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Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

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To cite this article: Aranya Manosroi, Hiroyuki Akazawa, Kassara Pattamapun, Worapong Kitdamrongtham, Toshihiro Akihisa, Worapaka Manosroi & Jiradej Manosroi (2015) Potent anti-proliferative effects against oral and cervical cancers of Thai medicinal plants selected from the Thai/Lanna medicinal plant recipe database "MANOSROI III", Pharmaceutical Biology, 53:7, 1075-1081, DOI: 10.3109/13880209.2014.959613

To link to this article: https://doi.org/10.3109/13880209.2014.959613

Pharmaceutical Biology

http://informahealthcare.com/phb
ISSN 1388-0209 print/ISSN 1744-5116 online
Editor-in-Chief: John M. Pezzuto
Pharm Biol, 2015; 53(7): 1075–1081
 DOI: 10.3109/13880209.2014.959613

informa
healthcare

ORIGINAL ARTICLE

Potent anti-proliferative effects against oral and cervical cancers of Thai medicinal plants selected from the Thai/Lanna medicinal plant recipe database ''MANOSROI III''

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Abstract

Context: Thai/Lanna medicinal plant recipes have been used for the treatment of several diseases including oral and cervical cancers.

Objective: To investigate anti-proliferative activity on human cervical (HeLa) and oral (KB) cancer cell lines of medicinal plants selected from Thai/Lanna medicinal plant recipe database ''MANOSROI III''.

Materials and methods: Twenty-three methanolic plant crude extracts were tested for phytochemicals and anti-proliferative activity on HeLa and KB cell lines for 24 h by the sulforhodamine B (SRB) assay at the doses of 1×10^{1}–1×10^{-6} mg/ml. The nine extracts with the concentrations giving 50% growth inhibition (GI_{50}) lower than 100 μg/ml were further semi-purified by liquid/liquid partition in order to evaluate and enhance the anti-proliferative potency.

Results: All extracts contained steroids/triterpenoids, but not xanthones. The methanolic extracts of *Gloriosa superba* L. (Colchinaceae) root and *Albizia chinensis* (Osbeck) Merr. (Leguminosae–Mimosoideae) wood gave the highest anti-proliferative activity on HeLa and KB cell lines with the GI_{50} values of 0.91 (6.0- and 0.31-fold of cisplatin and doxorubicin) and 0.16 μg/ml (28.78- and 82.29-fold of cisplatin and doxorubicin), respectively. Hexane and methanol–water fractions of *G. superba* exhibited the highest anti-proliferative activity on HeLa and KB cell lines with the GI_{50} values of 0.15 (37- and 1.9-fold of cisplatin and doxorubicin) and 0.058 μg/ml (77.45- and 221.46-fold of cisplatin and doxorubicin), respectively.

Discussion and conclusion: This study has demonstrated the potential of plants selected from MANOSROI III database especially *G. superba* and *A. chinensis* for further development as anti-oral and cervical cancer agents.

Keywords

Anti-proliferative effects, cervical cancer, oral cancer, ''MANOSROI III'' database, thai medicinal plants

History

Received 15 April 2014
Revised 4 July 2014
Accepted 21 August 2014
Published online 23 January 2015

Introduction

Globally, cervical and oral cancers are the third and sixth most common cancers in 2007. Cervical cancer is caused by several types of human papillomavirus that is spread through sexual contact. Cisplatin, doxorubicin, and vincristine, which are the standard chemotherapeutic drugs, are widely used to treat cancers including cervical and oral cancers. However, these chemotherapeutic drugs have severe side effects, such as hair loss, stomach upset, and fatigue (Guo et al., 2007; Kim, et al., 2011; Yang et al., 2003). Therefore, many medicinal plants have been investigated as alternatives for these chemotherapeutic drugs. Generally, higher safety and efficacy of medicinal plants can be obtained than the synthetic agents due to their traditional uses.

The Lanna region includes several provinces in China, Laos, Myanmar, and Thailand. For Thai Lanna, the region includes seven provinces, i.e., Chiang Mai, Chiang Rai, Lamphun, Lampang, Phayao, Phrae, and Nan. Lanna has its own folklore wisdoms including traditional medicinal plant recipes. These recipes have been recorded and used for over 700 years. Interestingly, some of them are still currently used in the northern part of Thailand for the treatment of several diseases such as fever, diarrhea, diabetes, tuberculosis, arthritis, and cancers. Prof. Dr. Jiradej Manosroi et al. (2006, 2012) at the Natural Products Research and Development Center (NPRDC), Science and Technology Research Institute (STRI), Chiang Mai University in

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Thailand, have collected the medicinal plant recipes from several institutions, temples, and folklore doctors in the Thai Lanna area and other regions all over Thailand and put them in the database called ''MANOSROI III''. Presently, this database contains 83 000 recipes for the treatment of several diseases including more than 600 recipes for cancer treatment (Kitdamrongtham et al., 2013).

Some plants selected from recipes in the ''MANOSROI III'' database have been previously shown to have potent anti-proliferative activities *via* apoptotic action (Manosroi et al., 2012). In this study, phytochemistry and anti-proliferative activity on human cervical and oral cancer cell lines of the methanolic crude extracts of 23 Thai medicinal plants selected from the Thai medicinal recipes database ''MANOSROI III'' were investigated. The crude extracts with strong anti-proliferative activity were further semi-purified by liquid/liquid partition in order to evaluate the potential for further development as anticancer drugs.

Materials and methods

Materials and chemicals

Twenty-three plants appeared with high frequency in the anticancer recipes were selected from the Thai/Lanna medicinal recipe database ''MANOSROI III'', by searching with the keywords of Ma-reng and San in Thai which mean cancer. The plant samples were collected from Chiang Mai, Thailand, during March–May 2010 and authenticated by, a botanist, Ms. Suda Saowakhon. The voucher specimens were deposited at Natural Product Research Development Center (NPRDC), Faculty of Pharmacy, Chiang Mai University in Chiang Mai, Thailand. Methanol was obtained from Labscan Asia Co. Ltd. (Bangkok, Thailand). Fetal bovine serum (FBS) was from Gibco BRL (Ontario, Canada). Dulbecco's Modified Eagle Medium (DMEM) cell culture medium, dimethylsulfoxide (DMSO), and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (St. Louis, MO). Cisplatin and doxorubicin hydrochloride were from Dabur Pharma Ltd (Hamshire, UK).

Extraction and fractionation

Crude extract preparation

The dried and powdered plants (10 g) were extracted with methanol under reflux three times for 2 h of each extraction. The mixture was filtered and the methanol filtrate was evaporated under reduced pressure using a rotary evaporator (Buchi, Switzerland) to give the methanolic crude extract (ME). The yield was represented as percentages of the crude extracts in comparing with the dried plant.

Liquid/liquid partition

Nine MEs which gave the GI_{50} values of less than 100 μg/ml [(active (A) or moderately active (MA)] were fractionated by liquid/liquid partition to obtain four soluble fractions each including hexane-soluble fraction (HF), methanol–water-soluble fraction (MF), *n*-butanol-soluble fraction (BF), and water-soluble fraction (WF) with the total of 36 fractions. Briefly, 1 g of ME was dissolved in 100 ml of water and partitioned with 100 ml of ethyl acetate to obtain the ethyl acetate and water layer. The water layer was again extracted twice with ethyl acetate. The ethyl acetate layer was pooled and concentrated to ca. of 5 ml and suspended in 95 ml of methanol and extracted with hexane three times to obtain the HF and MF fractions. The water layer was extracted with *n*-butanol three times to get the BF and WF fractions.

Phytochemical determination

The extracts and fractions were tested for the presence of flavonoids, tannins, saponins, alkaloids, steroids/triterpenoids, anthraquinones, and xanthones using standard procedures as previously described (Egwaikhide & Gimba, 2007; Manosroi et al., 2010). Shinoda test was used for the detection of flavonoids. Quercetin from Sigma (St. Louis, MO) was used as a positive control. Tannins were detected by the presence of blue-black precipitate of the sample solution after the addition of 15% $FeCl_3$ solution. Tannic acid (Sigma Chemical Co., St. Louis, MO) was used as a positive control. Frothing test was performed for the detection of saponins. For alkaloids, Dragendorff's reagent was used, and quinine sulfate (Sigma Chemical Co., St. Louis, MO) was used as a standard. Salkowski test was performed for steroids/triterpenoids detection. Stigmasterol and ursolic acid (Sigma Chemical Co., St. Louis, MO) were used as positive controls. For anthraquinones, after hydrolysis with hydrochloride, the sample was extracted with chloroform. After addition of ammonia solution, the rose-pink color indicated the presence of anthraquinone. Emodin (Sigma Chemical Co., St. Louis, MO) was used as a positive control. Xanthones were detected by the presence of yellow precipitate of the sample solution after the addition of dilute aqueous potassium hydroxide solution. *Garcinia mangostana* Linn. (Guttiferae) extract from NPRDC, STRI, Chiang Mai University, was used as a positive control.

Anti-proliferative activity

Sample preparation

The MEs and fractions were dissolved in the DMEM medium containing 0.5% v/v of DMSO (Houghton et al., 2007) and DMSO, respectively. The samples were centrifuged at $2000 \times g$ at room temperature 28 °C for 5 min and sterilized by filtering through 0.2 μm cellulose acetate membranes (Sartorius, Göttingen, Germany). The MEs and fractions were diluted with the DMEM medium containing 0.5% v/v of DMSO and DMSO to the concentration of 1×10^{1}–1×10^{-5} and 1–1×10^{-6} mg/ml and stored at −20 °C.

Human cervical and oral cancer cell culture

The human cervical adenocarcinoma (HeLa) and mouth epidermal carcinoma (KB) cells were sub-cultured into 25-cm^2 plastic flasks containing DMEM supplemented with 10% of FBS, 100 U/ml of penicillin, and 100 μ/ml of streptomycin. The flask was incubated at 37 °C in a humidified air incubator containing 5% carbon dioxide (CO_2).

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incubated at 37 °C in a humidified air incubator containing 5% carbon dioxide (CO_2).

Anti-proliferative assay by the sulforhodamine B method

Effects of extracts and fractions on the growth of HeLa and KB cell lines were evaluated according to the procedure of the American National Cancer Institute (NCI) for the *in vitro* anticancer drug screening using the protein-binding dye sulforhodamine B (SRB) to assess cell growth (Skehan et al., 1990). The assay was performed as described previously with some modifications (Manosroi et al., 2007). Briefly, cells were harvested, seeded into 96-well plates (Gibthai Co. Ltd., Bangkok, Thailand) at the density of 1×10^4 cells/well and incubated for 24 h at 37 °C in a 5% CO_2 in an incubator. The cells were treated with various sample concentrations (1×10^1–1×10^{-5} and 1–1×10^{-6} mg/ml for MEs and fractions, respectively) for 24 h. After incubation, the cells were fixed with 50% trichloroacetic acid and dyed with SRB solution. An amount of 100 µl of the Tris-solution was added to each well and incubated for 30 min. The absorbance was measured at 540 nm by a microplate reader (Bio-Rad, model 680, Philadelphia, PA). In the assay system, the final concentration of DMSO in each well was less than 1% v/v. The dose–response curve was prepared, and the GI_{50} values which were the concentrations of the samples giving 50% growth inhibition were determined. Two anti-cancer drugs including cisplatin and doxorubicin were used as the standards. Folds of anti-proliferative activity in comparing with the standard drugs were calculated as follows: folds = (GI_{50} value of the standard drug)/(GI_{50} value of the samples).

Statistical analysis

All assays were performed in triplicate of three independent and separate experiments. The means of each test were calculated.

Results

Preparation of 23 medicinal plant crude extracts (MEs)

Botanical and family names, part used, traditional uses, and yields of MEs of the 23 Thai medicinal plants are shown in Table 1. *Aegle marmelos* (L.) Correa ex Roxb. (Rutaceae) fruits and *Ventilago denticulata* Wild. (Rhamnaceae) stems gave the highest and lowest yields of 43.88 and 4.97% w/w, respectively. Several traditional uses including cancer treatment of the medicinal plants have been reported (Coe & Anderson, 2005; Houghton et al., 2007; Manaharan et al., 2011; Manosroi et al., 2009; Prachayasittikul et al., 2009; Siriwatanametanon et al., 2010; Su et al., 2008; Trakulsomboon et al., 2006; Ueda et al., 2002).

Anti-proliferative activity and phytochemical screening of the 23 plant crude extracts

The anti-proliferative activities on HeLa and KB cell lines and phytochemical characteristics of the 23 MEs are presented in Table 2. According to the criteria established by the NCI, the activity with the GI_{50} value lower than 20 µg/ml, in the range of 20–100 µg/ml, and more than 100 µg/ml are regarded as A, MA, and inactive (IA), respectively (Homan, 1972). The *G. superba* root extract showed the highest anti-proliferative activity on the HeLa cell line with the GI_{50} value of 0.91 µg/ml which was 6.04- and 0.31-fold of cisplatin and doxorubicin, respectively. For 23 extracts in the HeLa cell line, five were classified as A, while two were MA and 16 were IA. In KB cell line, ME of *A. chinensis* wood gave the highest anti-proliferative activity with the GI_{50} value at 0.16 µg/ml of 28.00- and 80.06-fold of cisplatin and doxorubicin, respectively. Two out of 23 extracts were regarded as A according to the NCI criteria, while four extracts were MA and 17 extracts were IA.

All extracts were positive for steroids/triterpenoids, whereas most extracts gave positive results for flavonoids, tannins, saponins, and alkaloids. Among 23 plants, *Cassia fistula* L. (Leguminosae), *Rhinacanthus nasutus* (L.) Kurz (Acanthaceae), and *Smilax corbularia* Kunth (Smilaceae) extracts were strongly positive for flavonoids, whereas *S. corbularia* was also strongly positive for tannins. *Hydnophytum formicarum* Jack (Rubiaceae), *Peltophorum pterocarpum* (DC.) Backer ex K.Heyne (Leguminosae–Caesalpinoideae), *Stemona collinsae* Craib (Stemonaceae), and *Tiliacora triandra* Diels. (Menispermaceae) were strongly positive for tannins. *Albizia chinensis*, *Pouzolzia pentandra* Benn. (Urticaceae), and *S. collinsae* were strongly positive for saponins. Only *Senna alata* (L.) Roxb. (Leguminosae–Caesalpinoideae) roots' extract was positive for anthraquinones. None was positive for xanthones. Nine plants which gave anti-proliferative activity with the GI_{50} value of less than 100 µg/ml on HeLa or KB cell lines were further fractionated.

Anti-proliferative activity and phytochemical screening of the crude extracts and fractions of the nine selected plants

Anti-proliferative activities of the MEs and fractions of the selected nine plants on HeLa and KB cell lines together with their phytochemical properties are summarized in Table 3. HF of *G. superba* extracts exhibited the highest anti-proliferative activity on HeLa cell line with the GI_{50} value of 0.15 µg/ml which was higher than its ME (6.07-fold) and 36.77- and 1.87-fold higher activity than cisplatin and doxorubicin, respectively. MF of *G. superba* gave the highest anti-proliferative activity on KB cell line with the GI_{50} value at 0.058 µg/ml of 77.45- and 221.46-fold of cisplatin and doxorubicin, respectively. HF of *G. superba* which indicated the highest anti-proliferative activity on HeLa cell line was positive for only alkaloids, while MF of *G. superba* was positive for flavonoids, alkaloids, and steroids/triterpenoids.

Discussion

Twenty-three plants were selected from the Thai/Lanna medicinal recipe database ''MANOSROI III'' due to their high-frequency appeared in the selected anticancer recipes. These recipes were traditionally used for anti-cancer activity or supporting the anticancer effects such as anti-inflammation, longevity, antimicrobial, and anti-pain. Usually, most recipes were traditionally prepared by boiling with water. However, the 23 selected plants in this study were extracted with methanol under reflux because the methanol extraction

Table 1. The botanical names, local names, parts use, traditional uses, and yields of the 23 medicinal plants selected from the Thai/Lannna medicinal plant recipe database ''MANOSROI III''.

Botanical names	Families	Local names	Parts use	Traditional uses	Yields (% w/w)	Voucher specimen no.
Acanthus ebracteatus Vahl	Acanthaceae	Ngueak Pla Moh	Stem	Anti-inflammatory, laxative, longevity, and skin diseases	11.50	MANOSROI#033
Aegle marmelos (L.) Correa ex Roxb.	Rutaceae	Ma Toom	Fruit	Anticancer, appetite enhancer, diarrhea, fever, hypochondriasis, and melancholia	43.88	MANOSROI#034
Albizia chinensis (Osbeck) Merr.	Leguminosae-Mimosoideae	Kang Luang	Wood	Ingredient of recipe for cancer, urine disorder, and hemorrhoid	5.79	MANOSROI#021
Caesalpinia sappan L.	Caesalpiniaceae	Nam Khong, Fang	Wood	Antihypercholesteremic, anti-inflammatory, antimicrobial, immunomodulation, and improvement of blood circulation	14.23	MANOSROI#022
Cassia fistula L.	Leguminosae	Ratcha Phruek	Wood	Diabetes, hematemesis, prunitus, recipe for cancer, and leucoderma	6.71	MANOSROI#012
Fibraurea tinctoria Lour.	Menispermaceae	Kamphaeng Ched Chan	Wood	Analgesic, antidote, antimalarial, antipyretic, diuretic, dye producing, and dysentery	7.04	MANOSROI#035
Gloriosa superba L.	Cochinaceae	Dong Dueng	Root	Anthelmintic, anticancer, antimalarial, colic, recipe for cancer, and gout	7.92	MANOSROI#036
Hydnophytum formicarum Jack	Rubiaceae	Hua Roi Ru	Bulb	Anti-inflammatory, chest pains, diarrhea, heart problems, hepatitis, rheumatism, recipe for cancer, and diabetes	17.06	MANOSROI#037
Nymphoides indica Ktze. = *Nymphoides indicum* Ktze.	Gentianaceae	Tab Tao Yai	Wood	Antidote and treatment for side effect by snake bite	8.37	MANOSROI#025
Peltophorum pterocarpum (DC.) Backer ex K.Heyne	Leguminosae-Caesalpinoideae	San Ngoen	Wood	Dye producing and wound treatment	10.52	MANOSROI#023
Polyalthia debilis Pierre Finet et Gagnep.	Annonaceae	Kluay Tao, Tab Tao	Wood	Abdominal pain, tuberculosis, febrifuge, and galactogogue	5.96	MANOSROI#024
Pouzolzia pentandra Benn.	Urticaceae	Kob Cha Nang Dang	Leaf	Anticancer, dermatological and urological disease, and malignancy	5.54	MANOSROI#045
Psophocarpus tetragonolobus (L.) DC.	Leguminosae-Papilionoideae	Thua Phu	Rhizome	Recipe for cancer, flatulence, mouth ulcer, and sore throat	10.07	MANOSROI#003
Rhinacanthus nasutus (L.) Kurz	Acanthaceae	Thong Phan Chang	Whole	Anticancer, antihypertension, anti-inflammatory, detoxicant, and skin disorder	8.61	MANOSROI#038
Senna alata (L.) Roxb.	Legumnosae-Caesalpinioideae	Chumhet Thet	Root	Asthma bronchiale, astringent, gastroenteritis, hepatitis, laxative, skin disease, recipe for laxative, antiworm, antihelmintic, kidney, and liver disorders	21.52	MANOSROI#030
Sida rhombifolia L.	Malvaceae	Ya Khat, Khat Mon	Root	Abscess, acne, anti-inflammatory, antibacterial, aphrodisiac, diuretic, fever, gonorrhea, hemorrhoid, nutritive, tonic, and rheumatism	7.68	MANOSROI#020
Smilax glabra Wall ex Roxb.	Smilaceae	Khao Yen Tai	Bulb	Antibacterial, anticancer, anti-inflammatory, arthritis, dysentery, hepatitis, leptospirosis, mercury poisoning, nephritis, rheumatoid, syphilis, and recipe for antibacterial and antivirus	17.05	MANOSROI#039
Smilax corbularia Kunth	Smilaceae	Khao Yen Nuea	Bulb	Acquired immunological deficiency syndrome, anti-inflammatory, arthritis, and diabetes	17.22	MANOSROI#040
Stemona collinsae Craib	Stemonaceae	Non Tai Yak	Stem	Bronchitis, insect pests, pulmonary tuberculosis, and respiratory disorders	23.00	MANOSROI#041
Suregada multiflora (A. Juss.) Baill. = *Suregada multiflorum* (A. Juss.) Baill	Euphorbiaceae	Khan Thong Pha Ya Bat	Wood	Anticancer, antifungal, anti-inflammation, antipain, antivirus, detoxify, gum disease, hepatic disorder, laxative, and skin diseases	6.35	MANOSROI#042
Tiliacora triandra Diels.	Menispermaceae	Thao Ya Nang	Leaf	Recipe for antipyretic	12.90	MANOSROI#043
Urceola minutiflora Pierre. (D.J. Middleton)	Apocynaceae	Thao Muak Khao	Vine	Analgesic, antipain, detoxify, diarrhea, and insect poison	9.64	MANOSROI#026
Ventilago denticulata Wild.	Rhamnaceae	Thaowal Lek	Stem	Antiorgan-aging, appetite, diuretic, recipe for loose motion, and red discharge from vagina	4.97	MANOSROI#044

Table 2. Anti-proliferative activity on human cervical (HeLa) and oral (KB) cancer cell lines and phytochemical properties of the 23 methanolic plant extracts selected from the Thai/Lanna medicinal recipe database ''MANOSROI III''.

	Anti-proliferative activity										Phytochemicals						
	HeLa					KB											
Medicinal plants	GI_{50} (μg/ml)	Fold of C	Fold of D	NCI criteria	Rank	GI_{50} (μg/ml)	Fold of C	Fold of D	NCI criteria	Rank	Fla	Tan	Sap	Alk	St/T	Ant	Xan
Acanthus ebracteatus	>1000	ND	ND	IA	17	>1000	ND	ND	IA	16	–	–	–	+	–	–	–
Aegle marmelos	>1000	ND	ND	IA	17	327.11	0.01	0.04	IA	11	+	–	–	–	++	–	–
Albizia chinensis	12.11	0.45	0.02	A	3	0.16	28.00	80.06	A	1	++	++	+++	–	++	–	–
Caesalpinia sappan	13.34	0.41	0.02	A	4	25.79	0.17	0.50	MA	3	+	++	+	+	++	–	–
Cassia fistula	184.11	0.03	0.00	IA	12	534.12	0.01	0.02	IA	14	+++	++	–	–	++	–	–
Fibraurea tinctoria	39.61	0.14	0.01	MA	7	31.50	0.14	0.41	MA	4	+	++	++	+	++	–	–
Gloriosa superba	0.91	6.04	0.31	A	1	0.22	20.36	58.23	A	2	+	+	+	++	++	–	–
Hydnophytum formicarum	652.08	0.01	0.00	IA	16	584.22	0.01	0.02	IA	15	++	+++	–	+	++	–	–
Nymphoides indica	>1000	ND	ND	IA	17	>1000	ND	ND	IA	16	++	++	++	++	++	–	–
Peltophorum pterocarpum	276.23	0.02	0.00	IA	14	>1000	ND	ND	IA	16	–	+++	–	–	++	–	–
Polyalthia debilis	181.15	0.03	0.00	IA	11	109.13	0.04	0.12	IA	7	+	–	++	++	++	–	–
Pouzolzia pentandra	37.28	0.15	0.01	MA	6	317.55	0.01	0.04	IA	10	–	–	+++	++	++	–	–
Psophocarpus tetragonolobus	125.31	0.04	0.00	IA	10	68.03	0.07	0.19	MA	5	–	+	++	++	++	–	–
Rhinacanthus nasutus	5.14	1.07	0.05	A	2	>1000	ND	ND	IA	16	+++	+	+	++	++	–	–
Senna alata	>1000	ND	ND	IA	17	70.11	0.06	0.18	MA	6	++	++	++	++	++	+++	–
Sida rhombifolia	>1000	ND	ND	IA	17	>1000	ND	ND	IA	16	++	–	+	++	++	–	–
Smilax glabra	>1000	ND	ND	IA	17	>1000	ND	ND	IA	16	–	+	+	–	++	–	–
Smilax corbularia	>1000	ND	ND	IA	17	428.33	0.01	0.03	IA	13	+++	+++	–	–	++	–	–
Stemona collinsae	104.89	0.05	0.00	IA	8	>1000	ND	ND	IA	16	+	+++	+++	++	++	–	–
Suregada multiflora	238.31	0.02	0.00	IA	13	337.41	0.01	0.04	IA	12	–	+	++	++	++	–	–
Tiliacora triandra	427.89	0.01	0.00	IA	15	110.11	0.04	0.12	IA	8	–	+++	+	+++	++	–	–
Urceola minutiflora	119.22	0.05	0.00	IA	9	>1000	ND	ND	IA	16	+	++	+	++	++	–	–
Ventilago denticulata	19.93	0.28	0.01	A	5	298.75	0.01	0.04	IA	9	++	++	–	++	++	–	–
Standard																	
Cisplatin (C)	5.50	1	ND	–	–	4.48	1	ND	–	–							
Doxorubicin (D)	0.28	ND	1	–	–	12.81	ND	1	–	–							

NCI criteria: the criteria indicated three types of activity. A, active (GI_{50}<20 μg/ml); MA, moderately active (20≤GI_{50}≤100 μg/ml); IA, inactive (GI_{50}>100 μg/ml). The percentages of plant numbers gave the GI_{50} values of less than 100 μg/ml (A/MA) were 30.43 and 26.09% for HeLa and KB cell lines, respectively. Fla, flavonoids; Tan, tannins; Sap, saponins; Alk, alkaloids; St/T, steroids/triterpenoids; Ant, anthraquinones; X, xanthones. ND, not detected; +++, copiously present; ++, moderately present; +, slightly present; – absent.

can give a wide range of constituent polarities in comparing with the water extraction.

The extract of *G. superba* gave the highest anti-proliferative activity on HeLa cell line followed by those of *R. nasutus* and *A. chinensis*, *C. sappan* and *V. denticulata*, while that of *A. chinensis* gave the highest anti-proliferative activity on KB cell line followed by those of *G. superba*, *C. sappan*, *Fibraurea tinctoria* Lour. (Menispermaceae), *Psophocarpus tetragonolobus* (L.) DC. (Leguminosae–Papilionoideae), and *S. alata*. These nine plant crude extracts were further semi-purified since they were regarded as A or MA according to the NCI criteria. Each extract was partitioned into four soluble fractions with a total of 36 fractions. All fractions from the selected nine MEs except that from *A. chinensis* indicated an increase anti-proliferative activity in comparing with their corresponding MEs. The ME and fractions of *A. chinensis* were positive to flavonoids, tannins, saponins, and steroids/triterpenoids. But, *A. chinensis* fractions showed the decrease anti-proliferative activity in comparing with its ME. Saponins which have been isolated from *A. chinensis* stem bark extract has been previously shown to have cytotoxic activity on human colon, hepatoma, lung, and gastric cancer cell lines (Liu et al., 2009). Thus, the phytochemicals found in *A. chinensis* may have a synergistic effect in anti-proliferative activity on HeLa and KB cell lines.

After partition, HF and MF of *G. superba* gave the highest anti-proliferative activity on HeLa and KB cell lines, respectively. *Gloriosa superba* seemed to be the best candidate to be developed as anti-proliferative agents for HeLa and KB cell lines. In fact, *G. superba* has been reported to have anticancer activity due to the contents of colchicinoidal compounds (Reuter et al., 2010). Flavonoids were positive in MF of *G. superba* which gave the highest GI_{50} value in KB cell lines. But, flavonoids were negative in other fractions.

For *P. tetragonolobus*, its BF gave higher activity than its ME on HeLa and KB cell lines of 3.63- and 8.40-fold, respectively. Saponin may be responsible for the activity enhancement after fractionation. Presently, there is no report of saponins from the *P. tetragonolobus* extract. Thus, the flavonoid contents in *G. superba* and saponin contents in *P. tetragonolobus* may be the active constituents for anti-cervical and oral cancers, respectively, which are certainly interesting for further development as novel anticancer drugs.

Conclusion

Potent anti-proliferative activity on HeLa and KB cancer cell lines of 23 Thai medicinal plants selected from the ''MANOSROI III'' database was demonstrated. *Gloriosa superba* root and *A. chinensis* wood ME extracts showed

Table 3. Anti-proliferative activity on HeLa and KB cell lines and phytochemical properties of the methanolic extracts (MEs) and 36 fractions of the nine plants selected from the Thai/Lanna medicinal plant recipe database ''MANOSROI III''.

Medicinal plants	Anti-proliferative activity										Phytochemicals						
	HeLa					KB											
	GI_{50} (μg/ml)	Fold of		NCI criteria	Rank	GI_{50} (μg/ml)	Fold of		NCI criteria	Rank	Fla	Tan	Sap	Alk	St/T	Ant	Xan
		C	D				C	D									
Albizia chinensis	12.11	0.45	0.02	A	10	0.16	28.78	82.29	A	4	++	++	+++	–	++	–	–
A. Chinensis HF	>100	ND	ND	IA	45	>100	ND	ND	IA	43	–	–	–	–	++	–	–
A. Chinensis MF	31.8	0.17	0.01	MA	26	16.0	0.28	0.80	A	16	+	++	+	–	++	–	–
A. Chinensis BF	19.9	0.28	0.01	A	17	4.7	0.96	2.75	A	6	+	+	+	–	++	–	–
A. Chinensis WF	80.6	0.07	0.00	MA	35	>100	ND	ND	IA	43	+	+	+	–	+++	–	–
Caesalpinia sappan	13.34	0.41	0.02	A	11	25.79	0.17	0.50	MA	21	+	++	+	+	++	–	–
C. sappan HF	>100	ND	ND	IA	45	30.3	ND	ND	IA	23	–	–	–	–	+	–	–
C. sappan MF	17.8	0.31	0.02	A	15	16.9	0.26	0.76	A	17	++	++	–	–	+	–	–
C. sappan BF	73.7	0.07	0.00	MA	34	23.3	0.19	0.55	A	19	+	+	+	–	+	–	–
C. sappan WF	>100	ND	ND	IA	45	>100	ND	ND	IA	43	+	–	–	–	–	–	–
Fibraurea tinctoria	39.61	0.14	0.01	MA	30	31.50	0.14	0.41	MA	25	+	++	++	+	++	–	–
F. tinctoria HF	16.9	0.32	0.02	A	13	27.7	0.16	0.46	MA	22	–	–	–	–	+	–	–
F. tinctoria MF	29.4	0.19	0.01	MA	23	24.3	0.18	0.53	MA	20	+	++	++	–	+	–	–
F. tinctoria BF	23.3	0.24	0.01	MA	20	51.9	0.09	0.25	MA	27	+	+	+	–	–	–	–
F. tinctoria WF	27.9	0.20	0.01	MA	22	11.0	0.41	1.16	A	10	–	–	–	–	+	–	–
Gloriosa superba	0.91	6.04	0.31	A	4	0.220	20.36	58.23	A	5	+	+	+	++	++	–	–
G. superba HF	0.15	36.77	1.87	A	1	0.15	30.25	86.51	A	2	–	–	–	+	–	–	–
G. superba MF	0.57	9.63	0.49	A	3	0.058	77.45	221.46	A	1	+	–	–	++	+	–	–
G. superba BF	0.44	12.49	0.64	A	2	0.15	29.07	83.12	A	3	–	–	–	++	+	–	–
G. superba WF	45.2	0.12	0.01	MA	31	31.5	0.14	0.41	MA	25	–	–	–	+	+	–	–
Psophocarpus tetragonolobus	125.31	0.04	0.00	IA	38	68.03	0.07	0.19	MA	30	–	+	++	++	++	–	–
P. tetragonolobus HF	>100	ND	ND	IA	45	>100	ND	ND	IA	43	–	–	+	+	+	–	–
P. tetragonolobus MF	>100	ND	ND	IA	45	76.7	0.06	0.17	MA	32	+	–	–	+	++	–	–
P. tetragonolobus BF	34.5	0.16	0.16	MA	27	8.1	0.55	1.58	A	8	–	–	+++	+	++	–	–
P. tetragonolobus WF	>100	ND	ND	IA	45	>100	ND	ND	IA	43	+	–	–	++	–	–	–
Rhinacanthus nasutus	5.14	1.07	0.05	A	7	>1000	ND	ND	IA	43	+++	+	+	++	++	–	–
R. nasutus HF	36.6	0.15	0.01	MA	28	53.5	0.08	0.24	MA	28	+	–	–	+	–	–	–
R. nasutus MF	14.6	0.38	0.02	A	12	7.1	0.63	1.80	A	7	+++	–	+	+	++	–	–
R. nasutus BF	>100	ND	ND	IA	45	13.2	0.34	0.97	A	12	–	–	–	++	+	–	–
R. nasutus WF	>100	ND	ND	IA	45	19.7	0.23	0.65	A	18	–	–	–	+	+	–	–
Senna alata	>1000	ND	ND	IA	45	70.11	0.06	0.18	MA	31	++	++	++	++	++	+++	–
S. alata HF	>100	ND	ND	IA	45	>100	ND	ND	IA	43	–	–	–	–	++	++	–
S. alata MF	23.8	0.23	0.01	MA	21	13.6	0.33	0.94	A	14	++	+++	++	+	++	+++	–
S. alata BF	>100	ND	ND	IA	45	>100	ND	ND	IA	43	+	–	+	+	++	++	–
S. alata WF	>100	ND	ND	IA	45	>100	ND	ND	IA	43	+	–	–	–	–	–	–
Ventilago denticulata	19.93	0.28	0.01	A	18	299	0.01	0.04	IA	36	++	++	–	++	++	–	–
V. denticulata HF	22.2	0.25	0.01	MA	19	8.2	0.55	1.57	A	9	++	+	–	–	++	++	–
V. denticulata MF	30.3	0.18	0.01	MA	25	>100	ND	ND	IA	43	++	+++	–	+	++	+	–
V. denticulata BF	>100	ND	ND	IA	45	>100	ND	ND	IA	43	+	++	–	–	++	–	–
V. denticulata WF	>100	ND	ND	IA	45	>100	ND	ND	IA	43	–	+	–	–	++	–	–
Standard																	
Cisplatin (C)	5.50	1	ND	–	–	4.48	1	ND	–	–							
Doxorubicin (D)	0.28	ND	1	–	–	12.81	ND	1	–	–							

Folds of the standard drugs were calculated as the ratio between the GI_{50} value of standard drugs and the samples. NCI criteria: the criteria indicated three types of activity. A, active (GI_{50}<20 μg/ml); MA, moderately active (20≤GI_{50}≤100 μg/ml); IA, inactive (GI_{50}>100 μg/ml); Fla, flavonoids; Tan, tannins; Sap, saponins; Alk, alkaloids; St/T, steroids/triterpenoids; Ant, anthraquinones; X, xanthones; ND, not detected; +++, copiously present; ++, moderately present; +, slightly present; – absent.

potent with the highest anti-proliferative activity on HeLa and KB cell lines of 6.0- and 0.31-fold and 28.00- and 80.06-fold higher than cisplatin and doxorubicin, respectively. After liquid–liquid partition, the HF and MF of *G. superba* gave the highest anti-proliferative activity on HeLa and KB cell lines of 36.77- and 1.87-fold and 77.45- and 221.46-fold higher than cisplatin and doxorubicin, respectively. This study has not only confirmed the traditional use of the Thai/Lanna medicinal plant recipes for cancer treatments but also the anti-cervical and oral cancer treatment potential of the

plants appeared in the recipes, especially *G. superba* and *A. chinensis* which will be beneficial for further development as modern anticancer drugs.

Declaration of interest

The authors report that they have no conflicts of interest. The authors alone are responsible for the content and writing of the article. The authors would like to thank the Agricultural Research Development Agency (Public Organization) the investment funds following the Royal Decree (ARDA) in Thailand; the Institute of Thai Traditional Medicine, Ministry of Public Health, Nonthaburi, Thailand and Manose Health and Beauty Research Center, in Chiang Mai, Thailand for their financial supports of this study.

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