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Cytotoxic activity of Thai medicinal plants for cancer treatment

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Abstract

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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 IC_{50}. The ethanolic extracts of six plants (*Bridelia ovata, Curcuma zedoaria*, *Derris scandens*, *Dioscorea membranacea*, *Nardostachys jatamansi* and *Rhinacanthus nasutus*) showed cytotoxic activity (IC_{50}< 30 µg/ml) against lung and prostate cancer cell lines. *Dioscorea membranacea* roots showed the highest cytotoxic activity against lung cancer cell lines (IC_{50}= 4.6 µg/ml) but it exhibited low cytotoxic activity against prostate cancer cell lines (IC_{50}= 17.55 µg/ml) and less cytotoxic activity against normal cell lines (IC_{50}= 66.05 µg/ml). *Curcuma zedoaria* showed cytotoxic activity against COR L-23 and PC3 but less cytotoxic activity against 10FS (IC_{50} = 6.05, 17.84 and 55.50 µg/ml respectively) *Rhinacanthus nasutus* root extract showed the highest cytotoxic activity against PC3 (IC_{50} = 2.01 µg/ml) and this extract also showed high activity against COR L-23 and 10FS (IC_{50}=5.05 and 10.95 µg/ml respectively). The water extract

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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

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การศึกษาฤทธิ์ต้านเซลล์มะเร็งของสมุนไพรไทยที่ใช้ในการรักษาโรคมะเร็ง

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

[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 (Itharat *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 μg/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 adencarcinoma (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 μg/ml streptomycin (Keawpradub *et al.,* 1997). The human fibroblast cell, which is a

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

Species (Family)	Places for specimen collection (Amphor, Province)	Voucher specimen number	Common name*	Plant part	Use in Thai traditional medicine
Bridelia ovata Decne (Euphorbiaceae)	Hat Yai, Songkhla	SKP0710205	Maka[a,b]	lf	Cathartic (5) antipyretic (3)
Curcuma zedoaria (Berg) Roscoe (Zingiberaceae)	Muang, Yala	SKP2060326	Kaminooi[a,b]	rh	Cancer (1,3,5)
Derris scandens (Roxb.) Benth (Papilionaceae)	Chana, Songkhla	SKP1410419	Thaowanpreing[a,b]	st	Anti-inflammation of bone and joint, analgesic(5)
Dioscorea membranacea Pierre (Dioscoreaceae)	Na Thawi, Songkhla	SKP0620413	Man mu[a] Hua-Khao-Yen[b]	rh.	Cancer (1,4)
Drynaria quercifolia Linn. (Polypodiaceae)	Chana, Songkhla	SKP1520417	Huawaw[a,b]	rh	Diuretic (3)
Erythrophleum teysmannii Craib (Caesalpiniaceae)	Muang, Nakhon Ratchasima	SKP0340520	Sark[a,b]	st	Antipyretic (5)
Moringa oleifera Lamk. (Moringaceae)	Hat Yai, Songkhla	SKP1181315	Marum[a,b]	b	Antipyretic, cardiotonic (3)
Nardostachys jatamansi DC (Valerianaceae)	-	SKP2011410	Kodchadamansi[a,b]	fl	Abscess (3)
Rhinacanthus nasutus (L.) Kurz. (Acanthaceae)	Hat Yai, Songkhla	SKP0011814	Thongpunchang[a,b]	r	Cancer (1,3,5)
Sapindus rarak DC. (Sapindaceae)	Muang, Krabi	SKP1701918	Makumdeecwai[a,b]	fr	Decrease toxic (5)
Smilax corbularia Kunth (Smilacaceae)	Wichian Buri, Phetchabun	SKP1791903	Khao-yen-neua[a,b]	rh.	Cancer (1,2,3)
Strychnos nux-vomica 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 Vimonkunakorn, 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% CO_2 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 ($1x10^3$, $1x10^3$ and $4x10^3$ 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
Bridelia ovata Decne	Et	BO2	12.54	4.65±0.09	1.16±0.25
	W	BO1	15.84	107±0.17	117.82±1.98
Curcuma zedoaria (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
Derris scandens (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
Dioscorea membranacea 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
Drynaria quercifolia 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
Erythrophleum teysmannii 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
Moringa oleifera 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
Nardostachys jatamansi 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
Rhinacanthus nasutus (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
Sapindus rarax 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
Smilax corbularia 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
Strychnos nux-vomica 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 $1x10^4$, $1x10^4$ and $4x10^4$ 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 ml 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 IC_{50} values were calculated from the Prism program obtained by plotting the percentage of surviving cells versus the concentrations, interpolated by cubic spine. According to National Cancer Institute guidelines (Boyd, 1997) the extracts with IC_{50} values < 20 µg/ml were considered "active". The IC_{50} of active plants against cancer cells were compared with IC_{50} 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 Institue (NCI) (IC_{50} <20 µg/ml for crude extract) (Boyde, 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 IC_{50} 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. IC_{50} of DM2 against CORL-23, PC3 and 10FS were 4.63, 17.55 and 66.05 µg/ml respectively, and IC_{50} 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 (IC_{50} 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
Bridelia ovata Decne	Et	BO2	9.11±0.58	7.11±0.004(**1.28**)	6.29±0.59(**1.45**)
	W	BO1	>100	>50	>50
Curcuma zedoaria (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
Derris scandens (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
Dioscorea membranacea Pierre	Et	DM2	66.05±1.25	4.63±0.20(**14.27**)	7.55±1.98(**3.76**)
	W	DM1	>100	>50	>50
Drynaria quercifolia Linn.	Et	DQ2	>100	>50	>50
	W	DQ1	>100	>50	>50
Erythrophleum teysmannii Craib	Et	ET2	>100	37.76±0.01	>50
	W	ET1	>100	>50	>50
Moringa oleifera Lamk.	Et	MO2	>100	>50	>50
	W	MO1	>100	>50	>50
Nardostachys jatamansi DC.	Et	NJ2	>100	45.7±0.70	22.97±1.89
	W	NJ1	>100	>50	>50
Rhinacanthus nasutus (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
Sapindus rarak DC.	Et	SR2	>100	37.02±1.21	44.34±1.44
	W	SR1	>100	>50	>50
Smilax corbularia Kunth	Et	SM2	>100	>50	>50
	W	SM1	>100	>50	>50
Strychnos nux-vomica 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 IC_{50} value were the ratio of IC_{50} (µg/ml)normal cells(10FS): IC_{50} (µg/ml) cancer cells of extracts at exposure time 72 h.

activity against prostate cancer (IC_{50} =2.01 µg/ml) and the ethanolic extract of *Bridelia ovata* leaves (BO2) was second in effectiveness against prostate cancer cells (IC_{50} =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

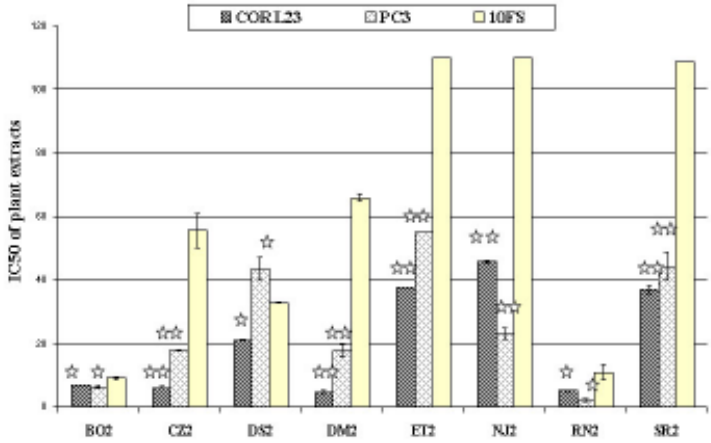

Figure 1. IC_{50} (µg/ml) of active crude extracts against cell lines (n=3) with exposure time of 72h and used student t-test from Prism to compare significant difference between normal cell (10FS) and each cancer cell (CORL-23 and PC3) by* for P<0.05 and ** for P<0.0001. The alphabet of X axis are the ethanolic extract of *Bridelia ovata* (BO2), *Curcuma zedoaria* (CZ2), *Derris scandens* (DS2), *Dioscorea membranacea* (DM2), *Erytropheum 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 (IC_{50} 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 activites (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 result 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.

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