
15	Haewmu (nutgrass)	rhizome	*Cyperus rotundus* L.	Cyperaceae	1
16	Hangnokyung daeng (red flower)	root	*Caesalpinia pulcherrima* (L.) Sw.	Fabaceae	1
17	Hangnokyung lueang (yellow flower)	root	*Caesalpinia pulcherrima* (L.) Sw.	Fabaceae	1

No	Local name (other name)	Part used	Scientific name	Family	Frequency (FC)
18	Hanumanprasankai	leaf	*Schefflera leucantha* R.Vig.	Araliaceae	1
19	Homdaeng (shallot)	young shoot	*Allium ascalonicum* L.	Amaryllidaceae	2
20	Huayang, thaowanyang (kumarika)	stem	*Smilax ovalifolia* Roxb. ex D.Don	Smilacaceae	1
21	Kamphaengchetchan	wood	*Salacia chinensis* L.	Celastraceae	1
22	Kanphlu (clove)	flower	*Syzygium aromaticum* (L.) Merr. & L.M.Perry	Myrtaceae	1
23	Kaprao (holy basil)	leaf	*Ocimum tenuiflorum* L.	Lamiaceae	1
24	Kasalong (Indian cork tree)	stem bark	*Millingtonia hortensis* L.f.	Bignoniaceae	2
25	Katangbai (bandicoot berry)	leaf	*Leea indica* (Burm. f.) Merr.	Vitaceae	1
26	Kha (galangal, Thai ginger)	rhizome	*Alpinia galanga* (L.) Willd.	Zingiberaceae	1
27	Khamfoi (safflower)	flower	*Carthamus tinctorius* L.	Asteraceae	1
28	Khaminkhruea, nae khruea (yellow-fruit moonseed)	root	*Arcangelisia flava* (L.) Merr.	Menispermaceae	1
29	Khanghuamu	N/A	N/A	N/A	1
30	Khaotong, Phlukhao (fishwort)	whole plant	*Houttuynia cordata* Thunb.	Saururaceae	1
31	Khaoyennuea	rhizome	*Smilax* spp.	Smilacaceae	2
32	Khaoyentai	rhizome	*Smilax* spp.	Smilacaceae	2
33	Khaton	root, wood	*Cinnamomum ilicioides* A.Chev.	Lauraceae	1
34	Khing (ginger)	rhizome	*Zingiber officinale* Roscoe	Zingiberaceae	5
35	Khoklan	wood	*Mallotus repandus* (Rottler) Müll. Arg.	Euphorbiaceae	1
36	Khontha	root	*Harrisonia perforata* (Blanco) Merr.	Simaroubaceae	1
37	Kloi	tuber	*Dioscorea hispida* Dennst.	Dioscoreaceae	2
38	Kothuabua, (Szechwan lovage rhizome, Chuan Xiong)	rhizome	*Ligusticum striatum* DC.	Apiaceae	2
39	Kotkamao (atractylodes, Cang Zhu)	dried rhizome	*Atractylodes lancea* (Thunb.) DC.	Asteraceae	2
40	Kotkraduk (costus root, Mu Xiang)	dried root	*Saussurea costus* (Falc.) Lipsch.	Asteraceae	2
41	Kotphungpla, Samothai (terminalia gall, myrobalan gall)	fruit	*Terminalia chebula* Retz.	Combretaceae	4
42	Krachai (fingerroot)	rhizome	*Boesenbergia rotunda* (L.) Mansf.	Zingiberaceae	1
43	Kradaddaeng (red giant taro)	rhizome	*Alocasia macrorrhizos* (L.) G.Don	Araceae	1
44	Kradadkhao (white giant taro)	rhizome	*Alocasia macrorrhizos* (L.) G.Don	Araceae	1
45	Kradon (slow match tree)	young leaf	*Careya arborea* Roxb.	Lecythidaceae	1

No	Local name (other name)	Part used	Scientific name	Family	Frequency (FC)
46	Kradukkaidam	leaf	*Justicia fragilis* Wall.	Acanthacaea	1
47	Krathiam (garlic)	young shoot	*Allium sativum* Linn.	Amaryllidaceae	3
48	Krawan (Siam cardamom)	fruit	*Amomum compactum* Sol. ex Maton	Zingiberaceae	1
49	Lamchiak, toei-ta-le (umbrella tree)	root	*Pandanus odorifer* (Forssk.) Kuntze	Pandanaceae	1
50	Maduea chumpon (cluster fig)	root	*Ficus racemosa* L.	Moraceae	1
51	Maduk	root	*Siphonodon celastrineus* Griff.	Celastraceae	1
52	Mafai (Burmese grape)	heartwood, root, bark	*Baccaurea ramiflora* Lour.	Phyllanthaceae	1
53	Mafueang (carambola, starfruit)	heartwood, root, bark	*Averrhoa carambola* L.	Oxalidaceae	1
54	Magrud (kaffir lime)	leaf	*Citrus hystrix* DC.	Rutaceae	2
55	Mahahing (asafoetida, stinking gum, devil's dung)	oleo-gum-resin	*Ferula assa-foetida* L.	Apiaceae	3
56	Mahuad	stem bark	*Lepisanthes rubiginosa* (Roxb.) Leenh.	Sapindaceae	1
57	Makham (tamarind)	leaf	*Tamarindus indica* L.	Fabaceae	1
58	Makhamkai	leaf	*Putranjiva roxburghii* Wall.	Putranjivaceae	1
59	Makhampom (emblic myrobalan)	fruit	*Phyllanthus emblica* L.	Phyllanthaceae	3
60	Maklamtanu (crab's eye vine, American pea)	sap wood	*Abrus precatorius* L.	Fabaceae	1
61	Maliwanpa	root	N/A	N/A	1
62	Manao (lime)	leaf, juice from fruits	*Citrus* × *aurantiifolia* (Christm.) Swingle	Rutaceae	2
63	Maprang (marian plum, gandaria, plum mango)	heartwood, root, bark	*Bouea macrophylla* Griff.	Anacardiaceae	1
64	Mapring (Burmese plum, plum-mango)	heartwood, root, bark	*Bouea oppositifolia* (Roxb.) Adelb.	Anacardiaceae	1
65	Marum (horseradish tree, drumstick tree)	leaf	*Moringa oleifera* Lam.	Moringaceae	2
66	Matum (bael, golden apple)	fruit	*Aegle marmelos* (L.) Corrêa	Rutaceae	2
67	Muakdaeng	stem	*Wrightia coccinea* (Roxb. ex Hornem.) Sims	Apocynaceae	1
68	Muakkhao	stem	*Wrightia pubescens* subsp. pubescens	Apocynaceae	1
69	Ngueakplamo dokmuang	leaf	*Acanthus ilicifolius* L.	Acanthaceae	1
70	Nontaiyak	root tuber	*Stemona tuberosa* Lour.	Stemonaceae	1
71	Oi-dam (sugar cane)	stem	*Saccharum officinarum* L.	Poaceae	1
72	Phakbungdaeng (morning glory)	root	*Ipomoea aquatica* Forssk.	Convulvulaceae	1
73	Phakchiangda (Gymnema sylvestre)	young flower, young leaf	*Gymnema inodorum* (Lour.) Decne.	Apocynaceae	1

No	Local name (other name)	Part used	Scientific name	Family	Frequency (FC)
74	Phakkradhuawaen (para cress)	young leaf	*Acmella caulirhiza* Delile	Compositae	1
75	Phakpaewdaeng	whole plant	*Iresine diffusa f. herbstii* (Hook.) Pedersen	Amaranthaceae	1
76	Phaktaew	leaf	*Cratoxylum formosum* (Jack) Benth. & Hook.f. ex Dyer	Hypericaceae	1
77	Phakwanban	leaf	*Sauropus androgynus* (L.) Mer.	Phyllanthaceae	1
78	Phakwanpa	leaf	*Melientha suavis* Pierre	Opiliaceae	1
79	Phitsanad	root	*Sophora exigua* Craib	Fabaceae	1
80	Phrikpa, Phriknaiphran	N/A	N/A	N/A	1
81	Phrikthai, black pepper	fruit	*Piper nigrum* L.	Piperaceae	2
82	Pua-ki-nai (Huang Qin, Baikal skullcap)	root	*Scutellaria baicalensis* Georgi	Lamiaceae	1
83	Rangchued (laurel clock vine, blue trumpet vine)	stem, root	*Thunbergia laurifolia* Lindl.	Acanthaceae	6
84	Reonoi	fruit	*Amomum villosum* Lour.	Zingiberaceae	1
85	Rhubarb	rhizome	*Rheum Palmatum* L. Or *R. officinale* Bail. Or *R. tanguticum* (Maxim. Ex Regel) Maxim. Ex Balf.	Polygonaceae	1
86	Rong, rongthong (gamboge)	resin	*Garcinia hanburyi* Hook.f.	Clusiaceae	1
87	Sakhan	stem	*Piper* aff. *pendulispicum* C.DC.	Piperaceae	2
88	Samodingu	fruit	*Terminalia citrina* (Gaertn.) Roxb. ex Flem	Combretaceae	1
89	Samophiphek, naeton (beleric myrobalan)	fruit	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	3
90	Samothet	fruit	*Terminalia* spp.	Combretaceae	1
91	Sankham (Chinese albizia, silk tree)	twig	*Albizia chinensis* (Osbeck) Merr.	Fabaceae	2
92	San-ngoen, insi (copperpod, golden flamboyant)	twig	*Peltophorum pterocarpum* (DC.) Backer ex K.Heyne	Fabaceae	2
93	Somchin (mandarin orange)	root	*Citrus* × *aurantium* L.	Rutaceae	1
94	Sompoi (soap pod)	leaf, pod	*Senegalia rugata* (Lam.) Britton & Rose	Fabaceae	2
95	Ta-khlai (lemongrass)	rhizome	*Cymbopogon citratus* (DC.) Stapf	Poaceae	1
96	Tamlueng (ivy gourd)	whole plant	*Coccinia grandis* (L.) Voigt	Cucurbitaceae	2
97	Thao-en-on	stem	*Cryptolepis dubia* (Burm.f.) M.R.Almeida	Asclepiadaceae	1
98	Thaowandaeng	stem	*Ventilago denticulata* Willd.	Rhamnaceae	1
99	Thaowanpriang (jewel vine)	stem	*Derris scandens* (Roxb.) Benth.	Fabaceae	3
100	Thaoyaimom	root	*Clerodendrum indicum* (L.) Kuntze	Lamiaceae	1
101	Thiandam (fennel flower, black caraway)	seed	*Nigella sativa* L.	Ranunculaceae	1

No	Local name (other name)	Part used	Scientific name	Family	Frequency (FC)
102	Thiankao (cumin)	fruit	*Cuminum cyminum* L.	Apiaceae	1
103	Thua-phu (winged bean)	root	*Psophocarpus tetragonolobus* (L.) DC.	Fabaceae	1
104	Wan hokmokkhasak	root	N/A	N/A	1
105	Wan khothongkae	rhizome	*Curcuma* sp.	Zingiberaceae	2
106	Wan nakkharat, Wan hangnak (Ceylon bowstring hemp, devil's tongue)	rhizome	*Sanseviera zeylanica* (L.) Willd.	Asparagaceae	1
107	Wan thonmokkhasak	rhizome	*Kaempferia* sp.	Zingiberaceae	1
108	Wanmahakan	root	*Gynura hispida* Thwaites	Compositae	1
109	Wanphetchahueng (giant orchid, tiger orchid)	root	*Grammatophyllum speciosum* Blume	Orchidaceae	1
110	Wanphetchaklab	rhizome	*Boesenbergia thorelii* (Gagnep.) Loes	Zingiberaceae	1
111	Ya khaosan, Sanrangdid	whole plant	N/A	N/A	1
112	Ya nuadmaew (cat's whisker)	whole plant	*Orthosiphon aristatus* (Blume) Miq.	Lamiaceae	1
113	Ya nuadruesi (black speargrass, tanglehead)	whole plant	*Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult.	Poaceae	1
114	Ya tudma	whole plant	*Paederia pilifera* Hook. f.	Rubiaceae	1
115	Yadam	dried latex	*Aloe* spp.	Xanthorrhoeaceae	2
116	Yakha (blady grass, cogon grass)	root	*Imperata cylindrica* (L.) P.Beauv.	Poaceae	2
117	Yanang	root, stem	*Tiliacora triandra* (Colebr.) Diels	Menispermaceae	3
118	Yapakkhwai (Egyptian crowfoot grass)	whole plant	*Dactyloctenium aegyptium* (L.) Willd.	Poaceae	1
119	Yo (Indian mulberry)	fruit	*Morinda citrifolia* L.	Rubiaceae	2

Table 2-13 shows frequency of citation (FC) and relative frequency of citation (RFC) of preventive plants that were mentioned by at least two informants. The species were ranked according to their total FC values. The first species in the ranking is *rangchued* (*Thunbergia laurifolia*) which was cited by six out of thirty-three informants. It was used as a single herb and in combination. However, it was mentioned by informants from two locations only, which were Lamphun (RFC = 0.44) and Bangkok Metropolitan Region (RFC = 0.33). Even though the total RFC is low, 0.18, its RFC values for each area show the spread of knowledge about the usefulness of the plant in each area. The second species in the ranking is *khing* (*Zingiber officinale* or ginger) which was mentioned by five informants from all locations. This might be because it is a food item and very easy to find. It was used in combination with other

ingredients. While *rangchued* is not recorded in *Khamphi Sappakhunya*[13], *khing* appears in this famous textbook in several important herbal formulae, such as *benchakool*[14] and *trikatuk*[15] (Foundation for the promotion of Thai traditional medicine and Ayurved Thamrong School Center of applied Thai traditional medicine, 2007a). The third in the ranking is *samothai* (*Terminalia chebula*) which was mentioned by four informants from Bangkok Metropolitan Region. Its RFC value is quite high showing that most TTM practitioners in Bangkok Metropolitan Region have an agreement in its usefulness in *mareng* prevention. It was used only in combination with other raw materials. *Samothai* is recorded in *Khamphi Sappakhunya* as an ingredient in the popular herbal medicine, *triphala,* and many others (Foundation for the promotion of Thai traditional medicine and Ayurved Thamrong School Center of applied Thai traditional medicine, 2007a).

It is acceptable to state that the cultural importance of a plant depends on the number of informants who cited its usefulness (FC) and the number of uses. Plants that are easy to use and can be adapted for many uses are more widespread and would have higher FC (Tardío and Pardo-de-Santayana, 2008). *Rangchued* is easy to use because it can be used as a single herb or in a combination. It is also easy to find and grow. *Khing* is used in *benchakool* which is used to re-balance the four elements. Maintain such balance is the key strategy in TTM.

Figure 2-12 shows the steps of potential plants selection for further pharmacological study. Table 2-14 shows the list of plants used in the *in vitro* screening in Chapter 3.

[13] A famous textbook about uses of medicinal plants and many herbal formulae. It is in *Phaetthasat songkhro*.

[14] Benchakool which comprises *dipli, chettamunploeng, sakhan, chaphlu*, and *khing* is used to fix the four element abnormality.

[15] Trikatuk which comprises *phrikthai, khing*, and *dipli* is used especially in rainy season to fix abnormality of fire or wind or water element.

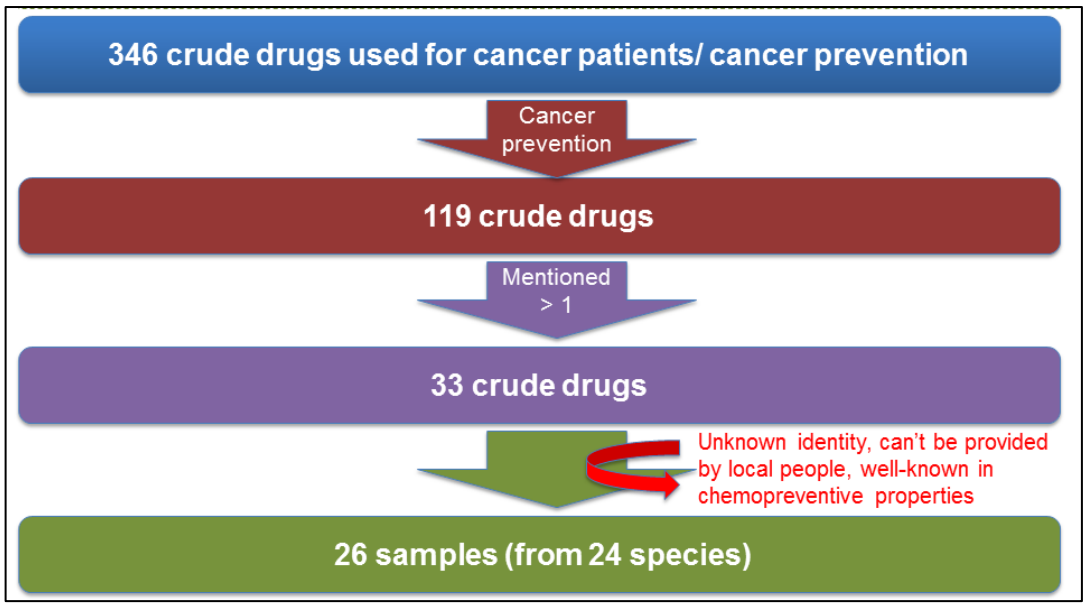

Figure 2-12 Selection of plants for further study. Seven plants were excluded from the study. Curcuma spp. was excluded due to the problem in identity and supply of the plants by local herbal dispensaries. A. marmelos, P. emblica, P. nigrum, S. glabra, T. chebula, and Z. officinale were excluded because there were many reports about their chemopreventive properties.

Table 2-13 FC and RFC of plants used in prevention of cancer/ mareng by the informants in each province

No	Scientific name	Family	Local name (english/ other name)	Part used	FC					RFC				
					SU	CH	LA	BK	*Total*	SU	CH	LA	BK	*Total*
1	*Thunbergia laurifolia* Lindl.	Acanthaceae	Rangchued (laurel clock vine, blue trumpet vine)	leaf, stem, root	0	0	4	2	**6**	0.00	0.00	0.44	0.33	0.18
2	*Zingiber officinale* Roscoe	Zingiberaceae	Khing (ginger)	rhizome	2	1	1	1	**5**	0.22	0.11	0.11	0.17	0.15
3	*Terminalia chebula* Retz.	Combretaceae	Kotphungpla, samothai (terminalia gall, myrobalan gall)	fruit	0	0	0	4	**4**	0.00	0.00	0.00	0.67	0.12
4	*Allium sativum* L.	Amaryllidaceae	Krathiam (garlic)	young shoot	0	2	1	0	**3**	0.00	0.22	0.11	0.00	0.09
5	*Ferula assa-foetida* L.	Apiaceae	Mahahing (asafoetida, stinking gum, devil's dung)	oleo-gum-resin	0	1	0	2	**3**	0.00	0.11	0.00	0.33	0.09
6	*Cladogynos orientalis* Zipp. ex Span.	Euphorbiaceae	Chetphangkhi	root	0	0	2	1	**3**	0.00	0.00	0.22	0.17	0.09
7	*Derris scandens* (Roxb.) Benth.	Fabaceae	Thaowanpriang (Jewel vine)	stem	1	0	1	1	**3**	0.11	0.00	0.11	0.17	0.09
8	*Tiliacora triandra* (Colebr.) Diels	Menispermaceae	Yanang	root, stem	0	1	0	2	**3**	0.00	0.11	0.00	0.33	0.09
9	*Phyllanthus emblica* L.	Phyllanthaceae	Makhampom (emblic myrobalan)	fruit	1	0	0	2	**3**	0.11	0.00	0.00	0.33	0.09
10	*Piper sarmentosum* Roxb.	Piperaceae	Chaphlu (wild betel)	leave, whole plant	0	1	1	1	**3**	0.00	0.11	0.11	0.17	0.09
11	*Plumbaco indica* L.	Plumbaginaceae	Chettamunploengdaeng (rose-coloured leadwort)	root	0	1	1	1	**3**	0.00	0.11	0.11	0.17	0.09
12	*Allium ascalonicum* L.	Amaryllidaceae	Homdaeng (shallot)	young shoot	0	2	0	0	**2**	0.00	0.22	0.00	0.00	0.06
13	*Ligusticum striatum* DC.	Apiaceae	Kothuabua (Szechwan lovage rhizome, Chuan Xiong)	rhizome	1	0	0	1	**2**	0.11	0.00	0.00	0.17	0.06
14	*Atractylodes lancea* (Thunb.) DC.	Asteraceae	Kotkamao (atractylodes, Cang Zhu)	dried rhizome	1	0	0	1	**2**	0.11	0.00	0.00	0.17	0.06

No	Scientific name	Family	Local name (english/ other name)	Part used	FC					RFC				
					SU	CH	LA	BK	*Total*	SU	CH	LA	BK	*Total*
15	*Saussurea costus* (Falc.) Lipsch.	Asteraceae	Kotkraduk (costus root, Mu Xiang)	dried root	0	0	0	2	**2**	0.00	0.00	0.00	0.33	0.06
16	*Millingtonia hortensis* L.f.	Bignoniaceae	Kasalong (Indian cork tree)	stem bark	0	0	2	0	**2**	0.00	0.00	0.22	0.00	0.06
17	*Capparis micracantha* DC.	Capparaceae	Chingchi, Saemathalai	root	0	1	0	1	**2**	0.00	0.11	0.00	0.17	0.06
18	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Samophiphek, Naeton (beleric myrobalan)	fruit	1	0	0	1	**2**	0.11	0.00	0.00	0.17	0.06
19	*Coccinia grandis* (L.) Voigt	Cucurbitaceae	Tamlueng (ivy gourd)	whole plant	0	0	0	2	**2**	0.00	0.00	0.00	0.33	0.06
20	*Dioscorea hispida* Dennst.	Dioscoreaceae	Kloi	tuber	0	0	0	2	**2**	0.00	0.00	0.00	0.33	0.06
21	*Senegalia rugata* (Lam.) Britton & Rose	Fabaceae	Sompoi (soap pod)	leaf, pod	1	0	1	0	**2**	0.11	0.00	0.11	0.00	0.06
22	*Peltophorum pterocarpum* (DC.) Backer ex K.Heyne	Fabaceae	San-ngoen, insi (copperpod, golden flamboyant)	twig	0	0	2	0	**2**	0.00	0.00	0.22	0.00	0.06
23	*Albizia chinensis* (Osbeck) Merr.	Fabaceae	Sankham (Chinese albizia, silk tree)	twig	0	0	2	0	**2**	0.00	0.00	0.22	0.00	0.06
24	*Tinospora crispa*(L.) Hook. f. & Thomson	Menispermaceae	Boraphet (putarwali)	stem	0	0	1	1	**2**	0.00	0.00	0.11	0.17	0.06
25	*Piper retrofractum* Vahl	Piperaceae	Dipli (long pepper)	fruit	0	1	1	0	**2**	0.00	0.11	0.11	0.00	0.06
26	*Piper nigrum* L.	Piperaceae	Phrikthai (black pepper)	fruit	0	0	0	2	**2**	0.00	0.00	0.00	0.33	0.06
27	*Piper ribesioides* Wall.	Piperaceae	Sakhan	stem	0	1	1	0	**2**	0.00	0.11	0.11	0.00	0.06
28	*Aegle marmelos* (L.) Corrêa	Rutaceae	Matum (bael, golden apple)	fruit	0	1	0	1	**2**	0.00	0.11	0.00	0.17	0.06
29	*Smilax* spp.	Smilacaceae	Khaoyennuea	rhizome	1	0	1	0	**2**	0.11	0.00	0.11	0.00	0.06

No	Scientific name	Family	Local name (english/ other name)	Part used	FC					RFC				
					SU	CH	LA	BK	*Total*	SU	CH	LA	BK	*Total*
30	*Smilax* spp.	Smilacaceae	Khaoyentai	rhizome	1	0	1	0	**2**	0.11	0.00	0.11	0.00	0.06
31	*Aloe* spp.	Xanthorrhoeaceae	Yadam	dried latex	0	1	0	1	**2**	0.00	0.11	0.00	0.17	0.06
32	*Curcuma* sp.	Zingiberaceae	Wan khothongkae	rhizome	0	0	0	2	**2**	0.00	0.00	0.00	0.33	0.06

SU = Suratthani, CH = Chanthaburi, LA = Lamphun, BK = Bangkok Metropolitan Region

Table 2-14 Total samples to be studied

No	Scientific name	Family	Local name	Part used	Abbr.	Voucher number*
1	*Allium ascalonicum*	Alliaceae	Homdaeng	young shoot	AA	-
2	*Allium sativum*	Alliaceae	Krathiam	young shoot	AS	-
3	*Aloe* spp.	Asphoderaceae	Yadam	processed resin from leaf	AL	NL-0028
4	*Atractylodes lancea*	Asteraceae	Kotkamao	dried rhizome	AT	NL-0029
5	*Capparis micracantha*	Capparidaceae	Chingchi	root	CM	PBM05194
6	*Citrus hystrix*	Rutaceae	Magrud	leave	CH	NL-0030
7	*Cladogynos orientalis*	Euphorbiaceae	Chetphangkhi	root	CO	PBM05196
8	*Coccinia grandis*	Cucurbitaceae	Tamlueng	Whole plant	CG	PBM05195
9	*Derris scandens*	Fabaceae	Thaowanpriang	stem	DS	PBM05189
10	*Ferula assa-foetida*	Apiaceae	Mahahing	resin from root	FA	NL-0031
11	*Imperata cylindrica*	Poaceae	Yakha	root	IC	NL-0032
12	*Ligusticum striatum* DC.	Apiaceae	Kothuabua	dried rhizome	LS	NL-0033
13	*Peltophorum pterocarpum*	Fabaceae	San-ngoen, Nonsi	twig	PP	NL-0034
14	*Piper* sp.	Piperaceae	Sakhan	stem	PA	PBM05190
15	*Piper retrofractum*	Piperaceae	Dipli	dried mature unripe fruit	PR	PBM05191
16	*Piper sarmentosum*	Piperaceae	Chaphlu	whole plant	PS1	NL-0035
17	*Piper sarmentosum*	Piperaceae	Chaphlu	leaf	PS2	NL-0035
18	*Plumbaco indica*	Plumbaginaceae	Chettamunploengdaeng	root	PI	PBM05192
19	*Saussurea costus*	Asteraceae	Kotkraduk	dried root	AU	NL-0036
20	*Senegalia rugata*	Leguminosae	Sompoi	leaf	SR1	NL-0037
21	*Senegalia rugata*	Leguminosae	Sompoi	pod	SR2	NL-0037
22	*Smilax* spp.	Smilacaceae	Khaoyennuea	root	SM	NL-0038
23	*Terminalia bellirica*	Combretaceae	Samophiphek, Naeton	fruit	TB	PBM05198
24	*Thunbergia laurifolia*	Acanthaceae	Rangchued	leave	TL	PBM05178
25	*Tiliacora triandra*	Menispermaceae	Yanang	stem	TT	PBM05193
26	*Tinospora crispa*	Menispermaceae	Boraphet	stem	TC	PBM05197

*PBM – voucher specimens deposited at Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. NL – collector's number for materials purchased from the market without fruits / flowers for taxonomic identification. The verification of scientific name was performed by comparison with authentic materials. Common food plants eg. garlic and shallot were not collected.

2.4. Conclusion

Mareng is commonly used to refer to cancer by Thai people nowadays. In TTM scriptures, it also refers to other diseases, mainly skin problems. Herbal medicines used in TTM, especially the ones for *mareng*, have been used to treat cancer. However, there was no study comparing *mareng* and cancer before. In this study, TTM's emic concept of cancer was analyzed, compared to biomedicine and, for the first time, the main characteristics of *mareng* are proposed. Four characteristics; waste accumulation, chronic illnesses, inflammation, and bad *luead* and *namlueang*, are useful in planning pharmacological assays to investigate bioactivities of herbal medicines. Cancer/*mareng* is thought to be preventable by herbal medicine, of which the use should be based on individuals' risk. Herbal formulae and medicinal plants used in cancer management were documented. Plants frequently cited to have benefits in cancer/ *mareng* prevention were common and easy to find. Then twenty-four potential plants were selected for further pharmacological investigation.

This study provides a useful resource for identifying promising plants for the development of chemopreventive agents. Pharmacological and phytochemical evaluations of these medicinal plants were carried out in the next steps. Furthermore, the information could be beneficial for the integration of TTM in cancer care because the link between TTM and biomedicine was reported, thus, provide a better understanding of TTM to main health care providers.

2.5. Study limitations and future work

There were some limitations of this study that need to be highlighted. Clearly, there is no clinical evidence for any of the treatments, and this is beyond the scope of this contribution. Instead it demonstrates plausible mechanisms which can be investigated further.

The number of participants (33) is sufficiently large to understand the basic concepts but it is too small to conclude that the data obtained represented an overall perspective of TTM practitioners' knowledge. However, we interviewed informants from different parts of the country to make sure that the overall views, as well as influences of local culture, herbal medicine resources and the indigenous medical knowledge on the healing practice, are covered. Secondly, there was no clinical evidence to confirm practitioners' diagnosis of cancer/*mareng*. The informants knew that their patients have cancer because the patients informed them so. Thirdly, we did not observe any real diagnosis or consults or treatment intervention as these were not included in the ethical approval.

For future studies, a larger number of informants should be included to ascertain the findings. Clinical observational studies and studies combining the treatments of TTM practitioners and mainstream medical doctors would be an ideal next step as part of a strategy to better understand treatment outcomes for a set of diseases which is both devastating and of great importance both in TTM and in biomedicine.

Chapter 3. Screening for chemopreventive properties of plant extracts: cytotoxicity, protective effect against oxidative stress, and effects on glutathione and NQO1

3.1. Introduction

Previously in chapter 2, five characteristics of cancer in TTM were presented and compared to Western medicine concepts. Together with the information on the uses of herbal remedies for *mareng* prevention from the informants, a TTM cancer prevention strategy was proposed. Furthermore, related models for pharmacological activities investigation were also suggested (Figure 3-1). In this thesis, three topics, which were removal of waste from the body, liver protection, and prevention from *krasai*, were the main focus. Therefore, *in vitro* screening assays were dealing with antioxidant enzymes, which have roles in the removal of toxic substances or carcinogens from cells, and oxidative stress.

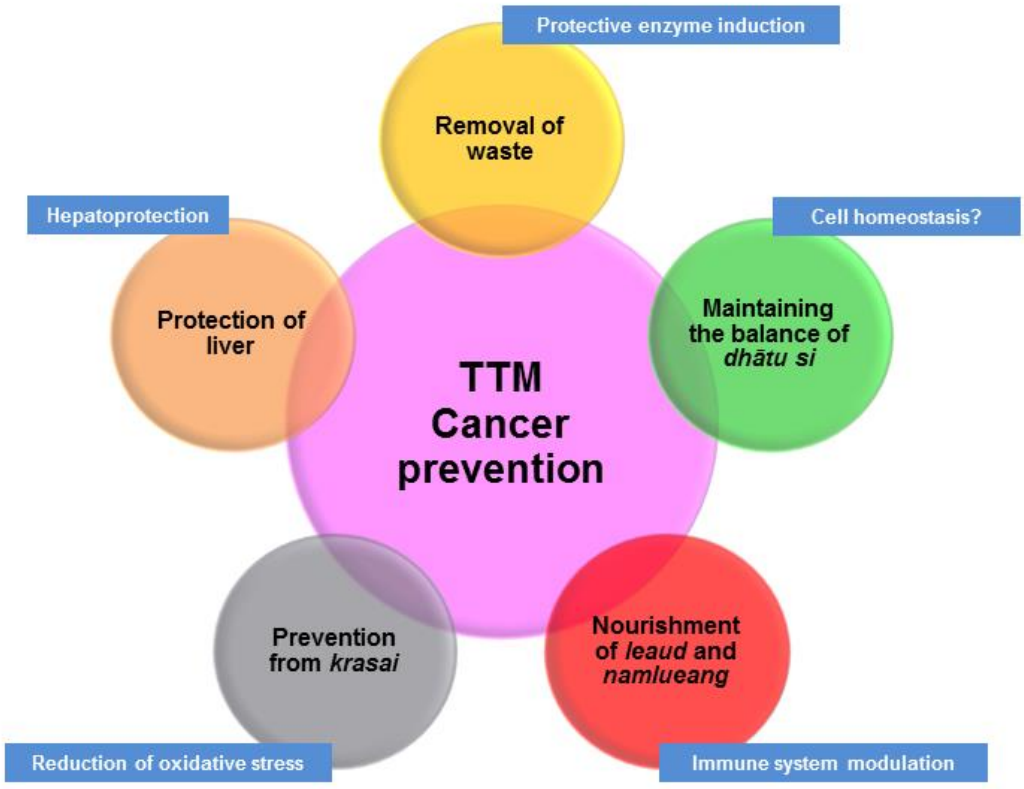

Figure 3-1 Cancer prevention strategy from Thai traditional medicine and its possible pharmacological models. Bioassay models for the four strategies; protection of liver, prevention from krasai, nourishment of leaud & namlueang, and removal of waste, can be suggested. The balance of dhātu si is unclear in biomedical sense. It is possible to involve with the cell homeostasis.

3.1.1. Oxidative stress and carcinogenesis

Yoshikawa and Naito (2002) defined oxidative stress as "*a state where oxidative forces exceed the antioxidant systems due to loss of the balance between them*". Oxidative stress is well-known for the associated risk because of the damages that reactive oxygen species (ROS) and free radicals produce to biological molecules, such as DNA, lipids, and proteins. It is associated with the causation and development of many diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and cancer. However, oxidative stress is useful in some events, such as triggering apoptosis to prepare the birth canal for delivery and strengthening the defence mechanisms during appropriate physical exercise and ischemia. In normal conditions, ROS are produced during the utilisation process of oxygen and are removed by antioxidant systems in the body. Under some circumstances ROS or free radicals are excessive and cause the loss of the balance between production and elimination, resulting in oxidative stress. Consequently, ROS and free radicals can attack biological molecules and cause DNA damage, lipid peroxidation, and lead to many diseases (Yoshikawa and Naito, 2002). It is important to note that ROS and free radicals are not synonymous. Table 3-1 shows major ROS and indicates which one is a free radical.

Table 3-1 Major reactive oxygen species (Source: Yoshikawa and Naito, 2002)

Reactive oxygen species	Remarks
O_2 (Singlet oxygen)	-
H_2O_2 (Hydrogen peroxide)	-
LOOH (Alkylhydroperoxide)	-
ClO^- (Hypochlorite ion)	-
$Fe4^+O$ (Ferryl ion)	-
$Fe5^+O$ (Periferryl ion)	-
$O_2^\bullet$ (Superoxide radical)	A free radical
$HO^\bullet$ (Hydroxyl radical)	A free radical
$HOO^\bullet$ (Hydroperoxyl radical)	A free radical
$LOO^\bullet$ (Alkylperoxyl radical)	A free radical
$LO^\bullet$ (Alkoxyl radical)	A free radical
$NO^\bullet$ (Nitric oxide)	A free radical

Oxidative stress has been proved to be associated with many of the hallmarks of cancer (Hanahan and Weinberg, 2011). It has an important role in cancer initiation and progression. Elevated ROS levels can initiate DNA damage. ROS also helps cancer cells to acquire proliferative signals and resist apoptosis, which result in uncontrolled cell growth. They also

promote the invasion and metastasis by activation of metalloproteinase (MMP) and epithelial mesenchymal transition, as well as angiogenesis (Fiaschi and Chiarugi, 2012). Many promising cancer chemopreventive natural products, such as (−)-epigallocatechin-3-gallate (EGCG) from tea, resveratrol from grapes and its analogs, genistein from soy, and curcumin, have been reported to possess abilities in ROS scavenging and protection against oxidative damage (Darvesh and Bishayee, 2013, Acharya and Ghaskadbi, 2013, Kim et al., 2015, Zhu et al., 2004, Gibellini et al., 2011).

3.1.2. *Krasai*: degenerative conditions in Thai traditional medicine and correlation with oxidative stress

Chronic illnesses/*krasai* is among the main characteristics of cancer recognised in TTM. According to the official Thai dictionary, *krasai* is characterised by emaciation, yellow skin, and cold feet and results in deterioration of the body (Royal Society of Thailand, 2011). It is also defined as a condition with symptoms generally located in the lower abdominal area including symptoms recognised in biomedicine as hernia, urino-genital afflictions, ulcers, and possibly gallbladder illness (Bamber, 1987). However, the most widely accepted definition among Thai traditional practitioners is the deterioration of the four elements or a decline of the body (Foundation for the promotion of Thai traditional medicine and Ayurved Thamrong School Center of applied Thai traditional medicine, 2007b). As such in TTM it can be interpreted as a type of degenerative diseases.

Degenerative diseases are non-infectious diseases whose incidence increases with age. This type of diseases develops as a result of the continuous degeneration of cells and tissues which later affects organs. Oxidative stress has been linked to the pathogenesis of many common degenerative diseases, such as cardiovascular diseases, diabetes mellitus, and rheumatoid arthritis (Islam et al., 2016). Denham Harman proposed that an accumulation of free radical damage was one factor in aging (Harman, 1956). Later, scientific evidence revealed that oxidative stress causes cell apoptosis, necrosis, and an accumulation of DNA and mitochondrial DNA (mtDNA) mutations, as well as showed the correlation between ROS and degenerative diseases. Therefore, it is now accepted that oxidative damage plays an important role in the loss of physiological functions (Kadenbach et al., 2009).

There are several reasons why *krasai* was considered as degenerative diseases. Firstly, the incidence of *krasai* increases with age because it takes a long time to develop the condition. Secondly, a patient will be diagnosed with *krasai* when an effect in an organ can be observed. Thirdly, patients with chronic illnesses would develop *krasai* eventually because it is at a terminal stage. Lastly, it is a result of the continuous degeneration of the body. Based on the link between *krasai*, degeneration, and cancer, *in vitro* assays using oxidative stress as an important marker were used in this project

3.1.3. Oxidative stress models in *in vitro*

Protective effect against oxidative stress-induced cell death was used to screen for potential extracts as it allows to screen many samples using a microplate format. Two chemicals widely used in oxidative stress assays in living cells are hydrogen peroxide (H_2O_2) and *tert*-butyl hydroperoxide (*t*-BHP) (Figure 3-2). H_2O_2 and *t*-BHP induce cell death by damaging DNA by a free radical/peroxidation mechanism, depletion of glutathione, and activation of calcium-dependent endonucleases. While H_2O_2 forms hydroxyl radicals which cause damage to DNA, lipids, and proteins, *t*-BHP causes little damage to DNA but mainly causes damage to the cell membrane by superoxide or superoxide-like radical formation. However, the mechanisms of *t*-BHP damage also involve lactate dehydrogenase (LDH) leakage, mitochondrial damage, the release of cytochrome c, the loss of calcium homeostasis, the induction of arachidonic acid release from membrane phospholipids, and malondialdehyde formation (Slamenova et al., 2013). *T*-BHP is also widely used in cytoprotective effect evaluation models. Therefore, t-BHP was used in this project to induce cell death/damage due to the fact that it attacks cells via several mechanisms.

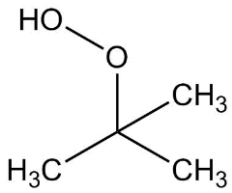

Figure 3-2 Tert-butyl hydroperoxide (t-BHP)

Based on the fieldwork, we selected detoxification and prevention of degeneration (oxidative stress) to be our focus to explore chemopreventive properties of the samples because it is difficult to avoid carcinogens and oxidative stress. Protection from oxidative damage caused by free radicals is one of blocking agents' mechanisms. This activity of such agents can be tested *in vitro* by assessment of the ability to induce detoxifying enzymes and glutathione. The activation of phase II enzymes facilitates the clearance of toxicants and is among main mechanisms of chemopreventive agents (De Flora and Ferguson, 2005).

3.1.3.1. NAD(P)H:quinone oxidoreductase 1 (NQO1)

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a key enzyme involved in the defence against the toxicity and carcinogenicity of quinones and inhibition of neoplasia. Quinones are widespread in our environment and human are exposed to them extensively. Many quinones have been reported to be carcinogens, such as dioxins (NTP (National Toxicology Program), 2016), tetrachloro-1, 4-benzoquinone (TCBQ) (Zhu et al., 2010), and quinone metabolites from polycyclic aromatic hydrocarbons (PAHs) in the air (Ran et al., 2008) (Figure 3-3). NQO1 also reacts to other reactive species, such as methylene blue, dichlorophenolindolphenol, azo, and nitro compounds (Atia et al., 2014). Therefore, it shows ability in chemoprotection.

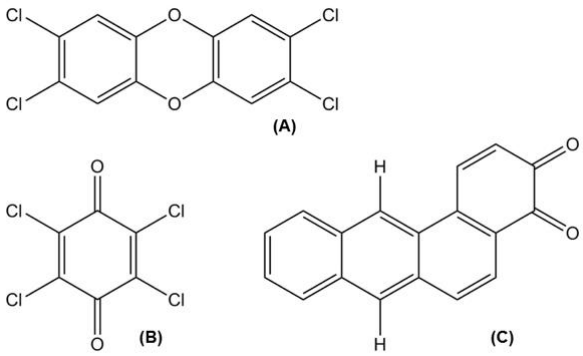

Figure 3-3 (A) TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin), also known as dioxins, a well-known human carcinogen which is a contaminant in herbicides that were widely used in 1960s and 1970s to control weeds and as a defoliant during the Vietnam War. (B) Tetrachloro-1,4-benzoquinone (TCBQ) is one of the major carcinogenic quinoid metabolites of the widely used wood preservative pentachlorophenol (PCP). (C) O-quinone metabolite of benz[a]anthracene, a polycyclic aromatic hydrocarbon.

NQO1 is induced under the oxidative stress conditions which results in the prevention of carcinogenesis by several activities. Firstly, the catalytic activity of NQO1 in the complete reduction and detoxification of highly reactive quinones allows the protection against DNA and other cellular macromolecules damage (Figure 3-4). Secondly, NQO1 maintains the endogenous lipid-soluble antioxidants (co-enzyme Q and α-tocopherol-quinone) in their reduced and active forms. The active forms of such lipid-soluble antioxidants protect against lipid peroxidation and damage to the plasma membrane. Thirdly, NQO1 is required for the stabilisation of p53 protein in response to DNA-damaging stimuli (Nioi and Hayes, 2004).

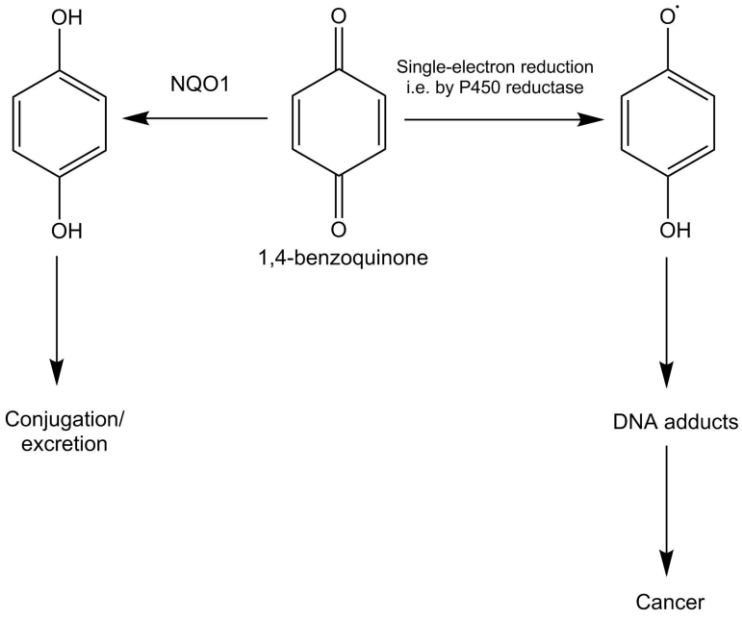

Figure 3-4 A hypothetical scheme for the carcinogenicity of a quinone. Simple quinones can undergo single-electron reduction and produces highly reactive semiquinone intermediates with an unpaired electron. Semiquinones can form adducts directly with cellular macromolecules including DNA and proteins, and may lead to carcinogenesis. NQO1 protects against toxicity and carcinogenicity of quinones by bypassing the semiquinone intermediates (modified from (Nioi and Hayes, 2004)).

3.1.3.2. Glutathione

Glutathione (L-gamma-glutamyl-L-cysteinglycine) has several functions in the body, including detoxification of reactive metabolites which mostly are toxic or carcinogenic, thiol-disulfide exchange which is involved in certain metabolic pathways, and acts as main plasma and tissue storage and transfer of cysteine because simple cysteine would auto-oxidize to its toxic form. In normal condition, glutathione reductase will rapidly reduce oxidized form of glutathione (GSSG) to its reduced form (GSH). Therefore, 98% of intracellular glutathione is GSH. GSH detoxifies toxic electrophiles by random conjugation or via a reaction catalysed by glutathione-S-transferase (GST). Then GSH conjugates, which are more water soluble, are metabolized to mercapturic acid and then excreted via the bile. GSH also has roles in the defence against oxidative stress which is a consequence of aerobic metabolism. Superoxide and hydrogen peroxide formed during the metabolism are reduced by GSH (DeLeve and Kaplowitz, 1991, Melino et al., 2011).

3.1.4. Objectives

The objectives of this chapter are to investigate cytotoxicity, protective effect against oxidative stress, and effects on glutathione and NQO1 enzyme activities of extracts from the selected plants used for cancer/*mareng* prevention in cell cultures.

3.2. Materials and methods

3.2.1. Materials

AlamarBlue and PrestoBlue were bought from Abd Serotec. Primary rat hepatocytes from Sprague-Dawley rats (RTCP10, Lot number RS874), Dulbecco's Modified Eagle Medium (DMEM), Foetal Bovine Serum (FBS), PBS, Penicillin-Streptomycin (10,000 U/mL), Williams E Medium, dexamethasone, human recombinant insulin, GlutaMAX™, and HEPES were purchased from Life technologies. HepG2 cells (ACC No 85011430, Lot 11C013), (-)-Epigallocatechin gallate (EGCG) (E4268), paclitaxel (T1912), Albumin from bovine serum (A2058), β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (NADPH) (N1630), L-Glutathione reduced (G4251), 5-5'-dithiobis (2-nitrobenzoic acid) (DTNB) (D218200), thiazolyl blue tetrazolium bromide (MTT) (M5655), glutathione reductase from baker's yeast (*S. cerevisiae*) (G3664), 5-Sulfosalicylic acid (SSA) (S2130), DMSO, Complete Mini protease inhibitor cocktail (11836170001), and other reagents were from Sigma Aldrich. NADP monosodium salt (sc-202724) and dicoumarol (sc-205647A) were purchased from Santa Cruz Biotechnology. Glucose 6-phosphate disodium salt was from Bio Basic Canada Inc. Yeast glucose 6-phosphate dehydrogenase (J61181) and flavin adenine dinucleotide disodium salt (FAD) (A14495) were from Alfa Aesar. RIPA lysis buffer 10X was from Merck Millipore. *DC* Protein Assay kit (500-0116) was from Bio-Rad Laboratories, Inc.

3.2.2. Plant extracts preparation

Plant materials were washed with deionized water, oven-dried between 40-60 ^{o}C, and ground. Water extracts were prepared according to Thai traditional methods for decoctions. Briefly, 60 g of herbal powder was boiled with 600 ml of water until the volume reached about 200 ml. The decoction was filtered through No.1 and No.4 filter paper (Whatman®) and the water was then removed by freeze drying. To prepare a 70% ethanolic extract, 60 g of herbal powder was added into a glass bottle. Then 70% ethanol was added until it covered the powder surface. The bottle was shaken for 15 minutes to mix the sample powder with the solvent. Later, the bottle was wrapped with aluminium foil to protect from light and allowed to stand for 7 days with a 15-minutes shake every day. After 7 days, the extract was filtered through No.1 and No.4 filter paper (Whatman®) and the solvent was then removed using a rotary evaporator and a freeze dryer. The dry extracts were stored in a cool, dry place and protected from light until use. Prior to cell-based assays, the extracts were prepared into stock solutions in water or DMSO. The water stock solution was then filtered through 0.22 µM syringe filter. All stock solutions were kept at -20 ^{o}C until use.

3.2.3. Cells

It is impractical to prevent specific forms of cancer, unless in increased risk population. Therefore, liver cells are selected based on some informants' cancer prevention concept that many forms of cancer could be prevented by liver protection. The HepG2 (human hepatocellular carcinoma) cell line has been used in many studies investigating chemopreventive properties of plant extracts. The advantages of HepG2 cell line are many. It is easy to maintain and immortal, so it provides an unlimited number of cell divisions. The cell line has morphology similar to that of liver parenchymal cells and secretes many proteins which resemble normal human liver cells, especially phase I and phase II enzymes which are important in the activation/detoxification of procarcinogens, which reflect the metabolism of xenobiotics in the human body (Knasmüller et al., 2004). Rat hepatocytes have been used as an alternative to whole rats in the assessment of genotoxic potential and cytotoxicity mechanism (Muakkassah Kelly et al., 1988), as well as other *in vitro* studies of liver physiology and pathophysiology (Arterburn et al., 1995). The advantages of normal hepatocytes are that they express *in vivo*-like enzyme and show *in vivo*-like sensitivity to apoptosis, unlike liver cancer cell lines which acquired high resistance against apoptosis (Vinken et al., 2014).

HepG2 cells were maintained in DMEM supplemented by 10% FBS and 1% Penicillin/Streptomycin in 75 cm^3 cell culture flasks at 37 ^{o}C in 5% CO_2/95% air. Fresh complete medium was changed every 3 days. The cells were discarded after 15th subculturing.

Primary rat hepatocytes were thawed and maintained in collagen I-coated 96 well plates. The thawing and plating medium was Williams E Medium supplemented with 5% FBS, 1 µM Dexamethasone, 1% Penicillin/Streptomycin, 4 µg/ml Human Recombinant Insulin, 2 mM GlutaMAX™, and 15 mM HEPES, pH 7.4. The serum-free medium was refreshed every 24 hours to maintain the hepatocytes. The hepatocytes were discarded after five days.

3.2.4. Cytotoxicity of plant extracts in HepG2 cells

AlamarBlue is a dye that fluoresces and changes its colour due to oxidation and reduction. It is very stable, water soluble, and has low toxicity. The AlamarBlue assay is a resazurin-based cell proliferation and viability assay which relies on the mitochondrial activity to reduce non-fluorescent resazurin to pink fluorescent compound, resorufin. Therefore, the mitochondrial damage results in the decrease of the fluorescence. The reduction of AlamarBlue dye may be related to mitochondrial enzymes, such as flavin mononucleotide dehydrogenase, flavin adenin nucleotide dehydrogenase, nicotinamide adenine dinucleotide dehydrogenase, nicotinamide adenine phosphate dehydrogenase, and cytochromes (Miret et al., 2006). The reduction of resazurin depends on the cellular metabolism and this varies among different type of cells (Nakayama et al., 1997). Therefore, the experimental parameters, such as the cell density and the incubation time with the dye must be optimized. Based on the results from the method optimization, the cell density of 5,000 cells/ well and 2 hour-incubation was used.

HepG2 cells were seeded at the density of 5,000 cells/well in 96-well black microplates and allowed to attach for 24 hours. The extracts (3.125 – 100 or 6.25 – 200 µg/ml), or paclitaxel (0.001 nM – 10 µM) or EGCG (50 – 400 µM) as positive control, or fresh medium as control were then added. After 48 hours, 100 µl of diluted AlamarBlue solution (1:10 in complete medium) was replaced and incubated for 2 hours at 37 ^{o}C. After that, the fluorescence intensity was measured at 560 nm excitation and 590 nm emission using a microtiter plate reader (Infinite M200, Tecan). The percentage difference between treated and control cell was calculated as follows;

$$\%\text{control} = \frac{\text{mean FI}_{\text{sample}} - \text{mean FI}_{\text{blank}}}{\text{mean FI}_{\text{control}} - \text{mean FI}_{\text{blank}}} \times 100$$

3.2.5. Protective effect against oxidative stress-induced cell death

Prior to the experiment with plant extracts, suitable concentration and incubation time of *t*-BHP were optimized. After the viability of HepG2 cells after different incubation time with various concentrations of *t*-BHP were tested, incubation with 0.5 mM *t*-BHP for 3 hours was used.

HepG2 cells were seeded in 96-well black plate at the density of 10,000 cells/well. After 24 hours, the medium was replaced with fresh complete medium as control, positive control (EGCG 50 µM), and plant extracts at maximum non-toxic concentration (MNTC). After incubation with the treatment for 24 hours, the medium was discarded and cell death was induced by addition of 200 µl of 0.5 mM *t*-BHP to each well. After 3 hours, AlamarBlue assay was performed to determine the cell viability.

3.2.6. NQO1 activity assay

NQO1 activity was measured following (Fahey et al., 2004). The principle of the assay is shown in Figure 3-5.

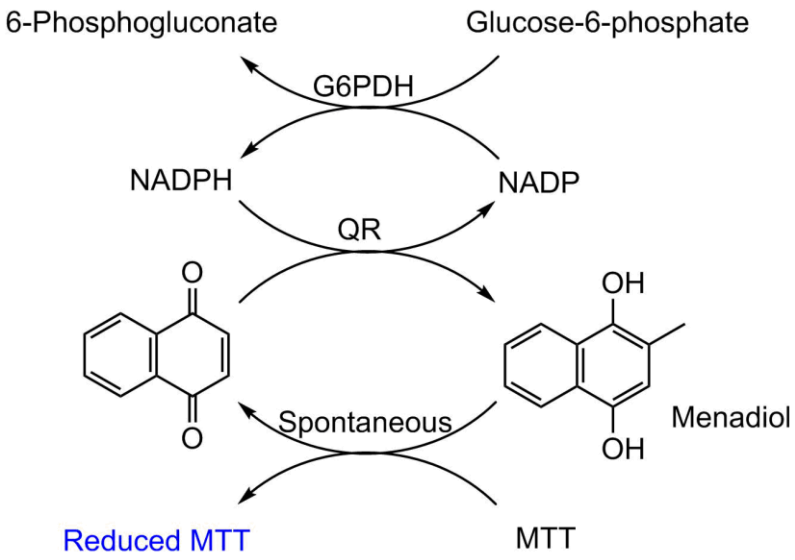

Figure 3-5 NQO1 assay principle. Glucose 6-phosphate and glucose-6-phosphate dehydrogenase (G6PDH) generate NADPH continually. NADPH is used by NQO1 (quinone reductase, QR) to transfer electrons to menadione and from menadiol. Then menadiol reduces MTT to the purple formazan which can be measured between 490 to 640 nm. Both NADPH and menadione are regenerated, which obviates problems encountered with substrate depletion (Prochaska and Santamaria, 1988).

The cells were seeded at the density of 10,000 cells per well in 96-well transparent plate and allowed to attach for one night. Then the cells were incubated with extracts at MNTC or DMSO or menadione (positive control) or dicoumarol (negative control). After the incubation, the cells were washed twice with PBS. Then the cells were lysed with 30 µl of RIPA buffer supplemented with 1 mM PMSF and shaken on a plate shaker for 20 minutes. Five µl of the cell lysate was transferred to a new plate for quantification of total protein. Just before the addition, 1 ml of reaction mixture was mixed with 1 µl of 50 mM menadione. Then 200 µl of the complete reaction mixture was added to each well. The absorbance of the product was measured immediately at 610 nm and every one minute up to five minutes with a microtiter plate reader (Infinite M200, Tecan). The reaction mixture was made according to Table 3-2 and kept at 2-8 ^{o}C not exceeding 4 days to ensure the stability of MTT. NQO1 specific activity of treated cells were reported as percentage of the control (Prochaska, 1994). Optimum incubation time was optimized by incubating HepG2 cells with various concentrations of menadione and dicoumarol for 24, 48, and 72 hours. 72-hours incubation was used throughout the experiment because it gave the highest response and most reproducible results.

Table 3-2 Composition of NQO1 assay reaction mixture

	Reagents	Amount/volume per 10 ml of reaction mixture
1	0.5 M Tris-Cl (pH 7.4)	500 µl
2	Bovine serum albumin	6.67 mg
3	1.5% Tween-20	67 µl
4	7.5 mM FAD	6.7 µl
5	150 mM glucose 6-phosphate	67 µl
6	50 mM NADP	6 µl
7	Yeast glucose 6-phosphate dehydrogenase	20 units
8	MTT	3 mg
9	Distilled water	to10 ml

3.2.7. Intracellular GSH assay

Glutathione measurement is based on the enzymatic recycling method (Figure 3-6) which was modified from (Allen et al., 2001).

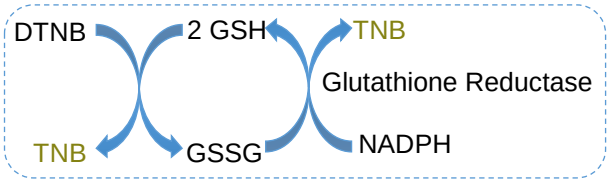

Figure 3-6 Enzymatic recycling method for GSH measurement. Reduced form of glutathione (GSH) reacts with DTNB and produce 2-nitro-5-thiobenzoic acid (TNB) and oxidized form of glutathione (GSSG). In the present of NADPH, GSSG is then reduced by glutathione reductase and form GSH and TNB. TNB absorbance is measured at 405 nm. The absorbance is proportional to GSH amount in the sample (Shaik and Mehvar, 2006).

The cells were seeded at the density of 6 x 10^5 cells per well in 6-well transparent plate and allowed to attach for one night. Then the cells were incubated with extracts at MNTC or DMSO. After 24-hour incubation, the cells were treated with 0.7 mM *t*-BHP for 4 hours. To prepare the cell lysate, the cells were washed twice with ice-cold PBS. After the addition of 150 µl of ice-cold RIPA buffer containing cOmplete Mini tablets (150 µl of 7X cOmplete tablet stock solution was added to every 900 µl of RIPA buffer) to each well, the cells were scraped off quickly and transferred to 1.5 ml reaction tubes and incubated in ice for 30 minutes. Then the tubes were ultrasonicated for 10 seconds and kept in ice for 10 seconds to help lysing the cells completely. This step was repeated three times. The supernatant (cell lysate) was transferred to new

reaction tubes after centrifugation at 8000 x g for 10 minutes at 4 °C. The cell lysate was diluted with 5% SSA at 1:2 or 1:5 to precipitate proteins and to inhibit γ-glutamyl transferase, which leads to the loss of GSH (Rahman et al., 2007). After centrifugation at 10,000 rpm for 10 minutes at 4 °C, 25 µl of supernatant was added to 96-well plates (3 replicates/sample). Then 125 µl of ice-cold complete GSH reaction mixture (Table 3-3) was added to the supernatant. The plates were then briefly shaken at 500 rpm on a plate shaker. The absorbance at 405 nm was immediately measured and every 30 seconds up to 5 minutes (11 cycles) by a microtiter plate reader (Infinite M200, Tecan). A GSH standard curve (0.012 – 25 µM) was performed together with each assay.

Table 3-3 Complete GSH reaction mixture composition for 6 samples; including GSH standard curve

	Reagents	Volume
1	143 mM Sodium Phosphate Buffer containing 6.3 mM EDTA (Prepare by dissolving 0.470 g disodium EDTA in a solution consisting of 168 ml of 286 mM dibasic sodium phosphate and 32 ml of 286 mM monobasic sodium phosphate)	7.5 ml
2	2.39 mM NADPH solution (Prepare by dissolving 2 mg NADPH in 1 ml of Sod. Phosphate Buffer)	1 ml
3	Glutathione reductase	31.5µl
4	0.01 M DTNB **(add just before the assay)** (Prepare freshly by dissolving 3.96 mg DTNB in 1 ml of Sod. Phosphate Buffer)	500 µl

Calculation of GSH levels in samples

The first curve was plotted by using the absorbance of each concentration of GSH standard (Y) against time (seconds, X) and slopes were calculated using MS Excel 2010. The second curve was done by plotting the slopes of each GSH standard (Y) against GSH concentration (X) (Figure 3-7). Then the sample curve was plotted in the same way with the first curve. To calculate GSH amount (x) in each sample, replacing y with the sample's slope (Allen et al., 2001). The results were expressed as GSH level per 1 mg of protein (µM/mg). The amount of protein was quantified using Lowry method (described in 3.2.8).

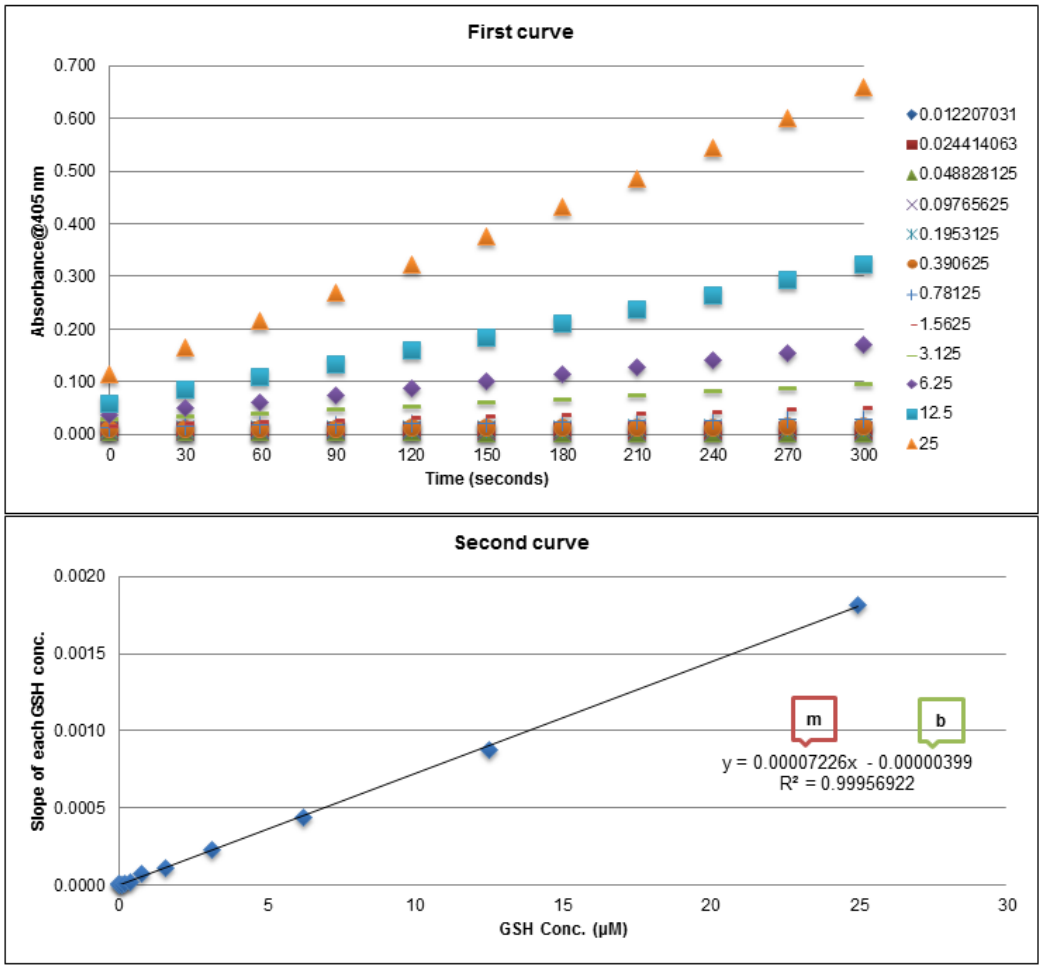

Figure 3-7 Example of GSH level calculation. From the second curve, m value was obtained from its slope and b value was obtained from y-intercept. GSH amount (x) was calculated by replacing y with the sample's slope.

3.2.8. Protein measurement

Bio-Rad DC™ Protein Assay kit, which utilizes Lowry method, was used to determine the amount of protein. Before the assay was performed, working reagent A was prepared by addition of 20 µl of reagent S to 1 ml of reagent A. Five µl of cell lysate was added into 96-well plate. Then twenty-five µl of working reagent A was added to each well followed by 200 µl of reagent B. After that the plate was shaken and incubated at room temperature for 15 minutes, protected from light, before an absorbance measurement at 750 nm with a microtiter plate reader (Infinite M200, Tecan). A BSA standard curve was generated and used to quantify the amount of protein in cell lysate. The curve was linear in the range of 0 – 1 mg/ml with R^2 > 0.99.

3.2.9. Cytotoxicity of plant extracts in primary rat hepatocytes

PrestoBlue® is a resazurin-based solution used to determine cell viability and proliferation. PrestoBlue can be reduced to highly fluorescent red colour solution by reducing environment

in living cells cytosol. There are several advantages to this assay. Firstly, it is non-toxic and does not require cell lysis. Cells can be allowed to continue to proliferate after the removal of the reagent and replacement with the fresh medium. Therefore, it is very suitable to be used with rat hepatocytes because they do not proliferate and cannot be sub-cultured. After one assay, the hepatocytes can be simply washed and then maintained in the serum-free medium with or without treatment to prepare them for the next assay. Secondly, the incubation time is very short, from 10 minutes. Thirdly, it is convenient and saves time because the reagent is ready to use. Lastly, the changes in colour can be detected using either fluorescence or absorbance measurements. So it is very flexible in any laboratory.

The hepatocytes were plated at the density of 20,000 cells/well in collagen I-coated 96 well plates and left for initial attachment for 6 hours. Then the hepatocytes were treated with CGe, CHe, PS1e, TLe, or SR1e in various concentrations for 24 and 48 hours. Ethanol (0.0625 – 10% v/v) was used as a positive control and fresh serum-free medium served as control. After the indicated incubation time, the medium was replaced by 10% PrestoBlue medium. The fluorescent intensity was scanned after 20 minutes at excitation wavelength of 535$\pm$9 nm and emission wavelength of 590$\pm$20 nm. Cytotoxicity was presented as % viability compared to the control.

3.2.10. Statistical analysis

Calculation of average, SD values, IC_{50} (the concentration of the extracts that inhibit the cell viability for 50%), and maximum non-toxic concentration (MNTC) (the concentration of the extracts that inhibit the cell viability for less than 20%), and one-way ANOVA analysis were performed using GraphPad Prism 5.01 software (GraphPad Software, Inc.). All experiment was performed at least N = 3. The level of significance was set at $P < 0.05$.

3.3. Results and discussion

3.3.1. Yield of plant extracts

After the freeze drying, the percent yield of each extract was calculated. The yield ranged from 0.63 to 85.14% (Table 3-4). Ethanol extract of *Yadam* (the resin from the leaf of *Aloe* spp.) gave the highest yield.

Table 3-4 Percent yield of plant extracts.

No	Scientific name	Local name	Part	Abbr.	Extracts	%Yield
1	*Allium ascalonicum*	Homdaeng	young shoot	AAe	Ethanol	1.61
2	*Allium ascalonicum*	Homdaeng	young shoot	AAw	Water	7.05
3	*Aloe* spp.	Yadam	processed resin from leaf	ALe	Ethanol	85.14
4	*Aloe* spp.	Yadam	processed resin from leaf	ALw	Water	27.97
5	*Allium sativum*	Krathiam	young shoot	ASe	Ethanol	1.88
6	*Allium sativum*	Krathiam	young shoot	ASw	Water	2.95
7	*Atractylodes lancea*	Kotkamao	dried rhizome	ATe	Ethanol	9.06

No	Scientific name	Local name	Part	Abbr.	Extracts	%Yield
8	*Atractylodes lancea*	Kotkamao	dried rhizome	ATw	Water	11.70
9	*Saussurea costus*	Kotkraduk	dried root	AUe	Ethanol	21.89
10	*Saussurea costus*	Kotkraduk	dried root	AUw	Water	9.95
11	*Coccinia grandis*	Tamlueng	leaf	CGe	Ethanol	8.53
12	*Coccinia grandis*	Tamlueng	leaf	CGw	Water	11.30
13	*Citrus hystrix*	Magrud, Kaffir lime	leaf	CHe	Ethanol	11.53
14	*Citrus hystrix*	Magrud, Kaffir lime	leaf	CHw	Water	12.41
15	*Capparis micracantha*	Chingchi	root	CMe	Ethanol	4.20
16	*Capparis micracantha*	Chingchi	root	CMw	Water	4.37
17	*Cladogynos orientalis*	Chetphangkhi	root	COe	Ethanol	5.40
18	*Cladogynos orientalis*	Chetphangkhi	root	COw	Water	2.68
19	*Derris scandens*	Thaowanpriang	stem	DSe	Ethanol	3.34
20	*Derris scandens*	Thaowanpriang	stem	DSw	Water	18.22
21	*Ferula assa-foetida*	Mahahing	resin from root	FAe	Ethanol	4.34
22	*Ferula assa-foetida*	Mahahing	resin from root	FAw	Water	22.20
23	*Imperata cylindrica*	Yakha	root	ICe	Ethanol	3.15
24	*Imperata cylindrica*	Yakha	root	ICw	Water	7.25
25	*Ligusticum striatum*	Kothuabua	dried rhizome	LSe	Ethanol	5.00
26	*Ligusticum striatum*	Kothuabua	dried rhizome	LSw	Water	1.90
27	*Piper ribesioides*	Sakhan	stem	PAe	Ethanol	5.01
28	*Piper ribesioides*	Sakhan	stem	PAw	Water	4.25
29	*Plumbaco indica*	Chettamunploengdaeng	root	PIe	Ethanol	10.49
30	*Plumbaco indica*	Chettamunploengdaeng	root	PIw	Water	18.99
31	*Peltophorum pterocarpum*	San-ngoen, Nonsi	stem bark	PPe	Ethanol	10.91
32	*Peltophorum pterocarpum*	San-ngoen, Nonsi	stem bark	PPw	Water	2.61
33	*Piper retrofractum*	Dipli	dried mature unripe fruit	PRe	Ethanol	6.05
34	*Piper retrofractum*	Dipli	dried mature unripe fruit	PRw	Water	11.20
35	*Piper sarmentosum*	Chaphlu	whole plant	PS1e	Ethanol	5.32
36	*Piper sarmentosum*	Chaphlu	whole plant	PS1w	Water	8.31
37	*Piper sarmentosum*	Chaphlu	leaf	PS2e	Ethanol	8.59
38	*Piper sarmentosum*	Chaphlu	leaf	PS2w	Water	3.18
39	*Smilax* sp.	Khaoyennuea	root	SMe	Ethanol	5.41
40	*Smilax* sp.	Khaoyennuea	root	SMw	Water	7.94
41	*Senegalia rugata*	Sompoi	leaf	SR1e	Ethanol	10.56
42	*Senegalia rugata*	Sompoi	leaf	SR1w	Water	10.95
43	*Senegalia rugata*	Sompoi	pod	SR2e	Ethanol	20.97
44	*Senegalia rugata*	Sompoi	pod	SR2w	Water	16.41
45	*Terminalia bellirica*	Samophiphek, Naeton	fruit	TBe	Ethanol	23.08
46	*Terminalia bellirica*	Samophiphek, Naeton	fruit	TBw	Water	18.55
47	*Tinospora crispa*	Boraphet	stem	TCe	Ethanol	1.49
48	*Tinospora crispa*	Boraphet	stem	TCw	Water	0.63
49	*Thunbergia laurifolia*	Rangchued	leave	TLe	Ethanol	7.84
5	*Thunbergia laurifolia*	Rangchued	leave	TLw	Water	4.37
51	*Tiliacora triandra*	Yanang	stem	TTe	Ethanol	5.54
52	*Tiliacora triandra*	Yanang	stem	TTw	Water	5.04

3.3.2. Cytotoxicity of plant extracts in HepG2 cells

Paclitaxel and EGCG had IC_{50} values of 5.63 nM and 178.4 μM, respectively. The MNTC value of EGCG was 119.3 μM. Paclitaxel was used to validate the assay. After that EGCG served as positive control because it is a well-known potential chemopreventive agent (Landis-Piwowar and Iyer, 2014). Table 3-5 shows IC_{50} and MNTC values of all extracts. According to National Cancer Institute's criteria, cytotoxicity of plant extracts can be categorized into three groups; potent activity ($\log IC_{50} < 0$), moderate activity ($0 < \log IC_{50} < 1.10$), and weak activity ($1.10 < \log IC_{50} < 1.5$) (Fouche et al., 2008). After cytotoxicity screening of 52 plant extracts (from 24 species), only SR2e exhibited moderate activity with $\log IC_{50} = 0.85$. A hit rate of 4.17% was obtained based on the number of species with moderate activity expressed as a percentage of the 24 species tested. Interestingly, SR1e (ethanol extract of SR leaves) did not show comparable effect with SR2e (ethanol extract of SR pods). Until now, *Senegalia rugata* (synonym *Acacia concinna*) has never been reported for its cytotoxicity in HepG2 cells or anti-cancer activity before. Three extracts; SR2w, AUe, and ASe, showed weak activity with $\log IC_{50}$ values of 1.16, 1.21, and 1.49, respectively. There was no report for cytotoxicity in HepG2 for extract from young shoots of *Allium ascalonicum* (AS). Unlike SR and AS, *Saussurea costus* (AU) was reported for anti-cancer activity of its isolated compounds; alantolactone, isoalantolactone, and contunolide (Rasul et al., 2013a, Khan et al., 2013). However, as this PhD project focused on chemopreventive agents, the main attention was not on finding extracts with potent cytotoxicity, but rather on other activities related to chemoprevention. For example, EGCG did not show a strong cytotoxic effect in previous studies, with concentration that inhibited cancer cell growth ranging from 5 μM to 44 mM (48 hours incubation) (Darvesh and Bishayee, 2013). Cytotoxicity assay was an important step in order to determine the MNTC as cell death had to be avoided in other experiment.

Table 3-5 IC_{50} and MNTC values of plant extracts in HepG2 cells

No	Extract codes	IC_{50} (µg/ml)		MNTC (µg/ml)	
		Ethanol extract (e)	Water extract (w)	Ethanol extract (e)	Water extract (w)
1	AA	>200	>200	31.25	200
2	AS	31.04	>200	25.08	200
3	AL	>200	>200	50	200
4	AT	51.57	>200	12.15	200
5	AU	16.19	>200	7.954	200
6	CM	>200	>200	200	200
7	CH	88.39	>200	50.17	200
8	CO	50.07	>200	24.71	200
9	CG	>200	>200	100	100
10	DS	98.57	>200	65.84	150
11	FA	196.6	>200	12.5	200
12	IC	62.24	>200	33.12	200
13	LS	100.5	>200	36.02	200
14	PA	>200	>200	44.44	200
15	PI	48.65	>200	37.06	89.81
16	PP	65.78	70.73	37.63	42.97
17	PR	154.3	>200	111.3	50
18	PS1	>200	>200	100	200
19	PS2	>200	>200	100	200
20	SR1	>200	>200	100	200
21	SR2	7.101	14.67	1.219	7.221
22	SM	>200	>200	200	200
23	TB	115.0	198.5	10	23.85
24	TC	>200	>200	150	200
25	TL	>200	>200	50	200
26	TT	81.06	>200	53.47	18.04

3.3.3. Plant extracts possessed protective effect against oxidative stress-induced cell death

After a three hours-incubation with 0.5 mM *t*-BHP, the cell viability was reduced to 31.47%. Pre-treatment with 50 μM EGCG and 15 extracts significantly reduced *t*-BHP-induced cell death (Figure 3-8). TBw at 30 μg/ml showed the most potent activity. It could restore the cell viability to 59.3%, which was higher than EGCG. This might due to the antioxidant activity of the water extracts that showed comparable DPPH radical scavenging activity to vitamin C (Chalise et al., 2010). Previous studies have reported some activities which might contribute to the protective effect of these plant extracts. Isoalantolactone isolated from AU activated Nrf2 (Rasul et al., 2013b). CH extracts exhibited hydroxyl radicals scavenging activity and inhibited lipid peroxidation in HepG2 cells (Laohavechvanich et al., 2010). Hydromethanolic extract of CG showed free radical scavenging and antioxidant activities (Umamaheswari and Chatterjee, 2007). TL extracts exhibited DPPH radical scavenging activity (Rojsanga et al., 2012) and protective activity against ethanol-induced liver damage in rats and rat hepatocytes (Pramyothin et al., 2005). Water extract of DS showed antioxidant effect (Laupattarakasem et al., 2003). Ethanol extracts of the stem, leaf, and fruit and water extracts of the fruit and stem of PS showed weak antioxidant activity in DPPH assay (Hussain et al., 2009). Therefore, its protective effect might largely depend on other mechanisms. For AS, the protective effect might due to the ability of organosulfur compounds and allyl derivatives in the induction of GST, which is an important defensive enzyme (Bianchini, 2001).

Fifteen extracts that showed significant protective effect were then investigated in NQO1 activity assay.

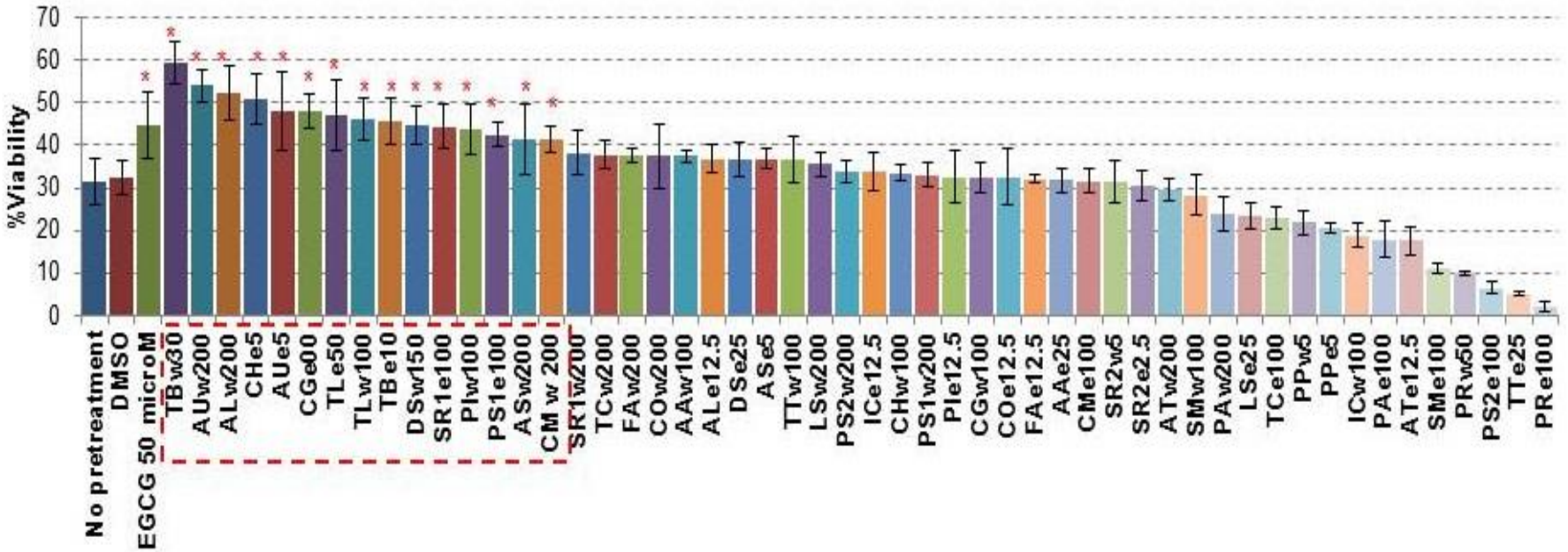

*Figure 3-8 Protective effects of plant extracts against tBHP-induced cell death. Cell viability of HepG2 cells was measured by AlamarBlue assay after an induction of cell death by 0.5 mM t-BHP for three hours. One-way ANOVA analysis showed that pre-treatment with 15 extracts for 24 hours significantly protected HepG2 cells from t-BHP-induced cell death, *P < 0.05 (N ≥3).*

3.3.4. Plant extracts induced NQO1 activity

Fifteen extracts; namely TBw, AUw, ALw, CHe, AUe, CGe, TLe, TLw, TBe, DSw, SR1e, PIw, PS1e, ASw, and CMw, were tested for the ability to induce NQO1 activity. After 72-hour incubation, five extracts; CHe, CGe, TLe, SR1e, and PS1e, significantly increased NQO1 level in a dose-dependent manner ($p < 0.01$) (Figure 3-9). Dicoumarol, an NQO1 inhibitor, reduced NQO1 activity by 50%. Menadione, an NQO1 inducer, enhanced NQO1 activity significantly in a dose-dependent manner. The use of negative and positive controls showed that the assay was working properly. 0.1% and 0.2% DMSO, which were equal to the amount of DMSO in the extract treatment, did not affect the enzyme activity. NQO1 activity induction might be one of the main protective mechanisms of CHe, CGe, TLe, SR1e, and PS1e. Our result is in agreement with a previous study which reported that *T.laurifolia* extracts induced NQO1 activity (Oonsivilai et al., 2007). This is the first time that NQO1 induction activity of ethanol extracts from *C.hystrix, C.grandis, S.rugata,* and *P.sarmentosum* was reported.

Even though potential cancer chemopreventive compounds must be proven to prevent tumour induction in animal models, phase II enzyme assays in cell cultures have been used for rapid screening for such compounds. The induction of phase II enzymes, such as GST and NQO1, is a major mechanism of a large number of anti-neoplastic and anti-mutagenic agents (Prochaska, 1994). NQO1 is important for prevention from toxic quinones, oxidative damage, and carcinogenesis (Nioi and Hayes, 2004). One of the protective actions of NQO1 is scavenging of superoxide and superoxide-like radicals (Zhu et al., 2007). *T*-BHP produces superoxide which results in cell damage (Slamenova et al., 2013). Therefore, induction of NQO1 activity is relevant to the protective effect of the plant extracts against *t*-BHP induced cell death, as well as helping to select potential candidates for the discovery of chemopreventive agents.

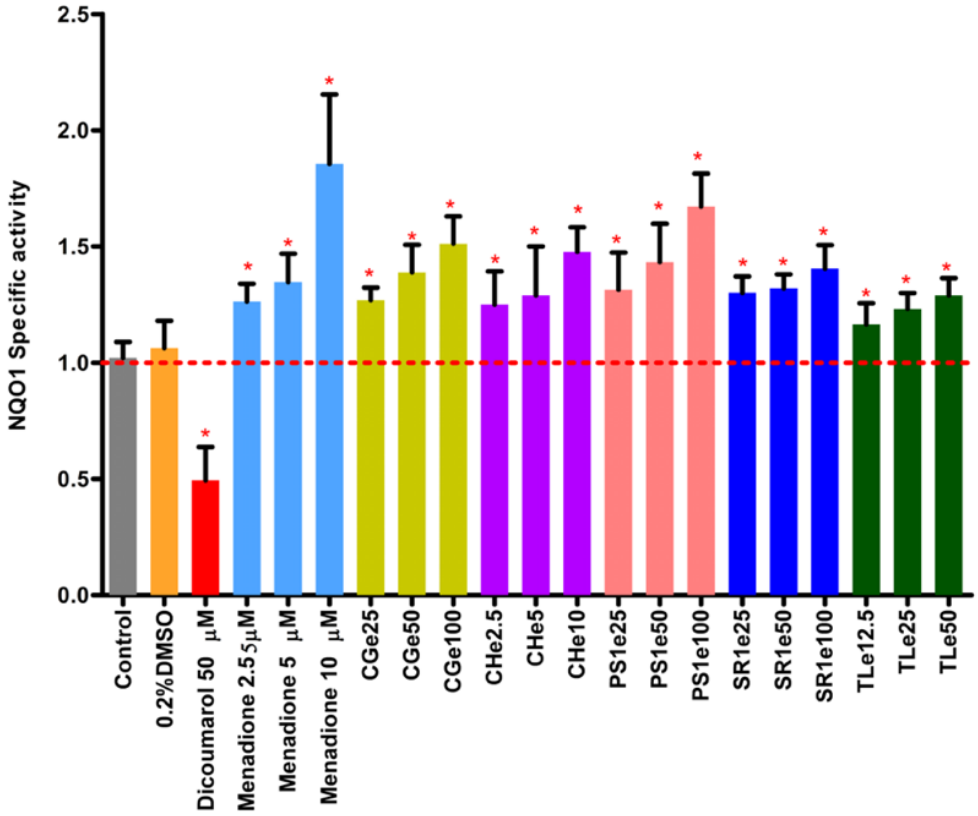

*Figure 3-9 NQO1 specific activity of HepG2 cells after 72 hour-treatment with indicated compounds (µM)/ extracts (µg/ml). One-way ANOVA analysis showed the significant effect of tested substances, *P < 0.05 (N ≥3).*

3.3.5. Plant extracts restored glutathione level after t-BHP treatment

After the treatment with 0.7 mM *t*-BHP for 4 hours, GSH level dropped from 32.22 ± 8.05 to 7.87 ± 3.08 µM/ mg protein. Pre-treatment with SR1e and TLe significantly restored GSH level in a dose-dependent manner ($P < 0.05$) (Figure 3-10). SR1e 50, 100 µg/ml, and TLe 50 µg/ml restored GSH level to 27.57 ± 3.60, 30.85 ± 4.88, and 22.72 ± 4.89 µM/ mg protein, respectively, which were almost equal to the baseline (no t-BHP). CHe also reversed the effect of *t*-BHP but not significantly. On the other hand, pre-treatment with PS1e and high concentration of CGe reduced GSH level more than *t*-BHP treatment alone, even though the effects were not significantly different. Prevention of the depletion of GSH might be one of the main protective mechanisms of SR1e and TLe against oxidative damage. *T.laurifolia* is well-known for its detoxifying properties and have shown hepatoprotective activity in several rat models, as well as in cell cultures (Junsi and Siripongvutikorn, 2016). However, this is the first time that ethanol extracts from *S.rugata* and *T.laurifolia* were reported for their ability to prevent GSH depletion by *t*-BHP.

Glutathione has roles in the defence against oxidative stress, which is an important factor in carcinogenesis. Many potential chemopreventive agents have been reported to induce GSH

level. For example, quercetin, a well-studied plant polyphenol found in onions, apples, berries, tea, and red wine, showed the ability to increase GSH level, as well as to block the reduction of GSH both *in vivo* and *in vitro* (Stagos et al., 2012). Therefore, GSH induction activity of the ethanol extract from *S.rugata* and *T.laurifolia* provide another evidence to support their traditional uses in cancer prevention and their role as candidates for cancer chemopreventive agent discovery.

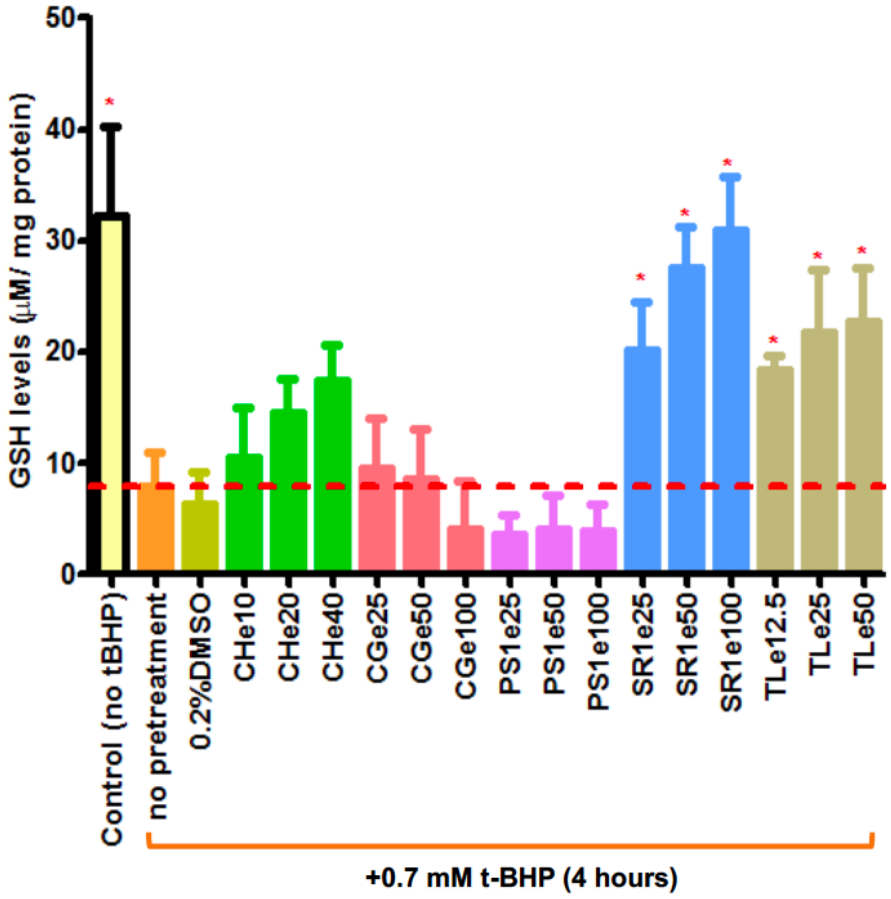

*Figure 3-10 GSH level after t-BHP treatment. One-way ANOVA analysis showed that pre-treatment with SR1e and TLe for 24 hours significantly attenuated GSH depletion effect of t-BHP, *P < 0.05 compared to no pre-treatment (N ≥3).*

3.3.6. The effects of plant extracts on cell viability in primary rat hepatocytes

The preliminary safety data of CHe, CGe, Ps1e, SR1e, and TLe (concentration 0 – 200 µg/ml) were assessed by cytotoxicity assay in primary rat hepatocytes. The % viability of hepatocytes treated with the extracts for 24 and 48 hours were between 77.8 – 104.52% and 73.1 – 102.38%, respectively. The IC_{50} values of all extracts were more than 200 µg/ml. This shows that all five extracts were not toxic to the hepatocytes (Figure 3-11). The cytotoxicity of these plant extracts in primary rat hepatocytes has never been published before, when searched using Web of Science and manual searches on Google. In addition, Pramyothin et al. (2005) reported that co-treatment of water extract from TL leaves at 2.5, 5.0 and 7.5 mg/ml with ethanol significantly reduced the cell death of primary rat hepatocytes, compared to ethanol-

treatment alone (Pramyothin et al., 2005). This helps to confirm that TL extracts produced protective effect rather than toxic effect in hepatocytes in *in vitro*.

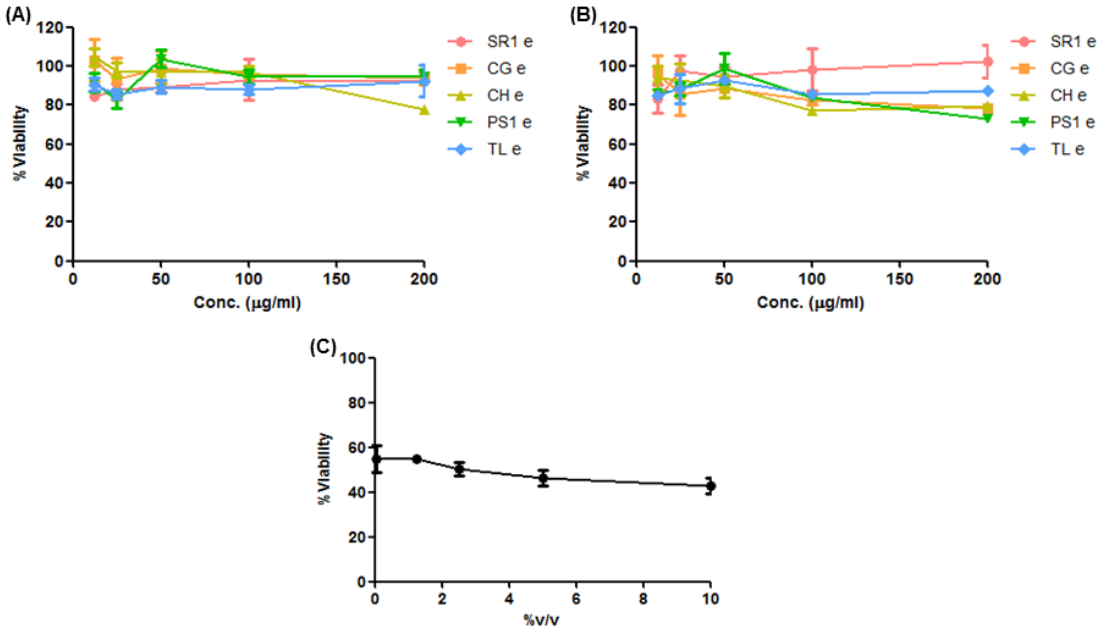

Figure 3-11 Cytotoxicity of CGe, CHe, PS1e, SR1e, and TLe in primary rat hepatocytes. (A) after 24 hours-incubation (B) after 48 hours-incubation (C) %viability of the hepatocytes after ethanol (0.06 – 10% v/v) treatment for 24 hours (N ≥ 3)

3.4. Conclusion

Thai traditional medicine (TTM) has become a popular alternative way for cancer patients in Thailand, especially those in terminal stages. In this chapter, cytotoxicity, protective effect against oxidative stress, and effects on glutathione and NQO1 enzyme activities of extracts from selected plants used for cancer/*mareng* prevention were studied in *in vitro*. The pharmacological assays were developed on the basis of characteristics of cancer as defined in TTM. Almost a third of the extracts tested showed protective effects against oxidative stress. Furthermore, 10% of them induced antioxidant enzyme activity and 4% restored GSH level from oxidative damage. These extracts also showed no toxicity in primary rat hepatocytes. Ethanol extracts from *S.rugata* and *T.laurifolia* showed the most promising protective activities by giving significant positive results from *t*-BHP induced cell death assay, NQO1 activity assay, GSH assay, and cytotoxicity in rat hepatocytes. These results show that TTM is a good source for chemopreventive drug discovery. Our work provides *in vitro* evidence that support these traditional uses of decoctions and macerated preparations of at least thirteen plants in cancer and disease prevention.

Oxidative stress plays a crucial role in carcinogenesis, as well as other chronic diseases. *Krasai* is an important factor for chronic illnesses development in TTM. In this report, *krasai* was linked to oxidative stress for the first time. The results from pharmacological investigation demonstrate that oxidative stress models can be utilized to test herbal medicine used for

krasai. Therefore, this basis can be applied in other areas wider than cancer, such as inflammation and most chronic conditions in TTM.

In this study, a cancer prevention strategy based on TTM concepts was proposed for the first time. The strategy is not only useful for TTM practitioners, but also helpful for scientists to develop bioassays to investigate herbal medicine that are used in a specific cultural context for conditions linked to cancerous diseases. We showed that TTM theory can provide useful information for planning pharmacological experiment. It also provides a model for developing *in vitro* assays in order to investigate TTM knowledge, especially in cancer, inflammation, and other chronic diseases. In the future, the strategy should be applied to *in vivo* and clinical studies in order to further confirm the validity of such a strategy. Other traditional medical systems that use holistic approach can also use our strategy as a model to develop traditional medicine with a better evidence-base.

Chapter 4. Chemopreventive profiles of *Senegalia rugata* and *Thunbergia laurifolia*: the effect on antioxidant gene expression in HepG2 cells, high-resolution radical scavenging profiles, and active phytochemicals

4.1. Introduction

According to the data gathered during the fieldwork (chapter 2), *Senegalia rugata* (Lam.) Britton & Rose (Fabaceae) and *Thunbergia laurifolia* Lindl. (Acanthaceae) were among medicinal plants used to prevent *mareng*. During the screening for pharmacological properties (chapter 3), their ethanol extracts showed the most promising *in vitro* activities by significantly protecting HepG2 cells from *t*-BHP induced cell death, inducing NQO1 activity, restoring glutathione level after *t*-BHP treatment, and showing no cytotoxicity to rat hepatocytes. These properties are crucial for cancer chemopreventive agents. Therefore, the ethanol extracts from these two species were selected for further investigation.

4.1.1. *Senegalia rugata* (Lam.) Britton & Rose: traditional uses, chemopreventive or related properties, and phytochemistry

4.1.1.1. Traditional uses

'*Sompoi*' or soap pod or *Senegalia rugata* (Lam.) Britton & Rose (synonym *Acacia concinna* (Willd.) DC.) from Fabaceae is a scandent shrub with thorny stem 2-5 m high. The leaves are bipinnate, alternate with pinnae 8-12 pairs, 7-20 cm long with 9-25 pairs of leaflets, linear or oblong. The inflorescence is in terminal and axillary, compound globose head. The flowers are yellowish white. The pods are thick, dark brown in colour, juicy, but rugose when dry (Saralamp et al., 1996).

Figure 4-1 Sompoi tree and dried pods (lower right)

In Thai traditional medicine, the stem is used to treat *Namtaphikan*[16]. The leaf is used for eye diseases and to cleanse the small and large intestines from diseases. While the flower is used to treat joint diseases, the fruit is used to treat fever in children, as well as thick mucus. Moreover, the root is used to treat fever. The plant is used in combination with other plants as anti-flatulent, expectorant, and externally to relieve abscess (Foundation for the promotion of Thai traditional medicine and Ayurved Thamrong School Center of applied Thai traditional medicine, 2007a). According to the national list of essential medicines of Thailand (2013), the leaf and pod are ingredients in a laxative formula. The pod is among the main ingredients of *Ya Faihakong* recipe which is used to repel lochia and help re-positioning the uterus in women after giving birth. Moreover, the leaf is used in *Ya Prakhob* (herbal compress) which is used externally to relieve muscle and joint pain (National Drug Committee, 2013).

As the plant is native to tropical Asia, it is also used in other traditional medicines. For instance, in India the pod is used as expectorant, shampoo, skin tonic, a purgative and as an emetic (Khory and Katrak, 1999).

4.1.1.2. Chemopreventive or related properties

Using Web of Science database there were 49 results after searching for '*Senegalia rugata*' or '*Acacia concinna*'. One article reported its immunological adjuvant activities. A methanol fraction from saponin fractions of the pod extract (MAC) induced splenocyte proliferations at the concentrations of 200, 400, 800 and 1000 µg/ml. OVA-specific IgG, IgG1 IgG2a and IgG2b antibody levels in serum were significantly enhanced by MAC as compared with the control group. This finding suggested that MAC might affect Th1 and Th2 helper T cells (Kukhetpitakwong et al., 2006). Its water extract was reported to possess antimicrobial and anthelmintic activities (Medisetti et al., 2016, Todkar et al., 2010).

4.1.1.3. Phytochemistry

The seeds contain a monoterpenoidal carboxamide, concinnamide (Sekine et al., 1997). The beans without seeds contain a saponin glycoside, sonuside (Sharma and Walia, 1977). The pods contain saponins; including prosapogenols, concinnosides A-F (Abul Gafur et al., 1997, Kukhetpitakwong et al., 2006), kinmoonosides A and C (Kukhetpitakwong et al., 2006); monoterpenes, including menthiafolic acid, (2E)-6-hydroxy-2-hydroxymethyl-6-methyl-2,7-octadienoic acid, and their glycosides which are (6R)- and (6S)-menthiafolic acid-6-O-β-D-xyloside (Kiuchi et al., 1997); four glycosides which are acaciaside, julibroside A1, julibroside A3, albiziasaponin C, and their aglycone, acacic acid lactone (Kukhetpitakwong et al., 2006).

[16] The symptoms of *namtaphikan* are blurred vision and dry eyes (*Khamphi Roknithan*).

4.1.2. *Thunbergia laurifolia* Lindl.: traditional uses, chemopreventive or related properties, and phytochemistry

4.1.2.1. Traditional uses

'*Rangchued*' or laurel clock vine or *Thunbergia laurifolia* Lindl. from Acanthaceae is a climbing plant. The leaves are simple, opposite along the stem, oblong or ovate, 4-5 cm wide, 8-10 cm long, broad based, narrowing to a pointed tip, usually with scalloped lobes towards the base. The flowers are bluish purple, trumpet-shaped and the seed pod is cone-shaped, 1 cm long, with a round base. The bracts are green with reddish brown blotches. The fruits are capsule. In Thailand, *T. laurifolia* has several names, such as rangchued, yawkaew, kobshanang, gumlungchangpuak, or kruanannae. (Junsi and Siripongvutikorn, 2016, Saralamp et al., 1996)

Figure 4-2 Rangchued plant

In Thai traditional medicine, the leaf is used as universal antidote, treatment for fever and thirst (National Drug Committee, 2013). Moreover, it is also used to treat abnormal menstruation, ear pain, removal of toxins from the liver, and used externally for treatment of swelling. Nowadays it is a well-known detoxifying herbal medicine in Thailand. The most popular dosage form is herbal tea.

The plant is native to India, Indochina and Malaysia. In Malaysia, juice from the leaf is used for the treatment of menorrhagia, placed into the ears for deafness, and externally for cuts and burns (Wonkchalee et al., 2012).

4.1.2.2. Chemopreventive or related properties

There were 36 results when searched for 'thunbergia laurifolia' on Web of Science. Eight references were related to our focus, which are anti-inflammatory, detoxifying, and protective effects. These studies supported its traditional use in detoxification.

Protective and detoxifying effects

Multiple lines of evidence supported the use of *Thunbergia laurifolia* (TL) in the detoxification against toxic substances. Pre-treatment of TL extract reduced toxicity signs and organ

damages caused by cadmium in rats (Ruangyuttikarn et al., 2013). Food supplemented with 50% ethanol extract increased the growth rate of fish exposed to $Pb(NO_3)_2$. The extract also normalized blood chemistry, haematological and histological parameters in $Pb(NO_3)_2$-treated fish (Palipoch et al., 2011). Moreover, its water extract attenuated the effect of ethanol in the increase in ALT, AST, hepatic triglyceride, and centrilobular hydropic degeneration of hepatocytes in *in vivo*, as well as protected primary rat hepatocytes from ethanol toxicity (Pramyothin et al., 2005).

In vitro experiment showed some mechanisms related to cytoprotection. Acetone extract, ethanol extract, and water extract increased NQO1 activity in Hepa 1C1C7 cells by 2.8 fold, 1.35 fold, and 1.56 fold, respectively. In addition, all extracts also showed strong dose-dependent anti-mutagenic activity by inhibition of aminoanthracene-induced mutagenesis up to 87% in *Salmonella typhimurium* TA 98 (Oonsivilai et al., 2007). Its ethanol extract induced the mRNA expression of CYP1A1, CYP1A2, CYP2B6, CYP3A4, and PPAR gamma, while it inhibited the mRNA expression of CYP3A7, CYP2D6, and CYP2E1. The extract also increased the activity of P-glycoprotein, which accelerated the excretion of xenobiotic and toxic substances from HepG2 cells (Rocejanasaroj et al., 2014).

Anti-inflammatory effects

Administration of TL reduced the inflammation in Syrian hamsters infected with human liver fluke *Opisthorchis viverrini* or treated with N-nitrosodimethylamine (Wonkchalee et al., 2012). Rosmarinic acid isolated from the leaves (at the doses of 50, 100, and 150 mg/kg) exhibited significant anti-nociceptive effect in the hot-plate test. At doses of 50 and 100 mg/kg, it decreased acetic acid-induced writhing by 52% and 85%, respectively. At 100 mg/kg, it inhibited formalin-induced pain in the early and late phases and suppressed carrageenan-induced paw oedema at 3, 4, 5 and 6 h after carrageenan injection, and showed significant activity against PGE_2-induced paw oedema. It also inhibited cotton pellet-induced granuloma formation in mice (Boonyarikpunchai et al., 2014).

Antioxidant effects

Rosmarinic acid isolated from the leaves exhibited DPPH-scavenging property with an EC_{50} value of 2.71 µg/ml (Suwanchaikasem et al., 2014). The compound has also shown remarkable biological properties both *in vitro* and *in vivo*, such as antioxidant, anti-inflammatory, antimutagenic, antigenotoxic, cytotoxic, antimetastatic, antiangiogenic, neuroprotective, antimicrobial, and immunomodulatory effects. For instance, it reduced kidney injury in several mice models, including cisplatin-induced, diabetes-induced, and gentamicin sulphate-induced injury. It exhibited liver protection against cholestatic fibrosis, ischemia-reperfusion injury, CCl_4-induced, and lipopolysaccharide-induced injury *in vivo*. These effects have been linked to its antioxidant and radical scavenging effects (Amoah et al., 2016).

4.1.2.3. Phytochemistry

The leaf contains rosmarinic acid (Suwanchaikasem et al., 2014), sterols, including β-sitosterol, stigmasterol, alphaspinasterol; phenolics, including apigenin, caffeic acid, gallic acid and protocatechuic,; carotenoids, including lutein and steroids; and glycosides, including 8-epigrandifloric acid, 3'-O-β-glucopyranosyl-stilbericoside, grandifloric acid, benzyl β-glucopyranoside, benzyl β-(2'-O-β-glucopyranosyl)-glucopyranoside, 6-C-glucopyranosyl apigenin, 6,8-di-C-glucopyranosyl apigenin, (E)2-hexenyl-β-glucopyranoside, and hexanol-β-glucopyranoside (Junsi and Siripongvutikorn, 2016). The aerial part contains two iridoid glucosides, which are 8-epi-grandifloric acid and 3′-O-β-glucopyranosyl-stilbericoside; benzyl β-glucopyranoside, benzyl β-(2′-O-β-glucopyranosyl) glucopyranoside, grandifloric acid, (E)-2-hexenyl β-glucopyranoside, hexanol β-glucopyranoside, 6-C-glucopyranosyl apigenin, and 6,8-di-C-glucopyranosyl apigenin (Kanchanapoom et al., 2002).

Primary constituents of water extracts are caffeic acid and apigenin. Acetone and ethanol extracts contain chlorophyll a and b, pheophorbide a, pheophytin a, and lutein identified by HPLC analysis (Oonsivilai et al., 2007).

4.1.3. Cytoprotection: an important mechanism for cancer prevention

Modulation of cytoprotective enzyme activation is among important property of blocking agents. Nrf2 (NF-E2-related factor 2) is a transcription factor which is now recognized as a master regulator of antioxidant defences. Nrf2 is bound by Keap1 in normal condition within the cytoplasm. Under stress condition, Nrf2 is released and translocated into the nucleus. Then it forms a heterodimer with Maf and then binds to ARE (antioxidant-responsive elements), which then encodes detoxifying and antioxidant enzymes, such as GSTs (glutathione S-transferases) and NQO1 (NADPH: quinone oxidoreductase) (Figure 4-3). Therefore, any agents that activate Nrf2 signaling would be beneficial to prevent or reduce DNA damage by oxidative stress (Landis-Piwowar and Iyer, 2014). Many of Nrf2 activators are from natural origins, especially from plants, such as Protandim®- a dietary supplement containing five herbal extracts, including *Silybum marianum*, *Bacopa monniera*, *Withania somnifera*, *Camellia sinensis*, and *Curcuma longa*. Protandim was reported to reduce skin tumour incidence *in vivo* and p53 suppression and mitochondrial SOD induction might be the mechanism of the tumour prevention (Hybertson et al., 2011). This data show that medicinal plants are possible sources in a search for Nrf2 activator for chemoprevention.

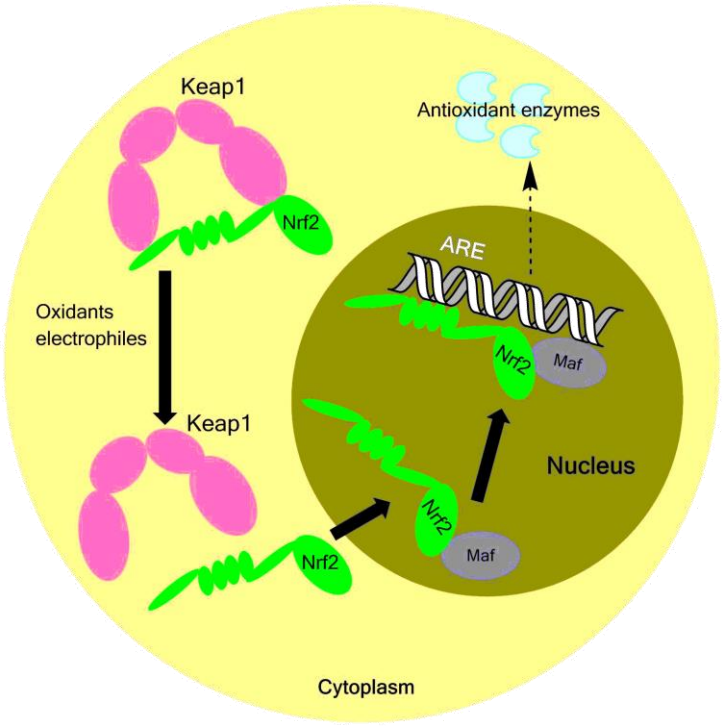

Figure 4-3 Mechanism of Nrf2 signalling. Modified from (Landis-Piwowar and Iyer, 2014)

When xenobiotics, including carcinogens, enter the body, they will be metabolized by phase I, or II, or III enzymes. Cytochrome P450s (CYPs) are phase I enzymes which initiate the detoxification process by transforming hydrophobic into hydrophilic compounds. However, sometimes CYPs reaction results in reactive intermediates which form covalent bonds with DNA and lead to the initiation of carcinogenesis. Among 18 families known to present in mammalian cells, CYP1A1 is well-known for its role in the activation of carcinogens, especially polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines. It is extra-hepatic, but present in the liver after induction (Kamenickova et al., 2013). Increase in mRNA expression of CYP1A1 has been associated with cancer initiation. Therefore, it becomes a target for chemopreventive agents (Badal and Delgoda, 2014).

Phase II enzymes, such as NQO1, and heme oxygenase-1 (HMOX-1) are enzyme that can inactivate reactive metabolites. NQO1 is a key enzyme involved in the defence against carcinogens (please see chapter 3 for more details). HMOX-1 protects from ROS by Fenton reaction. Firstly, it metabolizes pro-oxidant heme into antioxidant bilirubin which then increases the expression of ferritin. Ferritin sequesters ferrous ions which results in the prevention of the formation of ROS. Several cancer chemopreventive agents, such as dithiolethiones, possess the ability to induce the expression of HMOX-1 (Primiano et al., 1996).

Phase III enzymes comprise various ATP-binding cassette (ABC) transporter families which are involved in the exclusion of xenobiotics and their metabolites (Döring and Petzinger,

2014). However, this type of metabolizing enzymes is beyond the scope of this thesis. Therefore, they were not included in the experimental section.

4.1.4. Measurement of gene expression using real-time PCR technique

Gene expression comprises two major phases; transcription and translation (Figure 4-4). Studying how a gene is expressed can be done by studying of the RNA transcript or translated proteins (Dale and Schantz, 2002).

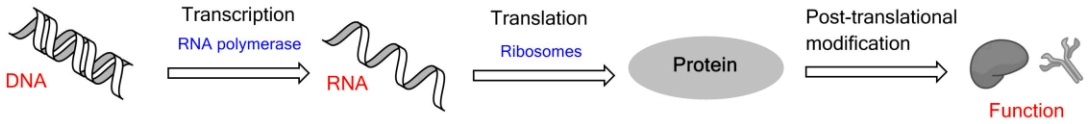

Figure 4-4 Fundamentals of gene expression. The first step is transcription of DNA to RNA. For most genes, RNA is then translated into a protein molecule to function. For some genes, such as tRNA or rRNA, RNA itself is the functional molecule. Modified from

Many techniques have been developed to study transcription. Polymerase chain reaction (PCR) is one of the major methods for studying genes because it is very sensitive and can detect extremely low level of expression (Dale and Schantz, 2002). The result of PCR is a selective amplification of a chosen region of a DNA molecule by the use of DNA polymerase enzyme which is thermostable. Any region of DNA can be selected as long as the sequences at the borders of the region are known. This can be done by the addition of specific oligonucleotide primers. After a mix of target DNA, DNA polymerase, forward and reverse oligonucleotide primers, and a supply of nucleotides, the PCR cycle is performed according to (Figure 4-5) (Brown, 2016). Normally these cycles are repeated 40 - 50 times to amplify the DNA template. Then the PCR products can be studied in various ways to gather information about the DNA template.

Nowadays quantitative PCR (qPCR) is usually performed using real-time PCR which measures the products over time. This method utilizes the use of a dye that emits fluorescent signal when it binds to double-stranded DNA or a probe that emits fluorescent signal when it hybridizes to the specific PCR product. The amount of RNA can also be measured by the use of reverse transcriptase PCR. In this technique, RNA is converted to complementary DNA (cDNA) by reverse transcriptase enzyme prior to standard real-time PCR reaction (Brown, 2016).

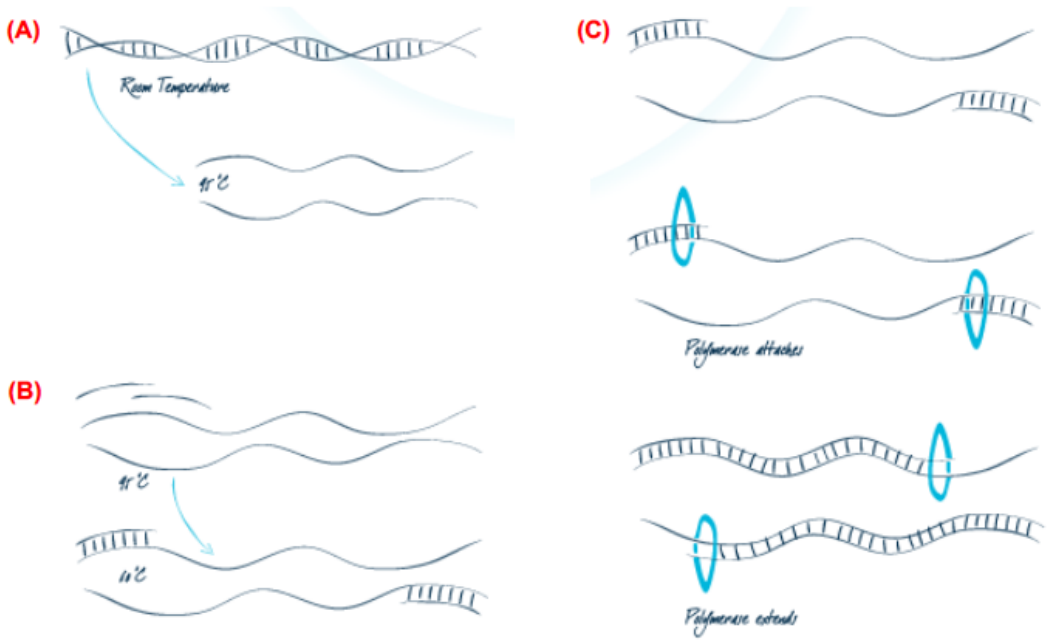

Figure 4-5 A PCR cycle comprises three steps; denaturation (A) – annealing (B) – synthesis (C). The PCR reaction starts by heating the mixture to 94-95ºC in order to break double-stranded DNA into single-stranded (A). Then the temperature is lowered to 50-60ºC to allow the primers to bind to gene of interest (B) before raised to 72-74ºC to make it suitable for the polymerase to copy the DNA strand (C). The image is obtained from Primerdesign® available from: http://www.primerdesign.co.uk/assets/files/beginners_guide_to_real_time_pcr.pdf

4.1.5. Studying protein expression by western blot analysis

DNA is the basic genetic material that carries information from one generation to the next. To affect the characteristics of the cell, it requires the production of the RNA copy of the DNA (transcription). Then the mRNA is translated into a polypeptide by ribosomes (Figure 4-4). Later processes called post-translational modifications; such as polypeptide folding or glycosylation or phosphorylation, are required in order to make the translated protein become functional. Studying of gene expression should be performed on both levels which are analysis of RNA transcription and protein translation.

Western blot analysis is the conventional way to study protein expressed in cells. Firstly, it involves protein separation by electrophoresis through a polyacrylamide gel in the presence of sodium dodecyl sulphate (SDS-PAGE). Then the proteins are transferred to a membrane; nitrocellulose or PVDF. Since there are too many proteins presented on the gel/membrane, the detection of interested proteins relies on the use of specific antibodies. These primary antibodies are commonly detected by the use of a secondary antibody followed by colorimetric or chemiluminescent detection. This technique allows researchers to study the presence or absence of interested proteins, as well as their sizes and relative levels of expression (Dale and Schantz, 2002). Figure 4-6 illustrates western blot steps.

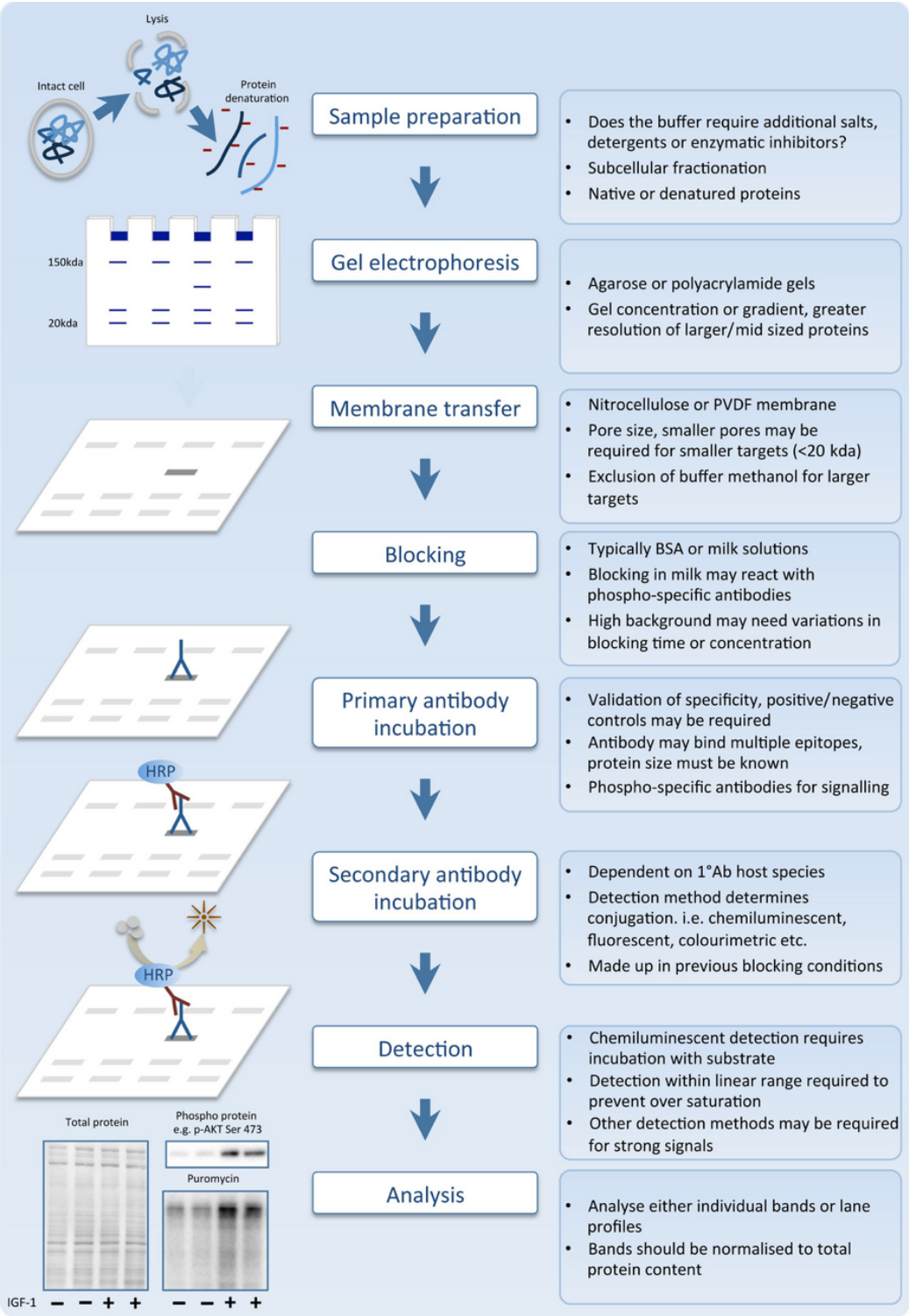

Figure 4-6 Western blot steps. The image is obtained from (Bass et al., 2017).

4.1.6. High-resolution radical scavenging assay-HPLC-HRMS-SPE-ttNMR technique

Conventional methods in the discovery of novel bioactive natural products are often labour and time consuming involving repeated bio-guided isolation steps and then structure elucidation. Regardless the effort investing in such isolation, there is still a high chance that

we would find already known compounds. Thus, knowing phytochemical profiles of interesting extracts prior to fractionation or isolation could save a lot of time and resources (Lambert et al., 2005). Dereplication by using hyphenated techniques based on the use of high-performance liquid chromatography (HPLC) system to perform separations and collections of micro-fractions directly into microtiter plates prior to high-throughput screening or bioassay has been used widely and can speed up the process (Gaudencio and Pereira, 2015).

HR-(bio)assay-HPLC-HRMS-SPE-*tt*NMR technique offers a fast and efficient analysis of the chemical composition of plant extracts, together with some information on bioactivities. It is a hyphenated system that combines high-resolution screening assays with high-performance liquid chromatography (HPLC), high-resolution mass spectrometry (HRMS), solid-phase extraction (SPE) and automated tube transfer nuclear magnetic resonance spectroscopy (*tt*NMR) (Figure 4-7). The system is used to identify bioactive chemical constituents with radical scavenging and α-glucosidase inhibition properties in sea aster and sea rocket (Wubshet et al., 2013a) and identify high-resolution radical scavenging profile and, for the first time, reported the direct detection of ^{13}C NMR spectra of metabolites from a crude extract of the endophytic fungus *Penicillium namyslowskii* (Wubshet et al., 2013b). Furthermore, the system was also used to identify antidiabetic constituents in *Radix Scutellariae* crude extract and its high-resolution triple aldose reductase, α-glucosidase, and radical scavenging profile (Tahtah et al., 2015).

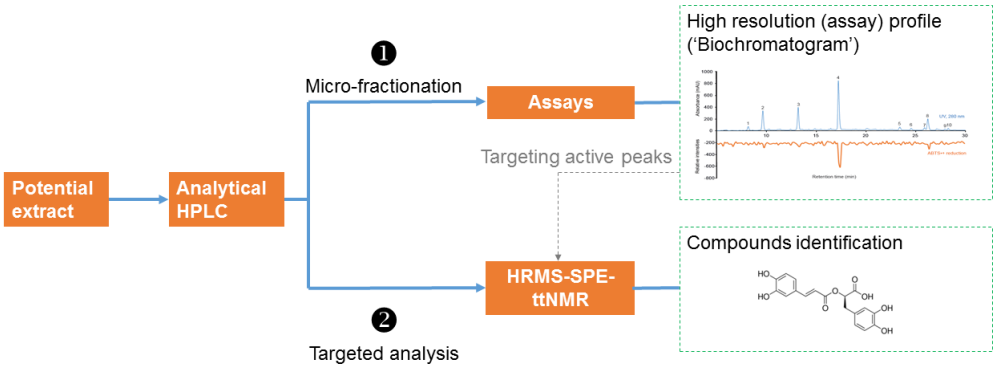

Figure 4-7 Workflow of HR-(bio)assay-HPLC-HRMS-SPE-ttNMR analysis. The first step is to separate and collect micro-fractions from a crude extract into 96-well plate(s). Then an assay is performed directly with the plates containing these fractions and a biochromatogram is created. Active peaks are then targeted and analysed with high-resolution mass spectrometry (HRMS) and NMR using a hyphenated and automated system; HPLC-HRMS-SPE-ttNMR. Modified from (Wubshet et al., 2013b).

4.1.7. Objectives

The objectives of this chapter are to investigate mechanisms related to the protective effects of ethanol extracts of *S.rugata* and *T.laurifolia*, to identify the potential fractions of the extracts, and to provide information on their phytochemistry.

4.2. Materials and methods

4.2.1. Materials

TRIzol™ (15596018), UltraPure™ DNase/RNase-free distilled water (10977035), qPCR master mix (PowerUp™ SYBR Green Master Mix), pre-cast SDS PAGE gels (Bolt 4-12% Bis-Tris Plus), LDS Sample Buffer (4X Bolt), reducing agent dithiothreitol (10X Bolt), transfer buffer for semi-dry transfer (Pierce 1-Step), Novex® Sharp Pre-Stained Protein Standard (LC5800), and MES SDS running buffer (20X Bolt) were ordered from Thermo Fisher Scientific. NQO1 (A180, sc-32793, Lot B0116) and β-actin (C4, sc-47778, Lot C0916) monoclonal antibodies were purchased from Santa Cruz Biotechnology, INC. Sodium chloride (S/3160/53) was from Fisher Scientific. *DC* Protein Assay kit (500-0116) was from Bio-Rad Laboratories, Inc. ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) (A1888), potassium persulfate (60489), sodium phosphate dibasic dehydrate (S0876), sodium phosphate monobasic dihydrate (71500), Trolox (238813), HPLC-grade acetonitrile, Trizma® base (T6066), tween-20 (P-1379), and protease inhibitor cocktail (EDTA-free mini cOmplete™ tablets, 11836170001 Roche) were purchased from Sigma–Aldrich. Formic acid and 10X RIPA buffer (0.5M Tris-HCl, pH 7.4, 1.5M NaCl, 2.5% deoxycholic acid, 10% NP-40, 10mM EDTA) were obtained from Merck. Other solvents were HPLC grade. MilliQ water was purified by deionization and 0.22 μm membrane filtration (Millipore, Billerica, MA). DNase treatment kit (Precision DNase) and reverse transcription kit (nanoScript 2) were purchased from Primerdesign Ltd.

4.2.2. Real-time PCR analysis

4.2.2.1. Cell treatment

HepG2 3 x 10^5 cells were seeded into a 6-well plate. After two days to allow the cells to proliferate, fresh medium containing 0.1% DMSO (control), or menadione 10 μM (positive control), or SR1e at 25, 50, 100 μg/ml, or TLe 12.5, 25, and 50 μg/ml were replaced and incubated for 24 hours. Then the treatment was discarded and RNA isolation was performed.

4.2.2.2. RNA isolation

Total RNA was isolated with TRIzol reagent according to the company's protocol. Briefly, 1 ml of TRIzol was added to each well, mixed with the cells by a cell scraper, and transferred into a reaction tube. Then 0.2 ml of chloroform was added to each tube and vortex to mix the content together. To separate the chloroform from TRIzol, the tube was centrifuged at 11,300xg at 4°C for 15 minutes. Then the clear upper layer containing RNA was transferred to a new clean reaction tube. 0.5 ml of isopropanol was added, vortex, and incubated for 10 minutes to precipitate RNA. Centrifugation at 11,300 x g at 4 °C for 10 minutes was then applied to remove the solvent. The RNA pellet was washed with 1 ml 75% ethanol (freshly prepared) twice. After centrifugation at 11,300 x g at 4 °C for 10 minutes and discard the ethanol, the pellet was left to air-dry. The RNA was kept at -80 °C until use.

4.2.2.3. RNAs quality assessment

RNAs were dissolved in 30 µl of the ultrapure water. Quality and quantity of the RNAs were determined using Agilent RNA ScreenTape assay with TapeStation system (Agilent Technologies) performed at UCL Genomics (London, UK). RIN^e is presented as a value between 1 and 10, where 10 represent the highest quality RNA sample. RNAs with RNA integrity number equivalent (RIN^e) above 8.0 were used for real-time PCR analysis in this project.

4.2.2.4. Real-time qPCR analysis

Elimination of genomic DNA contamination from RNAs was performed by DNase treatment according to Primerdesign protocol. Each 30 µl of RNA solution was mixed with 0.3 µl of DNase enzyme and 3 µl of the reaction buffer. DNase treatment was performed at 30 ^oC for 30 minutes and DNase inactivation was performed at 55 ^oC for 5 minutes in a thermal cycler (Bio-Rad MJ Mini). Then DNase-treated RNAs were diluted with Ultrapure water to obtain 1500 ng/9 µl. Reverse transcription was performed according to Primerdesign protocol. After the addition of 1 µl of Oligo dT primer into each 9 µl of diluted solution of DNase-treated RNA, the reaction tubes were incubated at 65 ^oC for 5 minutes. Ten µl of RT reaction mix was prepared freshly by mixing 5 µl of 4x buffer, 1 µl of 10 mM dNTP mix, 3 µl of Ultrapure water, and 1 µl of nanoScript enzyme together. This RT mix was added to the tube containing the RNA and the primer, and then the tube was incubated at 42 ^oC for 20 minutes followed by 75 ^oC for 10 minutes to inactivate the reaction. The synthesized cDNA was stored at -20 ^oC until use.

Quantitative PCR was performed following the manufacturer protocol. After cDNAs were obtained, 140 ng of cDNAs (2 µL) was mixed with 10 µl of qPCR mastermix with SYBRgreen probes, 6 pmols of forward primer, 6 pmols of reverse primer, and filled up to 20 µl with Ultrapure water. Then 20 µl of the assay mixture was added into 96-well PCR plate and real-time PCR analysis was performed using PikoReal™ Real-Time PCR System (Thermo Scientific). The cDNAs amplification started with the activation of UDG and DNA polymerase at 50 ^oC for 2 minutes and 95 ^oC for 2 minutes, respectively. Then each PCR cycle comprises three steps; 95 ^oC (15 seconds) is applied to separate double-stranded DNA, 58 ^oC (15 seconds) to let primers anneal to the DNAs, and then 72 ^oC (1 minute) which is the suitable temperature for the polymerase to copy DNA strand. This temperature cycling is repeated 40 times to amplify the DNA template.

Forward and reverse primers were designed using NCBI Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome) (Table 4-1). Relative mRNA expression level was calculated by comparative C_T method ($2^{-\Delta\Delta CT}$) using the expression of GAPDH gene as an internal control described in (Livak and Schmittgen, 2001). The calculation is presented below where GOI is gene of interest eg. Nrf2, NQO1, HMOX-1, CYP1A1 and calibrator is untreated control. C_T (threshold cycle) is defined as the

PCR cycle at which the fluorescent signal of the SYBR green crosses an arbitrarily placed threshold.

$$C_{T\ GOI\ of\ sample} - C_{T\ GAPDH\ of\ sample} = \Delta C_{T\ Sample}$$

$$C_{T\ GOI\ of\ calibrator} - C_{T\ GAPDH\ of\ calibrator} = \Delta C_{T\ calibrator}$$

$$\Delta\Delta C_T = \Delta C_{T\ Sample} - \Delta C_{T\ calibrator}$$

$$\text{Fold change} = 2^{-\Delta\Delta Ct}$$

Table 4-1 Primer sequences for real-time PCR analysis

Gene	5'-3' Primer sequence	Product length (bp)	Tm (°C)
NQO1	Forward: CCG TGG ATC CCT TGC AGA GA	84	61.62
	Reverse: AGG ACC CTT CCG GAG TAA GA		59.29
Nrf2	Forward: AGT GGA TCT GCC AAC TAC TC	106	56.64
	Reverse: CAT CTA CAA ACG GGA ATG TCT G		57.14
HMOX1	Forward: AAG AGG CCA AGA CTG CGT TC	143	60.60
	Reverse: GGC AGA ATC TTG CAC TTT GTT G		58.95
GAPDH	Forward: ATG CTG GCG CTG AGT ACG TC	107	62.89
	Reverse: GGG CAG AGA GAT GAC CCT T		61.55
CYP1A1	Forward: AGA TGG TCA AGG AGG ACT ACA	113	57.53
	Reverse: CTG GAT ATT GGC GTT CTC AT		55.30

4.2.3. Western blot analysis

4.2.3.1. Cell treatment

HepG2 6 x 10^5 cells were seeded into a 25 cm^3 TC flask and left for three days to allow the cell proliferation. Then the cells were treated with 0.1% DMSO or TLe 12.5 µg/ml, or 25 µg/ml, or 50 µg/ml, or SR1e 25 µg/ml, or 50 µg/ml, or 100 µg/ml, or menadione 10 µM for 24 hours.

4.2.3.2. Whole cell lysate preparation

To prepare whole cell lysate, the cells were washed twice with ice-cold PBS. The cells were rapidly scraped off and suspend in 1 ml of PBS and transferred to a 1.5 ml reaction tube. The PBS was removed after centrifugation at 1000 x g for 5 minutes at 4 °C. Then 100 µl of RIPA buffer supplemented with protease inhibitor cocktail (100 µl of 7xComplete minitab solution for each 600 µl of RIPA buffer) was added to the cell pellet, vortex, and kept in ice for 30 minutes with vortexing to lyse the cells every five minutes. After allowing complete cell lysis, the tube was centrifuged at 17000 x g for 20 minutes at 4 °C. The clear supernatant was kept at -80 °C until use.

4.2.3.3. Protein measurement

Bio-Rad DC™ Protein Assay kit, which utilizes Lowry method, was used to determine the amount of protein. Before the assay was performed, working reagent A was prepared by addition of 20 µl of reagent S to 1 ml of reagent A. Five µl of cell lysate was added into 96-well plate. Then twenty-five µl of working reagent A was added to each well followed by 200 µl of reagent B. After that the plate was shaken and incubated at room temperature for 15 minutes, protected from light, before an absorbance measurement at 750 nm with a microtiter plate reader (Infinite M200, Tecan). A BSA standard curve was generated and used to quantify the amount of protein in cell lysate.

4.2.3.4. Western blot analysis

Prior to the experiment, the amount of protein loaded, transfer time, the strength of blocking buffer, and the concentration of antibodies were optimized. NQO1 protein was measured because it was abundantly expressed in the HepG2 cells.

Ten µg of protein was loaded into each well of SDS-PAGE gels along with molecular weight markers. Ice-cold running buffer was filled into the electrophoresis chamber (Mini gel tank, Thermo Fisher Scientific) and the gel was run at 50 V for 5 minutes and then at 125 V for 1 hour 10 minutes. After that the protein was transferred to a PVDF membrane using Pierce™ Power Blotter (Thermo Fisher Scientific) for 15 minutes with the maximum voltage at 25V and 1.3A. Then the membrane was blocked in 10 ml of 3% dried skim milk for 1 hour at room temperature on a rocking platform. Primary antibodies; NQO1 (1:500) and β-actin (1:1000) were then added and incubated with the membrane over night at 4 ^{o}C on a rocking platform. The membrane was washed with TBST three times before the incubation with secondary antibody (1:2500) in 3% dried skim milk for 1 hour at room temperature on a rocking platform. After the incubation, the membrane was washed with TBST three times. The chemiluminescent substrate was then applied to the blot for 5 minutes. Then the signal was captures using GeneGnome system (Syngene) with 3-minute exposure. ImageJ 1.50i software (Schneider et al., 2012) was used to read the band intensities and MS Excel 2013 was used to calculate the NQO1/β-actin relative ratio.

4.2.4. $ABTS^{\bullet+}$ radical scavenging assay

Crude extracts (concentration between 6.25 – 200 µg/ml) and micro-fractions were screened for their antioxidant property using $ABTS^{\bullet+}$ microplate assay (Wubshet et al., 2013a). An advantage of $ABTS^{\bullet+}$ assay is that it can be used to the study both water-soluble and lipid-soluble antioxidants, pure compounds, and herbal/food extracts (Re et al., 1999). Briefly, $ABTS^{\bullet+}$ stock solution consisting of 2.5 mM ABTS and 0.875 mM potassium persulfate in MilliQ water was prepared at least 16 hours before the assay. This is to allow complete $ABTS^{\bullet+}$ radicals forming which results in blue/green colour solution (Re et al., 1999). Before the assay began, the $ABTS^{\bullet+}$ stock solution was diluted five times with 0.1 M sodium phosphate buffer, pH 7.4. The diluted solution was then added to each well 200 µl. Then the plate was shaken

for 15 seconds and the absorbance was measured at 620 nm every one minute up to 20 minutes with a microplate reader (Thermo Scientific Multiskan FC, Waltham, MA, USA). Trolox (0 – 300 µM) was used as a standard. ABTS reduction values were calculated by;

$$\%\text{ABTS reduction} = \left(\frac{Absorbance\ of\ sample}{Absorbance\ of\ methanol}\right) \times 100$$

4.2.5. HPLC analysis

Prior to micro-fractionation, the extracts were injected to high-performance liquid chromatography (HPLC) to screen for common antioxidant compounds. The HPLC system was an Agilent 1200 series (Agilent Technologies) consisting of a G1311A quaternary pump, a G1322A degasser, a G1316A thermostatted column compartment, a G1315D diode array detector, and a G1329A thermostatted standard autosampler. Agilent ChemStation ver. B.04.01 software was used to control the instrument and acquire the data. The separation was performed using a reverse phase Luna C18 (Phenomenex, 150 x 2.0 mm, 3 µm, 100 Å) maintained at 40 ^{o}C. The mobile phase A was MilliQ water: Acetonitrile: Formic acid (95: 5: 0.1 % *v/v*) and B was Acetonitrile: MilliQ water: Formic acid (95: 5: 0.1 % *v/v*). The flow rate was 0.3 mL/minute. The injection volume was 0.8 µl. Single reference compounds (1 mg/ml) and a reference cocktail solution in methanol were run in the same sequence with the extracts. The samples were dissolved in mobile phase B for TLe and methanol for SR1e at the concentration of 100 mg/ml and 50 mg/ml, respectively. For TLe, the gradient profile was 5-60% B in 40 minutes. For SR1e, the gradient profile was 0-25% B in 40 minutes. The chromatograms were recorded at 254, 280, and 360 nm.

4.2.6. High-resolution radical scavenging assay

4.2.6.1. Micro-fractionations of plant extracts

Fractionations of TLe and SR1e crude extracts were performed using HPLC. The HPLC system was Agilent 1100 series (Agilent Technologies) consisting of a G1311A quaternary pump, a G1322A degasser, a G1316A thermostated column compartment, a G1315B photodiode array detector, a G1367C high-performance autosampler, and a G1364C fraction collector. The instrument was controlled using Agilent ChemStation ver. B.03.02 software. The separation was performed using a reverse phase Luna C18 (Phenomenex, 150 x 4.6 mm, 3 µm, 100 Å) maintained at 40 ^{o}C. The mobile phase A was MilliQ water: Acetonitrile: Formic acid (95: 5: 0.1 % *v/v*) and B was Acetonitrile: MilliQ water: Formic acid (95: 5: 0.1 % *v/v*). The flow rate was 0.8 mL/minute. The injection volume was 5 µl. The samples were dissolved in mobile phase B for TLe and methanol for SR1e at the concentration of 100 mg/ml. For TLe fractionation, the gradient profile was 5-60% B in 40 minutes. For SR1e, the gradient profile was 0-25% B in 40 minutes. The eluate from each sample was fractionated from 5 to 30 minute into two 96-well plates with the resolution approximately 7 points per minute. After that the mobile phase was removed immediately to avoid compound degradation by a vacuum concentrator (RVC 2-25 CDplus, Christ). Before starting the ABTS radical scavenging assay, 100 µl of methanol (HPLC grade) was added into each well and the plate was shaken for 10

minutes to reconstitute the fractions. The work was carried out at the Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark under the supervision of Professor Dan Stærk.

4.2.6.2. Biochromatogram plot

HPLC chromatogram at 280 nm was exported into DIF file and the fractions collection report was exported into text file. Average absorbance between 10 – 20 minutes of each well was obtained from the SkanIt software (Tahtah et al., 2015). Then ABTS reduction profile was plotted against its respective retention time underneath the HPLC chromatogram using MS Excel 2013. This plot is called 'Biochromatogram'.

4.2.7. Compounds identification of active fractions

After high-resolution radical scavenging assay was performed, the extracts were separated using the same HPLC condition, with the outlet connected to HRMS and SPE cartridges for compounds identification. The analysis was performed at the Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark under the supervision of Professor Dan Stærk.

4.2.7.1. HPLC-HRMS-SPE-NMR analysis

A small volume (0.1 %) of the HPLC eluate was subjected to a microTOF-Q II mass spectrometer (Bruker) equipped with an electrospray ionization interface. Mass spectra were acquired in positive-ion mode, using a drying temperature of 200 °C, a capillary voltage of 4100 V, a nebulizer pressure of 2.0 bar, and a drying gas flow of 7 L/min. A solution of sodium formate clusters was automatically injected in the beginning of each run to enable internal mass calibration. Data Analysis software (Bruker) was used to analyse MS data. The HPLC eluate was diluted with Milli-Q water at a flow rate of 1.0 mL/min prior to trapping in Resin GP SPE cartridges (10 × 2 mm ID, 5−15 µm, spherical shape, polydivinyl-benzenephase) (Spark), based on absorption thresholds at 280 nm and base peak chromatograms. Then the cartridges were dried with pressurized nitrogen gas for 30 minutes and the analytes were automatically eluted with MeOD into 1.7 mm NMR tubes using a Gilson Liquid Handler controlled by PrepGilson software 1.2 (Bruker Biospin). Chromatography, peak trapping, and solutions transfer from the SPE unit were controlled with HyStar ver. 3.2 software (Bruker Biospin).

4.2.7.2. NMR experiment

NMR experiment was performed with a Bruker Avance III 600 MHz NMR spectrometer (^{1}H operating frequency 600.13 MHz) equipped with a Bruker SampleJet sample changer and a cryogenically cooled gradient inverse triple-resonance 1.7 mm TCI probe-head (Bruker Biospin). Bruker standard pulse sequences were used. IconNMR 4.2 (Bruker Biospin) was used to control automated acquisition of NMR data. ^{1}H NMR spectra were recorded using 30° pulses and 64K data points, which were zero-filled to 128K and multiplied with an exponential

function (line-broadening = 0.3 Hz) prior to Fourier transformation. All samples were run in MeOD at 128 or 256 scan. Topspin 3.5 (Bruker Biospin) was used to process NMR data.

4.2.8. Statistical analysis

Mean, SD, student's t-test, and ANOVA analysis followed by Dunnett's test were performed using GraphPad Prism 5.01 software (GraphPad Software, Inc.). All experiment was performed at least N = 3. $P < 0.05$ was considered statistically significant.

4.3. Results and discussion

4.3.1. The effect of TLe and SR1e on antioxidant genes expression in HepG2 cells

All RNAs used for real-time PCR analysis were of good quality with RIN^e between 8.6 and 10 (Figure 4-8).

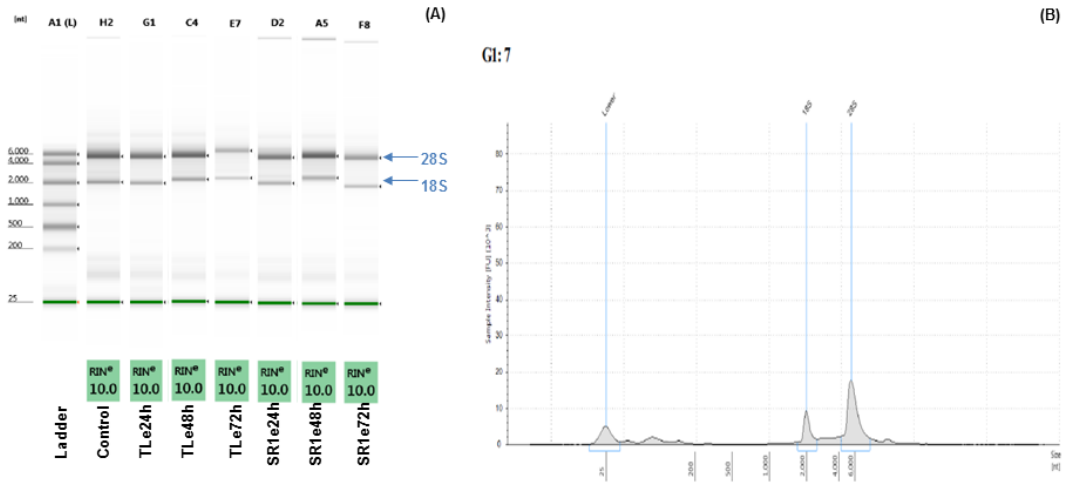

Figure 4-8 RNA analysis. (A) Representative gel image showing the separation profile of each RNA sample. RIN^e value for each sample is shown below the gel image. (B) Representative electropherogram of total RNA.

The effect of the extracts on the expression of important genes relevant for antioxidant effects; including Nrf2, NQO1, and HMOX-1 was investigated to determine their mechanisms. The level of CYP1A1 gene was also measured to assess the risk of DNA-adduct formation. Menadione (MQ), a toxic quinone known to induce NQO1 and cause DNA damage (Dietz et al., 2005) was used as a positive control. HepG2 cells were exposed to 10 µM of MQ for 24, 48, and 72 hours to find a suitable incubation time (appendix). After 24 hour-incubation, MQ significantly induced NQO1 and CYP1A1 genes and also inhibited Nrf2 genes (Figure 4-9). The strong induction effect of MQ on CYP1A1 (35.6 $\pm$ 5.0 folds for 5 µM and 33.9 $\pm$13.4 folds for 10 µM) illustrated its toxicity and this is in agreement with published literature (Sidorova et al., 2005). Therefore, 24 hours-incubation was used throughout the experiment. This time point also correlated with the assay investigating protective activity of the extracts against *t*-BHP-induced cell death which was presented previously in chapter 3.

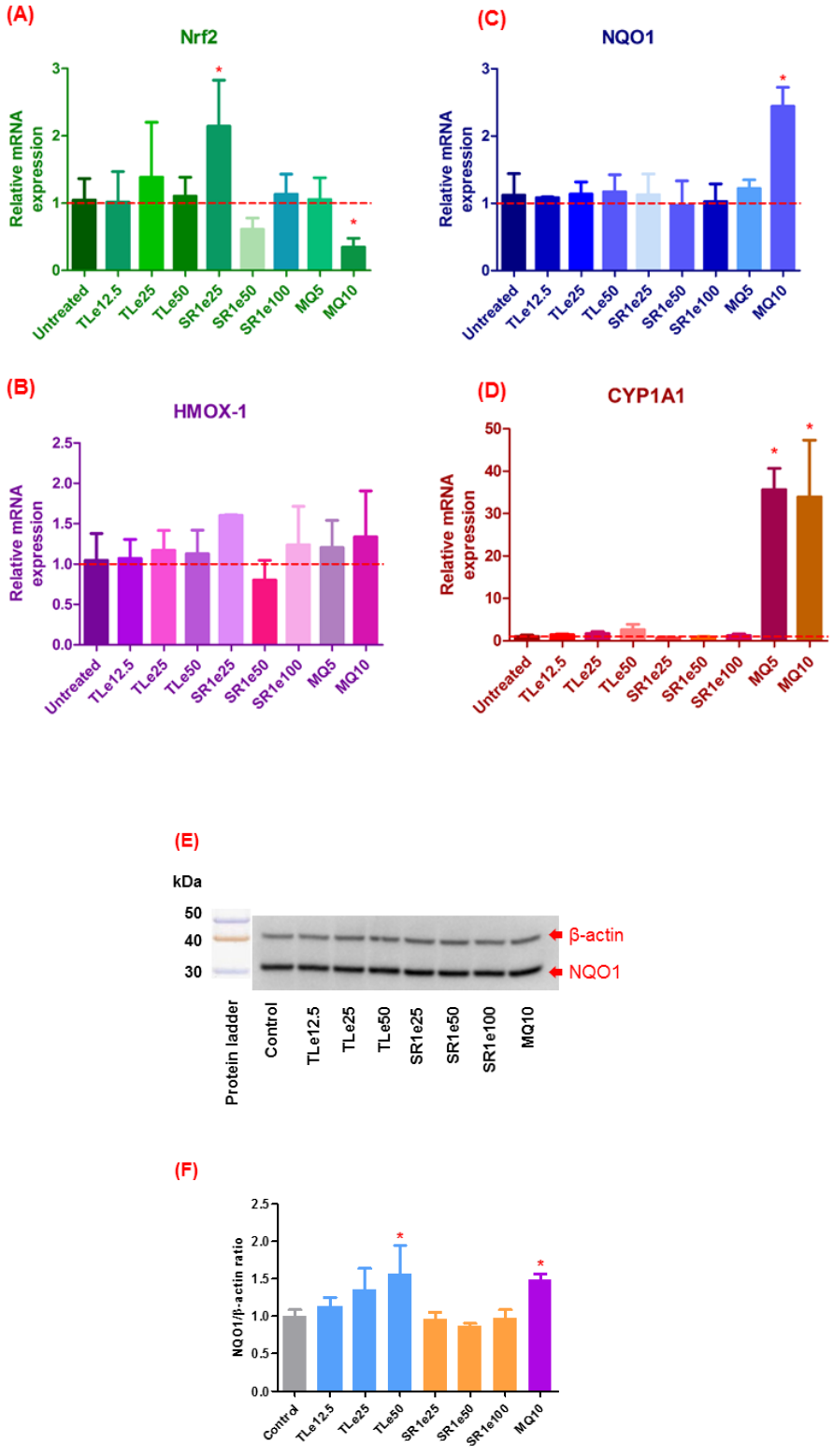

Figure 4-9 HepG2 cells were treated with various concentrations of TLe and SR1e for 24 hours. Level of mRNA expression of Nrf2 (A), HMOX-1 (B), NQO1 (C), and CYP1A1 (D) genes were measured by real time PCR using GAPDH as internal gene control. (E) and (F) Level of NQO1 protein expression was measured by western blot analysis. Results are mean ± SD (N ≥ 3). Unpaired 2-tailed t-test and one-way ANOVA were used to determine p values of PCR and western blot analyses, respectively. P < 0.05 was considered statistically significant.

Western blot analysis showed that NQO1 was abundantly expressed in HepG2 cells. As presented in chapter 3, both extracts enhanced NQO1 activity after 72 hours. We hypothesized that this might be a result of an increase in NQO1 expression and/or an activation of its master regulator Nrf2. Therefore, real-time PCR and western blot analyses were performed to investigate this matter.

SR1e and TLe did not change NQO1 mRNA expression (Figure 4-9 C). However, TLe exhibited a dose-dependent induction of NQO1 protein and significantly increased the expression to 1.6 $\pm$ 0.3 folds at higher dose of 50 µg/ml (maximum non-toxic concentration) which is comparable with MQ10 (1.5 $\pm$ 0.07 folds) (Figure 4-9 E and F). This illustrated that the increased activity of NQO1 enzyme in the cells exposed to TLe was due to NQO1 gene upregulation. A possible explanation that NQO1 mRNA level was not affected might be because of unsuitable incubation time, which should be less than 24 hours. For SR1e, NQO1 protein expression was not altered which correlated with the result from PCR analysis (Figure 4-9). In future work, various incubation times, especially between 0-24 hours, should be used in order to investigate the effect of the extracts on NQO1 gene expression in HepG2 cells. For instance, a lycopene metabolite and dieckol induced NQO1 gene and protein expression at 8 and 16 hours, respectively (Yang et al., 2012, Lee et al., 2015d).

SR1e at low dose of 25 µg/ml significantly upregulated Nrf2 mRNA expression (Figure 4-9 A). This might contribute to its protective effect against oxidative stress-induced cell death. However, the effect did not show a dose-response relationship in the concentration range that we investigated. Therefore, SR1e should be tested with broader concentration range and more time points in future work. TLe did not change the expression of Nrf2 after 24 hours-incubation (Figure 4-9 A). It might affect this gene at another time point. In addition, the protective effect and increased NQO1 activity might be due to an induction of Nrf2 translocation (Landis-Piwowar and Iyer, 2014) rather than its expression or act through another transcription factors that also bind to ARE, such as Nrf1 or Maf (Ross et al., 2000).

Low dose SR1e also induced HMOX-1 expression (Figure 4-9 B). This upregulation, although not statistically significant, correlated with the increase in Nrf2 expression. HMOX-1 is known to protect the cells from ROS (Primiano et al., 1996). This might contribute to the protective effect against oxidative stress-induced cell death of SR1e. To provide a clearer information, SR1e should be tested with broader concentration range and more time points in future work. On the other hand, TLe did not affect HMOX-1 expression after 24 hours-treatment (Figure 4-9 B). Therefore, its immediate protective effect (in 24 hours) was not a result from HMOX-1.

Exposure to both extracts resulted in the unchanged CYP1A1 mRNA expression (Figure 4-9 D). The result suggested that the extracts were likely to be non-toxic rather than toxic. On the other hand, a toxic substance menadione induced CYP1A1 expressions to 35 folds. A previous study by Rocejanasaroj et al. (2014) reported that CYP1A1 mRNA expression in HepG2 was upregulated by TLe. However, the concentration of TLe in that study was

extremely high, 200 to 600 µg/ml and it enhanced the mRNA expression 1.5 and 1.8 folds, respectively (Rocejanasaroj et al., 2014). Compared to our data, the concentration above 50 µg/ml produced some levels of cytotoxicity to the cells (see chapter 3). These results suggested that high dose of TLe might be able to produce some unwanted effects. *In vivo* experiment should be performed to investigate further safety profile.

4.3.2. Antioxidant properties of the crude extracts

ROS and free radicals can attack biological molecules and cause DNA damages, lipid peroxidation, and lead to many diseases, including cancer (Yoshikawa and Naito, 2002). Therefore, prevention of free radical formation is crucial to prevent oxidative stress which leads to carcinogenesis. This assay determines the ability of the extracts in the scavenging of stable cations formed through the reaction between ABTS and potassium persulfate (Re et al., 1999).

Trolox standard curves were created for the calculation of TEAC value of the samples (Figure 4-10). TEAC (Trolox equivalent antioxidant capacity) values of SR1e and TLe were 3.23±0.88 and 2.25±0.41 mM/ g extract, respectively. $ABTS^{\bullet+}$ reduction profiles of SR1e and TLe crude extracts are shown in Figure 4-11. IC_{50} values of Trolox, SR1e and TLe were 158.66 µM, 81.69 µg/ml, and 93.60 µg/ml, respectively. TEAC and IC_{50} values showed that SR1e exhibited slightly stronger ABTS radical scavenging activity than TLe.

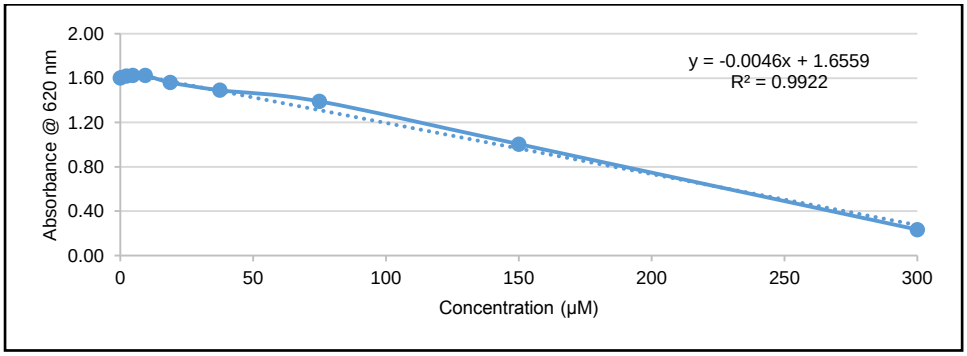

Figure 4-10 Representative Trolox standard curve. The linear equation (R^2 > 0.99) was used to calculate TEAC values of the samples. The calculation of TEAC value was performed by replacing y with the absorbance recorded from Skanit software.

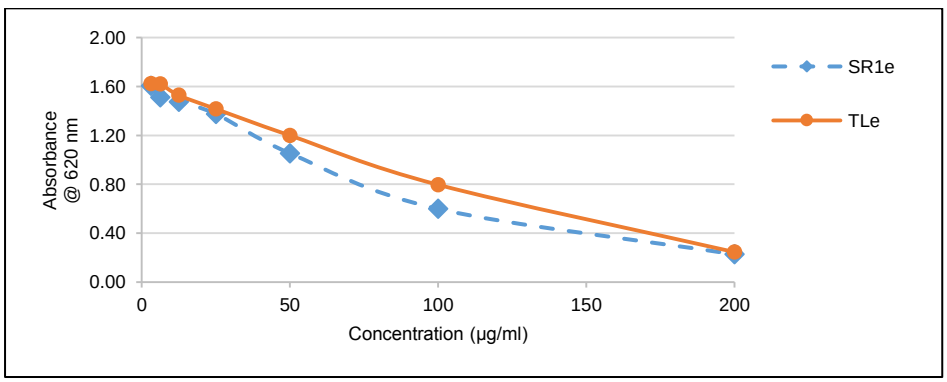

Figure 4-11 $ABTS^{\bullet+}$ reduction profiles of SR1e and TLe crude extracts. SR1e showed slightly stronger $ABTS^{\bullet+}$ radical scavenging activity compared to TLe.

4.3.3. HPLC analysis of SR1e and TLe

The HPLC conditions gave satisfying separations for SR1e, TLe, and standard cocktail. The standard cocktail comprises eight common antioxidants from plants which were gallic acid (GA), chlorogenic acid (ChA), caffeic acid (CA), vanillin (V), ferulic acid (FA), hesperidin (H), rutin (R), and quercetin (Q). These antioxidants were selected to be marker compounds because they are well-known antioxidants which are widely found in green plant parts, fruits, and vegetables (Ow and Stupans, 2003, Alía et al., 2006, Sato et al., 2011, Lee et al., 2015a, Devi et al., 2015, Tai et al., 2011). Since SR1e and TLe showed protective properties against oxidative stress, they might contain these common antioxidants. HPLC chromatograms of antioxidant mixture are shown in Figure 4-12 and Figure 4-13.

SR1e contained compounds with similar retention time (RT) to those of rutin and ferulic acid at 25.461 and 21.555, respectively. However, the UV spectra revealed that the peaks were not rutin and ferulic acid (Figure 4-14).

Figure 4-15 reveals HPLC chromatogram of TLe at 280 nm. The extract contains compounds with similar RT to caffeic acid, vanillin, and rutin at 7.712, 10.994, and 12.975, respectively. The UV spectra comparison of these peaks and those of the reference standards showed that the peak at 7.712 was caffeic acid. Signal at 360 nm illustrated one TLe peak with close RT to that of rutin, but the UV spectrum was not matched (Figure 4-16).

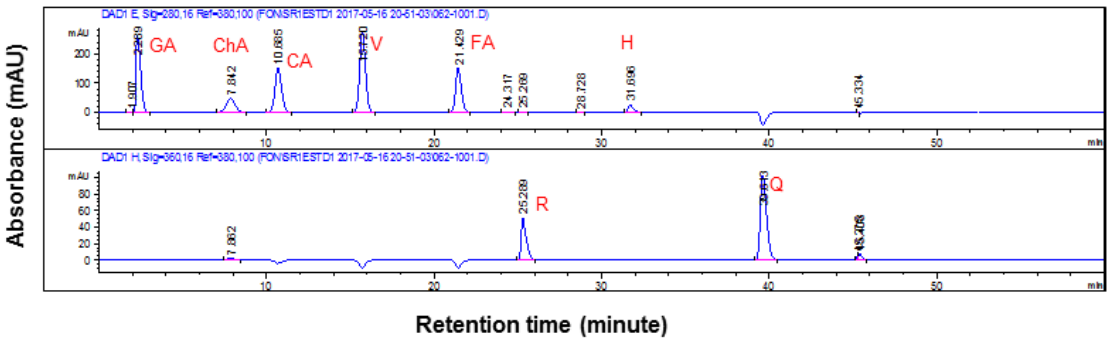

Figure 4-12 HPLC chromatogram at 280 nm (upper) and 360 nm (lower) of antioxidant mixture separated using a gradient mode; 0-25 % B in 40 minutes mobile phase system (SR1e condition). Gallic acid (GA), chlorogenic acid (ChA), caffeic acid (CA), vanillin (V), ferulic acid (FA), hesperidin (H), rutin (R), and quercetin (Q).

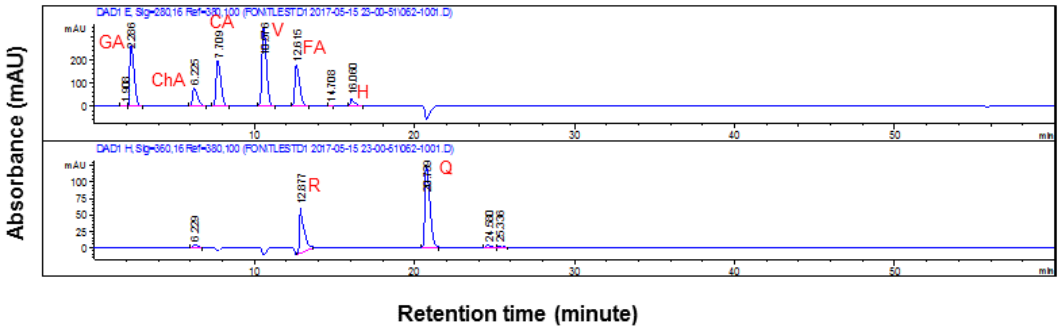

Figure 4-13 HPLC chromatogram at 280 nm (upper) and 360 nm (lower) of standard mixture separated using a gradient mode; 5-60 % B in 40 minutes mobile phase system (TLe condition). Gallic acid (GA), chlorogenic acid (ChA), caffeic acid (CA), vanillin (V), ferulic acid (FA), hesperidin (H), rutin (R), and quercetin (Q). Ferulic acid and rutin had very similar RT, but ferulic acid is not visible at 360 nm.

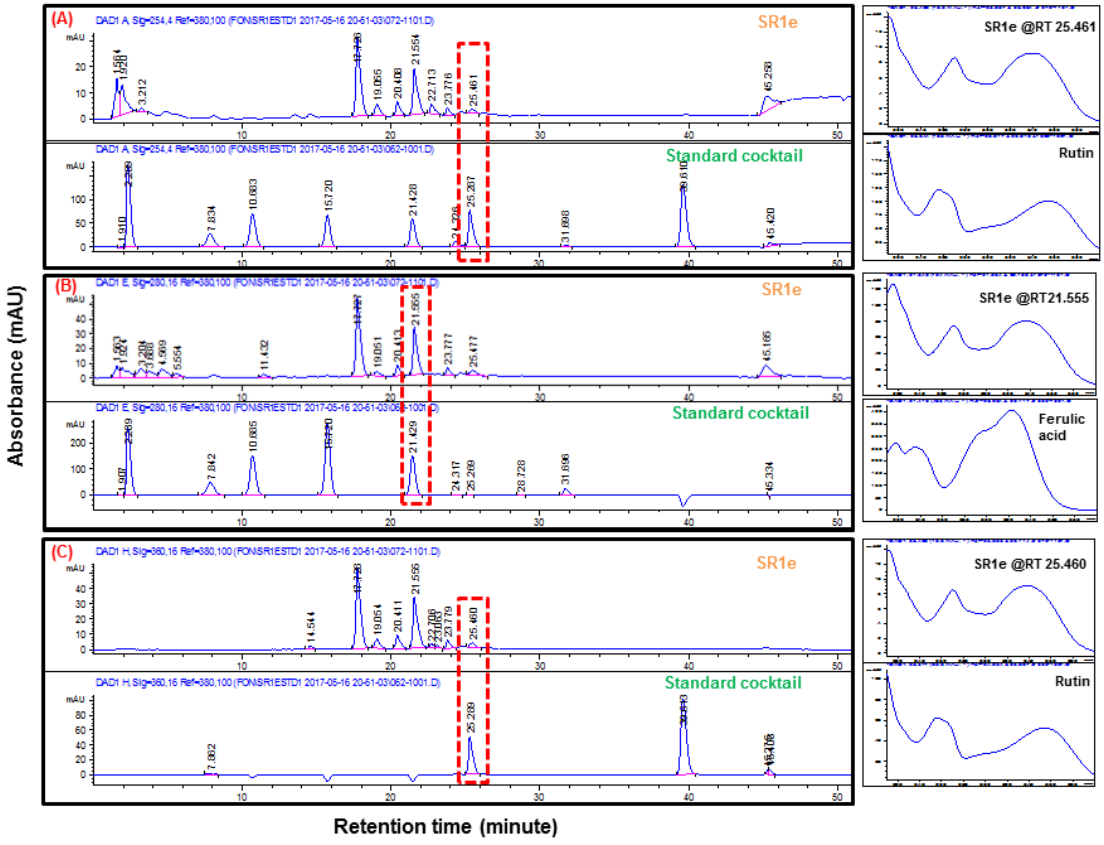

Figure 4-14 HPLC chromatograms of SR1e at 254 nm (A), 280 nm (B), and 360 nm (C). SR1e peaks with similar retention time with those of the standard cocktail are marked by red boxes and their absorption spectra are shown on the right for comparison purposes.

HPLC analysis shows that these common antioxidants were not present in SR1e, while caffeic acid was present in TLe. This finding is in agreement with a previous report that found caffeic acid in the leaf of *T.laurifolia* (Kanchanapoom et al., 2002). Caffeic acid (Figure 4-17) has been studied for its anti-tumour and anti-inflammatory activities. Chung et al. (2004) reported that it inhibited HepG2 growth both in *in vitro* and *in vivo* (Chung et al., 2004). Regarding the antioxidant activity, the compound is a scavenger for singlet oxygen $O_2^{-\bullet}$ and $OH^{\bullet}$ (Asano and

Iwahashi, 2014). Therefore, caffeic acid might contribute to the cytotoxic (at high concentration of the extract) and $ABTS^{\bullet+}$ radical scavenging effects of TLe.

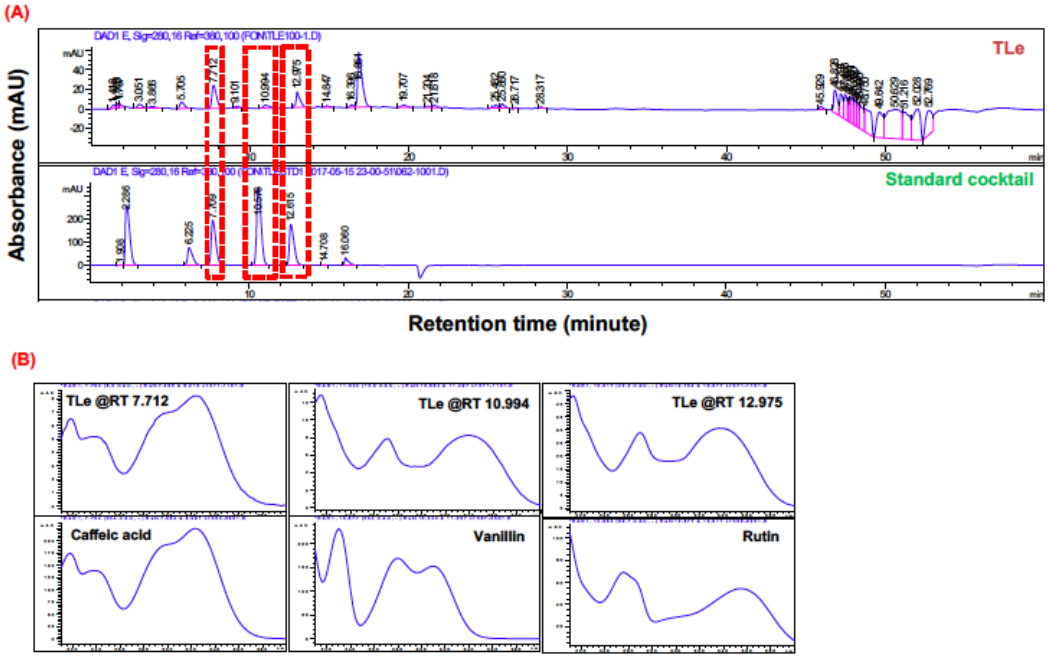

Figure 4-15 (A) HPLC chromatogram at 280 nm of TLe. The peaks with similar retention time with those of the standard cocktail are marked by red boxes and their absorption spectra are shown in (B) for comparison purposes.

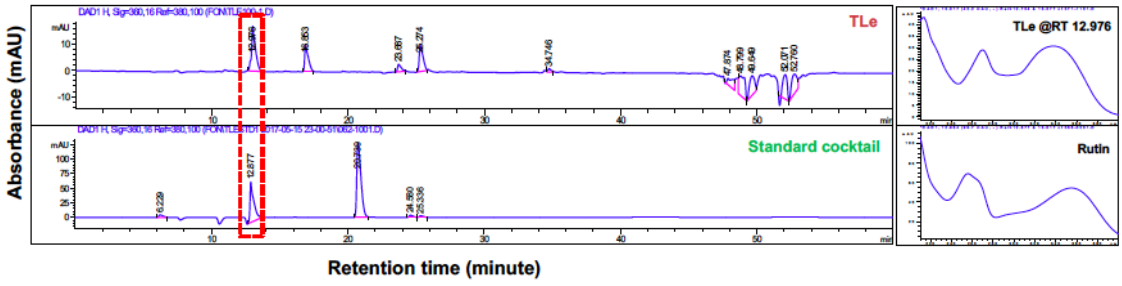

Figure 4-16 HPLC chromatogram at 360 nm of TLe. The peak with similar retention time with that of the standard cocktail is marked by a red box and their absorption spectra are shown on the right for comparison purposes.

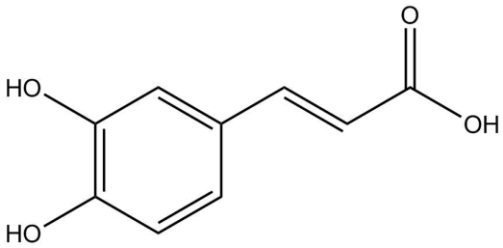

Figure 4-17 Caffeic acid

4.3.4. High-resolution radical scavenging profile

To identify active peaks that contribute to protective effect of the extracts, micro-fractionation and high-resolution radical scavenging assay were performed in the dereplication process. High-resolution radical scavenging profiles of TLe and SR1e extracts are presented in Figure 4-18 and Figure 4-19, respectively. $ABTS^{\bullet+}$ reduction profile of TLe was more discrete than that of SR1e. Four peaks from TLe with dominant scavenging property were selected for further analysis (Figure 4-18; Peak 2, 3, 4, and 8). SR1e contained three peaks which gave discrete responses (Figure 4-19; Peak 1, 5, and 6). Increasing the resolution of micro-fractions to more than 8 points per minute might improve the radical scavenging profile of SR1e.

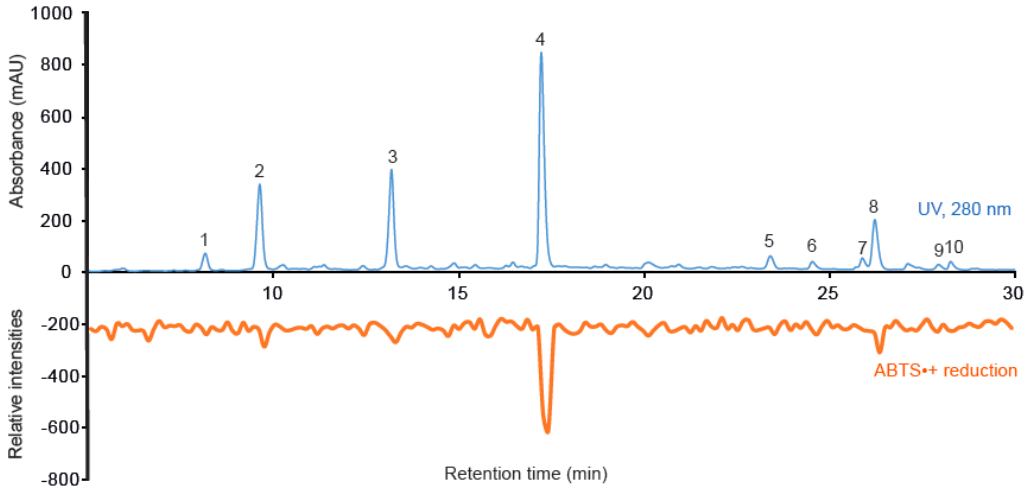

Figure 4-18 High-resolution radical scavenging profile of TLe extract with HPLC chromatogram at 280 nm. Peak 2 (RT 9.58), peak 3 (RT 13.22), peak 4 (RT 17.28), and peak 8 (RT 26.29) show the most discrete radical scavenging activity.

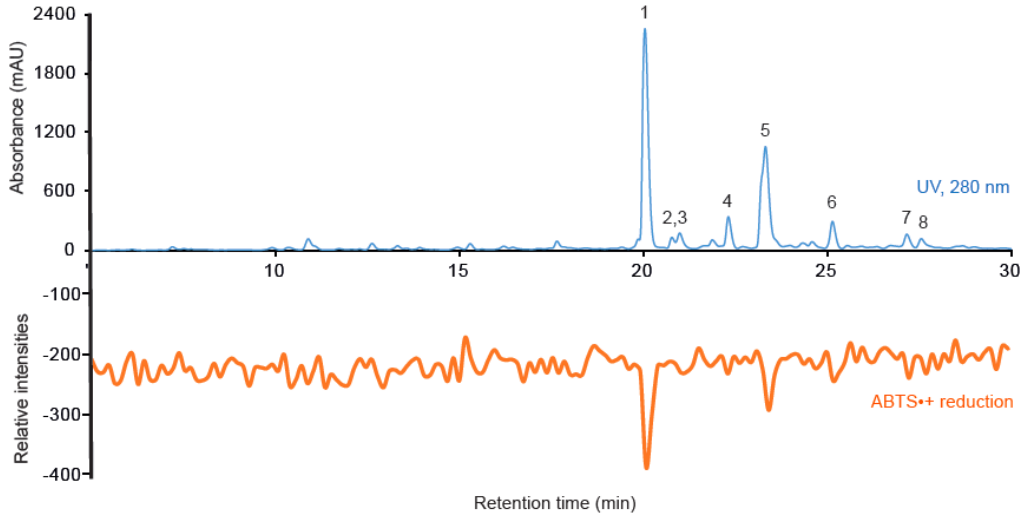

Figure 4-19 High-resolution radical scavenging profile of SR1e extract with HPLC chromatogram at 280 nm. Peak 1 (RT 20.02), peak 5 (RT 23.29), and peak 6 (RT 25.17) show the most discrete radical scavenging activity.

4.3.5. HPLC-HRMS-SPE-*tt*NMR analysis

4.3.5.1. TLe analysis

Peak 2, 3, 4, 8 of TLe crude extract were analysed by HRMS and NMR. Peak 3 (*m/z* 433.1150 $[M+H]^+$) could be identified as isovitexin (6-C-glucopyranosyl apigenin) (Figure 4-20) by comparison with the literature (Hao et al., 2016a). Peak 4 (*m/z* 361.0903 $[M+H]^+$) was assigned as rosmarinic acid (Figure 4-20) by comparison with the literature (Lu and Foo, 1999). Previous studies also reported the identification of these two compounds in the leaves and aerial parts of TL (Suwanchaikasem et al., 2014, Junsi and Siripongvutikorn, 2016). Rosmarinic acid is a common ester derivative of caffeic acid (Amoah et al., 2016). This confirms the presence of caffeic acid in TLe.

Peak 2 and 8 cannot be elucidated at this stage because the fractions were possibly mixtures and the concentrations were too low for structure elucidation even though the trapping was increased to 80 consecutive injections for each fraction. In future work, these two peaks should be purified prior to NMR analysis. HRMS and ^{1}H NMR analysis of peak 3 and 4 are reported below. Ar refers to aromatic and Glu refers to glucosyl ring.

Peak 3

HRMS (m/z): 433.1150 $[M+H]^+$; ^{1}H-NMR (600 MHz, MeOD): δ 7.86 (d, 2H, *J* = 9 Hz, CHAr H2', 6'), 6.94 (d, 2H, *J* = 8.4 Hz, CHAr H3', 5'), 6.63 (s, 1H, CHFlavone 3), 6.53 (s, 1H, CHFlavone 8), 4.91 (d, 1H, *J* = 9.6 Hz, CHGlu H1"), 4.15 (t, 1H, *J* = 9.3 Hz, CHGlu H2"), 3.87 (dd, 1H, *J* = 12, 2.4 Hz, CHGlu H6a"), 3.74 (dd, 1H, *J* = 12, 5.4 Hz, CHGlu H6b"), 3.49 – 3.46 (m, 2H, CHGlu H3", 4"). The spectral data presented are in agreement with published report on isovitexin (Hao et al., 2016a). However, one proton on glucosyl ring (δ 3.33, H5") reported in Hao et al. (2016) was absent in our data due to MeOD solvent peak (δ 3.31).

Peak 4

HRMS (*m/z*): 361.0903 $[M+H]^+$; ^{1}H-NMR (600 MHz, MeOD): δ 7.54 (d, 1H, *J* = 15.6 Hz, CH 7), 7.04 (d, 1H, *J* = 1.8 Hz, CHAr 2), 6.95 (dd, 1H, *J* = 8.4, 2.4 Hz, CHAr 6), 6.79 - 6.75 (m, 2H, CHAr 2', 5'), 6.69 (d, 1H, *J* = 8.4 Hz, CHAr 5), 6.61 (dd, 1H, CHAr 6', *J* = 7.8, 2.0 Hz), 6.26 (d, 1H, *J* = 16.2 Hz, CH 8), 5.17 (dd, 1H, *J* = 9.0, 4.2 Hz, CH 8'), 3.09 (dd, 1H, *J* = 14.4, 4.2 Hz, CH 7a'), 2.99 (dd, 1H, *J* = 14.4, 9.0 Hz, CH 7b'). The spectral data presented are in agreement with published report on rosmarinic acid (Lu and Foo, 1999).

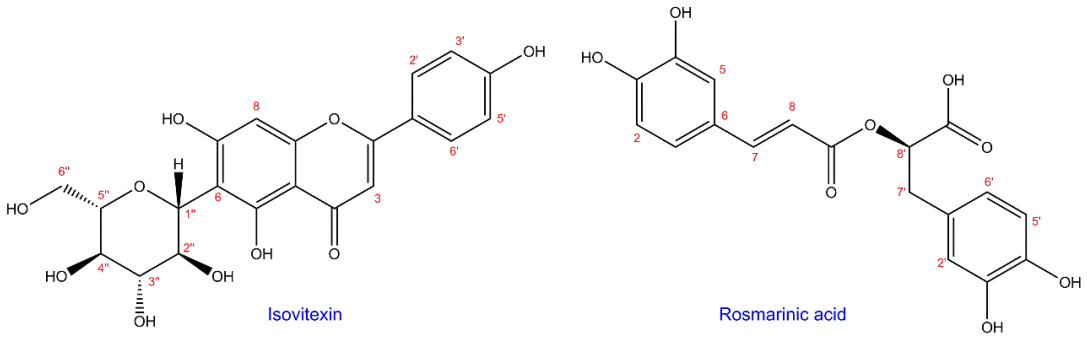

Figure 4-20 Structures of isovitexin and rosmarinic acid

Isovitexin, an isomer of vitexin, has been studied for its protective and antioxidant effects. Isovitexin exhibited a significant protective effect against amyloid β-induced toxicity in PC12 rat adrenal pheochromocytoma cells by increasing the cell viability for 21% compared to the cells exposed to amyloid β-peptide alone. As amyloid β toxicity involves ROS generation, isovitexin might possess free radical scavenging properties (Guimarães et al., 2015). However, the compound showed weak DPPH scavenging activity with IC_{50} value of 370 µg/ml, weak total antioxidant capacity assessed by FRAP assay with TEAC value of 129.12 mg/g (Zhang et al., 2011), and weak ABTS radical scavenging property with IC_{50} value of 1224 µM (Shibano et al., 2008). Even though in another study its IC_{50} from DPPH assay was 18.9 µg/ml, but it also showed weak superoxide radical scavenging activity with IC_{50} value > 385 µg/ml (Shibano et al., 2008). Our result is in agreement with these published literature as can be seen from the biochromatogram of TLe that peak 3 did not give a narrow and sharp ABTS inhibition peak (Figure 4-18).

Rosmarinic acid has been reported for its remarkable pharmacological activities, especially the protective effects in various tissues and cell lines. For instance, it decreased acute liver damage and fibrogenesis induced by CCl_4 in mice. One of the protective mechanism was the ability in the upregulation of Nrf2 and HMOX-1 protein expression (Domitrović et al., 2013). Pre-treatment with rosmarinic acid protected from *t*-BHP damages by recovery of GSH level and reduction of malondialdehyde (MDA) formation *in vitro* and *in vivo*, as well as increased cell viability of HepG2 cells (Yang et al., 2013). Moreover, rosmarinic acid also illustrated protection against oxidative stress in various models, such as induced diabetic rats (Mushtaq et al., 2015, Govindaraj and Sorimuthu Pillai, 2015), neuronal cells (Ghaffari et al., 2014), HaCaT keratinocytes (Fernando et al., 2016), and etc. As this compound presented in TLe in high proportion, it is possible that rosmarinic acid largely contributed to the protective effect of TLe.

4.3.5.2. SR1e analysis

Peak 1, 5, 6 of SR1e crude extract were analysed by HRMS and NMR. Peak 1 (*m/z* 595.1682 $[M+H]^+$) was elucidated as 3,6-di-C-glucosylapigenin (Figure 4-21) by comparison with the literature (Matsubara et al., 1985). Peak 5 (*m/z* 565.1563 $[M+H]^+$) was identified as isoschaftoside (Figure 4-21) by comparison with the literature (Oliveira et al., 2013). Peak 6 (*m/z* 565.1553 $[M+H]^+$) was assigned as schaftoside (Figure 4-21) by comparison with the literature (Norbaek et al., 2000). This is the first time that these compounds were found in *S.rugata*. HRMS and ^{1}H NMR analysis of peak 1, 5, and 6 are reported below. Ar refers to aromatic, Glu refers to glucosyl ring, and Ara refers to arabinosyl ring.

Peak 1

HRMS (*m/z*): 595.1682 $[M+H]^+$; ^{1}H-NMR (600 MHz, MeOD): δ 8.11 (d, 1H, CHAr 6', *J* = 8.4 Hz), 8.02 (d, 1H, CHAr 2', *J* = 7.2 Hz), 7.06 (d, 2H, *J* = 8.4 Hz, CHAr 3', 5'), 6.76 (s, 1H, CHFlavone 8), 5.15 (d, 1H, CHGlu 1'''', *J* = 9.6 Hz), 5.07 (d, 1H, CHGlu 1'', *J* = 9.9 Hz), 4.22 (t, 1H, CHGlu 2''', *J* = 9 Hz), 4.05 (d, 1H, CHGlu 6b''', *J* = 10.9 Hz), 3.99 – 3.95 (m, 2H, CHGlu 6a''', 6b''), 3.93 (dd, 1H, CHGlu 6a", *J* = 13.8, 7.5 Hz), 3.77 (t, 1H, *J* = 12, 5.4 Hz, CHGlu H2"), 3.70 – 3.65 (m, 3H, CHGlu 3''', 5''', 3''), 3.63 – 3.56 (m, 2H, CHGlu 4''', 5''), 3.45 (t, 1H, CHGlu 4'', *J* = 1.8 Hz). The spectral data showed are in agreement with published report on 3,6-di-C-glucosyl apigenin (Matsubara et al., 1985).

Peak 5

HRMS (*m/z*): 565.1563 $[M+H]^+$; ^{1}H-NMR (600 MHz, MeOD): δ 7.99 (d, 2H, CHAr 2', 6', *J* = 8.4 Hz), 6.94 (dd, 2H, CHAr 3', 5', *J* = 9, 4.2 Hz), 6.65 (s, 1H, CHFlavone 3), 5.03 (d, 1H, CHAra 1''', *J* = 10.2 Hz), 4.88 (d, 1H, CHGlu 1'', *J* = 10.2 Hz), 4.08 (d, 1H, CHAra 5b''', *J* = 10.2 Hz), 4.04 – 3.99 (m, 2H, CHGlu 6b'', CHAra 2'''), 3.95 (d, 1H, CHGlu 6a'', *J* = 10.2 Hz), 3.87 (m, 2H, CHGlu 2'', CHAra 4'''), 3.77 (m, 2H, CHGlu 3'', 5''), 3.65 (m, 1H, CHAra 3'''), 3.54 (t, 2H, CHGlu 4'', CHAra 5a''', *J* = 8.7 Hz). The spectral data showed are in agreement with published report on isoschaftoside or apigenin 6-C-arabinoside-8-C-glucoside (Oliveira et al., 2013).

Peak 6

HRMS (*m/z*): 565.1553 $[M+H]^+$; ^{1}H-NMR (600 MHz, MeOD): δ 7.91 (d, 2H, CHAr 2', 6', *J* = 9 Hz), 6.96 (d, 2H, CHAr 3', 5', *J* = 9 Hz), 6.65 (s, 1H, CHFlavone 3), 5.00 (d, 1H, CHAra 1'', *J* = 10.2 Hz), 4.96 (d, 1H, CHGlu 1''', *J* = 9.6 Hz), 4.09 (dd, 1H, CHAra 5b'', *J* = 10.8, 5.4 Hz), 4.05 (t, 1H, *J* = 9.3 Hz, CHAra 2''), 3.88 – 3.86 (m, 2H, CHGlu 2''', CHAra 4''), 3.82 – 3.78 (m, 2H, CHAra 5a'', CHGlu 6b'''), 3.73 – 3.69 (m, 1H, CHAra 3''), 3.53 (d, 1H, CHGlu 6a''', *J* = 9 Hz), 3.48 (t, 2H, CHGlu 3''', 5''', *J* = 9 Hz), 3.23 (t, 1H, CHGlu 4'''*J* = 10.5 Hz). The spectral data presented are in agreement with published report on schaftoside or apigenin 6-C-glucoside-8-C-arabinoside (Norbaek et al., 2000).

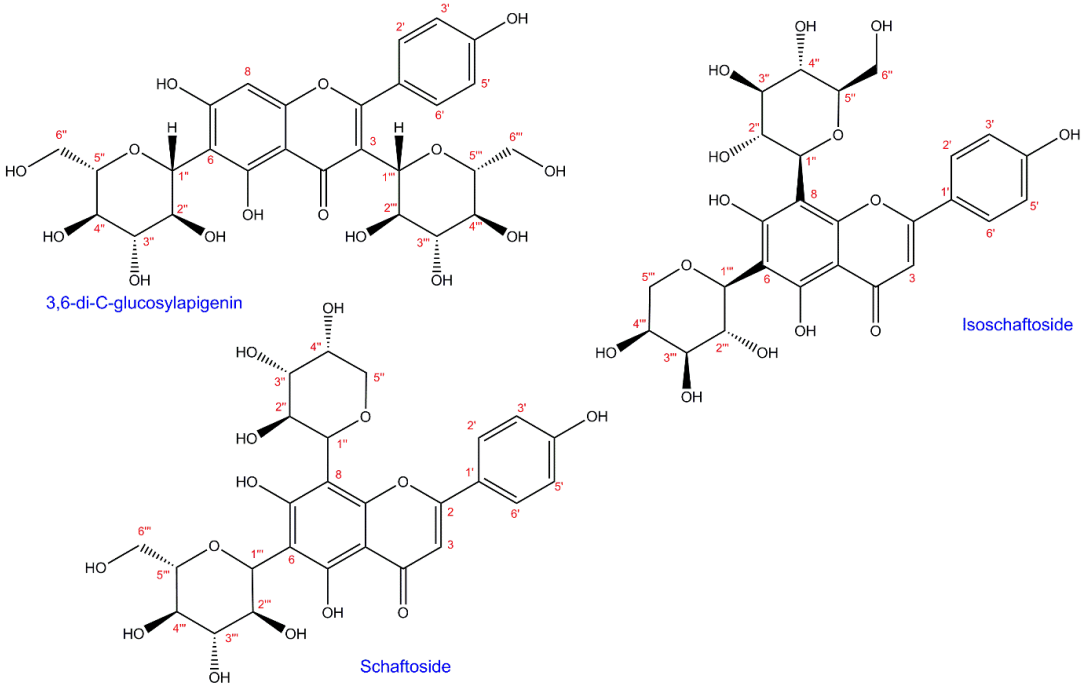

Figure 4-21 Structures of 3,6-di-C-glucosylapigenin, isoschaftoside, and schaftoside

Previous study regarding bioactivity of 3,6-di-C-glucosylapigenin, isoschaftoside, and schaftoside was limited. Peak 1 or 3,6-di-C-glucosylapigenin was the major constituent of SR1e and presented a distinctive free radical scavenging activity in our study (Figure 4-19). Therefore, it possibly plays an important role in ABTS scavenging property of SR1e, which might be a contribution from its apigenin skeleton. Previous study suggested that glucose substitution at 6-C can increase the antioxidant activity, while glucose substitution at 8-C can decrease the activity (Zielińska and Zieliński, 2011). Our result is in agreement with this statement as peak 1 (3,6-di-C-glucosylapigenin) exhibited stronger ABTS scavenging activity than peak 5 (apigenin 6-C-arabinoside-8-C-glucoside). For peak 6 (apigenin 6-C-glucoside-8-C-arabinoside), even though it contains 6-C substitution, its proportion in SR1e was much lower than that of peak 5. This explains why peak 6 had lower ABTS scavenging activity than peak 5. However, there was no available report regarding the protective or antioxidant effect of these compounds to aid the confirmation. Only the group who discovered 3,6-di-C-glucosylapigenin from the peels of *Citrus unshiu* (presumably a synonym of *Citrus reticulata* Blanco) reported its hypotensive effect *in vivo* (Matsubara et al., 1985). To confirm whether these compounds have a contribution in SR1e effect or not, they should be isolated and further investigated.

4.4. Conclusion

Antioxidant genes play a crucial role in the protection against oxidative stress, which is one of the main targets in cancer chemoprevention. In this chapter, we reported the effect of SR1e and TLe on NQO1 and HMOX-1 expression, as well as the expression of their regulator; Nrf2. The study revealed that the protective activity of TLe was due to the upregulation of NQO1 expression and radical scavenging property, while that of SR1e might be the result from the induction of Nrf2 and HMOX-1 expression and radical scavenging activity. This is the first time that the effect of TLe and SR1e on these antioxidant genes was reported. The effect on CYP1A1 expression suggested their low risk of toxicity from DNA damage. Future work using wider range of concentrations of the extracts and incubation times is needed to confirm these effects. Dereplication using HPLC-ABTS assay-HRMS-NMR technique identified that the major constituent of TLe is rosmarinic acid and that it contributed to the protective activities of the extract. Three known apigenin glucosides were present in SR1e. However, their contribution to the protective and antioxidant effect of SR1e deserves further research.

The findings from this chapter help to conclude the whole thesis. The study of the protective mechanisms and dereplication lead to the identification of active constituents, which explained the effects from the screening steps and supported traditional uses of the plants. Even though some results are remained unclear and needed further study, we have provided several important and new information for the first time.

General conclusion

Cancer chemoprevention is an approach to tackle cancer, a global health problem. This approach aims to prevent, delay, or reverse carcinogenesis, rather than killing cancer cells. Since cancer is heterogeneous, our basic assumption is that it is important to prevent cancer development via several mechanisms at the same time, as well as to maintain cell homeostasis. Thai Traditional Medicine (TTM) is a holistic medicine which emphasises maintaining the balance of the body. It has been used among cancer patients in Thailand and has shown positive results, such as prolonged survival and improved quality of life. Therefore, we argue that TTM is a potential source for cancer preventive agents and – more broadly – that it could play a role in prevention. Using an ethnopharmacology approach this thesis aims to discover new extracts or compounds which could be useful in the prevention of cancer.

From biomedical and TTM perspective, cancer has been on different 'pages'. Currently available scientific evidence illustrates possible pharmacological actions that are involved in the benefits of medicinal plants used in cancer patients, such as anti-proliferation, anti-metastasis, anti-angiogenesis, apoptosis induction, or immunomodulation. However, these could not describe 'TTM actions'. Our fieldwork revealed the characteristics of cancer (or *mareng*) within the context of the theory and practice of TTM. Differently from the 'hallmarks of cancer' given by Hanahan and Weinberg (2011), *mareng* is associated with an accumulation of waste (*khong sia*), chronic inflammation (*kan aksep*), chronic illnesses (*krasai*), bad condition of body fluid (mainly '*luead*' and '*namlueang*'), and imbalance of the four elements (*dhātu si*). Treatment and prevention strategies are based on these characteristics. Further analysis of preventive methods and herbal medicine considered to be useful in cancer prevention led to understanding strategies for *mareng* prevention, which are removal of waste, liver protection, prevention from *krasai*, maintaining the balance of *dhātu si*, and nourishment of *leaud* and *namlueang*. In an attempt to link TTM actions to pharmacology, we propose that the first three topics of *mareng* prevention strategy are related to the antioxidant system.

After the initial screening of fifty-two extracts (from twenty-six herbal drugs frequently mentioned), fifteen (from twelve drugs) exhibited significant protective effect against oxidative stress-induced cell death in a liver cancer cell line. This result suggested that *khong sia* and *krasai* were possibly associated with oxidative stress as almost half of selected plant materials showed positive effect in the assay investigated. Next, these fifteen extracts were studied for the ability to induce NQO1 enzyme and glutathione as they are important targets in chemoprevention. Ethanol extracts from the leaf of *Thunbergia laurifolia* (TLe) and the leaf of *Senegalia rugata* (SR1e) exhibited the most potent activities and were then studied for their protective mechanisms and phytochemical profiles.

Upregulation of antioxidant genes and radical scavenging were among protective mechanisms of TLe and SR1e. While TLe induced NQO1 expression, SR1e upregulated the expression of Nrf2, the master regulator of antioxidant genes, including NQO1. However, the results should be confirmed by performing time-dependent experiment. Both extracts did not induce CYP1A1 expression and did not reduce cell viability of primary rat hepatocytes. These results could provide preliminary safety profiles of the extracts. Later phytochemical analysis revealed possible active constituents of TLe, with caffeic acid, isovitexin, and rosmarinic acid being identified tentatively, while SR1e contained several glycosyl apigenins. In conclusion, this is the first time that the above chemopreventive properties of TLe and SR1e have been revealed.

This thesis presents, for the first time, TTM characteristics of cancer and TTM cancer prevention strategy. We also present the analysis of TTM knowledge and the link to pharmacological models using a scientific strategy to understand the effects of TTM plants using biomedical approaches. In a next step it will be essential to use this approach again with a higher number of TTM practitioners to further develop the results presented herein. Moreover, this thesis also contains other novel information, including chemical constituents of the leaf of *S.rugata*, promising cytotoxicity in HepG2 of water and ethanol extracts of the pod of *S.rugata*, and NQO1 induction activity of ethanol extracts from *Citrus hystrix, Coccinia grandis,* and *Piper sarmentosum*. These *in vitro* findings provide evidence that supports the promising use of these medicinal plants in cancer prevention, but in the future physiologically more relevant models will be important. As the medical uses of the plants investigated are different from the experiment, further studies to collect more information from the actual uses by TTM practitioners and to investigate the effects in *in vivo* should be performed.

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Appendix

Table A-1 Plant-derived materials used in the treatment or prevention of cancer by TTM practitioners

No	Local name	Other name	Family	Scientific name
1	Ang-kab-lueang	Philippine violet	Acanthaceae	*Barleria cristata* L.
2	Banmairuroi Kao	Everlasting, Globe Amaranth	Amaranthaceae	*Gomphrena globosa* L.
3	Boraphet	Putarwali	Menispermaceae	*Tinospora crispa*(L.) Hook. f. & Thomson
4	Boraphet Phungchang	-	Menispermaceae	*Stephania suberosa* Forman
5	Buabok	Asiatic pennyworth, Gotu kola	Apiaceae	*Centella asiatica* (L.) Urb.
6	Bualuang	Lotus	Nelumbonaceae	*Nelumbo nucifera* Gaertn.
7	Buk	Stanlet's water tub	Araceae	*Amorphophallus paeoniifolius* (Dennst.) Nicolson
8	Bunnak	Iron wood	Calophyllaceae	*Mesua ferrea* L.
9	Cha-em-thet	Licorice	Fabaceae	*Glycyrrhiza glabra* L.
10	Chan	Nutmeg tree	Myristicaceae	*Myristica fragrans* Houtt.
11	Chang-nga-diaw	Lavang Lata	Rutaceae	*Luvunga scandens* (Roxb.) Buch.-Ham. ex Wight & Arn.
12	Chanthana	-	Rubiaceae	*Tarenna hoaensis* Pit.
13	Cha-om	Pennata wattle	Fabaceae	*Senegalia pennata* (L.) Maslin
14	Chaphlu	Wild betel	Piperaceae	*Piper sarmentosum* Roxb.
15	Cheng-chu-chai	White mugwort Guizhou Group	Asteraceae	*Artemisia lactiflora* Wall. ex DC.
16	Chetphangkhi	-	Euphorbiaceae	*Cladogynos orientalis* Zipp. ex Span.
17	Chettamunplo engdaeng	Rose-colored leadwort	Plumbaginaceae	*Plumbaco indica* L.
18	Chingcha Chali	Heart-leaved moonseed	Menispermaceae	*Tinospora baenzigeri* Forman
19	Chingchi	-	Capparaceae	*Capparis micracantha* DC.
20	Chong-ra-aa	-	Acanthacaea	*Barleria lupulina* Lindl.
21	Chum-het-thet	Ringworm bush, candle bush	Fabaceae	*Senna alata* (L.) Roxb.
22	Di-pli	Long pepper	Piperaceae	*Piper retrofractum* Vahl
23	Do-mai-ru-lom	-	Asteraceae	*Elephantopus scaber* L.
24	Dongdueng	Climbing lily	Colchicaceae	*Gloriosa superba* L.
25	Duea-din	-	Apocynaceae	*Amphineurion marginatum* (Roxb.) D.J.Middleton
26	Dueai-hin	Job's tears	Poaceae	*Coix lachryma-jobi* L.
27	Fa Thalaai	Andrographis herb	Acanthaceae	*Andrographis paniculata* (Burm. f.) Wall. ex Nees
28	Fang	Sappan tree	Fabaceae	*Caesalpinia sappan* L.
29	Haem	False calumba, Tree turmeric	Menispermaceae	*Coscinium fenestratum* (Goetgh.) Colebr.
30	Haewmu	Nutgrass	Cyperaceae	*Cyperus rotundus* L.
31	Hangnokyung	-	Fabaceae	*Caesalpinia pulcherrima* (L.) Sw.
32	Hanuman prasankai	-	Araliaceae	*Schefflera leucantha* R.Vig.
33	Hinghai	-	Convulvulaceae	*Blinkworthia lycioides* Choisy
34	Homchang	-	Acanthaceae	*Phlogacanthus curviflorus* (Wall.) Nees
35	Homdaeng	Shallot	Alliaceae	*Allium ascalonicum* L.

No	Local name	Other name	Family	Scientific name
36	Hora Khaokrabuea	-	Dennstaedtiaceae	*Microlepia platyphylla* (Don) J.Sm.
37	Horathaosunak	-	Balanophoraceae	*Balanophora abbreviata* Blume
38	Huaprohom	Aromatic ginger, Sand ginger	Zingiberaceae	*Kaempferia galanga* L.
39	Huaroiru	Ant plant	Rubiaceae	*Hydnophytum formicarum* Jack
40	Huawao	Basket fern, Oak-leaf fern, Squirrel head	Polypodiaceae	*Drynaria quercifolia* (L.) J. Sm.
41	Huayang, Thaowanyang	Kumarika	Smilacaceae	*Smilax ovalifolia* Roxb. ex D.Don
42	Hu-kwang	-	Combretaceae	*Terminalia catappa* L.
43	Kae-lae	Cockspur thorn	Moraceae	*Maclura cochinchinensis* (Lour.) Corner
44	Ka-kruea	Gac	Cucurbitaceae	*Momordica cochinchinensis* (Lour.) Spreng.
45	Kamchad	Indian prickly ash	Rutaceae	*Zanthoxylum rhetsa* DC.
46	Ka-meng-tua-mia	-	Asteraceae	*Eclipta prostrata* (L.) L.
47	Kamphaengchetchan	-	Celastraceae	*Salacia chinensis* L.
48	Kancha	Marihuana	Cannabidaceae	*Cannabis sativa* L.
49	Kanchathet	Motherwort	Lamiaceae	*Leonurus sibiricus* Linn.
50	Kang-hua-mu	-	Annonaceae	*Miliusa velutina* (A.DC.) Hook.f. & Thomson
51	Kangpladaeng	-	Phyllanthaceae	*Phyllanthus pulcher* Wall. ex Müll. Arg.
52	Kangplakhao	-	Phyllanthaceae	*Flueggea virosa* (Roxb. ex Willd.) Royle.
53	Kanplu	Clove	Myrtaceae	*Syzygium aromaticum* (L.) Merr. & L.M.Perry
54	Kaoki	Wolfberry	Solanaceae	*Lycium chinens*e Mill.
55	Kapiadchang	-	Stemonaceae	*Stemona collinsae* Craib.
56	Kaprao	Holy basil	Lamiaceae	*Ocimum tenuiflorum* L.
57	Kasalak	-	Solanaceae	*Datura metel* L. var. *fastuosa* Safford
58	Kasalong	Indian cork tree	Bignoniaceae	*Millingtonia hortensis* L.f.
59	Ka-tang-bai	Bandicoot Berry	Vitaceae	*Leea indica* (Burm. f.) Merr.
60	Kha	Galangal, Thai ginger	Zingiberaceae	*Alpinia galanga* (L.) Willd.
61	Khadkhao	-	Rubiaceae	*Oxyceros horridus* Lour.
62	Khamfoi	Safflower	Asteraceae	*Carthamus tinctorius* L.
63	Khamin Khruea, Nae Khruea	Yellow-fruit moonseed	Menispermaceae	*Arcangelisia flava* (L.) Merr.
64	Kha-min-chan	Turmeric	Zingiberaceae	*Curcuma longa* L.
65	Kha-min-oi	Wild turmeric, Aromatic turmeric	Zingiberaceae	*Curcuma aromatica* Salisb.
66	Khangpoi	-	Euphorbiaceae	*Alchornea rugosa* (Lour.) Müll. Arg.
67	Khanthongphayabat	-	Euphorbiaceae	*Suregada multiflora* (A.Juss.) Baill.
68	Kha-nun	Jackfruit	Moraceae	*Artocarpus heterophyllus* Lam.
69	Kha-nun-sam-pa-lo	Breadfruit	Moraceae	*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg
70	Khao Fang, Khao Fanghangchang	Sorghum, Broomcorn, Milo	Poaceae	*Sorghum bicolor* (L.) Moench
71	Khao Pod	Corn	Poaceae	*Zea mays* L.
72	Khaochao	Rice	Poaceae	*Oryza sativa* L.

No	Local name	Other name	Family	Scientific name
73	Khaoyennuea	-	Smilacaceae	*Smilax* spp.
74	Khaoyentai	-	Smilacaceae	*Smilax* spp.
75	Kha-pia	-	Lamiaceae	*Hymenopyramis parvifolia* Moldenke
76	Kha-ton	-	Lauraceae	*Cinnamomum ilicioides* A.Chev.
77	Khi-ka	-	Cucurbitaceae	*Gymnopetalum scabrum* (Lour.) W.J.de Wilde & Duyfjes
78	Khi-lek	Cassod tree, Thai copper pod	Fabaceae	*Senna siamea* (Lam.) Irwin et Barneby
79	Khing	Ginger	Zingiberaceae	*Zingiber officinale* Roscoe
80	Khinghang	-	Zingiberaceae	*Zingiber kerrii* Craib
81	Khlu	Indian marsh fleabane	Asteraceae	*Pluchea indica* (L.) Less.
82	Khoi	Siamese rough bush, Toothbrush tree	Moraceae	*Streblus asper* Lour.
83	Khok-kra-om	Balloon vine	Sapindaceae	*Cardiospermum halicacabum* L.
84	Khok-kra-sun	Bullhead, Devil's thorn	Zygophyllaceae	*Tribulus terrestris* L.
85	Kho-klan	-	Euphorbiaceae	*Mallotus repandus* (Willd.) Müll. Arg.
86	Khontha	-	Rutaceae	*Harrisonia perforata* (Blanco) Merr.
87	Khui	-	Apocynaceae	*Willughbeia edulis* Roxb.
88	Kloi	-	Dioscoreaceae	*Dioscorea hispida* Dennst.
89	Kloi-chuet	-	Dioscoreaceae	*Dioscorea daemona* Roxb. Var. mollicima Prain.
90	Klongkleng	Malabar Gooseberry, Malabar Melastome, Straits Rhododendron	Melastomataceae	*Melastoma malabathricum* L.
91	Kot Chadamangsi	Spikenard, Nard, Nardin, Muskroot.	Caprifoliaceae	*Nardostachys jatamansi* (D.Don) DC.
92	Kot Namtao	Rhubarb	Polygonaceae	*Rheum Palmatum* L. Or *R. officinale* Bail. Or *R. tanguticum* (Maxim. Ex Regel) Maxim. Ex Balf.
93	Kotchiang	Chinese angelica branching root, guiwei	Apiaceae	*Angelica sinensis* (Oliv.) Diels
94	Kotchulalamph a	Sweet wormwood herb, Qinghao	Asteraceae	*Artemisia annua* L.
95	Kothuabua	Szechwan lovage rhizome	Apiaceae	*Ligusticum striatum* DC.
96	Kotkamao	Atractylodes	Asteraceae	*Atractylodes lancea* (Thunb.) DC.
97	Kotkraduk	Costus root, Mu Xiang	Asteraceae	*Saussurea costus* DC.
98	Kotphungpla, samothai	Terminalia gall, myrobalan gall	Combretaceae	*Terminalia chebula* Retz. var. chebula
99	Kotso	Dahurian angelica root	Apiaceae	*Angelica dahurica* var. *formosana* (Boissieu) Yen
100	Kra-bao	-	Achariaceae	*Hydnocarpus anthelminthicus* Pierre ex Laness.
101	Krachai	Fingerroot	Zingiberaceae	*Boesenbergia rotunda* (L.) Mansf.
102	Krachaidam	Thai Ginseng, Black galingale	Zingiberaceae	*Kaempferia parviflora* Wall. ex Baker
103	Krachiap	Roselle	Malvaceae	*Hibiscus sabdariffa* L.
104	Kradaddaeng	Red giant taro	Araceae	*Alocasia macrorrhizos* (L.) G.Don
105	Kradadkhao	White giant taro	Araceae	*Alocasia macrorrhizos* (L.) G.Don
106	Kradom	-	Cucurbitaceae	*Gymnopetalum chinense* (Lour.) Merr.
107	Kra-don	Slow Match Tree	Lecythidaceae	*Careya arborea* Roxb.

No	Local name	Other name	Family	Scientific name
108	Kradukkaidam	-	Acanthacaea	*Justicia fragilis* Wall.
109	Krathiam	Garlic	Alliaceae	*Allium sativum* Linn.
110	Krathomlueat, Sa-bu-lueat	-	Menispermaceae	*Stephania venosa* (Blume) Spreng.
111	Kratungmaba	-	Apocynaceae	*Dregea volubilis* (L.f.) Benth. ex Hook.f.
112	Krawan	Siam cardamom	Zingiberaceae	*Amomum testaceum* Ridl.
113	Kritsana	Eagle wood, calambour	Thymelaeaceae	*Aquilaria* spp.
114	Krungkhamao	Icevine, Velvet leaf pareira	Menispermaceae	*Cissampelos pareira* L. var. hirsuta (Buch ex DC.) Forman
115	Lamchiak, Toei Ta-le	Fragrant Screw Pine, Umbrella tree	Pandanaceae	*Pandanus odorifer* (Forssk.) Kuntze
116	Lamphong	Devil's trumpet, Angel's trumpet	Solanaceae	*Datura metel* L.
117	Lanthom	Plumeria, Temple tree	Apocynaceae	*Plumeria* spp.
118	Lian	Bastard Cedar, Persian Lilac	Meliaceae	*Melia azedarach* L.
119	Lodthanongdaeng	-	Euphorbiaceae	*Trigonostemon reidioides* (Kurz) Craib
120	Luead Khwai, Kamlangluead ma	-	Myristicaceae	*Knema cinerea* var. *glauca* (Blume) Y.H. Li
121	Luktaibai	-	Phyllanthaceae	*Phyllanthus amarus* Schum. et Thonn.
122	Ma-duea Chumpon	Cluster Fig	Moraceae	*Ficus racemosa* L.
123	Ma-duea-hom	-	Moraceae	*Ficus hirta* Vahl.
124	Ma-duea-plong	Hairy Fig, Devil fig	Moraceae	*Ficus hispida* L.f.
125	Maduk	-	Celastraceae	*Siphonodon celastrineus* Griff.
126	Ma-fai	Burmese grape	Phyllanthaceae	*Baccaurea ramiflora* Lour.
127	Ma-fai-dueanha	-	Apocynaceae	*Asclepias curassavica* L.
128	Mafueang	Carambola, Starfruit	Oxalidaceae	*Averrhoa carambola* L.
129	Magrud	Kaffir lime	Rutaceae	*Citrus hystrix* DC.
130	Mahahing	Asafoetida, Stinking gum, Devil's dung	Apiaceae	*Ferula assa-foetida* L.
131	Mahat	Lakoocha, Monkey Jack	Moraceae	*Artocarpus lakoocha* Roxb.
132	Mahuad	-	Sapindaceae	*Lepisanthes rubiginosa* (Roxb.) Leenh.
133	Maiyarab	Sensitive plant	Fabaceae	*Mimosa pudica* L.
134	Maka	-	Phyllanthaceae	*Bridelia ovata* Decne.
135	Ma-ka-mong, Ma-ka-hua-kham	-	Fabaceae	*Afzelia xylocarpa* (Kurz) Craib
136	Ma-kham	Tamarind	Fabaceae	*Tamarindus indica* L.
137	Makham Pom	Emblic myrobalan	Phyllanthaceae	*Phyllanthus emblica* L.
138	Makhamkai	-	Putranjivaceae	*Putranjiva roxburghii* Wall.
139	Maklam Tanu	Crab's eye vine/ American pea	Fabaceae	*Abrus precatorius* L.
140	Ma-kluea	Ebony tree	Ebenaceae	*Diospyros mollis* Griff.
141	Ma-kra-thuep-rong	-	Moraceae	*Ficus sarmentosa* Buch.-Ham. ex Sm.
142	Maktaek	-	Celastraceae	*Celastrus paniculatus* Willd.
143	Manao	Lime	Rutaceae	*Citrus aurantifolia* (Christm. et Panz.) Swingle

No	Local name	Other name	Family	Scientific name
144	Maprang	marian plum, gandaria, and plum mango	Anacardiaceae	*Bouea macrophylla* Griff.
145	Mapring	Burmese plum, plum-mango	Anacardiaceae	*Bouea oppositifolia* (Roxb.) Adelb.
146	Mara	Bitter cucumber	Cucurbitaceae	*Momordica charantia* L.
147	Ma-ra-khi-nok	Bitter cucumber	Cucurbitaceae	*Momordica charanthia* L. ssp. *charanthia* var. *minima* Williams et Ng
148	Marum	Horseradish tree, Drumstick tree	Moringaceae	*Moringa oleifera* Lam.
149	Matum	Bael, Golden apple	Rutaceae	*Aegle marmelos* (L.) Corrêa
150	Mawaengkhom, Mawaengton	Talong-siam	Solanaceae	*Solanum sanitwongsei* Craib
151	Mawaengkhruea, Mawaengthao	Purple fruited Pea Egg plant	Solanaceae	*Solanum trilobatum* L.
152	Mon	White mulberry	Moraceae	*Morus alba* Linn.
153	Muakdaeng	-	Apocynaceae	*Wrightia coccinea* (Roxb. ex Hornem.) Sims
154	Muakkhao	-	Apocynaceae	*Wrightia pubescens* subsp. pubescens
155	Nad	-	Asteraceae	*Blumea balsamifera* (L.) DC.
156	Nadkham	-	Asteraceae	*Inula cappa* (Buch.-Ham. ex D.Don) DC.
157	Namchai	Teripof plant	Fabaceae	*Caesalpinia digyna* Rottler
158	Namkong	Fever nut	Fabaceae	*Caesalpinia crista* L.
159	Nammanmapr ao	Coconut oil	Arecaceae	*Cocos nucifera* L. var. nucifera
160	Namtao	Bottle gourd	Cucurbitaceae	*Lagenaria siceraria* (Molina) Standl.
161	Nao-nai	-	Aquifoliaceae	*Ilex umbellulata* Loes.
162	Ngueak Pla Mo Dok Khao	Sea holly	Acanthaceae	*Acanthus ebracteatus* Vahl
163	Ngueak Pla Mo Dok Muang	-	Acanthaceae	*Acanthus ilicifolius* L.
164	Noi-na	Sugar apple	Annonaceae	*Annona squamosa* L.
165	Nomsawan Tuamia	-	Lamiaceae	*Clerodendrum schmidtii* Clarke
166	Nontaiyak	-	Stemonaceae	*Stemona tuberosa* Lour.
167	Nun	-	Malvaceae	*Ceiba pentandra* (L.) Gaertn.
168	O	-	Gramineae	*Arundo donax* Linn.
169	Oi-dam	Sugar cane	Poaceae	*Saccharum officinarum* L.
170	Op-choei	-	Lauraceae	*Cinnamomum* spp.
171	Op-choei-thet	Ceylon cinnamon	Lauraceae	*Cinnamomum verum* J. Presl.
172	Pae-tuk	Baizhu, Rhizoma Atractylodis Macrocephalae	Asteraceae	*Atractylodes macrocephala* Koidz.
173	Phai Pa	Giant thorny bamboo	Poaceae	*Bambusa bambos* (L.) Voss
174	Phai Ruak	Monastery bamboo	Poaceae	*Thyrsostachys siamensis* Gamble
175	Phai Sisuk	Spiny Bamboo	Poaceae	*Bambusa blumeana* Schult.f.
176	Phakbungdaeng	Morning Glory	Convulvulaceae	*Ipomoea aquatica* Forsk.
177	Phakchi	-	Apiaceae	*Oenanthe javanica* subsp. *javanica*
178	Phakchiangda	Gymnema sylvestre	Apocynaceae	*Gymnema inodorum* (Lour.) Decne.
179	Phakkhaotong	Lizard tail, Chameleon plant, Fishwort	Saururaceae	*Houttuynia cordata* Thunb.

No	Local name	Other name	Family	Scientific name
180	Phakkrachet	Water mimosa, Sensitive neptunia	Fabaceae	*Neptunia oleracea* Lour.
181	Phak-krad-hua-waen	Para cress	Asteraceae	*Acmella caulirhiza* Delile
182	Phakpaewdaeng	-	Amaranthaceae	*Iresine herbstii* Hook.
183	Phakpeddaeng	Joy weed	Amaranthaceae	*Alternanthera bettzickiana* (Regel) G.Nicholson
184	Phakpomdin, Krodnam	Goatweed	Plantaginaceae	*Scoparia dulcis* L.
185	Phaksianphi	-	Cleomaceae	*Cleome viscosa* L.
186	Phaktaew	-	Hypericaceae	*Cratoxylum formosum* subsp. *pruniflorum* (Kurz) Gogelein
187	Phakwanban	-	Phyllanthaceae	*Sauropus androgynus* (L.) Mer.
188	Phakwanpa	-	Opiliaceae	*Melientha suavis* Pierre
189	Phangphuai	Water Primrose	Onagraceae	*Ludwigia adscendens* (L.) H. Hara
190	Phaya Rakdam	-	Ebenaceae	*Diospyros defectrix* Fletcher
191	Pha-ya-yor	-	Acanthaceae	*Clinacanthus nutans* (Burm. f.) Lindau
192	Philangkasa	-	Primulaceae	*Ardisia polycephala* Wall. ex A.DC.
193	Phitsanad	-	Fabaceae	*Sophora exigua* Craib
194	Phlai, Pu-loei	Cassumunar ginger, Thai ginger	Zingiberaceae	*Zingiber montanum* (J. König) Link ex A.Dietr.
195	Phlu Chin	Betel vine	Piperaceae	*Piper betle* L.
196	Phrik Thai	Pepper	Piperaceae	*Piper nigrum* L.
197	Phumsamngam, Makmok	-	Opiliaceae	*Lepionurus sylvestris* Blume
198	Phungdo	-	Salvadoraceae	*Azima sarmentosa* (Blume) Benth. & Hook.f.
199	Plalaiphueak	Tongkat ali	Simaroubaceae	*Eurycoma longifolia* Jack
200	Po-bit	Indian Screw Tree, Deer's Horn	Malvaceae	*Helicteres isora* L.
201	Pua-ki-nai, Huang qin (Chinese)	Baikal skullcap	Lamiaceae	*Scutellaria baicalensis* Georgi
202	Quinin	Neem	Meliaceae	*Azadirachta indica* A. Juss.
203	Rangchued	Laurel clock vine, Blue trumpet vine	Acanthaceae	*Thunbergia laurifolia* Lindl.
204	Reo noi	-	Zingiberaceae	*Amomum villosum* Lour.
205	Rong	Gamboge	Clusiaceae	*Garcinia lateriflora* Blume
206	Sa-dao	Neem	Meliaceae	*Azadirachta indica* A. Juss. var. siamensis Valeton
207	Sak	Teak	Lamiaceae	*Tectona grandis* L.f.
208	Sak	-	Fabaceae	*Erythrophleum teysmannii* (Kurz) Craib
209	Sakae	-	Combretaceae	*Combretum quadrangulare* Kurz
210	Sakaesaeng	-	Annonaceae	*Cananga latifolia* (Hook. f. & Thomson) Finet & Gagnep.
211	Sakhan	-	Piperaceae	*Piper* aff. *pendulispicum* C.DC.
212	Sa-mae-san	-	Fabaceae	*Senna garrettiana* (Craib) H.S.Irwin & Barneby
213	Sa-mae-ta-le	-	Acanthaceae	*Avicennia marina* (Forsk.) Vierh.
214	Samedkhao	Shore Euginia	Myrtaceae	*Syzygium antisepticum* (Blume) Merr. & L.M.Perry
215	Sam-ma-li-nga	Garden quinine	Lamiaceae	*Cleodendrum inerme* (L.) Gaertn.
216	Sa-mo-di-ngu	-	Combretaceae	*Terminalia citrina* (Gaertn.) Roxb. ex Flem
217	Samophiphek, naeton	Beleric myrobalan	Combretaceae	*Terminalia bellirica* (Gaertn.) Roxb.

No	Local name	Other name	Family	Scientific name
218	Samothet	-	Combretaceae	*Terminalia* spp.
219	Samsib	-	Asparagaceae	*Asparagus racemosus* Willd.
220	Sa-mun-la-waeng, Dun ye gui (Chinese)	-	Lauraceae	*Cinnamomum bejolghota* (Buch.-Ham.) Sweet
221	Sa-niad	-	Acanthaceae	*Justicia adhatoda* L.
222	Sankham	Chinese albizia, silk tree	Fabaceae	*Albizia chinensis* (Osbeck) Merr.
223	San-ngoen, In-si	Copperpod, Golden Flamboyant	Fabaceae	*Peltophorum pterocarpum* (DC.) K.Heyne
224	Singhamora	-	Araceae	*Cyrtosperma johnstonii* (N.E.Br.) N.E.Br.
225	Si-siad-thet	Gambir	Rubiaceae	*Uncaria gambir* (Hunter) Roxb.
226	Som Poi	Soap-pod	Fabaceae	*Acacia concinna* (Willd.) DC.
227	Somchin	Orange	Rutaceae	*Citrus sinensis* (L.) Osbeck
228	Tamlueng	-	Cucurbitaceae	*Coccinia grandis* (L.) Voigt
229	Tamsao	Tembusu	Gentianaceae	*Fagraea fragrans* Roxb.
230	Tandam	-	Ebenaceae	*Diospyros montana* Roxb.
231	Tankamoy	-	Boraginaceae	*Tournefortia ovata* Wall. ex. G. Don.
232	Tanmon	-	Asteraceae	*Tarlmounia elliptica* (DC.) "H.Rob., S.C.Keeley, Skvarla & R.Chan"
233	Tansian	-	Salicaceae	*Flacourtia indica* (Burm. f.) Merr.
234	Tantanod	Fan palm	Arecaceae	*Borassus flabellifer* L.
235	Tao-khiad	-	Araceae	*Homalomena aromatica* (Spreng.) Schott
236	Taorang	Fistail palm	Arecaceae	*Caryota mitis* Lour.
237	Thao-en-on	-	Apocynaceae	*Cryptolepis buchanani* Roem. & Schult.
238	Thaowandaeng	-	Rhamnaceae	*Ventilago denticulata* Willd.
239	Thao-wan-priang	Jewel vine	Fabaceae	*Derris scandens* (Roxb.) Benth.
240	Thao-yai-mom	-	Lamiaceae	*Clerodendrum indicum* (L.) Kuntze
241	Theptharo	Safrol laurel	Lauraceae	*Cinnamomum porrectum* (Roxb.) Kosterm.
242	Thiandaeng	Cress	Brassicaceae	*Lepidium sativum* Linn.
243	Thiandam	Fennel flower, Black caraway	Ranunculaceae	*Nigella sativa* L.
244	Thiankao	Cumin	Apiaceae	*Cuminum cyminum* L.
245	Thiankaopluea k	Dill	Apiaceae	*Anethum graveolens* L.
246	Thing Thon	White siris	Fabaceae	Albizia procera (Roxb.) Benth.
247	Thonglang	-	Fabaceae	*Erythrina subumbrans* (Hassk.) Merr.
248	Thongpanchang	-	Acanthaceae	*Rhinacanthus nasutus* (L.) Kurz
249	Thua-phu	Winged bean	Fabaceae	*Psophocarpus tetragonolobus* (L.) DC.
250	Thua-rae	Pigeonpea	Fabaceae	*Cajanus cajan* (L.) Millsp.
251	Toei	Pandan leaves	Pandanaceae	*Pandanus amaryllifolius* Roxb.
252	Wa	Black plum	Myrtaceae	*Syzygium cumini* (L.) Skeels
253	Waan Nam	Myrtle grass	Acoraceae	*Acorus calamus* L.
254	Wan Chakmodluk	-	Zingiberaceae	*Curcuma comosa* Roxb.
255	Wan Keep-raet	Giant Fern	Marattiaceae	*Angiopteris evecta* (G. Forst.) Hoffm.
256	Wan Kho-thong-kae	-	Zingiberaceae	*Curcuma* sp.

No	Local name	Other name	Family	Scientific name
257	Wan Nakkharat, Wan Hangnak	Ceylon Bowstring Hemp, Devils Tongue	Asparagaceae	*Sanseviera zeylanica* (L.) Willd.
258	Wan Phetchaklab	-	Zingiberaceae	*Boesenbergia thorelii* (Gagnep.) Loes
259	Wan Thonmokkhasak	-	Zingiberaceae	*Kaempferia* sp.
260	Wankainoi	-	Cibotiaceae	*Cibotium barometz* (L.) J. Sm.
261	Wanmahakan	-	Asteraceae	*Gynura hispida* Thwaites
262	Wan-nga-chang (Hoksurakan)	-	Agavaceae	*Sansevieria cyclindrica* Bojer
263	Wanphetchahueng	Giant orchid, Tiger orchid	Orchidaceae	*Grammatophyllum speciosum* Blume
264	Ya Faran	Saffron	Iridaceae	*Crocus sativus* L.
265	Ya Khobchanang	-	Urticaceae	*Gonostegia pentandra* (Roxb.) Miq.
266	Ya Lin-ngu	Flat-top mille graines	Rubiaceae	*Oldenlandia corymbosa* L.
267	Ya Nuadmaew	Cat's whisker	Lamiaceae	*Orthosiphon aristatus* (Blume) Miq.
268	Ya Nuad-rue-si	Black speargrass, Tanglehead	Poaceae	*Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult.
269	Ya Pakkhwai	Egyptian crowfoot grass	Poaceae	*Dactyloctenium aegyptium* (L.) Willd.
270	Ya Pakking	-	Commelinaceae	*Murdannia loriformis* (Hassk.) R. S. Rao et Kammathy
271	Ya Taibai	Chamber bitter	Phyllanthaceae	*Phyllanthus urinaria* L.
272	Ya Thotplong	-	Equisetaceae	*Equisetum ramosissimum* subsp. debile (Roxb. ex Vaucher) Hauke
273	Ya Tudma	-	Rubiaceae	*Paederia pilifera* Hook. f.
274	Yadam	-	Asparagaceae	*Aloe* spp.
275	Ya-kha	Blady grass, Cogon grass	Poaceae	*Imperata cylindrica* (L.) Raeusch.
276	Yamornoi, Yadokkhao	-	Asteraceae	*Vernonia cinerea* (L.) Less.
277	Yanang	-	Menispermaceae	*Tiliacora triandra* (Colebr.) Diels
278	Yo	Indian mulberry	Rubiaceae	*Morinda citrifolia* L.

Table A-2 Animal-derived materials used in the treatment or prevention of cancer by TTM practitioners

No	Local name	English name	Family	Scientific name	Part used
1	Khwai-phueak	Swamp buffalo	Bovidae	*Bubalus bubalis*	bone
2	Khangkhok	Toad (natural death)	Bufonidae	*Bufo* spp.	whole death body
3	Kwang	Deer	Cervidae	*Cervus* spp.	antler
4	Chang	Elephant	Elephantidae	*Elephas maximus indicus*	bone
5	Kaiban	Chicken	Phasianidae	*Gallus gallus* domesticus	feather
6	Tao Lueang	Elongated tortoise	Testudinidae	*Indotestudo elongata* (Blyth,1853)	shell
7	Tao Ta	Malayan snail - eating turtle	Bataguridae	*Malayemys subtrijuga* (Schlegel and Muller, 1844)	shell
8	Ngu Hao	Cobra	Elapinae	*Naja* spp.	bone
9	Linglom	Slow loris	Lorisidae	*Nycticebus* spp.	bone
10	Suea	Tiger	Felidae	*Panther tigris* Linn.	bone

No	Local name	English name	Family	Scientific name	Part used
11	Ngu Lueam	Python	Phytoninae	*Python reticulatus* Schneider	bone
12	Raet	Rhino	Rhinocerotidae	*Rhinoceros sondaicus* Dermarest, 1822	jawbones
13	Pu	Crab	Portunidae	*Scylla serrata* (Forsskål)	shell
14	Hoi-khraeng	Blood cockle	Arcidae	*Tegillarca granosa* (Linnaeus, 1758)	shell
15	Mi	Bear	Ursidae	*Ursus* spp.	bone

Table A-3 Chemicals or minerals used in the treatment or prevention of cancer by TTM practitioners

No	Local name	English name
1	Ka-ra-bun	Camphor
2	Kammathandaeng	Realgar, Arsenic disulfide
3	Kammathanlueang	Sulfur
4	Kluea	Salt, Sodium chloride
5	Chunnasi	Copper (II) sulphate
6	Di-kluea	Magnesium sulphate
7	Parot	mercury
8	Phongfu	baking powder
9	Phimsen	Borneol camphor
10	Sannu	Arsenic

Table A-4 Mushrooms used in the treatment or prevention of cancer by TTM practitioners

No	Local name	English name	Family	Scientific name
1	Hed Danmi	King Alfred's cakes	Xylariaceae	*Daldinia concentrica* (Bolt. ex Fr) Ces et De Not.
2	Hed Linchue	Reishi, Lingzhi Mushroom	Ganodermataceae	*Ganoderma lucidum* (Curtis) P. Karst., 1881
3	Hed Dang	unresolved	unresolved	unresolved
4	Hed Daeng	unresolved	unresolved	unresolved

Table A-5 Unresolved plant-derived materials used in the treatment or prevention of cancer by TTM practitioners

No	Local name
1	Banyaem
2	Batthayak
3	Dangdid
4	Fai
5	Hora Mahura
6	Khanghumu

No	Local name
7	Kra-duk-kai-dam
8	Kutti
9	Maklek
10	Maliwan Pa
11	Muakkhao
12	Nommaeo
13	Phaengphuai
14	Phaya Muelek
15	Phow-kong-aen
16	Phrik Pa, Phrik Naiphran
17	Phuttharaksa
18	Rak-kak-aen
19	Reo Pa
20	Rok
21	Sabadaeng
22	Sabanu
23	Samkhwai
24	Sanhuak
25	Siao-sua-hong
26	Son
27	Sonthet
28	Suraphitkham
29	Ta-phiad
30	Thao-khui-daeng
31	Thao-wa-ti
32	Tra
33	Tungkwang
34	Wan Hokmokkhasak
35	Wan Saklek
36	Ya Khaemhom
37	Ya Khaosan (Sanrangdid)
38	Ya Lakna
39	Ya Pluaknam

Figure A-1 Images of plant materials used for pharmacological activity screening

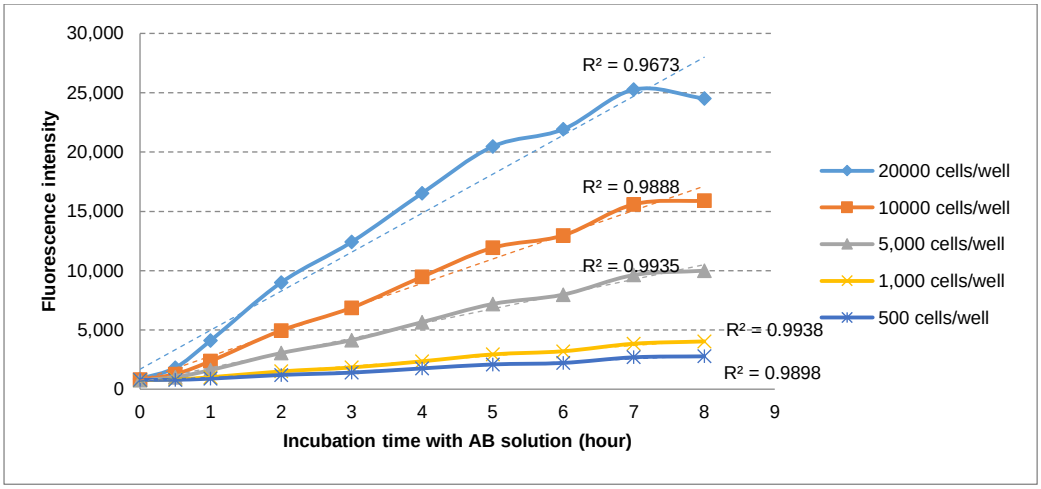

Figure A-2 Fluorescence intensity of resofurin from different cell density at different incubation time. The cell density at 5,000 cells per well has the best linearity between 0.5 – 8 hours.

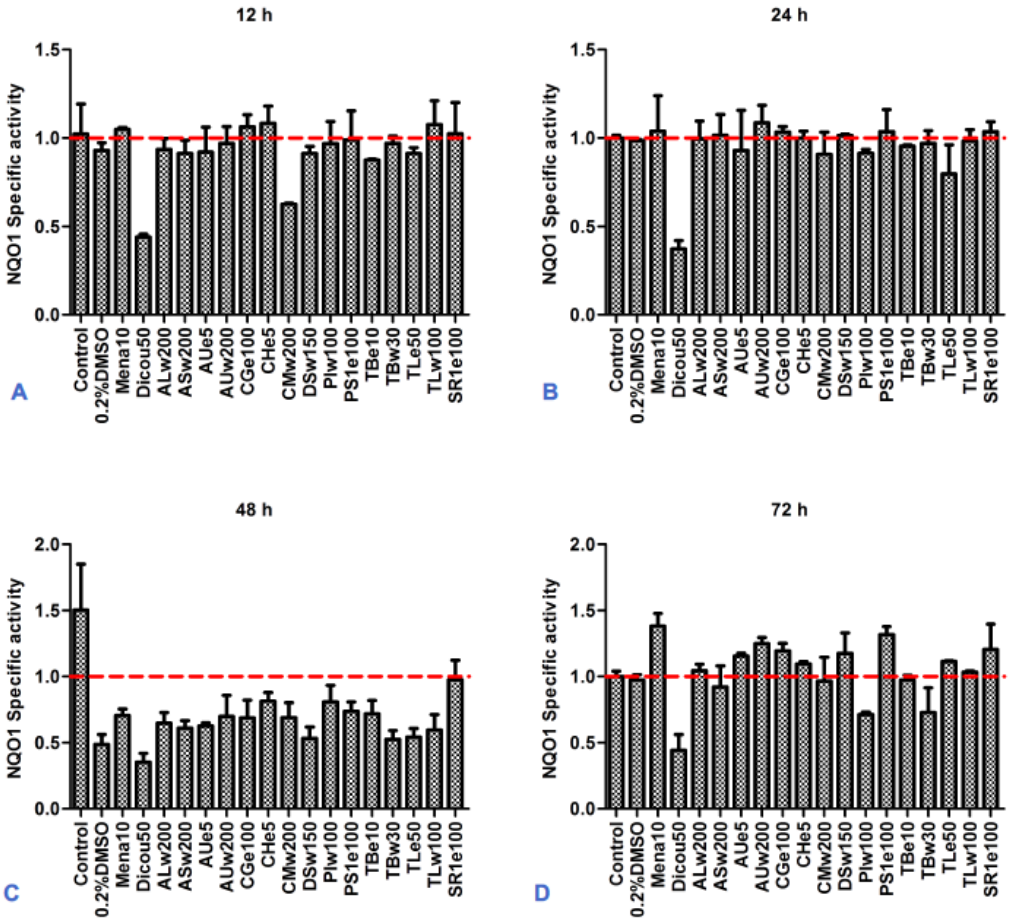

Figure A-3 The effects of plant extracts at maximum non-toxic centration (as indicated in the figure) on NQO1 activity.

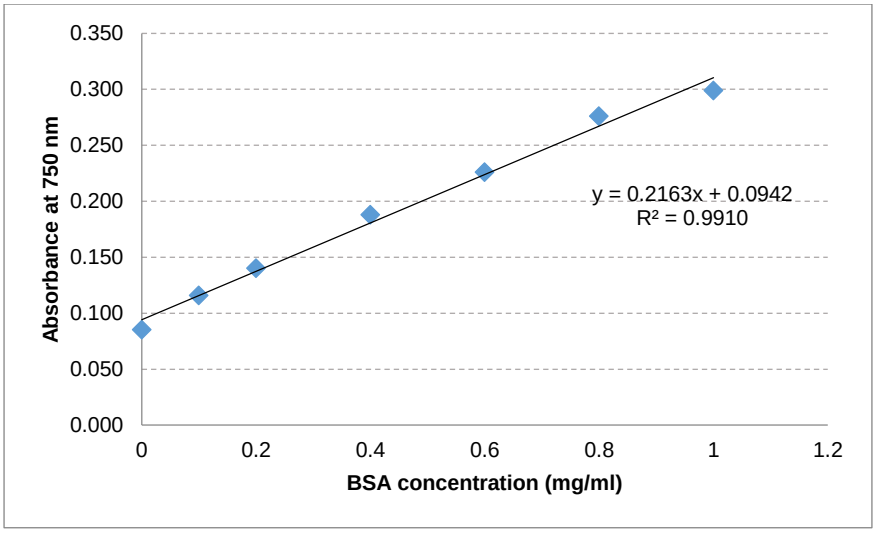

Figure A-4 Representative BSA standard curve for calculation of protein amount in cell lysates used in NQO1 and GSH assays

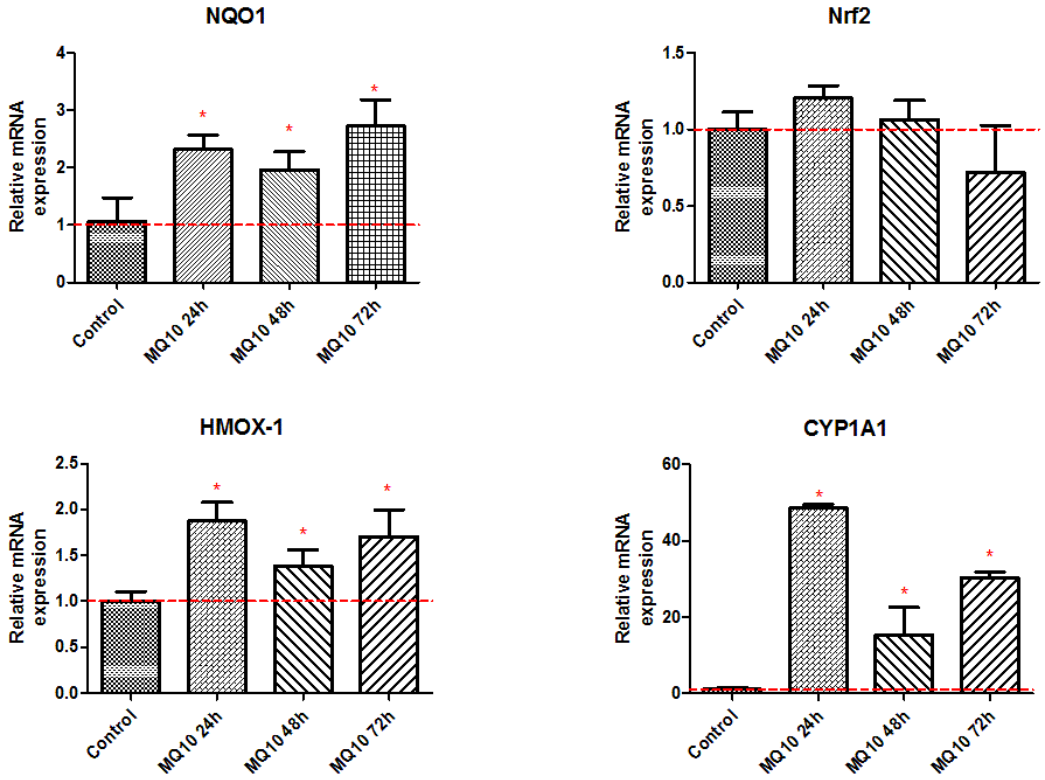

Figure A-5 Optimisation of incubation time for qPCR experiment by the use of 10 µM of menadione. Twenty-four showed the highest responses. Results are mean ± SD (N ≥ 3). Unpaired 2-tailed t-test was used to determine p values. P < 0.05 was considered statistically significant.

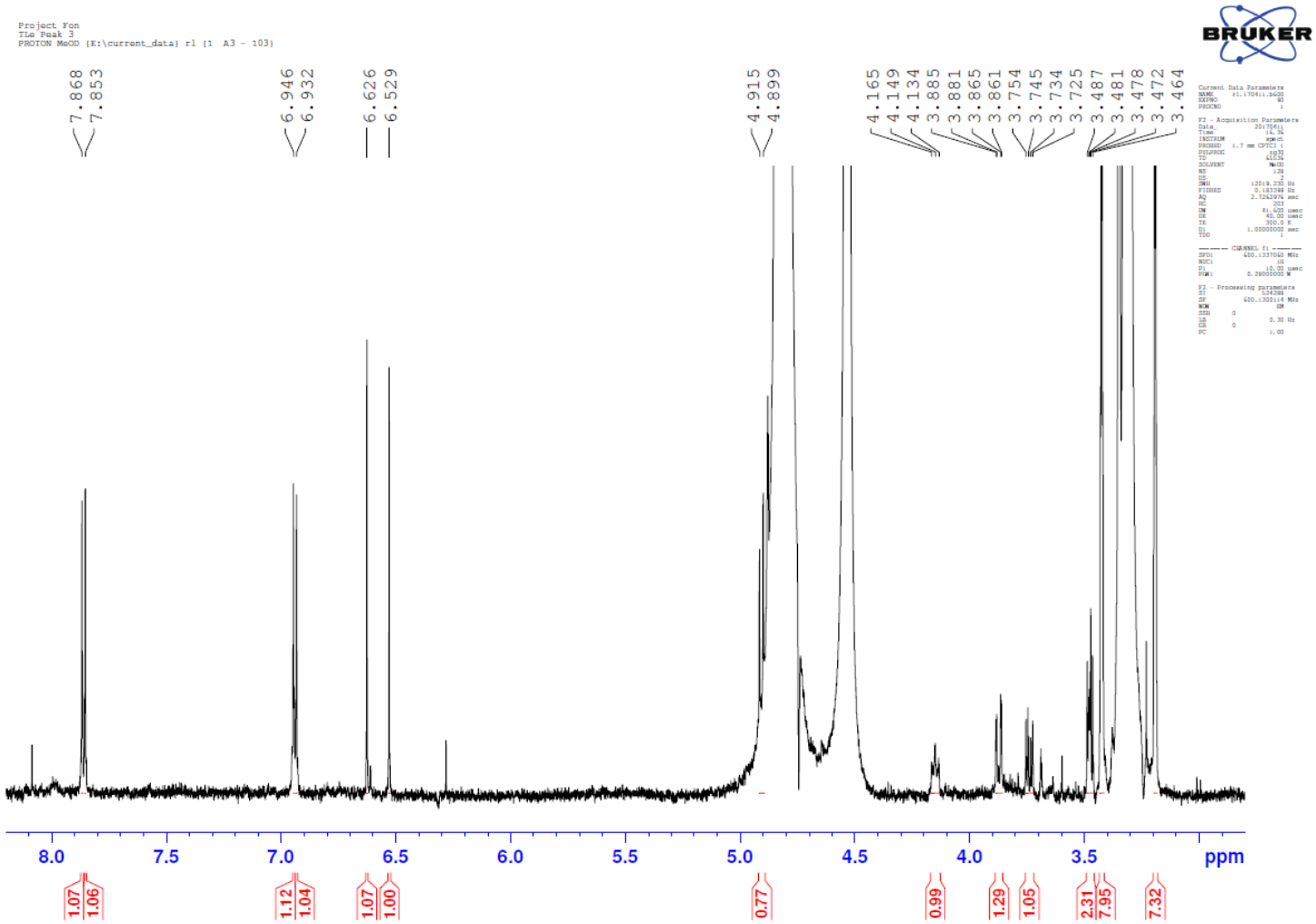

Figure A-6 ^{1}H-NMR spectra for TLe peak 3, which was later identified as isovitexin

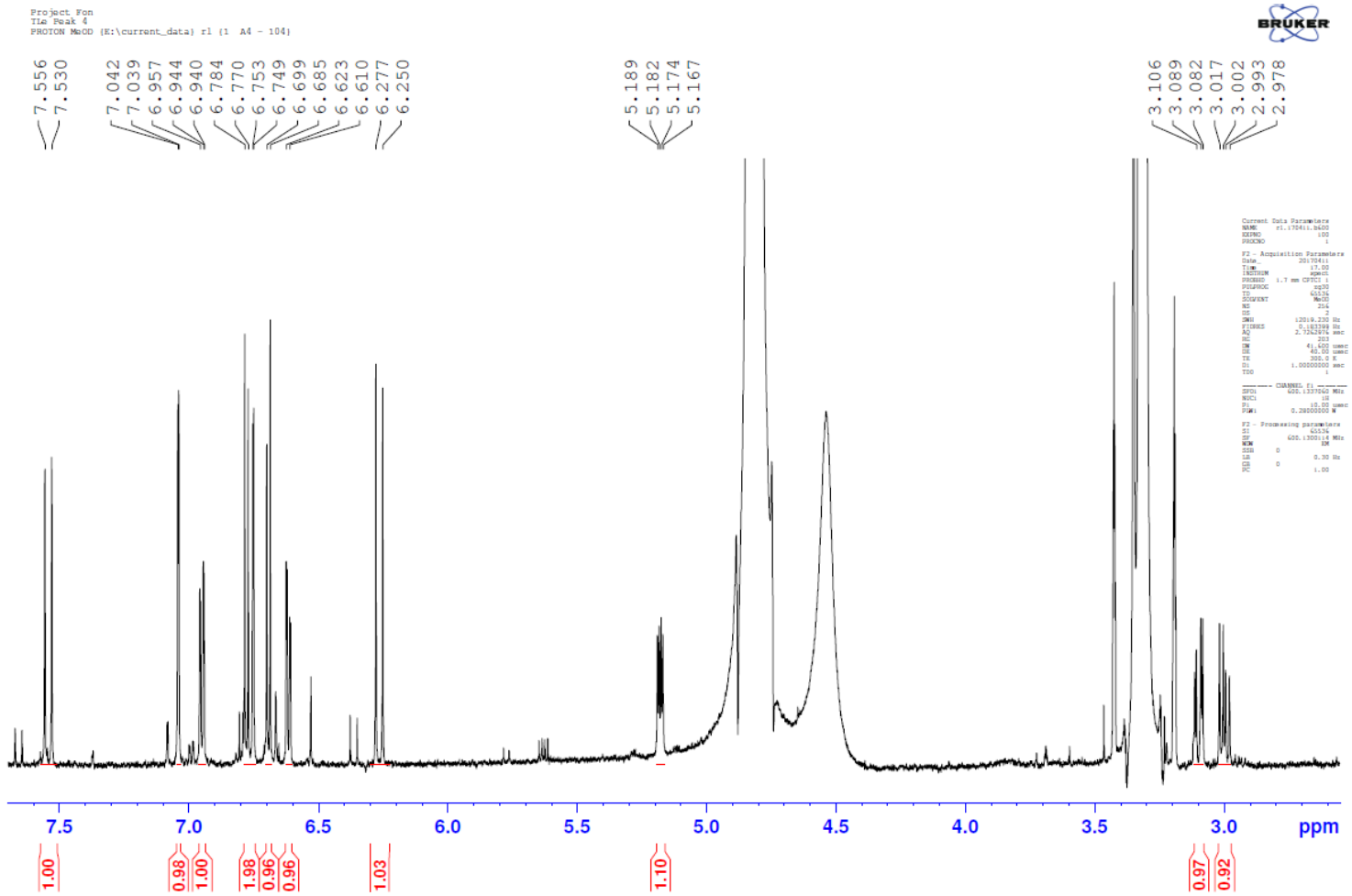

Figure A-7 ^{1}H-NMR spectra for TLe peak 4, which was later identified as rosmarinic acid

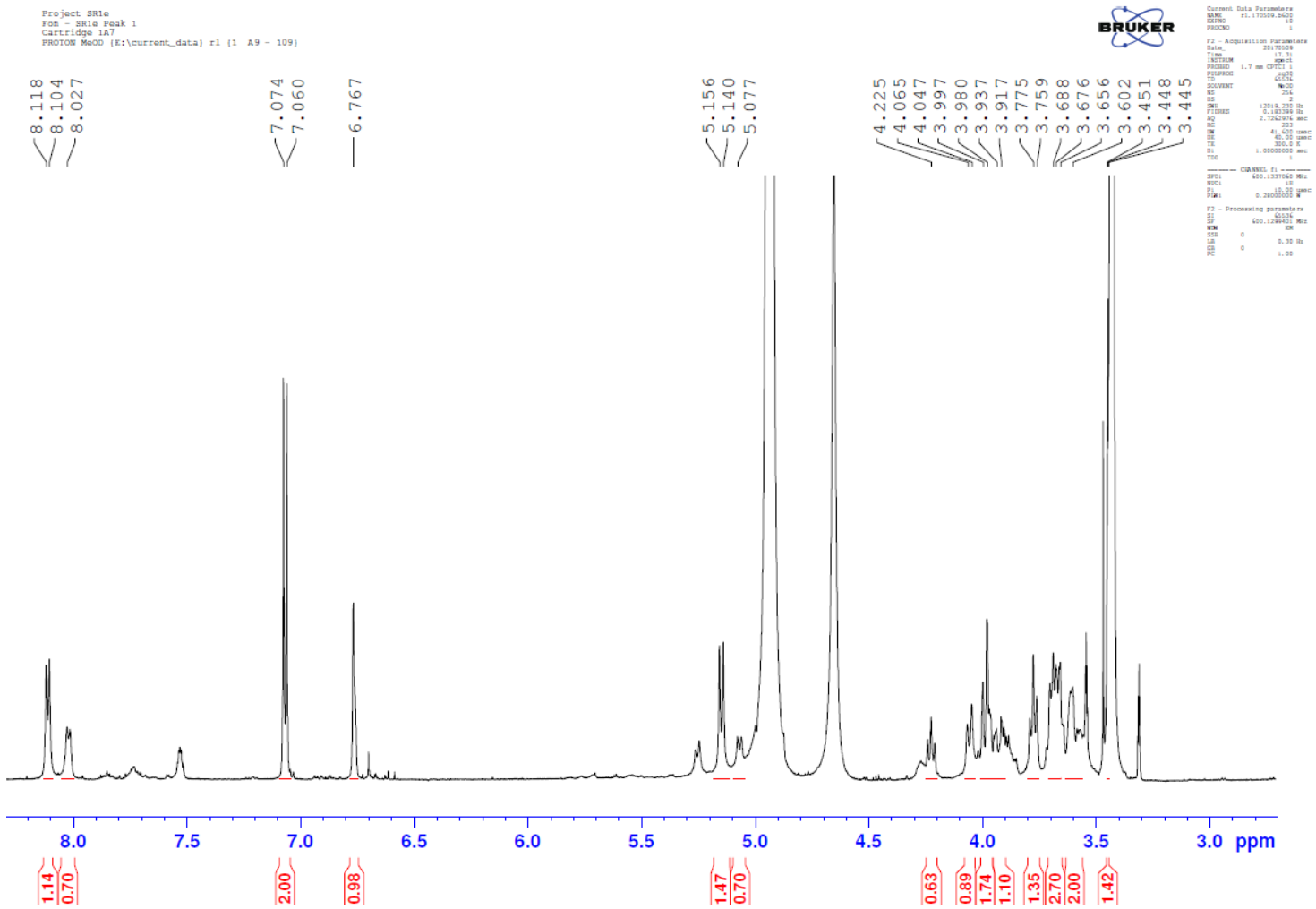

Figure A-8 ^{1}H-NMR spectra for SR1e peak 1, which was later identified as 3,6-di-C-glucosyl apigenin

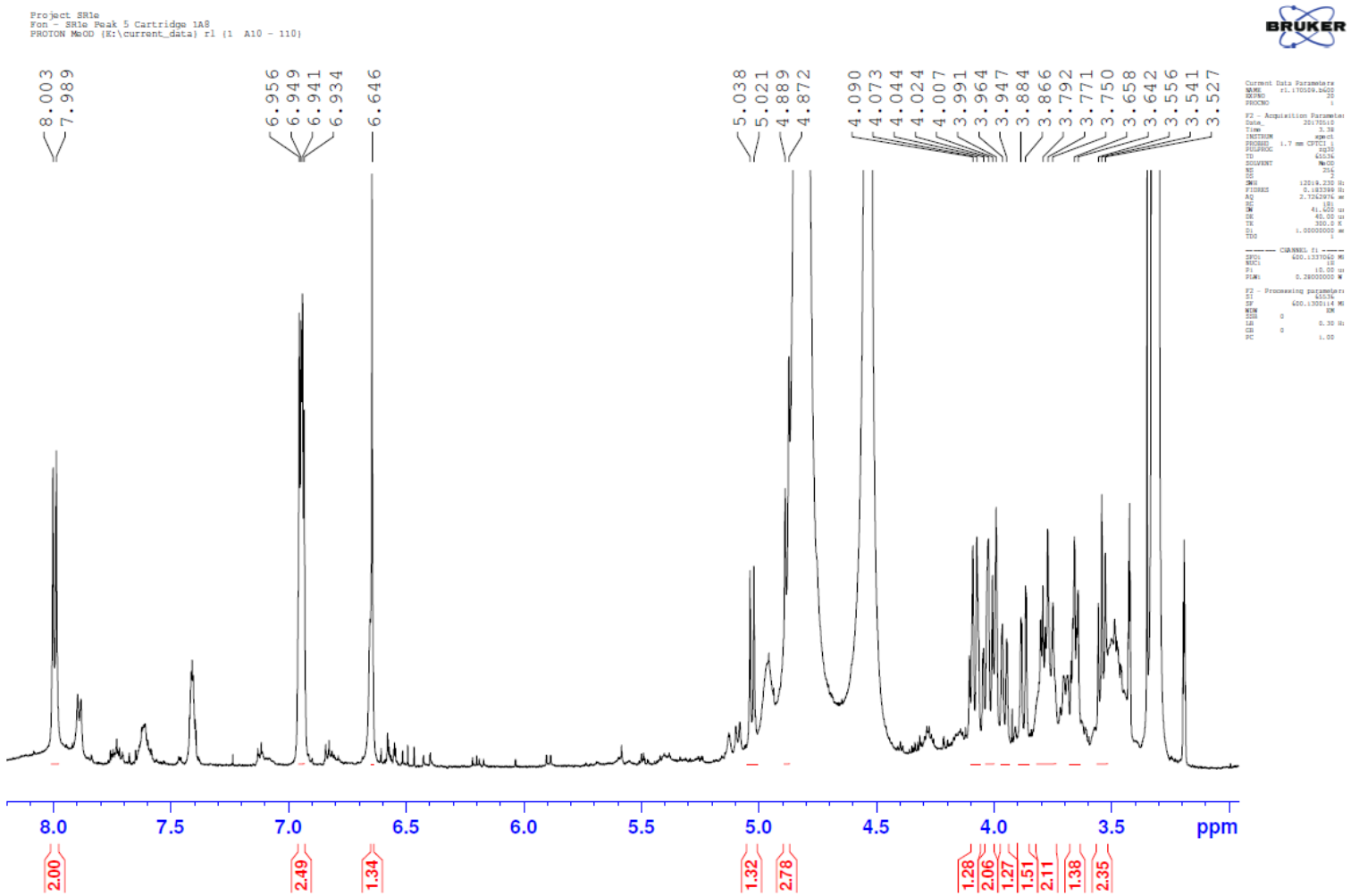

Figure A-9 ^{1}H-NMR spectra for SR1e peak 5, which was later identified as isoschaftoside

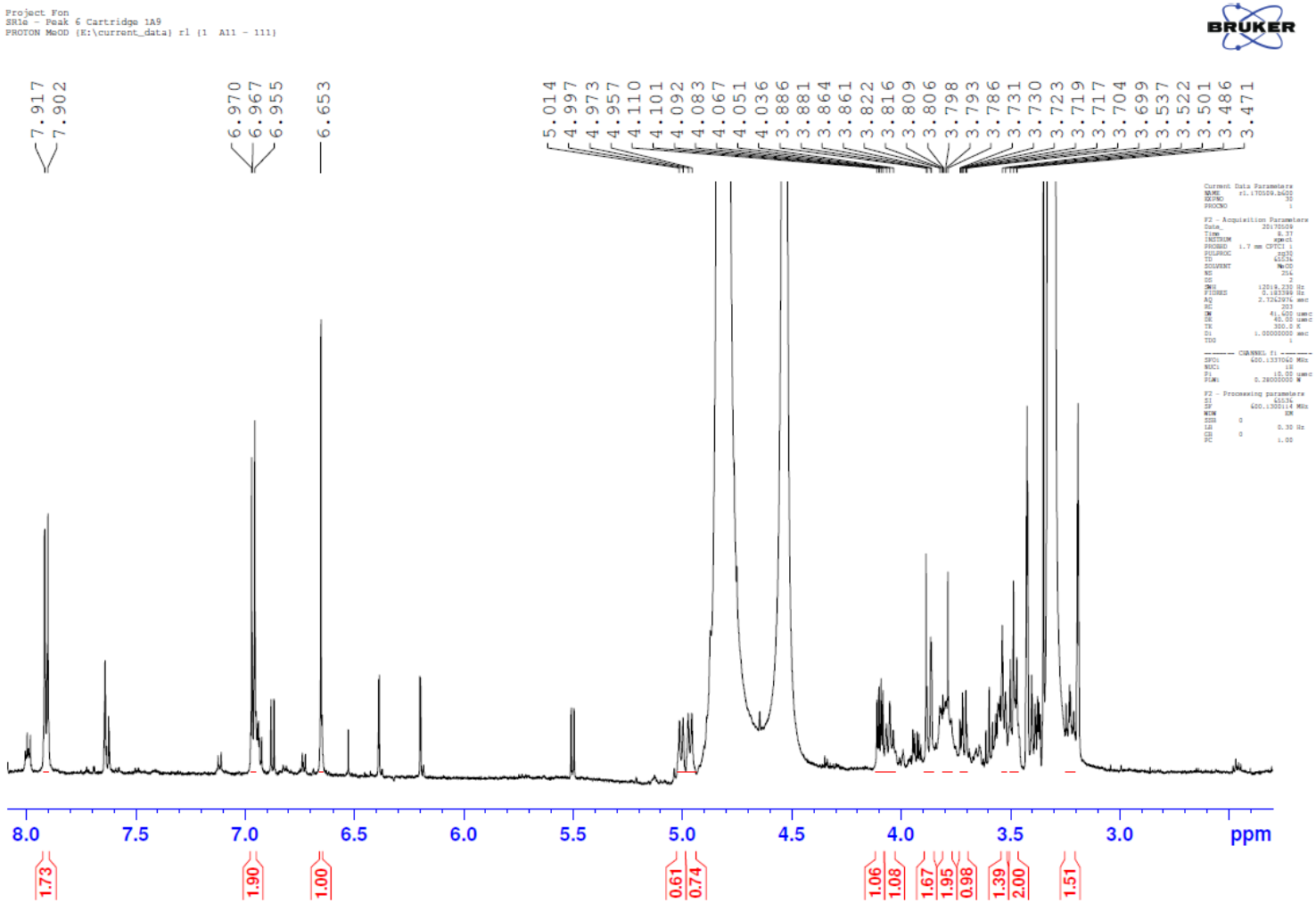

Figure A-10 ^{1}H-NMR spectra for SR1e peak 6, which was later identified as schaftoside

List of publications

Abstract publications

Lumlerdkij N, Boonrak R, Pongsitthichok S, Sriruk N, Wipanso H, Heinrich M. Cytotoxicity of medicinal plants used in cancer prevention in Thailand. Planta Medica 2015; 81(16): 363.

Lumlerdkij N, Boonrak R, Heinrich M. Herbal medicines for treating cancers used by local healers in Thailand. Abstract book for the 15th International Congress of the International Society for Ethnopharmacology; 5-8 May 2015; Petra, Jordan.

Presentation

Lumlerdkij N, Boonrak R, Pongsitthichok S, Wipanso H, Akarasereenont P, Heinrich M. Protective effect of medicinal plants used in cancer prevention by traditional healers in Thailand. 9th Joint Natural Products Conference 2016, Copenhagen, July 24-27, 2016. (Poster)

Lumlerdkij N, Boonrak R, Pongsitthichok S, Sriruk N, Wipanso H, Heinrich M. Cytotoxicity of medicinal plants used in cancer prevention in Thailand. 63rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA2015), Budapest, August 23-27, 2015. (Poster)

Lumlerdkij N, Boonrak R, Heinrich M. Herbal medicines for treating cancers used by local healers in Thailand. 15th International Congress of the International Society for Ethnopharmacology, Petra, May 05-08, 2015. (Poster)

Lumlerdkij N, Sangsumart P, Boonrak R, Tantiwongse J, Khlainok W, Sriruk N, Akarasereenont P, Laohapand T, Heinrich M. Role of herbalists/ traditional practitioners in cancer treatment and prevention in Thailand. 7th European Congress for integrative medicine, Belgrade, October 10 – 11, 2014. (Oral)