

Cytotoxic activity of Thai medicinal plants for cancer treatment

Athima Saetung¹, Arunporn Itharat², Chawaboon Dechsukum³,
Chatchai Wattanapiromsakul⁴, Niwat Keawpradub⁵, and
Pranee Ratanasuwan⁶

Abstract

Saetung, A., Itharat, A., Dechsukum, C., Keawpradub, K.,
Wattanapiromsakul, C., and Ratanasuwan, P.

Cytotoxic activity of Thai medicinal plants for cancer treatment

Songklanakarin J. Sci. Technol., 2005, 27(Suppl. 2) : 469-478

Twelve Thai medicinal plants as the ingredients of a Southern Thai traditional formula for cancer treatment were selected to test cytotoxicity activity against two types of human cancer cell lines ; large cell lung carcinoma (CORL-23) and prostate cancer cell lines (PC3) and one type of normal human cell line, fibroblast cells (10FS). SRB assay was used to test cytotoxic activity against all the cell types. Two of the extracts (water and ethanolic extracts) procedures used were similar to those practised by Thai traditional doctors. One concentration (50 µg/ml) of two different extracts was tested first against cell lines and the active plant extracts were diluted and tested for calculating IC₅₀. The ethanolic extracts of six plants (*Bridelia ovata*, *Curcuma zedoaria*, *Derris scandens*, *Dioscorea membranacea*, *Nardostachys jatamansi* and *Rhinacanthus nasutus*) showed cytotoxic activity (IC₅₀ < 30 µg/ml) against lung and prostate cancer cell lines. *Dioscorea membranacea* roots showed the highest cytotoxic activity against lung cancer cell lines (IC₅₀ = 4.6 µg/ml) but it exhibited low cytotoxic activity against prostate cancer cell lines (IC₅₀ = 17.55 µg/ml) and less cytotoxic activity against normal cell lines (IC₅₀ = 66.05 µg/ml). *Curcuma zedoaria* showed cytotoxic activity against COR L-23 and PC3 but less cytotoxic activity against 10FS (IC₅₀ = 6.05, 17.84 and 55.50 µg/ml respectively) *Rhinacanthus nasutus* root extract showed the highest cytotoxic activity against PC3 (IC₅₀ = 2.01 µg/ml) and this extract also showed high activity against COR L-23 and 10FS (IC₅₀ = 5.05 and 10.95 µg/ml respectively). The water extract

¹Graduate student in Pharmacognosy, ²Ph.D. (Pharmacognosy), Assoc. Prof., ³Ph.D. (Phytochemistry), ⁴Ph.D. (Pharmacognosy), Asst. Prof, ⁵B.Sc. (Biology), Scientist, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, ⁶Ph.D. (Pathology), Department of Pathology, Faculty of Medicine, Prince of Songkhla University, Hat Yai, Songkhla 90112, Thailand.

Corresponding e-mail: arunporn.i@psu.ac.th

Received, 12 August 2004 Accepted, 17 November 2004

of all plants exhibited no activity against all types of human cells. Two ethanolic plant extracts (*Dioscorea membranacea* and *Curcuma zedoaria*) which showed specific activity against lung cancer cell lines and less cytotoxic activity against normal cells should be further investigated for active compounds against lung cancer cell.

Key words : cytotoxic activity, lung cancer, prostate cancer, *Dioscorea membranacea*, *Curcuma zedoaria*, *Rhinacanthus nasutus*

บทคัดย่อ

อาทิมา แซ่ตั้ง¹ อรุณพร อิฐรัตน์¹ ชวบูลย์ เดชสุขุม² ฉัตรชัย วัฒนากิริมย์สกุล¹
นิวัติ แก้วประดับ¹ และ ปราณี รัตนสุวรรณ
การศึกษากฤทธิ์ต้านเซลล์มะเร็งของสมุนไพรไทยที่ใช้ในการรักษาโรคมะเร็ง
ว.สงขลานครินทร์ วทท. 2548 27(ฉบับพิเศษ 2) : 469-478

การศึกษากฤทธิ์ความเป็นพิษต่อเซลล์ของพืชสมุนไพรจำนวน 12 ชนิด ที่เป็นส่วนประกอบในตำรับยารักษาโรคมะเร็งของภาคใต้ โดยทำการทดสอบฤทธิ์ความเป็นพิษต่อเซลล์มะเร็ง 2 ชนิด ได้แก่ เซลล์มะเร็งปอด (COR L-23) และเซลล์มะเร็งต่อมลูกหมาก (PC3) และเซลล์ปกติอีก 1 ชนิด ได้แก่ เซลล์ fibroblast (10FS) วิธีการศึกษาโดยการนำสมุนไพรมาสกัดด้วยน้ำและเอทานอลตามวิธีการสกัดที่หมอบ้านใช้ในการเตรียมยาเพื่อรักษาผู้ป่วย เตรียมสารสกัดความเข้มข้น 50 µg/ml มาทดสอบความเป็นพิษต่อเซลล์ในเบื้องต้น สารสกัดที่มีฤทธิ์จะนำไปทดสอบต่อโดยใช้หลักการเจือจางในความเข้มข้นต่างๆ เพื่อหาค่า IC₅₀ ผลการทดลองพบว่าสารสกัดชั้นเอทานอลของใบมะกอก (*Bridelia ovata*), เหง้าขมิ้นอ้อย (*Curcuma zedoaria*), เถาวัลย์เปรียง (*Derris scandens*), หัวข้าวเย็นใต้ (*Dioscorea membranacea*) และรากทองพันชั่ง (*Rhinacanthus nasutus*) มีฤทธิ์ในการต้านเซลล์มะเร็งทั้ง 2 ชนิด (IC₅₀ < 30 µg/ml) ซึ่งหัวข้าวเย็นใต้มีฤทธิ์ต้านเซลล์มะเร็งปอดสูงสุด (IC₅₀ = 4.6 µg/ml) และมีฤทธิ์ต้านเซลล์มะเร็งต่อมลูกหมากมีค่า IC₅₀ เท่ากับ 20.8 µg/ml ขมิ้นอ้อยมีฤทธิ์ต้านเซลล์มะเร็งปอดที่ IC₅₀ เท่ากับ 6.05 µg/ml และฤทธิ์ต้านเซลล์มะเร็งต่อมลูกหมากที่ IC₅₀ เท่ากับ 17.84 µg/ml โดยสารสกัดทั้งสองชนิดนี้มีความเป็นพิษต่อเซลล์ปกติน้อย (IC₅₀ = 66.05 และ 55.05 µg/ml ตามลำดับ) ส่วนสารสกัดของรากทองพันชั่งมีฤทธิ์ต้านเซลล์มะเร็งต่อมลูกหมากสูงที่สุด (IC₅₀ = 2.01 µg/ml) รวมทั้งยังมีฤทธิ์ต้านเซลล์มะเร็งปอดและเซลล์ปกติสูงอีกด้วย (IC₅₀ = 5.05 และ 10.95 µg/ml ตามลำดับ) ดังนั้นสารสกัดชั้นเอทานอลของหัวข้าวเย็นใต้และขมิ้นอ้อยเป็นสารสกัดที่มีฤทธิ์จำเพาะกับเซลล์มะเร็งปอด ดังนั้นพืชทั้งสองชนิดควรมานำมาแยกสารสำคัญที่ออกฤทธิ์ต้านเซลล์มะเร็งปอดต่อไป

¹ ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ ² ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์
อำเภอหาดใหญ่ จังหวัดสงขลา 90112

Cancer is the third leading cause of death worldwide, only preceded by cardiovascular disease, infectious and parasitic disease (Mathers et al., 2001). Interestingly, cancer has been the leading cause of death in Thailand for several years, with an increase in the death rate every year (National statistical office, 2003). In Thailand, many people use traditional medicine as an alternative treatment for cancer (Subchareon, 1998).

Folk doctors of Southern Thailand have used many medicinal plants in cancer drug formulae (Itharath et al., 1998). These traditional medicines preparation are made by boiling the plant material in water or soaking in alcohol; the commonly methods used by Thai people to prepare oral drugs. A traditional drug formula for treatment of cancer, was selected to investigate cytotoxic activity against lung and prostate cancer. It is composed of twelve plants :

Bridelia ovata Decne, *Curcuma zedoaria* (Berg) Roscoe, *Derris scandens* (Roxb.) Benth., *Dioscorea membranacea* Pierre, *Drynaria quercifolia* Linn., *Erythrophleum teysmannii* Craib, *Moringa oleifera* Lamk., *Nardostachys jatamansi* DC., *Rhinacanthus nasutus* (L.) Kurz, *Sapindus rarak* DC., *Smilax corbularia* Kunth and *Strychnos nux-vomica* L. Surprisingly, in spite of the fact that all of these species were reported to be used as ingredients of Thai traditional medicine for treatment of cancer, only three plants are recorded as so far having been tested for cytotoxicity against cancer cell lines. The ethanolic extract of *Curcuma zedoaria* rhizomes has cytotoxic activity against liver cancer cell lines (HepG2) (Matthes *et al.*, 1980) and has an inhibitory activity against the human ovarian cancer cell line (OVCAR-3 cells) (Syu *et al.*, 1998). The methanolic extract of *Rhinacanthus nasutus* roots has cytotoxic activity against KB cell lines ($IC_{50}=3.0 \mu\text{g/ml}$) (Wu *et al.*, 1988). The ethanolic extraction of *Dioscorea membranacea* has cytotoxic activity against human breast adenocarcinoma (MCF-7) and lung cancer (CORL-23) but *Smilax corbularia* has no cytotoxic activity on MCF-7, CORL-23 and human colon adenocarcinoma (LS-174T) (Itharat *et al.*, 2004). However, the activities of some plants against lung cancer cell lines and all plants against prostate cancer cell lines have not been reported. Cytotoxicity screening models are the preliminary methods for selection of active plant extracts against cancer (Cardellina *et al.*, 1999). In the present study, the twelve Thai medicinal plant extracts which are ingredients of cancer the formula were tested for their cytotoxic activity against large cell lung carcinoma (CORL-23), prostate cancer (PC3) and human fibroblast cell (10FS). The comparison of cytotoxic activity against cancer cells and normal cells is discussed. These results could also support the use of these plants by folk doctors to treat cancer patients.

Method and Materials

Plant materials

The part of the plants which were reported to be used against cancer by folk doctors in

Thailand, were collected from all parts of Thailand from January to March 2003. The place of collection and plant parts are shown in Table 1. Authentication of plant materials was carried out at the herbarium center of the Department of Forestry, Bangkok, Thailand where the herbarium vouchers have been kept identifying the plant species. Voucher specimens of these plants were kept in the Herbarium of Southern Center of Thai Medicinal plant at Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand.

Preparation of plant extracts

Plant materials were dried at 50°C, powdered and extracts obtained by methods similar to those practised by Thai traditional doctors, e.g. water extraction and ethanolic extraction. In brief, for water extraction, dried ground plant material (100 g) was boiled for 30 minutes in distilled water (300 ml), the extracts were then filtered and freeze dried. For ethanolic extraction, dried ground plant material (100 g) was percolated with 95 % ethanol for 3 days. The ethanolic extracts were then filtered and concentrated to dryness under reduced pressure. The percentage of yields is shown in Table 2. The water extracts were dissolved in sterile water and the ethanolic extracts were dissolved in DMSO to make a stock solution, sterilized by filtration (pore size as 0.2 μm) before testing.

In vitro assay for cytotoxic activity

Human cell lines

Two different kinds of human cancerous cell lines and one normal cell line were used: large cell lung carcinoma (CORL-23), human prostate cancer (PC3), and one type of normal cell line i.e. human fibroblast cell line (10FS). CORL-23 cells, were established and kindly provided by Dr. P. Twentyman and Dr.P.Rabbitts of MRC Clinical Oncology & Radiotherapeutics Unit, Cambridge, UK. PC3 were cultured in RPMI 1640 medium supplement with 10% heat-inactivated foetal bovine serum, 1% of 2 mM L-glutamine, 50 IU/ml penicillin and 50 $\mu\text{g/ml}$ streptomycin (Keawpradub *et al.*, 1997). The human fibroblast cell, which is a

Table 1. Plant used to treat conditions consistent with cancer symptomatology

Species (Family)	Places for specimen collection (Amphor, Province)	Voucher specimen number	Common name*	Plant part	Use in Thai traditional medicine
<i>Bridelia ovata</i> Decne (Euphorbiaceae)	Hat Yai, Songkhla	SKP0710205	Maka ^{a,b}	lf	Cathartic (5) antipyretic (3)
<i>Curcuma zedoaria</i> (Berg) Roscoe (Zingiberaceae)	Muang, Yala	SKP2060326	Kaminooi ^{a,b}	rh	Cancer (1,3,5)
<i>Derris scandens</i> (Roxb.) Benth (Papilionaceae)	Chana, Songkhla	SKP1410419	Thaowanpreing ^{a,b}	st	Anti-inflammation of bone and joint, analgesic(5)
<i>Dioscorea membranacea</i> Pierre (Dioscoreaceae)	Na Thawi, Songkhla	SKP0620413	Man mu ^a Hua-Khao-Yen ^b	rh.	Cancer (1,4)
<i>Drynaria quercifolia</i> Linn. (Polypodiaceae)	Chana, Songkhla	SKP1520417	Huawaw ^{a,b}	rh	Diuretic (3)
<i>Erythrophleum teysmannii</i> Craib (Caesalpiniaceae)	Muang, Nakhon Ratchasima	SKP0340520	Sark ^{a,b}	st	Antipyretic (5)
<i>Moringa oleifera</i> Lamk. (Moringaceae)	Hat Yai, Songkhla	SKP1181315	Marum ^{a,b}	b	Antipyretic, cardiotonic (3)
<i>Nardostachys jatamansi</i> DC (Valerianaceae)	-	SKP2011410	Kodchadamansi ^{a,b}	fl	Abscess (3)
<i>Rhinacanthus nasutus</i> (L.) Kurz. (Acanthaceae)	Hat Yai, Songkhla	SKP0011814	Thongpunchang ^{a,b}	r	Cancer (1,3,5)
<i>Sapindus rarak</i> DC. (Sapindaceae)	Muang, Krabi	SKP1701918	Makumdeecwai ^{a,b}	fr	Decrease toxic (5)
<i>Smilax corbularia</i> Kunth (Smilacaceae)	Wichian Buri, Phetchabun	SKP1791903	Khao-yen-neua ^{a,b}	rh.	Cancer (1,2,3)
<i>Strychnos nux-vomica</i> L. (Strychnaceae)	-	SKP1851914	Salangjai, Kodkakling ^{a,b}	se	Bitter tonic (5)

* ^a name from Thai Plant Names Book. (Smitinand, 2001); ^b name from Southern Thai Traditional doctors.;
– buy specimens from folk medicine shop.

Plant parts : b=bark, fl=flower, fr=fruit, lf = leaves, st = stem , r = root , rh = rhizome, se=seed

References for use: (1) Prakru Vimokunakorn, 1979; (2)Boonyatanakornkit and Chantaptavan,1993;
(3) Boonyaprapatsorn et al., 2000; (4) Itharat et al.,1999; (5)Pongboonrod, 1979

non-cancerous cell line, was kindly provided by Associate Professor Dr. Aureeporn Kejaroon of the Faculty of Dentistry, Prince of Songkla University, Thailand, and was grown in an incubator with 10% CO₂ at 37°C in DMEM culture medium containing 10% foetal bovine serum and 1% of 10,000 U penicillin and 10 mg/ml streptomycin. According to their growth profiles, the optimal plating density of each cell line was determined (1x10³, 1x10³ and 4x10³ cells/well for CORL-23, PC3 and 10FS

respectively) to ensure exponential growth throughout the experimental period and to ensure a linear relationship between absorbance at 492 nm and cell number when analyzed by SRB assay.

Cytotoxicity assay

For the assay, cells were washed with magnesium-and calcium-free phosphate buffered saline (PBS) (Oxoid Ltd., UK). PBS was decanted and cells detached with 0.025% trypsin-EDTA (sigma). PBS was then added to a volume of 50 ml. The cell

Table 2. % yield of extracts and %survival of cancerous cells (lung adenocarcinoma cell line =CORL-23 and prostate cancer cell line =PC3) treated with extract at the concentration 50 µg/ml with exposure time of 72h ± SEM (n = 3)

Plant	solvent	code	% yied of extract	Cell line	
				COR L-23	PC3
<i>Bridelia ovata</i> Decne	Et	BO2	12.54	4.65±0.09	1.16±0.25
	W	BO1	15.84	107±0.17	117.82±1.98
<i>Curcuma zedoaria</i> (Berg) Roscoe	Et	CZ2	8.08	0.31±0.07	1.32±0.35
	W	CZ1	10.39	122.28±1.2	122.21±1.32
<i>Derris scandens</i> (Roxb.) Benth.	Et	DS2	9.14	0.04±0.10	42.42±1.04
	W	DS1	12.24	133.22±1.7	143.14±2.31
<i>Dioscorea membranacea</i> Pierre	Et	DM2	2.55	1.55±0.07	0.73±0.28
	W	DM1	24.9	131.23±1.7	137.27±3.6
<i>Drynaria quercifolia</i> Linn.	Et	DQ2	3.44	58.18±2.43	83.92±2.58
	W	DQ1	12.97	113.76±1.28	119.89±3.22
<i>Erythrophleum teysmannii</i> Craib	Et	ET2	2.02	7.39±0.85	98.95±2.30
	W	ET1	3.63	112.31±1.77	141.08±2.40
<i>Moringa oleifera</i> Lamk	Et	MO2	4.82	105.47±1.15	98.09±3.49
	W	MO1	10.28	128.32±1.77	126.66±2.79
<i>Nardostachys jatamansi</i> DC	Et	NJ2	2.73	37.27±0.99	24.73±1.33
	W	NJ1	6.93	114.12±1.81	133.63±3.47
<i>Rhinacanthus nasutus</i> (L.) Kurz.	Et	RN2	4.88	3.44±0.33	2.17±0.61
	W	RN1	16.58	124.23±8.27	140.57±2.74
<i>Sapindus rarax</i> DC.	Et	SR2	33.3	75.87±5.06	32.93±1.19
	W	SR1	30.2	115.0±1.56	124.66±2.01
<i>Smilax corbularia</i> Kunth	Et	SM2	12.05	101.93±2.36	124.86±2.12
	W	SM1	8.25	98.94±0.82	126.62±2.9
<i>Strychnos nux-vomica</i> L.	Et	SN2	635	94.34±1.14	103.29±2.48
	W	SN1	9.35	117.52±1.45	125.01±2.46

Et = ethanolic extract, W = water extract, n = 3 (n = Number of independent experiment which was performed in 6 replicates), SEM = Standard error mean.

pellets, obtained by centrifugation (1000g, 5 min) were resuspended in 10 ml of medium to make single cell suspension. Viable cells were counted by trypan blue exclusion using haemocytometer and diluted with medium to give a final concentration of 1×10^4 , 1×10^4 and 4×10^4 cells/ml for CORL-23, PC3 and 10FS respectively). Volumes 100 µl/well of these cell suspensions were seeded in 96-well microtiter plates and incubated to allow cell attachment. After 24h the cells were treated with the extracts or pure compounds. Each extract was

initially dissolved in DMSO for ethanolic extracts and Vinblastine sulphate (Sigma, Lot No.34H0447) and in sterile distilled water for water extracts. The first screening was 50 µg/ml of each extract, which was tested against all cancer cells and the result recorded as the percentage cell survival less than 50% at exposure time of 72 hours. Those extracts were evaluated as active. The active extracts were further serially diluted in medium to produce 8 concentrations and 100 µl/well of each concentration was added to the plates in 6 replicates to obtain

final concentrations of 0.1, 0.5, 1, 5, 10, 25, 50, 100 µg/ml for extract and 0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100 nM for vinblastine sulphate (a positive control of cancer cell). The final mixture used for treating the cell contained not more than 1% of the solvent, the same as in solvent control wells. The plates were incubated for selected exposure times of 72 hours. At the end of exposure time, the medium was removed. The wells were then washed with medium, and 200 µl of fresh medium were added. The plates were incubated for a recovery period of 6 days, and cell numbers analyzed by SRB assay. Three replicate plates were used to determine the cytotoxicity of each extract.

Sulphorhodamine B (SRB) assay

The antiproliferative assay, SRB (sulphorhodamine B) assay, performed according to the method of Skehan *et al.* (1990), was used to assess growth inhibition. This colorimetric assay estimates cell number indirectly by staining total cellular protein with the dye SRB (Skehan *et al.*, 1990). For the assay, cells were fixed by layering 100 µl of ice-cold 40% trichloroacetic acid (TCA, Aldrich Chemical) on top of the growth medium. Cells were incubated at 4°C for 1 hour, after which plates were washed five times with cold water, excess water drained off and the plates left to dry in air. SRB stain (50 µl; 0.4% in 1% acetic acid) (Sigma) was added to each well and allowed to be in contact with the cell for 30 minutes. Subsequently, to remove excess dye, they were washed with 1% acetic acid, rinsed 4 times until only dye adhering to the cells was left. The plates were dried and 100 µl of 10 mM Tris base [tris (hydroxy methyl) aminomethane, pH 10.5] (Sigma,) was added to each well to solubilise the dye. The plates were shaken gently for 20 minutes on a gyratory shaker. The absorbance (OD) of each well (6 replicate) was read on a Power Wave X plate reader (Bio-TEK instrument, Inc.) at 492 nm. Cell survival was measured as the percentage of the absorbance compared with the control (non-treated cells). The IC₅₀ values were calculated from the Prism program obtained by plotting the percentage of surviving cells versus the concentrations, interpolated by

cubic spline. According to National Cancer Institute guidelines (Boyd, 1997) the extracts with IC₅₀ values < 20 µg/ml were considered "active". The IC₅₀ of active plants against cancer cells were compared with IC₅₀ of normal cells and calculated by the student t-test using InStat program

Results and Discussion

Table 1 shows the ethnobotanical data of the investigated plant species, which include botanical name and popular use which correlate with cancer treatment in Thai traditional medicine as well as the plant parts employed in this study. The information sources as well as citation index for selected plants are also summarized. The percentage yields of plant extracts and the results of cytotoxicity evaluation of all plant extracts at 50 µg/ml concentration and exposure time 72 hours are also summarized in Table 2. This data showed that the water extract of all plant species exhibited no cytotoxic activity against the two cancer cell lines and normal cell (Table 3). The ethanolic extract of four plants showed cytotoxicity activity against lung and prostate cancer cells according to the American National Cancer Institute (NCI) (IC₅₀ < 20 µg/ml for crude extract) (Boyd, 1997). The ethanolic extract of *Dioscorea membranacea* rhizomes was the most cytotoxic against PC3 (% survival of cancer cell line on 50 µg/ml concentration at exposure time 72 hours was 0.73) and the ethanolic extract of *Curcuma zedoaria* against lung cancer CORL-23 (% survival at 50 µg/ml was 0.31). Studies of making dilutions of plant extracts (Table 3) showed that IC₅₀ of ethanolic extract of *Dioscorea membranacea* (DM2) showed the highest activity and *Curcuma zedoaria* rhizome (CZ2) showed the second most effective activity against the lung cancer cell line. They exhibited specific activity against lung cancer cells which was higher than prostate cancer cells but less active than normal cell. IC₅₀ of DM2 against CORL-23, PC3 and 10FS were 4.63, 17.55 and 66.05 µg/ml respectively, and IC₅₀ of CZ2 were 6.05, 17.84 and 55.50 µg/ml respectively. The ethanolic extract of *Rhinacanthus nasutus* root (RN2) showed the highest cytotoxic

Table 3. Cytotoxicity activity (IC₅₀ µg/ml±SEM) of plant extracts against two types of cancer cell (COR L-23, PC3) and one type of normal cells (10FS) with exposure time 72 hours and n=3

Plant	solvent	code	Cell line		
			10FS	COR L-23	PC3
<i>Bridelia ovata</i> Decne	Et	BO2	9.11±0.58	7.11±0.004(1.28)	6.29±0.59(1.45)
	W	BO1	>100	>50	>50
<i>Curcuma zedoaria</i> (Berg) Roscoe	Et	CZ2	55.50±1.32	6.05±0.40(9.17)	17.84±0.36(3.11)
	W	CZ1	>100	>50	>50
<i>Derris scandens</i> (Roxb.) Benth.	Et	DC2	32.98±0.07	21.04±0.57(1.57)	43.45±3.60(0.76)
	W	DS1	>100	>50	>50
<i>Dioscorea membranacea</i> Pierre	Et	DM2	66.05±1.25	4.63±0.20(14.27)	7.55±1.98(3.76)
	W	DM1	>100	>50	>50
<i>Drynaria quercifolia</i> Linn.	Et	DQ2	>100	>50	>50
	W	DQ1	>100	>50	>50
<i>Erythrophleum teysmannii</i> Craib	Et	ET2	>100	37.76±0.01	>50
	W	ET1	>100	>50	>50
<i>Moringa oleifera</i> Lamk.	Et	MO2	>100	>50	>50
	W	MO1	>100	>50	>50
<i>Nardostachys jatamansi</i> DC.	Et	NJ2	>100	45.7±0.70	22.97±1.89
	W	NJ1	>100	>50	>50
<i>Rhinacanthus nasutus</i> (L.) Kurz.	Et	RN2	10.95±2.46	5.05±0.25(2.17)	2.01±0.58 (5.45)
	W	RN1	>100	>50	>50
<i>Sapindus rarak</i> DC.	Et	SR2	>100	37.02±1.21	44.34±1.44
	W	SR1	>100	>50	>50
<i>Smilax corbularia</i> Kunth	Et	SM2	>100	>50	>50
	W	SM1	>100	>50	>50
<i>Strychnos nux-vomica</i> L.	Et	SN2	>100	>50	>50
	W	SN1	>100	>50	>50

Et = ethanolic extract, W = water extract, n = 3 (n = Number of independent experiment which was performed in 6 replicates), SEM = Standard error mean. the numbers which were in () behind IC₅₀ value were the ratio of IC₅₀ (µg/ml)normal cells(10FS): IC₅₀ (µg/ml) cancer cells of extracts at exposure time 72 h.

activity against prostate cancer (IC₅₀ =2.01 µg/ml) and the ethanolic extract of *Bridelia ovata* leaves (BO2) was second in effectiveness against prostate cancer cells (IC₅₀ =6.29 µg/ml). Although RN2 and BO2 exhibited the most activity against prostate cancer, they also showed activity against lung cancer cell lines and normal cell (Table 3). This indicates that they have nonspecific cytotoxic activity because they killed all types of cancer and normal cells.

The comparison between ratio of normal cell: lung cell found that the ethanolic extract of *Dioscorea membranacea* and *Curcuma zedoaria* had the highest difference ratio (14.27, 9.17 respectively) (Table 3) and P-value was extremely significant (p<0.0001) calculated by student t-test from Prism program. The ratio of normal cell: prostate cancer cell of the ethanolic extract of *Rhinacanthus nasutus* exhibited the highest ratio (5.45) (Table 3). This result suggested that the ethanolic

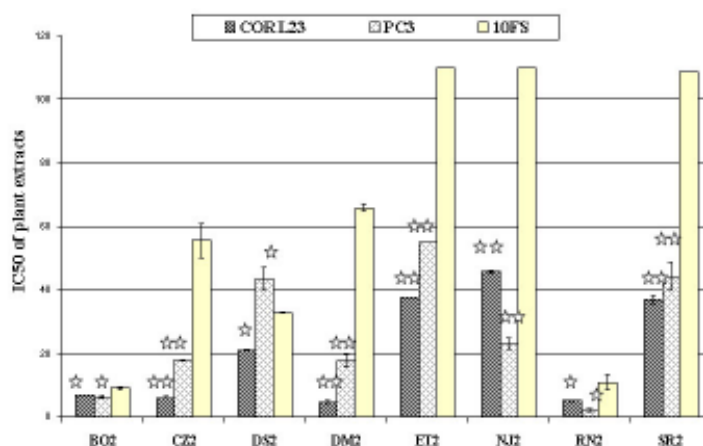


Figure 1. IC₅₀ (µg/ml) of active crude extracts against cell lines (n=3) with exposure time of 72h and used student t-test from Prism to compare significant difference between normal cell (10FS) and each cancer cell (CORL-23 and PC3) by* for P<0.05 and ** for P<0.0001. The alphabet of X axis are the ethanolic extract of *Bridelia ovata* (BO2), *Curcuma zedoaria* (CZ2), *Derris scandens* (DS2), *Dioscorea membranacea* (DM2), *Erythropheum teysmannii* (ET2), *Nardostachys jatamansi* (NJ2), *Rhinacanthus nasutus* (RN2), *Sapindus rarak* (SR2)

extracts of *Dioscorea membranacea* and *Curcuma zedoaria* were selectively toxic against lung cancer cells. This result indicated that *Dioscorea membranacea* and *Curcuma zedoaria* showed good results because they can kill cancer cells but do little damage to normal cell. These results are related with the objective of using the substance as cancer chemotherapy which they could kill cancer cells but do little damage to normal cells and should be selectively active (Halliwell and Gatteridge, 1988). Although the ethanolic extract of *Rhinacanthus nasutus* showed the highest ratio for comparison between normal and prostate cancer cell, it also showed high cytotoxic activity and was not selective of cells. This result is related with the previous investigation that found many compounds from *R. nasutus* against P388, HL-60, KB, HT-29 and A549 and all compounds killed all cells with no specific cytotoxic activity. Surprisingly, *Bridelia ovata* showed high cytotoxic activity against with all types of cell and has not been reported to have cytotoxic activity. The specific activity of cancer cells and less activity against normal cells will be first criterion for further investigation. Thus more emphasis should be given to the study of the

ethanolic extract of *Dioscorea membranacea* and *Curcuma zedoaria* than on the ethanolic extract of *Rhinacanthus nasutus*. However, nearly 50% of the ingredients in this formula showed cytotoxic activity according to the previous criteria of NCI on 1990 (IC₅₀ of the active plant extracts < 30 µg/ml) (Suffness and Pezzuto, 1990). These data could support using this traditional drug formula to treat cancer patients. From the previous data it was found that Dioscorealide B, isolated from the ethanolic extract of *Dioscorea membranacea*, also showed specific cytotoxic activity against lung cancer cell line CORL-23 (Itharat, *et al.*, 2004). This compound may be used as a marker for analysis of this preparation against lung cancer cells. However, there is no report that this compound exhibited cytotoxic activity against prostate cancer, and further phytochemical work on the isolation of this compound from *Dioscorea membranacea* is being proposed to test its cytotoxicity against prostate cancer cells. There is only one previous study for cytotoxic compounds isolated from *Curcuma zedoaria* against human ovarian cancer cell (Syu, *et al.*, 1998) but there is no report against human lung and prostate cancer. These results give

support to the use of these Thai folk medicine preparations for treating cancer patients (Itharat *et al.*, 1998). Moreover the results of three active plants (*Dioscorea membranacea*, *Curcuma zedoaria* and *Rhinacanthus nasutus*) are also related with folk medicine text books (Table 1). Some plants extracts, which were in this cancer preparation and had no cytotoxic activity, had been reported as having other activities related to cancer, such as *Smilax glabra* showed anti-inflammatory activities (Jiang *et al.*, 1997) and prevented immunological hepatocyte damage (Chen *et al.*, 1999), *Derris scandens* and *Strychnos nux-vomica* showed anti-inflammation and analgesic effects (Laupattarakasem *et al.*, 2003 and Yin *et al.*, 2003).

Conclusion

The result obtained in this work indicated that a third of the studied plants, which were the ingredients of Thai folk medicine to treat cancer patients, were active against cancer cells. The ethanolic extracts of *Dioscorea membranacea* rhizomes are the most cytotoxic against lung cancer cell line and showed no cytotoxicity against the normal cell line. The ethanolic extract of *Rhinacanthus nasutus* root showed the highest activity against prostate cancer and nonspecific cytotoxic activity because it could kill all cell types. These results support using this formula for treatment of cancer patients. Further studies are in progress involving the isolation of compounds from *Dioscorea membranacea* rhizome, *Curcuma zedoaria* rhizome, *Rhinacanthus nasutus* root and *Bridelia ovata* leaf extracts and testing them on the same cell lines.

Acknowledgements

The authors wish to thank three Thai traditional doctors; Dr.Prakob Ubongkhao Dr.Jon Phensuk and Dr.Pinkao Kungnuan for their assistance in the collection of these plants. We are grateful to Prince of Songkla University for financial support of Atima Saetung, Graduate student, project

and also give special thanks to Dr.Ureeporn Kajaroon for providing human fibroblast cells.

References

- Boonyaratanakornkit, L. and Chantaptavan, V. 1993. Identification and specification of Khao-Yen-Neua and Khao-Yen-Tai. Thai J. Pharm. Sci. 1: 79-90.
- Boonyaprapatsorn, N. and Chokchajaroenporn, A. 2000. Medicinal Plant. Medicinal Plant Data Center, Faculty of Pharmaceutical Sciences, Mahidol University, Bangkok.
- Boyd, MR. 1997. The NCI *in vitro* anticancer drug discovery screen. In Teicher, B.(Ed.).Anticancer drug development guide; preclinical screening, clinical trials and approval, Humana Press, Totowa, p.30.
- Cardellina II, J.H., Fuller, R.W., Gamble, W.R., Westergaard, C., Boswell, J., Munro, M.H.G., Currens, M., and Boyd, M.R. 1999. Evolving strategies for the selection, dereplication and prioritization of antitumor and HIV-inhibitory natural products extracts. In Bohlin, L., Bruhn, J.G.(Eds.) Bioassay Methods in Natural Product Research and Development, Kluwer Academic Publishers, Dordrecht, pp.25-36.
- Chen, T., Li, J., Cao, J., Xu, Q., Komatsu, K., and Namba, T. 1999. A new flavanone isolated from rhizome *Smilax glabra* and the structural requirements of its derivatives for preventing immunological hepatocyte damage. Planta Med., 65:56-9.
- Halliwell, B., and Gatteridge, J.M.C. 1988. Free radicals in biology and medicine 2Ed. Charendon Press, Oxford, pp 481.
- Itharat, A., Supavita, T., Jusanit, P., Singchangchai, P., Subchareon, P., Deevisad, G., Ubongkaw, P., Ratanasuwan, P., Kajpunyapong, V., and Muangsrinun, R. 1999. Survey of medicinal plants and local folk wisdom in Southern Thailand: case study at Talebun National Parks. Research Report, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, p82-115.
- Itharat, A., Supavita, T., Singchangchai, P., Kajarun, U., Ratanasuwan, P., Pongpeerachote, S., and Chantaptawan, V. 1999. Identification and specification of Khao-Yen-Neua and Khao-Yen-Tai from traditional doctors in Thailand. Prince of Songkla University, Songkla., p.9.

- Itharat, A., Houghton, P.J., Eno-Amoquaye, E., Burke, P.J., Sampson, J.H., and Ramam, A. 2004. *In vitro* cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. *J. Ethnopharm.* 90:33-38.
- Itharat, A., Singchangchai, P., and Ratanasuwan, P. 1998. Wisdom of Southern Thai traditional doctors. Research report of Prince of Songkla University, Songkhla, p.126.
- Itharat, A., Singchangchai, P., Subchareon, P., Supavita, T., Ubokaw, P., Ratanasuwan, P., Kummee, S., and Srisuchatum, P. 2002. Folk wisdom about treatment of traditional doctors in Southern Thailand: focus groups discussion. Research Report of Prince of Songkla University, Songkla, p.83.
- Jiang, J.Y., Wu, F.H., Lu, J.F., Lu, Z.H., and Xu, Q.N. 1997. Anti-inflammatory activities of aqueous extract from rhizome *Smilax glabra*. *Pharmaco. Res.* 36:309-314
- Keawpradub, N., Amooqyaye, E.E., Burke, P.J., and Houghton, P.J. 1999. Cytotoxic activity of indole alkaloids from *Alstonia macrophylla*. *Planta Med.* 65: 311-315.
- Keawpradub, N., Houghton, P.J., Amooqyaye, E.E., and Burke, P.J. 1997. Activity of extracts and alkaloids of Thai *Alstonia* species against human lung cancer cell lines. *Planta Med.* 63: 97-101.
- Laupattarakasem, P., Houghton, P.J., Hoult, J.R.S., and Itharat, A. 2003. An evaluation of the activity related to inflammation of four plants used in Thailand to treat arthritis. *J. Ethnopharm.*, 85: 207-215.
- Mathers, C.D., Boschi-Pinto, C., Lopez, A.D., and Murray, C.J.L. 2001. Cancer incidence, mortality and survival by site for 14 regions of the world. World Health Organization, p3.
- Matthes, H.W.D., Luu, B., and Ourisson, G. 1980. Cytotoxic components of *Zingiber zerumbet*, *Curcuma zedoaria* and *C. domestica*. *Phytochem.* 19: 2643-2650.
- Mau, J.L., Lai, E.Y.C., Wang, N.P., Chen, C.C., Chang, C.H. and Chyau, C.C. 2003. Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*. *Food Chem.* 82: 583-591.
- National Statistical Office. 2003. Key Statistics of Thailand 2003. Ministry of Information and Communication Technology, Bangkok.
- Pongboonrod, S. 1976. Thai medicinal plants p107-108, Bangkok, Kasambanakit Press.
- Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, I., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., and Boyd, M.R. 1990. New colorimetric cytotoxicity assay for anticancer drug screening. *J Natl. Cancer Inst.* 82:1107-1112.
- Smithinan, T. 2001. Thai plant names. The Forest Herbarium Royal Forest Department, Bangkok .
- Subchareon, P. 1998. Handbook of anticancer : Thai traditional medicine : new concept for treated cancer. Thai traditional medicine Institute, Bangkok, p-3.
- Suffness, M., and Pezzuto, J.M. 1990. Assays Related to Cancer Drug Discovery. In K Hostettmann (ed), *Methods in Plant Biochemistry*. Vol. 6. Assays for Bioactivity. Academic Press, London, pp.71-133.
- Syu, W.J., Shen, C.C., Don, M.J., Ou, J.C., Lee, G.H. and Sun, C.M. 1998. Cytotoxicity of curcuminoids and some novel compounds from *Curcuma zedoaria*. *J. Nat. Prod.* 61:1531-1534.
- Vimolkhunakorn. 1979. Medicinal plant drug formula., Wat Phikul Ngen, Nonthaburi, p.7-10
- Wu, T.S., Hsu, H.C., Wu, P.L., Teng, C.M. and Wu, Y.C. 1998. Rhinacanthin-Q, A naphthoquinone from *Rhinacanthus nasutus* and its biological activity. *Phytochem.* 49: 2001-2003.
- Wu, T.S., Tien, H.J., Yeh, M.Y., and Lee, K.H. 1988. Isolation and cytotoxicity of rhinacanthin-A and B, two naphthoquinone from *Rhinacanthus nasutus*. *Phytochem.* 27: 3787-3788.
- Yin, W., Wang, T.S., Yin, F.Z., and Cai, B.C. 2003. Analgesic and anti-inflammatory properties of brucine and brucine N-oxide extracted from seeds of *Strychnos nux-vomica*. *J. Ethnopharm.* 88: 204-205.