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APPENDIX A

CYTOTOXICITY ASSAY*

The initial aqueous extract, the concentrate, the permeate at concentration step and at diafiltration step were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa (human cervical carcinoma) cell lines employing the colorimetric method.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Chemical Co., USA) was dissolved in saline solution to make the concentration of 5 mg/ml as a stock solution. Cancer cell ($3x10^3$ cells) suspended in 100 µg/well of MEM medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY, USA) were seeded onto 96-well culture plate (Costar, Corning Incorporated, NY 14831, USA) After 24h pre-incubation at 37°C in a humidified atmosphere of 5% $CO_2/95\%$ air to allow cells attachment, various concentrations of test solution ($10 \mu l/well$) were added and then incubated for 72 hours under above condition. At the end of the incubation, $10 \mu l$ of tetrazolium reagent was added into each well then incubated at 37° C for 3 hours. The supernatant was decanted and DMSO ($100 \mu l/well$) was added to allow formosan solubilization. The optical density (OD) of each wll was detected by a Microplate reader (Bio-Rad, Benchmark Microplate reader) at 550 nm and for correction at 595 nm. Each determination represents the average means of three replicates. The 50% inhibition concentration ($10 \mu l/c_0$) was determined by curve fitting.

^{*} The cytotoxicity assay was performed at Natural Products Section, Research Division, National Cancer Institute, Bangkok, Thailand.

APPENDIX B

THE FITTING CURVE TO DETERMINE THE VALUE OF IC50 AGAINST CELL LINES

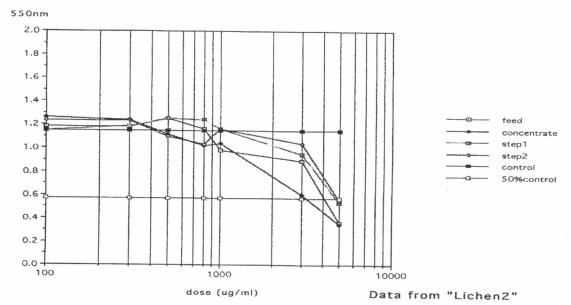


Figure B.1 The fitting curve for determination of value of IC_{50} against KB cell line. The feed is the initial aqueous extract and the permeate 1 and 2 are the permeate after the concentration and diafiltration step, respectively

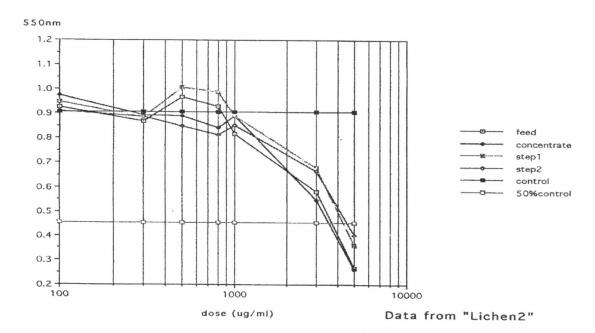


Figure B.2 The fitting curve for determination of the value of IC₅₀ against HeLa cell line. The feed is the initial aqueous extract and the permeate 1 and 2 are the permeate after the concentration and diafiltration step, respectively.