In Vitro Cytotoxic Activity of Benjakul Herbal Preparation and Its Active Compounds against Human Lung, Cervical and Liver Cancer Cells

Srisopa Ruangnoo MSc*, Arunporn Itharat PhD*,
Intouch Sakpakdeejaroen MSc*, Ruchilak Rattarom MSc**,
Pimonwan Tappayutpijarn MSc*, Kumman Kumarn Pawa MD***

** Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand*
*** Graduate School, Faculty of Medicine, Thammasat University, Pathumthani, Thailand*
**** Department of Medicine, Faculty of Medicine, Thammasat University, Pathumthani, Thailand*

Background: *Benjakul [BEN], a Thai Traditional medicine preparation, is composed of five plants: Piper chaba fruit [PC], Piper sarmentosum root [PS], Piper interruptum stem [PI], Plumbago indica root [PL] and Zingiber officinale rhizome [ZO]. From selective interviews of folk doctors in Southern Thailand, it was found that Benjakul has been used for cancer patients.*

Objective: *To investigate cytotoxicity activity of Benjakul preparation [BEN] and its ingredients against three human cancer cell lines, large lung carcinoma cell line (COR-L23), cervical cancer cell line (Hela) liver cancer cell line (HepG2) as compared with normal lung fibroblast cell (MRC-5) by using SRB assay.*

Material and Method: *The extraction as imitated the method used by folk doctors was done by maceration in ethanol and boiling in water. Bioassay guided isolation was used isolated cytotoxic compound.*

Results: *The ethanolic extracts of PL, ZO, PC, PS, BEN and PS showed specific activity against lung cancer cell (IC_{50} = 3.4, 7.9, 15.8 , 18.4, 19.8 and 32.91 μg/ml) but all the water extracts had no cytotoxic activity. Three active ingredients [6-gingerol, plumbagin and piperine as 0.54, 4.18 and 7.48% w/w yield of crude extract respectively] were isolated from the ethanolic extract of BEN and they also showed cytotoxic activity with plumbagin showing the highest cytotoxic activity against COR-L23, HepG2, Hela and MRC-5 (IC_{50} = 2.55, 2.61, 4.16 and 11.54 μM respectively).*

Conclusion: *These data results may support the Thai traditional doctors who are using Benjakul to treat cancer patients and three of its constituents (6-gingerol, plumbagin and piperine) are suggested to be used as biomarkers for standardization of this preparation.*

Keywords: *Cytotoxicity test, SRB assay, Thai medicinal plants, Benjakul preparation, Lung cancer, Liver cancer, Cervical cancer*

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Cancer is the leading cause of death in Thailand. Many Thai people still use traditional medicine as an alternative for cancer treatment[1]. The investigation of indigenous wisdom on cancer treatment by Thai traditional doctors as report by Itharat et al[2] revealed that a Benjakul preparation, which is composed of five Thai medicinal plants (*Piper chaba* Linn., *Piper sarmentosum* Roxb., *Piper interruptum* Opiz., *Plumbago indica* Linn. and *Zingiber officinale* Roscoe.) has been used as an adaptogen drug for cancer patients. Folk doctors would give Benjakul to cancer patients for 2 or 3 weeks before the treatment by cancer drugs, believing that the preparation can be an element balancing in the patient's body or increase their immunity. Benjakul extracts showed no toxicity and no effect on animal tissue when tested by a sub-chronic toxicity method[3]. Surprisingly, although this preparation is commonly used before drug treatment of many diseases in Thai traditional medicine, no research is reported into testing its cytotoxic activity against cancer and normal cells. There is only one study of testing the ethanolic extract of *Piper chaba* for cytotoxicity against cancer cells *i.e.* human lymphocytes, ovarian cells from Chinese hamster and

Correspondence to:
Itharat A, Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasart University, Klong Luang, Pathumthani 12120, Thailand.
Phone: 0-2926-9749, Fax: 0-2926-9705
E-mail: iarunporn@yahoo.com

lymphoma Dalton's cell (IC_{50} = 0.13, 0.145 and 0.3 µg/ml respectively)[4]. In the present study, the five Thai medicinal plant extracts which are ingredients of Benjakul formula and Benjakul preparation were tested for their cytotoxic activity against large cell lung carcinoma (COR-L23), liver cancer (HepG2), cervical cancer (Hela) and human lung fibroblast cells (MRC-5). The comparison of cytotoxic activity against cancer cells and normal cells was discussed. These results also support the use by folk doctors to treat cancer patients with Benjakul formula and the plants used in its preparation.

Method and Material

Plant material

Plants which have been recorded to be used against anticancer by folk doctors in Thailand, were collected from different location of Thailand during January to March 2006 as follows: *Piper chaba* fruit (Thongphaphoom, Kanjanaburee), *Piper sarmentosum* root (Hatyai, Sonkhla), *Piper interruptum* stem (Maerim, Chaingmai), *Plumbago indica* root (Bankoknoi, Bangkok), *Zingiber officinale* rhizome (Khaokho, Petchaboon). Authentication of plant materials was carried out at the herbarium of the Department of Forestry Bangkok, Thailand where herbarium vouchers have been kept. Another set of specimens were kept in the herbarium of Southern Center of Thai Medicinal Plants at Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla, Thailand and the relevant voucher numbers are shown in Table 1.

Preparation of plant extracts

Plant material was dried at 50°C in an oven, powdered and extracted in ways analogous to those practiced by Thai traditional doctors, *e.g.* water extraction and ethanolic extraction. For the water extract, dried ground plant material (100 g) was boiled for 30 minutes in distilled water (300 ml), filtered and freeze dried. For the alcoholic extract, dried plant powder (100 g) was percolated with 95% ethanol and concentrated to dryness under reduced pressure. The percentage yields are shown in Table 2. The water extracts were dissolved in sterile water and the ethanolic extracts were dissolved in DMSO and all the stock solutions were filtered by sterile filter paper (0.2 mm) before testing.

Isolation and purification of active ingredients

An aliquot of the ethanolic extract of Benjakul (40 g) was separated by vacuum liquid chromatography

Table 1. The ethnobotanical use of five plants which component of Benjakul as adaptogen for cancer patients

Species (Family)	Places for specimen collection (Amphor, Province)	Voucher specimen number	Common name [a]	Plant part	Use in Thai traditional medicine[b]
Piper chaba Linn. Piperaceae	Thongphaphoom, Kanjanaburee	SKP 146160301	Deeplee	fr	The herb for earth element and used for cancer and dyspepsia
Piper sarmentosum Roxb. Piperaceae	Hadyai, Songkhla	SKP 146161901	Chaplu	lf	The herb for water element and used for expectorant
Piper interruptum Opiz. Piperaceae	Maerim, Chaingmai	SKP146160901	Sakan	st	The herb for wind element and used for cerebral blood flow, anti-inflam mation of bone and joint.
Plumbago indica Linn. Plumbaginaceae	Bankoknoi, Bangkok	SKP148160901	Jedtamunplengdang	rt	The herb for fire element used for blood tonic and infectious disease
Zingiber officinale Roscoe. Zingiberaceae	Khaokho, Petchaboon	SKP206261501	Khing	rh	The herb for air element used for dyspepsia

Plant parts : fr = fruit, lf = leave, st = stem , r = root , rh = rhizome
References (a) Smitinand, 2001 [5] (b) Pongboonrod, 1979[6]

Table 2. Cytotoxicity activity (IC_{50} µg/ml ± SEM) of plant extracts against four types of cancer cell lines (Hela, HepG2, and COR-L23) and one type of normal cell line (MRC-5) at exposure time 72 hrs (n = 3)

Plant Species	Part	Extracts	Code	%yield	Cytotoxicity activity (IC_{50} µg/ml ± SEM)			
					Hela	HepG2	COR-L23	MRC-5
Piper chaba Linn.	Fruit	EtOH	PCE	12.3906	47.72 ± 2.11	34.54 ± 0.20	15.82 ± 0.80	91.71 ± 0.50
		Water	PCW	15.8965	>100	>100	>100	>100
Piper sarmentosum Roxb.	Root	EtOH	PSE	1.7449	69.35 ± 3.36	72.27 ± 0.9	32.91 ± 1.71	>100
		Water	PSW	8.5603	>100	>100	>100	>100
Piper interruptum Opiz.	Stem	EtOH	PIE	0.6596	28.65 ± 1.10	26.12 ± 0.65	18.40 ± 0.61	34.44 ± 1.61
		Water	PIW	5.0254	>100	>100	>100	>100
Plumbago indica Linn.	Root	EtOH	PLE	5.0071	8.71 ± 0.40	33.22 ± 0.24	3.43 ± 1.93	70.04 ± 2.16
		Water	PLW	20.3808	>100	>100	>100	>100
Zingiber officinale Roscoe.	Rhizome	EtOH	ZOE	8.5651	37.29 ± 0.23	51.63 ± 0.31	7.90 ± 1.62	83.45 ± 5.37
		Water	ZOW	13.7434	>100	>100	>100	>100
Benjakul preparation	-	EtOH	BENE	7.7332	47.72 ± 2.11	45.58 ± 1.26	19.80 ± 1.89	48.95 ± 0.34
		Water	BENW	16.2897	>100	>100	>100	>100

n = number of independent experiment which was performed in 3 replicates

(VLC), using hexane (10 x 200 ml), hexane:chloroform (10 x 200 ml), chloroform (10 x 200 ml), chloroform: MeOH (1:1) (10 x 200 ml), MeOH (10 x 200 ml). All of eluents were dried and evaporated into fractions yielding residues of 0.22, 0.46, 5.61, 21.72 and 6.26 g, denoted as FA, FB, FC, FD and FE respectively. These five fractions were tested for cytotoxic activity against the COR-L23 lung cancer cell line by the SRB assay because it was found that Benjakul preparation showed the highest cytotoxicity against that line. Results are given in Table 3. The result was found that FC showed the highest cytotoxic activity.

An aliquot (2 g) of fraction FC was separated by column chromatography (CC) on silica gel with a gradient of solvents, hexane:EtOAc (8:2); (350 ml); hexane:EtOAc (7:3) (100 ml); hexane:EtOAc (7:3) (100 ml); hexane:EtOAc (6:4) (200 ml); hexane:EtOAc (1:1) (200 ml); EtOAc:hexane (2:8) (300 ml), EtOAc (200 ml); EtOAc:MeOH (9.5:0.5) (200 ml), EtOAc:MeOH (9:1) (200 ml); EtOAc:MeOH (1:1) (200 ml) and finally MeOH (300 ml) respectively. Ten ml fractions were collected for each eluting solvent and the fractions were combined following TLC examination [silica gel/$CHCl_3$:MeOH (7:3)] using acidic anisaldehyde spray for detection. Compound 1 (158.5 mg, 7.81% w/w) was isolated from FC before loading onto and also from EtOAc:hexane (2:8) as light yellow crystals, further recrystallized from MeOH. Compound 2 (74.9 mg, 4.18% w/w) was obtained as orange crystals by crystallization from MeOH after isolation from fractions 12-31. Compound 3 (9.6 mg, 0.54% w/w) was a pale yellow oil isolated from fractions 66-73.

Structure elucidation

The structure of the isolates (Fig. 1) was determined by their NMR data [^1H and ^{13}C] on a Varian Unity Inova 500 spectrometer (500 MHz for ^1H; 125

Table 3. IC_{50} (µg/ml) ± SEM of the fractions from the ethanolic extract of Benjakul preparation separated by vacuum liquid chromatography against COR-L23 at exposure time 72 hours (n = 3)

Fraction	%Yield	IC_{50} ± SEM (µg/ml) COR-L23
FA (Hexane)	0.54	50.12 ± 1.54
FB (Hexane:$CHCl_3$ 1:1)	1.15	20.55 ± 2.25
FC ($CHCl_3$)	14.03	7.38 ± 0.21
FD ($CHCl_3$:MeOH 1:1)	54.30	62.81 ± 2.02
FE (MeOH)	15.65	>100

MHz for ^{13}C)], UV spectra [a Hewlett Packard 8452A Diode array spectrometer], IR spectra [Jasco IR-810 spectrometer], EI mass spectra, Low resolution were obtained from a JEOL JMS-AX505W spectrometer.

Compound 1 (Piperine): $C_{17}H_{19}NO_3$ (158.5 mg, 7.81% w/w); light yellow needle crystal solids; EIMS (low resolution) *m/z* (% relative intensity) 285 (M^+, 75), 201 (100), 173 (19), 143 (17), 115 (45). Compound 1 was the major compound isolated from the ethanolic extract of Benjakul preparation. This compound was compared with authentic sample of piperine (Merck) by TLC using 3 solvent systems and gave identical behavior. The ^{1}H-NMR spectrum, compared with the previous ^{1}H-NMR data of piperine, was the same as the spectrum recorded for piperine[7]. Thus compound 1 was identified as piperine, whose structure is shown in Fig. 1.

Compound 2 (Plumbagin): $C_{11}H_8O_3$ (74.9 mg, 4.18% w/w); orange needle crystal solid; EIMS (low resolution) *m/z* (% relative intensity) 188 (M^+, 100), 131 (54), 81 (62), 69 (98). Compound 2 was isolated from the ethanolic extract of Benjakul preparation. This compound was compared with authentic sample of plumbagin (Sigma) by TLC using 3 solvent systems and gave identical behavior. Its ^{1}H-NMR spectrum, compared with previous ^{1}H-NMR data of plumbagin, was the same[8]. Thus, compound 2 was identified as plumbagin, whose structure is shown in Fig. 1.

Compound 3 (6-gingerol): $C_{17}H_{26}O_4$ (9.6 mg, 0.54% w/w); a pale yellow oil; EIMS (low resolution) *m/z* (% relative intensity) 294 (M^+, 50), 150 (55), 137 (100). Compound 3 was isolated from the ethanolic extract of Benjakul preparation. This compound was compared with an authentic sample of 6-gingerol (Wako) by TLC using 3 solvent systems and gave identical behavior. The ^{1}H-NMR spectrum was the same as the spectrum recorded for 6-gingerol[9]. Thus compound 3 was identified as 6-gingerol, whose structure is shown in Fig. 1.

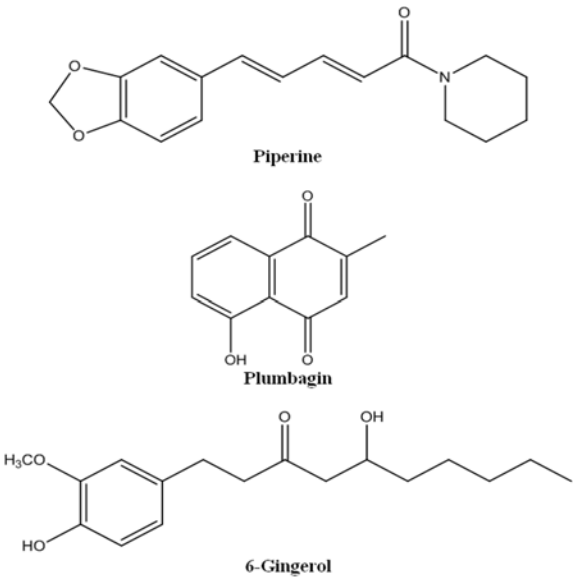

Fig. 1 Structure of the isolated compounds from ethanolic extract of Benjakul preparation

In vitro assay for cytotoxic activity

Human cell lines

Three different kinds of human cancerous cell lines *i.e* large cell lung carcinoma (COR-L23), human cervical cancer (HeLa) and liver cancer (HepG2) and one type of normal cell line *i.e.* human fibroblast cell line (MRC-5). COR-L23 cells established and kindly provided by Dr. Pintusorn Harnsakul, Faculty of Medicine, Thammasat University, were cultured in RPMI 1640 medium supplement with 10% heated foetal bovine serum, 1% 2 mM L-glutamine, 50 IU/ml penicillin and 50 mg/ml streptomycin. Hela and HepG2 cell lines were obtained from National Cancer Institute of Thailand and were cultured in Minimum Essential Media (MEM) with Earle Salt without glutamine medium supplement with 10% heated foetal bovine serum, 1% 2 mM L-glutamine, 50 IU/ml penicillin and 50 mg/ml streptomycin and hepes. The cell lines were maintained at 37°C in a 5% CO_2 atmosphere with 95% humidity The MRC-5 cell line was kindly provided by Professor Houghton, King's College, London, UK 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 were determined as 1 x 10^3, 3 x 10^3, 3 x 10^3 and 5 x 10^3 cells/well for COR-L23, Hela, HepG2 and MRC-5 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 buffer saline (PBS) (Oxoid Ltd., UK) PBS was decanted and cells detached with 0.025% trypsin-EDTA (Sigma). PBS was added to a volume of 50 ml and centrifuged. The cell pellet obtained by centrifugation (1,000 g, 5 min) was resuspensed in 10 ml of medium to make a single cell suspension and viable cells were counted by Trypan Blue exclusion in haemocytometer and diluted with

medium to give a final concentration of 1 x 10^3, 3 x 10^3, 3 x 10^3 and 5 x 10^3 cells/well for COR-L23, HeLa, HepG2 and MRC-5 respectively. 100 µl/well of these cell suspensions were seeded in 96-well microtiter plates and incubated to allow for cell attachment. After 24 h the cells were treated with the extracts or pure compounds. Each extract was initially dissolved in an amount of DMSO for ethanolic extracts and sterile distilled water for water extracts and vinblastine sulphate (Sigma, Lot No. 34H0447). The extracts were 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 and 100 µg/ml for extract and 0.05, 0.1, 0.5, 1,5, 10, 25, 50, 100 nM for vinblastine sulphate (the positive control cytotoxic substance). The final mixture used for treating the cell contained not more than 1% of the solvent, the same as in the solvent control wells. The plates were incubated for 72 hours as indicated. At the end of each exposure time, the medium was removed. The wells were then washed with medium and 200 ml of fresh medium were added. The plates were incubated for recovery period of 3 days and cell number was analyzed by SRB assay.

Sulphorhodamine B (SRB) assay

The anti-proliferative assay, SRB (sulphorhodamine B) assay, was performed according to 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[10]. The plates were dried and 100 ml of 10 mM Tris base [tris (hydroxy methyl) aminomethane, pH10.5] (Sigma) 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 replicates) was read on on a Power Wave X plate reader (Bio-TEK instrument, Inc.) at 492 nm is an indication of cell number. Cell survival was measured as the percentage absorbance compared the control (non-treated cells). The IC_{50} values were calculated from the Prism program obtained by plotting the percentage of survival versus the concentrations, interpolated by cubic spine. According to National Cancer Institute guidelines[11] extracts with IC_{50} values < 20 µg/ml were considered active.

Results and Discussion

Table 1 shows the ethnobotanical data of the investigated plant species, which include botanical name and popular use in Thai traditional medicine as well as the plant parts employed in the present study. The information sources as well as citation index for selected plants are also summarized. Percentage of yields of plant extracts are shown in Table 2 and the results of cytotoxicity evaluation of all plant extracts as IC_{50} (µg/ml) at exposure time 72 hours are also summarized in Table 2 and Fig. 2. This data showed that none of the water extracts of five plants and Benjakul showed any cytotoxic activity (IC_{50} > 100 µg/ml). The ethanolic extract of Benjakul preparation gave the strongest effect against lung cancer cells (IC_{50}= 19.8 µg/ml) but less cytotoxicity towards normal lung cells (IC_{50}= 48.9 µg/ml). The different ratio between IC_{50} of lung cancer and IC_{50} of normal lung cell was 2.5 times. Four constituent plants of Benjakul *i.e.* PLE, ZOE, PCE and PIE showed high activity against lung cancer cells (IC_{50} = 3.4, 7.9, 15.8 and 18.4 µg/ml respectively) and little activity against normal lung cells, except PIE which showed less cytotoxic (IC_{50} = 34.4 µg/ml). The different ratio between lung cancer and normal lung cell of PLE showed the highest was 20.4 times. The ethanolic extract of Benjakul was less active against liver and cervical cancer cells and normal cancer cells (IC_{50} = 45.6 and 47.7 and 48.9 µg/ml respectively). It is concluded that the ethanolic extract of Benjakul preparation showed the most selective cytotoxic activity against lung cancer cells compared with normal lung cells. Its ingredients of Benjakul which showed high different ratio between lung cancer and normal lung cell were *Plumbago indica* and *Zingiber officinale*

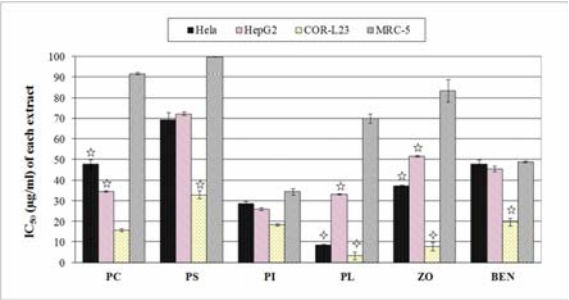

Fig. 2 Cytotoxic activity [IC_{50} (µg/ml)] of the ethanolic extract of five plants and Benjakul preparation on four types of cell lines exposure time 72 hrs using student t-test from prism to compare the significant difference between normal cell (MRC-5) and each cancer cell (Hela, HepG2 and COR-L23) (n = 3) ☆p < 0.05, ✧p < 0.01, PC = *Piper chaba,* PS = *Piper sarmentosum*, PI = *Piper interruptum,* PL = *Plumbago indica* and ZO = *Zingiber officinale*

(20.4 and 10.6 times respectively). This result agrees with the objective of cancer chemotherapy, which is to kill cancer cells with as little damage as possible to normal cells[12]. Bioassay guided fractionation was used to isolate pure compound from Benjakul preparation and it was found that FC (chloroform fraction) showed the highest activity against COR-L23 cell line (IC_{50} = 7.3 µg/ml and % yield = 14.03%) (Table 3).

Piperine [1], plumbagin [2] and 6-gingerol [3] were cytotoxic against all cell lines at exposure times 72 h (Table 4). All compounds showed a significant difference in effects on cancer cells and normal cells (Table 4 and Table 5). The comparison of cytotoxicity activity of the compounds against four cell types concluded that plumbagin showed the highest activity and the selectivity with lung cancer cell lines since it had less effect on normal cell, especially the MRC-5 normal lung cells (IC_{50} = 2.55 and 11.54 µM). From the comparison of ratio of IC_{50} (µM) lung normal cells/IC_{50} (µM) lung cancer cells of the three cytotoxic compounds and the crude extracts at exposure time 72 h, it was found that plumbagin showed the highest ratio (4.5) for COR-L23 and MRC-5 cells. A difference was also seen with the crude extract of Benjakul which was 2.5 times more active against cancer cell line than normal lung cell MRC-5 (Table 5). These results agree with previous data which found that plumbagin had IC_{50} = 14.6 µM against small lung carcinoma (A549)[13].

This work has also shown that plumbagin, isolated from *Plumbago indica*[14] occurs in a high percentage in Benjakul (4.18% w/w) so it could be a marker for chemical analysis of the ethanolic Benjakul extract. Piperine was seen to occur as 7.81% in Benjakul preparation and it occurs in *Piper longum*[15] and many other species of *Piper*. Piperine had a selectivity index of greater than 4 for lung cancer cells compared with normal lung cells. Thus, piperine is also an active cytotoxic component of Benjakul preparation. A previous report showed that piperine inhibited the solid tumor development in mice induced with DLA cells and increase the life span of mice bearing Ehrlich ascites carcinoma tumor to 58.8%[16] but, perhaps surprisingly,

Table 4. Cytotoxicity activity (IC_{50} µg/ml ± SEM and µM) of isolated compounds against four types of cancer cell lines (Hela, HepG2 and COR-L23) and one type of normal cell line (MRC-5) at exposure time 72 hrs (n = 3)

Compounds	% yield	IC_{50} (µg/ml ± SEM) (µM)			
		Hela	HepG2	COR-L23	MRC-5
Piperine [1]	7.81	23.12 ± 1.53 (81.12)	17.56 ± 2.32 (61.61)	12.38 ± 1.45 (43.44)	> 50 (> 175.44)
Plumbagin [2]	4.18	0.78 ± 0.06 (4.15)	0.49 ± 0.01 (2.61)	0.48 ± 0.02 (2.55)	2.17 ± 0.77 (11.54)
6-gingerol [3]	0.54	29.12 ± 2.30 (99.05)	14.69 ± 1.47 (49.97)	25.31 ± 0.90 (86.09)	> 50 (> 170.07)
Vinblastine sulphate (nM)		3.40 ± 0.21	2.80 ± 0.17	1.28 ± 0.07	> 50

Table 5. Different ratio of cytotoxicity activity (IC_{50} µg/ml ± SEM) of the ethanolic extracts and compounds between cancer cell lines (Hela, HepG2 and COR-L23) and cytotoxicity activity (IC_{50} µg/ml ± SEM) of normal cell line (MRC-5)

Plant Species	Different ratio (IC_{50} of cancer cell : IC_{50} of normal cell)		
	Hela	HepG2	COR-L23
Piper chaba Linn.	1.9	2.7	5.8
Piper sarmentosum Roxb.	1.4	1.4	>3.0
Piper interruptum Opiz.	1.2	1.3	1.9
Plumbago indica Linn.	8.0	2.1	20.4
Zingiber officinale Roscoe.	2.2	1.6	10.6
Benjakul preparation	1.0	1.1	2.5
Piperine [1]	2.2	2.8	>4.0
Plumbagin [2]	2.8	4.4	4.5
6-gingerol [3]	1.7	3.4	2.0

nothing is known about the mechanism of piperine agianst lung cancer cells. 6-Gingerol has previously been isolated from *Zingiber officinale*[9] and is a major component in Benjakul Preparation. It showed cytotoxicity against liver cancer cell lines (IC_{50} = 49.9 μM respectively) and this agrees with a previous report that showed its cytotoxic effect against some human cancer cells but not COR-L23, HepG2 and HeLa cell lines[9]. Thus, further work is needed to investigate the mechanism of the ethanolic extract of Benjakul against cancer cell lines.

In summary, Benjakul as a Thai traditional medicine, which is normally used as an adaptogen in cancer treatment, but also shows selective cytotoxicity against lung cancer cell lines. This appears to be due to at least three of the compounds present *i.e*. plumbagin, piperine and 6-gingerol. The use of Benjakul by Thai traditional doctors to treat cancer patients is therefore supported by these findings. Further studies are needed to investigate the molecular mechanisms of cytotoxicity of the isolated compounds from Benjakul extracts.

Potential conflicts of interest

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ฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งปอด ปากมดลูก และตับ ของตำรับเบญจกูลและสารสำคัญ

ศรีโสภา เรืองหนู, อรุณพร อิฐรัตน์, อินทัช ศักดิ์ภักดีเจริญ, รุจิลักขณ์ รัตตะรมย์, พิมลวรรณ ทัพยุทธพิจารณ์, กัมมาล กุมาร ปาวา

ภูมิหลัง: *เบญจกูลเป็นตำรับยาไทยที่ประกอบด้วยพืช 5 ชนิด คือ ผลดีปลี รากช้าพลู เถาสะค้าน รากเจตมูลเพลิงแดง และเหง้าขิงแห้ง จากการสัมภาษณ์หมอพื้นบ้านภาคใต้พบว่าเบญจกูลใช้ในการรักษาผู้ป่วยมะเร็ง*

วัตถุประสงค์: *เพื่อทดสอบฤทธิ์ความเป็นพิษของตำรับเบญจกูลและสารสำคัญต่อเซลล์มะเร็ง 3 ชนิด คือมะเร็งปอด ปากมดลูก และ ตับ และทดสอบกับเซลล์ปกติปอด โดยใช้วิธีการทดสอบด้วย เอส อาร์ บี*

วัสดุและวิธีการ: *วิธีการสกัดใช้เลียนแบบการใช้ของหมอพื้นบ้านคือการหมักด้วยเอทธานอลและการต้มน้ำ และใช้วิธีการแยกสารที่มีฤทธิ์ความเป็นพิษต่อเซลล์ด้วยวิธี bioassay guided isolation*

ผลการศึกษา: *สารสกัดชั้นเอทานอลของเจตมูลเพลิงแดง ขิง ดีปลี สะค้าน เบญจกูล และ ช้าพลู มีค่า IC_{50} เท่ากับ 3.4, 7.9, 15.8, 18.4, 19.8 และ 32.91 ไมโครกรัมต่อมิลลิลิตร แต่สารสกัดชั้นน้ำของพืชทุกชนิดและเบญจกูลไม่มีความเป็นพิษต่อเซลล์ สาร 3 ชนิด แยกจากสารสกัดชั้นเอทธานอล ชื่อ gingerol, plumbagin และ piperine สกัดได้ปริมาณ 0.54, 4.18 และ 7.48 % ของน้ำหนักสารสกัดตามลำดับ เมื่อทดสอบฤทธิ์ความเป็นพิษต่อเซลล์ พบว่า plumbagin มีฤทธิ์ต้านมะเร็งปอด ตับ ปากมดลูก และเซลล์ปกติ มีค่า IC_{50} เท่ากับ 2.55, 2.61, 4.16 และ 11.54 ไมโครโมล*

สรุป: *ผลการศึกษานี้สนับสนุนการใช้ตำรับเบญจกูลในการรักษามะเร็งและสารที่แยกได้จะเป็นสารที่ใช้สำหรับวิเคราะห์มาตรฐานของตำรับ*