In vitro protective effects of plants frequently used traditionally in cancer prevention in Thai traditional medicine: an ethnopharmacological study

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Abstract

Ethnopharmacological relevance: Thai traditional medicine (TTM) has been used widely in cancer management in Thailand. Although several Thai medicinal plants were screened for pharmacological activities related to cancer treatment, such evidence still suffers from the lack of linking with TTM knowledge.

Aim of the study: To document knowledge and species used in cancer prevention in TTM and to preliminary investigate pharmacological activities related to the documented knowledge of twenty-six herbal drugs used in cancer/*mareng* prevention.

Methods: Fieldwork gathering data on TTM concept and herbal medicines used in cancer prevention was performed with TTM practitioners across Thailand. Later, water and ethanol extracts from twenty-six herbal drugs mentioned as being used in cancer prevention were screened for their protective effect against *tert*-butyl hydroperoxide-induced cell death in HepG2 cells. Then active extracts were investigated for their effects on NQO1 activity, glutathione level, and safety in normal rat hepatocytes.

Results: The fieldwork helped in the development of TTM cancer prevention strategy and possible experimental models to test the pharmacological activities of selected medicinal

plants. Fifteen plant extracts showed significant protective effect by restoring the cell viability to 40 - 59.3%, which were comparable or better than the positive control EGCG. Among them, ethanol extracts from *S.rugata* and *T.laurifolia* showed the most promising chemopreventive properties by significantly increased NQO1 activity, restored GSH level from oxidative damage, as well as showed non-toxic effect in normal rat hepatocytes.

Conclusion: TTM knowledge in cancer prevention was documented and used in the planning of pharmacological experiment to study herbal medicines, especially in cancer, inflammation, and other chronic diseases. The proposed 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 integrated approaches could also apply our strategy to develop evidence that supports a more rational uses in traditional medicine.

Keywords: cancer prevention, traditional medicine, *Senegalia rugata*, *Thunbergia laurifolia*

1. Introduction

Cancer patients worldwide have increased their interests in using complementary or alternative medicines for cancer care. It was reported that 9 - 81% of cancer patients mentioned the uses of at least one type of complementary or alternative therapy, especially herbal medicines, after their cancer diagnosis (Damery et al., 2011). In Thailand, Thai traditional medicine (TTM) is an essential form of integrative medicine used by cancer patients. Generally, it is considered to be beneficial, especially in pain relief. However, the use of TTM in cancer patients lacks a systematic development of an evidence-based approach (Poonthananiwatkul et al., 2015). Although cytotoxicity of Thai medicinal plants were reported (Itharat et al., 2004; Lee and Houghton, 2005; Mahavorasirikul et al., 2010; Saetung et al., 2005), other pharmacological activities related to cancer treatment and prevention in TTM are still needed for developing a more rational use.

TTM is considered a holistic medical system focusing on maintaining the balance of the body, especially of the four fundamental elements (*dhātu si*) which are *dhātu din* (earth), *dhātu nam* (water), *dhātu lom* (wind), and *dhātu fai* (fire). When a person loses this balance, he/she will become ill (Chokevivat and Chuthaputti, 2005). Maintaining the balance of the elements is the main strategy for preventing illnesses.

In modern Thai, *mareng* is commonly used to refer to cancer. In TTM scriptures, it is also used to refer to other diseases, which mostly are severe skin conditions (Foundation for the

Promotion of Thai Traditional Medicine and Ayurved Thamrong School Center of Applied Thai Traditional Medicine, 2007). Therefore, *mareng* is not equal to cancer. We previously studied the Thai concept of *mareng* and proposed for the first time five characteristics of cancer in TTM and compared them to Western medical concepts. In the same report, we also proposed that a TTM condition called *krasai* could involve oxidative stress (Lumlerdkij et al., 2018). Oxidative stress has an important role in carcinogenesis. Elevated reactive oxygen species (ROS) levels can initiate DNA damage, help cancer cells to acquire proliferative signals and resist apoptosis, and promote the invasion, metastasis and angiogenesis (Fiaschi and Chiarugi, 2012). Therefore, oxidative stress can be an important target in cancer prevention in both biomedical and TTM senses.

Antioxidant systems are important in the prevention from toxic substances and carcinogens. NAD(P)H:quinone oxidoreductase 1 (NQO1) is involved in the defence against toxicity and carcinogenicity of quinones (Ross et al., 2000). Glutathione plays a crucial role in the detoxification of toxic or carcinogenic reactive metabolites. It also protects against superoxide and hydrogen peroxide formed during the metabolism (DeLeve and Kaplowitz, 1991; Melino et al., 2011). Therefore, we proposed that NQO1 and glutathione were possible pharmacological mechanisms to remove waste from the body in TTM sense (Lumlerdkij et al., 2018).

The objectives of this study are to document knowledge and species used in cancer prevention in TTM and to assess pharmacological activities related to the documented knowledge; including cytotoxicity, protective effect against oxidative stress, and effects on glutathione and NQO1 enzyme activities of twenty-six species used in cancer/*mareng* prevention.

2. Materials and methods

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.

2.2. Ethnopharmacological field survey

Interviews with 33 TTM practitioners were carried out during December 2013 – April 2014 in different regions of Thailand. The core questions used were 'can *mareng* be prevented?' or 'how can we prevent *mareng*?' The project was approved by the UCL Research Ethics Committee, Project ID: 5068/001, and Siriraj Institutional Review Board (Thailand), Protocol number 779/2556(EC4). Information on the interviews and further details on data collection are given in (Lumlerdkij et al., 2018). It followed the guidelines for such research (Heinrich et al., 2018).

Identification of voucher specimens was done by comparison with books, monographs, or authentic specimens from botanical gardens with helps from experienced TTM practitioners from Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand (CATTM). Imported crude drugs were identified by comparing macroscopic features with authentic materials obtained from Sun Ten Pharmaceutical Co., Ltd.. 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. The taxonomic validation of the species is based on http://mpns.kew.org/mpns-portal/ and www.theplantlist.org.

2.3. Species selection for pharmacological studies

The first step was to list all the species used in cancer/*mareng* prevention. Then the species which were not endangered species, can be sustainably supplied, were mentioned by at least two informants, and have not been studied extensively with regards to their chemopreventive activities were selected for further bioactivity assessment.

2.4. Preparation of plant extracts

Plant materials were washed with deionized water, oven-dried between 40-60 °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 then dried using a freeze dryer. To prepare a 70% ethanolic extract, 60 g of herbal powder was added into a glass bottle followed by 70% ethanol to cover the powder surface. The extraction was performed for seven 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, stock solutions of the extracts were prepared using deionized water or DMSO. The water stock solutions were filtered through 0.22 µM syringe filter under a sterile condition. All stock solutions were kept at -20 °C until use.

2.5. Cells

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

2.6. Cytotoxicity of plant extracts in HepG2 cells

HepG2 cells (5,000 cells/well) were seeded into 96-well black plates and allowed to attach overnight. 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 °C. After that, the fluorescence intensity was measured at 560 nm excitation and 590 nm emission using a microtiter plate reader (Infinite M200, Tecan). Cytotoxicity was presented as % viability compared to the control.

2.7. Protective effect against oxidative stress-induced cell death

HepG2 cells (10,000 cells/well) were seeded into 96-well black plates. 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.

2.8. NQO1 activity assay

NQO1 activity was measured following (Fahey et al., 2004). Briefly, HepG2 cells (10,000 cells/well) were seeded into 96-well transparent plates 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 (500 µl of 0.5 M Tris-Cl, pH 7.4, 6.67 mg of Bovine serum albumin, 67 µl of 1.5% Tween-20, 6.7 µl of 7.5 mM FAD, 67 µl of 150 mM glucose 6-phosphate, 6 µl of 50 mM NADP, 20 units of Yeast glucose 6-phosphate dehydrogenase, 3 mg of MTT, and fill deionized water to 10 ml) 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). NQO1 specific activity of treated cells were reported as percentage of the control (Prochaska, 1994).

2.9. Intracellular reduced glutathione (GSH) assay

Measurement of reduced form of glutathione (GSH) is based on the enzymatic recycling method modified from (Allen et al., 2001). HepG2 cells (6×10^5 cells/well) were seeded into 6-well transparent plates and allowed to attach overnight. 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 and then lysed with 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). Then 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 xg 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 xg 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 (7.5 ml of 143 mM Sodium Phosphate Buffer containing 6.3 mM EDTA, 1 ml of 2.39 mM NADPH solution, 31.5 µl of glutathione reductase, and 500 µl of 0.01 M DTNB) was added to the supernatant. The plates were then briefly shaken at 500 xg 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. Calculation of GSH levels in the samples were performed according to (Allen et al., 2001).

2.10. Protein measurement

Protein measurement was performed using Bio-Rad DC™ Protein Assay kit. The absorbance at 750 nm was measured 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 $R^2 > 0.99$.

2.11. Cytotoxicity of plant extracts in primary rat hepatocytes

The primary rat hepatocytes (20,000 cells/well) were seeded into 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 at 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 measured after 20 minutes at excitation wavelength of 535 ± 9 nm and emission wavelength of 590 ± 20 nm. Cytotoxicity was presented as % viability compared to the control.

2.12. 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. Results and discussion

3.1. Thai traditional medicine concept of cancer/*mareng*[1] prevention

Twenty-nine informants suggested three methods for prevention of *mareng* which were taking herbal medicines, eating proper food items (eg. local fresh vegetables), and life style modification. Herbal medicines included multi-herbal preparations or single herbs. The uses of herbal medicines were to remove waste from the body (detoxification), maintain the balance of the four elements, nourish *luead* and *namlueang*, prevent *krasai*, and protect the liver (for the details of *luead*, *namlueang* and *krasai*, see (Lumlerdkij et al., 2018)). In this study, we focus on the detoxification and liver protection. The TTM practitioners suggested detoxification for people with an increased risk, such as farmers and industrial workers who continuously exposed to insecticides or lead or mercury. ~~The TTM practitioners suggested detoxification for people with an increased risk, such as farmers who used a large amount of insecticides or industrial workers who were continuously exposed to lead or mercury.~~ They considered that it is important to protect the liver because the liver was among the most important organs of the body. If the liver becomes abnormal, *mareng* could show up in many organs, such as the liver itself, breast, uterus, ovary, or prostate gland. Figure 1 was developed from the uses of preventive herbal medicines suggested by the informants. It shows~~–~~ our representation of TTM cancer preventive strategy and related pharmacological assays (~~, i.e.~~ an etic interpretation).

~~The five characteristics of cancer and the uses of preventive herbal medicines suggested by the informants assisted in developing the strategy for pharmacological assays of the herbal medicines identified previously (Figure 1).~~

[1] Since the cases mentioned by the informants had no medically confirmed diagnosis of cancer, the term 'cancer/*mareng*' is used throughout this report indicating that the data are based on reported uses.

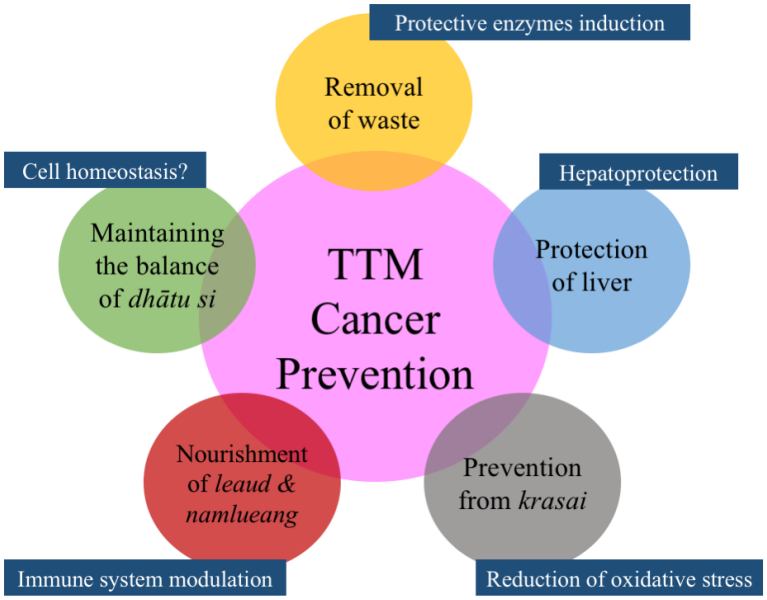

Figure 1 Cancer prevention strategy from Thai traditional medicine and its possible pharmacological models. Bioassay models for the four strategies; protection of liver, prevention from *krasai*, nourishment of *leaud* & *namlueang*, and removal of waste, can be suggested. The balance of *dhātu si* is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

3.2. Selected plant samples for pharmacological assays

The informants mentioned 41 herbal remedies used in the prevention of cancer/*mareng*. A total of 119 species belonging to 53 families were mentioned by TTM practitioners for their uses in cancer/*mareng* prevention (Appendix). Five species could not be verified scientifically. Species from the Fabaceae and Zingiberaceae were reported particularly frequently, with 11 % and 7 % of total species, respectively. After the selection criteria were applied (Methods 2.3), 26 species were selected for further analyses. Table 1 shows frequency of citation (FC) of the selected species reported to have preventive effects.

Table 1 Total samples in the pharmacological studies and their FC

No	Scientific name	Family	Local name	Part used	Abbr.	Voucher number	FC
1	*Allium ascalonicum* L.	Alliaceae	Homdaeng (shallot)	young shoot	AA	-	2
2	*Allium sativum* L.	Alliaceae	Krathiam (garlic)	young shoot	AS	-	3

No	Scientific name	Family	Local name	Part used	Abbr.	Voucher number	FC
3	*Aloe* spp.	Asphoderaceae	Yadam	processed resin from leaf	AL	NL-0028*	2
4	*Atractylodes lancea* (Thunb.) DC.	Asteraceae	Kotkhamao (atractylodes, Cang Zhu)	dried rhizome	AT	NL-0029[#]	2
5	*Capparis micracantha* DC.	Capparidaceae	Chingchi, Saemathalai	root	CM	PBM05194	2
6	*Citrus hystrix* DC.	Rutaceae	Magrud (kaffir lime)	leaf	CH	-	2
7	*Cladogynos orientalis* Zipp. ex Span.	Euphorbiaceae	Chetphangkhi	root	CO	PBM05196	3
8	*Coccinia grandis* (L.) Voigt	Cucurbitaceae	Tamlueng (ivy gourd)	whole plant	CG	PBM05195	2
9	*Derris scandens* (Roxb.) Benth.	Fabaceae	Thaowanpriang (jewel vine)	stem	DS	PBM05189	3
10	*Ferula assa-foetida* L.	Apiaceae	Mahahing (asafoetida)	resin from root	FA	NL-0031*	3
11	*Imperata cylindrical* (L.) P.Beauv.	Poaceae	Ya-kha (cogon grass)	root	IC	PBM05179	2
12	*Ligusticum striatum* DC. (*L. Sinense* Oliv. Cv. Chuanxiong)	Apiaceae	Kothuabua (Szechwan lovage, Chuan Xiong)	dried rhizome	LS	NL-0033[#]	2
13	*Peltophorum pterocarpum* (DC.) Backer ex K.Heyne	Fabaceae	San-ngoen, insi	twig	PP	PBM05180	2
14	*Piper ribesioides* Wall.	Piperaceae	Sa-khan	stem	PA	PBM05190	2
15	*Piper retrofractum* Vahl.	Piperaceae	Dipli (long pepper)	dried mature unripe fruit	PR	PBM05191	2
16	*Piper sarmentosum* Roxb.	Piperaceae	Chaphlu	whole plant	PS1	PBM05181	3
17	*Piper sarmentosum* Roxb.	Piperaceae	Chaphlu	leaf	PS2	PBM05181	
18	*Plumbaco indica* L.	Plumbaginaceae	Chettamunploeng daeng	root	PI	PBM05192	3
19	*Saussurea costus* (Falc.) Lipsch.	Asteraceae	Kotkraduk (costus root, Mu Xiang)	dried root	AU	NL-0036[#]	2
20	*Senegalia rugata* (Lam.) Britton & Rose	Leguminosae	Sompoi (soap pod)	leaf	SR1	NL-0037[§]	2
21	*Senegalia rugata* (Lam.) Britton & Rose	Leguminosae	Sompoi (soap pod)	pod	SR2	NL-0037[§]	
22	*Smilax* spp.	Smilacaceae	Khaoyennuea	root	SM	NL-0038[§]	2
23	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Samophiphek, Naeton (beleric myrobalan)	fruit	TB	PBM05198	2
24	*Thunbergia laurifolia* Lindl.	Acanthaceae	Rangchued (laurel clock vine, blue trumpet vine)	leaf	TL	PBM05178	6
25	*Tiliacora triandra* (Colebr.) Diels	Menispermaceae	Yanang	stem	TT	PBM05193	3
26	*Tinospora crispa*(L.) Hook. f. & Thomson	Menispermaceae	Boraphet (putarwali)	stem	TC	PBM05197	2

-, common species; [#], the identification was performed by comparison of macroscopic features with authentic samples from Sun Ten and Monographs of selected Thai Materia Medica Volume I; *, raw materials derived from plants; [§]; tentative identifications by comparison with Monographs of selected Thai Materia Medica Volume I & II

3.3. Cytotoxicity of plant extracts in HepG2 cells

This assay was performed to determine MNTC of the plant extracts. 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. EGCG, a well-known potential chemopreventive agent (Landis-Piwowar and Iyer, 2014), was used as positive control. Table 2 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, 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* (syn.: *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 (Khan et al., 2013; Rasul et al., 2013a). However, this study focused on chemopreventive properties rather than the ability to kill cancer cells. Cytotoxicity assay was an important step in order to determine the MNTC as cell death had to be avoided in other experiment.

Table 2 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

No	Extract codes	IC_{50} (µg/ml)		MNTC (µg/ml)	
		Ethanol extract (e)	Water extract (w)	Ethanol extract (e)	Water extract (w)
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

3.4. Plant extracts with protective effect against oxidative stress-induced cell death

This assay was performed to screen for potential extracts with abilities to prevent oxidative stress for further analysis. 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 2). Water extract of TB at 30 µg/ml (TBw30) 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 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.

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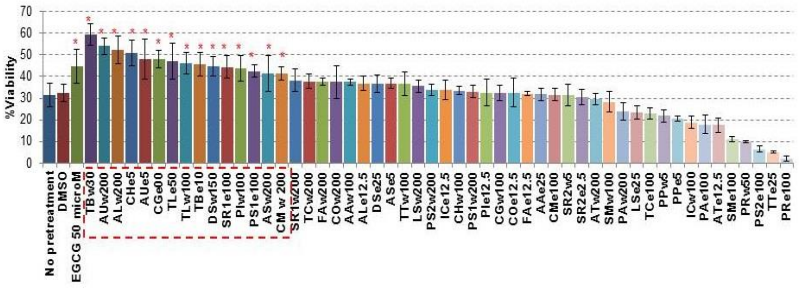

Figure 2 Protective effects of plant extracts against tBHP-induced cell death. Cell viability of HepG2 cells was measured by AlamarBlue assay after an induction of cell death by 0.5 mM t-BHP for three hours. One-way ANOVA analysis showed that pre-treatment with 15 extracts for 24 hours significantly protected HepG2 cells from t-BHP-induced cell death, *P < 0.05 (N ≥3). Where 'w' indicates water extract, 'e' indicates ethanol extract, the number indicates the concentration of the extract tested.

3.5. Plant extracts induced NQO1 activity

We proposed that NQO1 was involved in the removal of waste from the body in TTM sense. In this investigation, fifteen extracts that protected the cells from *t*-BHP; 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 ($p < 0.05$) (Figure 3). 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 or in clinical research, phase II enzyme assays in cell cultures have been used for rapid screening of 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.

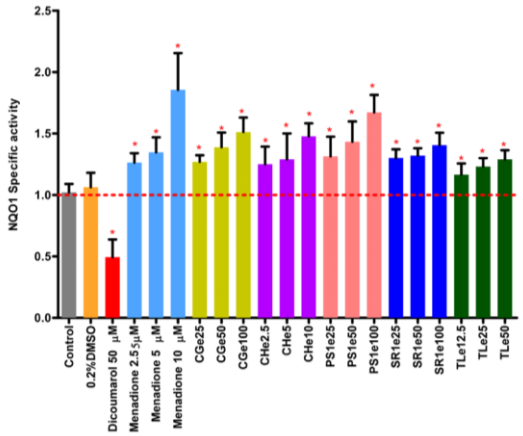

Figure 3 NQO1 specific activity of HepG2 cells after 72 hour-treatment with indicated compounds (µM)/ extracts (µg/ml). One-way ANOVA analysis showed the significant effect of tested substances, *P < 0.05 (N ≥3). Where 'e' indicates ethanol extract and the number indicates the concentration of the extract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

3.6. Plant extracts restored glutathione level after *t*-BHP treatment

Similar to NQO1, we proposed that glutathione was also involved in the removal of waste from the body. In this assay after the treatment with 0.7 mM *t*-BHP for 4 hours, GSH level of HepG2 cells 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 4). 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 (without~~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* (SR1e) and *T.laurifolia* (TLe) were reported for their ability to prevent GSH depletion by *t*-BHP.

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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* (SR1e) and *T.laurifolia* (TLe) provide another evidence to support their traditional uses in cancer prevention and their role as candidates for cancer chemopreventive agent discovery.

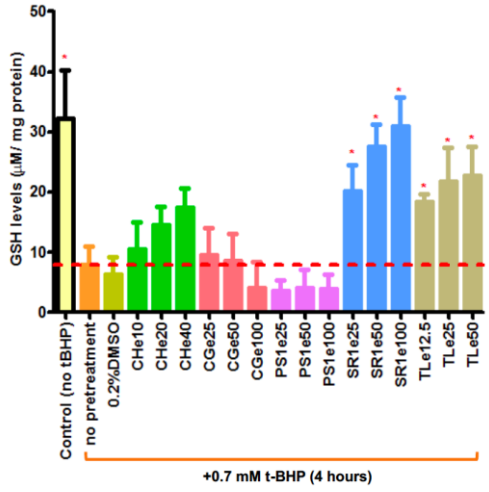

Figure 4 GSH level after t-BHP treatment. One-way ANOVA analysis showed that pre-treatment with SR1e and TLe for 24 hours significantly attenuated GSH depletion effect of t-BHP, *P < 0.05 compared to no pre-treatment (N ≥3). Where 'e' indicates ethanol extract and the number indicates the concentration of the extract tested.

3.7. 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. While ethanol (0.06 – 10 % V/V) reduced the cell viability to around 50%, 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 5). The cytotoxicity of these plant extracts in primary rat hepatocytes has never been published before. 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*.

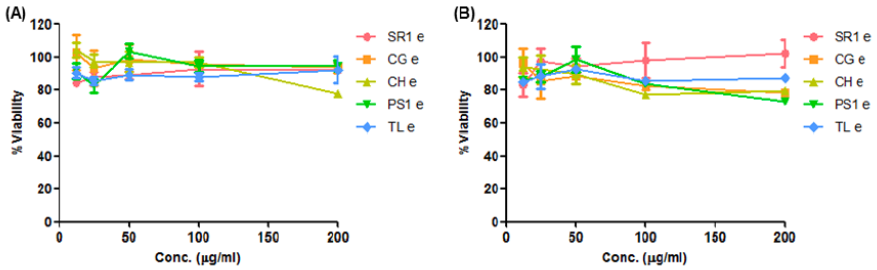

Figure 5 Cytotoxicity of CGe, CHe, PS1e, SR1e, and TLe in primary rat hepatocytes. (A) after 24 hours-incubation (B) after 48 hours-incubation (N ≥ 3)

4. Conclusion

In this study, we successfully developed a strategy for the evaluation of pharmacological activities based on TTM theory by using information from an ethnopharmacological fieldwork (cf. Heinrich et al, 2019). ~~In this study, we successfully developed a strategy going from ethnopharmacological fieldwork to pharmacological experiments evaluating possible activities based on TTM theory.~~ This forms the foundation for a cancer prevention strategy based on TTM concepts and its related pharmacological models as proposed here for the first time. We found that *S.rugata* and *T.laurifolia* showed promising activities related to chemoprevention. This could be additional information for using these herbs for preventive purposes. Our findings are not only useful for TTM practitioners, but also serve as a scientific model to investigate herbal medicine used in a cultural context for diseases, such as cancer, inflammatory conditions, as well as many chronic diseases. In the future, chemical profiles of the potential extracts should be studied in order to provide ~~more~~a better understandings in the biological effects. Furthermore, t~~t~~he strategy should be applied to *in vivo* and clinical studies in order to further confirm the validity of such a strategy~~.~~. This is an approach which could serve as a model for developing an evidence based of o~~O~~ther traditional medical systems~~ines that use holistic approach can also apply our strategy to~~ by developin~~m~~g evidence that supports more rational uses ~~in~~of specific traditional medicine.~~Other traditional medical systems that use~~

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~~holistic approach can also use our strategy as a model to develop traditional medicine with a better evidence base.~~

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Conflict of interest statement

The authors declare no conflict of interest.

Authors contributions

NL performed the fieldwork and all assays, analysed and interpreted the data, and wrote the manuscript.

RB performed the fieldwork, prepared the voucher specimens and extracts.

SB collected the plants, prepared the voucher specimens and extracts.

PA participated in the manuscript preparation.

MH performed the fieldwork, analysed and interpreted the data, and wrote the manuscript.

All authors read and approved the final manuscript.

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Appendix A. Medicinal plants mentioned for their uses in the prevention of cancer/*mareng*

No	Local name (other name)	Part used	Tentative scientific name	Family	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	Cha-em-thet (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	Fabaceae	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

No	Local name (other name)	Part used	Tentative scientific name	Family	FC
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
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.	Rutaceae	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

No	Local name (other name)	Part used	Tentative scientific name	Family	FC
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
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

No	Local name (other name)	Part used	Tentative scientific name	Family	FC
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
74	Phakkradhuawaen (para cress)	young leaf	*Acmella caulirhiza* Delile	Asteraceae	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	Phak-wan-pa	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	Reo-noi	fruit	*Amomum villosum* Lour.	Zingiberaceae	1
85	Kotnamtao (Rhubarb, Da Huang)	rhizome	*Rheum Palmatum* L., *R. officinale* Bail., *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

No	Local name (other name)	Part used	Tentative scientific name	Family	FC
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
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	Asteraceae	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.	Asphodelaceae	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

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