CHAPTER VI

BIOLOGICAL ACTIVITIES

6.1 Biological assay

6.1.1 Cytotoxicity test

Bioassay of cytotoxic activity against six cell lines, including HS 27 (fibroblast), Kato (gastric), BT 474 (breast), Chago (lung), SW 620 (colon) and Hep–G2 (hepatoma) cancer, *in vitro* was performed by MTT [3-(4,5–dimethylthiazol-2-yl)-2,5–diphenyl tetrazolium bromide] colorimetric method **[47].** In principle, the viable cell number per well is directly proportional to the production of formazan, which following solubilization, can be measured spectrophotometrically.

The isolated compounds were tested for cytotoxicity activity toward 5 cell line types as follow:

KATO-III: Gastric carcinoma, Human, ATCC No. HTB-103

BT474: Ductol carcinoma, breast, Human, ATCC No. HTB 20

SW620: Lymph node metastasis, colon adenocarcinoma, Human, ATCC CCL 227

HEP-G2: Liver hepatoblastoma, Human, ATCC No.HB 8065

Chago: Lung undifferentiated, Human, Nat. Cancer Inst.

Cell lines, (5 x 10^4 cells/ml) were cultured in RPMI 1640 culture medium, supplemented with 10%(v/v) fetal calf serum, penicillin and streptomycin. All cells were incubated in 5 % CO_2 humidified incubator at 37 °C. They grew as monolayer and 0.05 % trypsin was added to disaggregate cells.

Cell lines at the exponential growth phase were harvested and centrifuged at 200 x g for 5 min, counted under inverted microscope and resuspended in complete RPMI medium approximately 2.5 x10^4 cells /ml and 200 µl of the cells suspension was added to each well of a flat bottom 96–well microtiter plate with a multichannel pipette. Number of cells per well is 5 x 10^3 cells. After 24 h incubation in a 5 % CO_2 humidified incubator at 37 °C, 2 µl of sample solution was added in appropriate wells

to give various final concentrations of sample (control group, N=6, each samples treatment group, N=3). Peripheral wells of each plate (lacking cells) were utilized for sample blank (N=2) and medium/ tetrazolium reagent blank (N=6) "background" determinations. The concentration of DMSO used to dissolve the samples was adjusted to 0.5 % and this concentration of solvent was used in control wells. After 72 h incubation at 37 °C, MTT working solution was prepared as 5 mg MTT/ ml PBS and was filtered with 0.22 μm filtered units. MTT working solution (10μl) was added to each culture well resulting in 50 μg MTT/ 250 μl total medium volume and cultures were incubated at 37 °C for 4 h.

Incubation cell monolayers and formazan were then inspected microscopically. Culture plates containing suspension lines or any detached cells were centrifuged at low speed (200 x g) for 5 min. All of culture medium supernatant was removed from wells by slow aspiration through a blunt 18–gauge needle and replaced with 150 μl of DMSO using pipette and mixed wells by plate mixer. Following through the formazan solubilization, the absorbance of each well was measured using a microtiter plate reader at 540 nm (single wavelength, calibration factor = 1.00).

Cell lines growth and growth inhibition were expressed in terms of mean (± 1 SD) absorbance units and/or percentage of control absorbance (± 1 SD %) following subtraction of mean "background" absorbance. In addition, the IC_{50} was expressed as the sample concentration in μg/ml that caused a 50 % inhibition of growth compared with controls. Doxorubicin or Adiblastina was used as a possitive control in every experiment.

5.2 Result of biological assay

5.2.1 Cytotoxic activity against cancer cell lines

The *in vitro* activity of isolated compounds (10μg) of *Croton oblongifolius* from Sakolnakorn Province against six cell lines are reported in Table 21.

Table 21. Cytotoxicity activity against cancer cell lines of isolated compounds of *Croton oblongifolius* from Sakolnakorn Province

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Compound	%Survival (10 μg/ml)				
	Hep G2 hepatoma	SW 620 colon	Chago lung	Kato-3 gastric	BT 474 breast
1	29	25	28	19	69
2	22	19	19	12	47
3	15	14	17	12	42
4	21	20	26	20	70
5	24	8	9	10	31