

มหาวิทยาลัยเชียงใหม่
Chiang Mai University

APPENDIX

Appendix A

Samples of Voucher Specime Plants

Table 14. Scientific Name and Voucher Specimen Number

Scientific name	Parts used	Voucher specimen number
<i>Borreria alata</i> (Aubl.) DC.	roots, stem, leaves	18748
<i>Borrevia lavevis</i> (Lmk.) Griseb.	roots, stem, leaves	18755
<i>Canthium glabrum</i> Bl.	leaves	18750
<i>Catunaregam spathulifolia</i> Tirv.	leaves	18753
<i>Gardenia jasminoides</i> Ellis.	leaves	-
<i>Gardenia erythoclada</i> Kurz	leaves	-
<i>Gardenia obtusifolia</i> Roxb. ex Kurz	leaves	18749
<i>Gardenia sootepensis</i> Hutch.	leaves	18756
<i>Haldina cordiflora</i> (Roxb.) Ridsdale	leaves	18751
<i>Hymenodictyon oriexense</i> (Roxb.) Mabb.	leaves	18081
<i>Ixora cibdela</i> Craib var. <i>puberula</i> Craib	leaves	18186
<i>Ixora stricta</i> Roxb.	leaves	-
<i>Lasianthus kurzii</i> Hook. f.	leaves	18192
<i>Mitragyna hirsuta</i> Havil.	leaves	18188
<i>Mussaenda parva</i> Wall. ex G. Don	leaves	18190

Table 14. (cont.)

Scientific name	Parts used	Voucher specimen number
<i>Paederia pilifera</i> Hook. f.	roots, stem, leaves	18752
<i>Pavetta tomentosa</i> Roxb. ex Sm. var. <i>tomentosa</i>	leaves	18080
<i>Psychotria ophioxybides</i> Wall.	leaves	18189
<i>Tarennoidea wallichii</i> (Hk. f.) Triv.&Sastre	leaves	18195
<i>Uncaria macrophylla</i> Wall.	leaves	18194



Figure 27. Voucher specimen of *Gardenia sootepensis* Hutch.



Figure 28. Voucher specimen of *Gardenia obtusifolia* Roxb. ex Kurz

Appendix B

Preparation of Medium and Reagent

Preparation of Dulbecco's Modified Eagle Medium (Incompleted Medium)

DMEM powder	1	packet
HEPES	10	mM
NaHCO ₃	3.7	gm
mercaptoethanol	1.0	ml
deionized distilled water (DI) to make	1000.0	ml

Preparation of components mixture in volumetric flask 1 L. adjust pH 7.2-7.3 with 1N NaOH or 1N HCl and filter of medium solution with sterile filtration method (0.2 µm microporous filters).

Completed DMEM Medium

Fetal Calf Serum (FCS)	10.0	ml
Penicillin-Streptomycin	1.0	ml
Incompleted DMEM to make	100.0	ml

Preparation of completed DMEM medium with aseptic technique.

Preparation of Washing TD-EDTA Buffer Solution

1 M NaCl	73.5	ml
100 mM KCl	25.0	ml
325 mM Na ₂ HPO ₄	1.0	ml
1 M Tris	12.5	ml
100 mM EDTA	0.5	ml
DI to make	500.0	ml

Preparation of TD-EDTA buffer in volumetric flask 500.0 ml and adjust to pH 7.4 with conc. HCl. Sterile solution with autoclave. (15 lb/in² at 121 °C for 20 min)

Appendix C

Graph of Anticancer Drugs

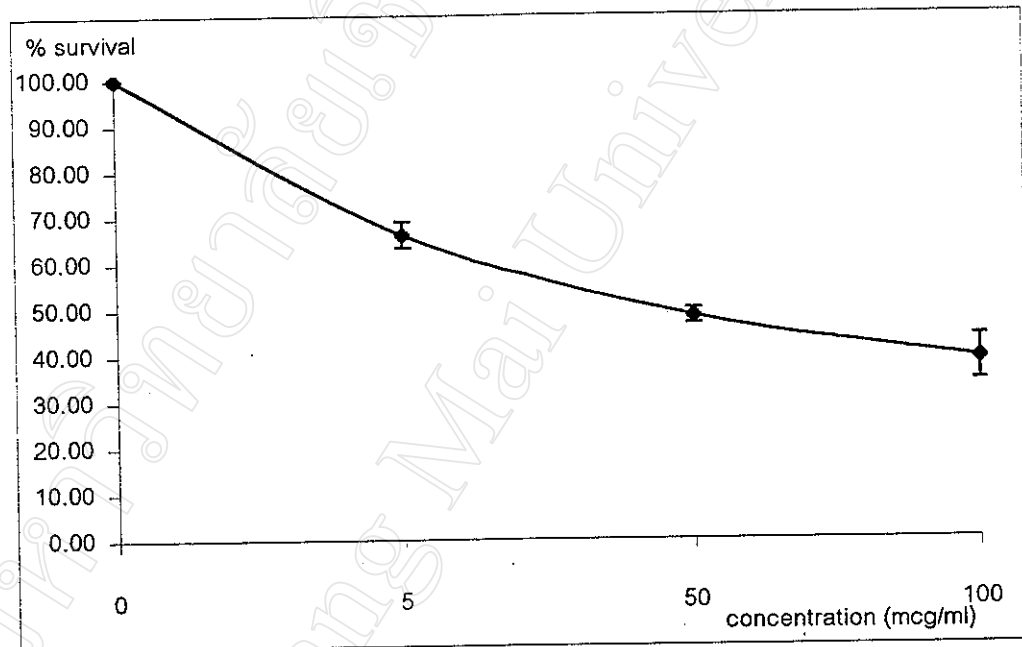


Figure 29. Concentration of 5-Fluorouracil (positive control) and percent survival of MCF-7 cell lines (n=6); IC_{50} (average) $47.8 \pm 7.6 \mu\text{g/ml}$

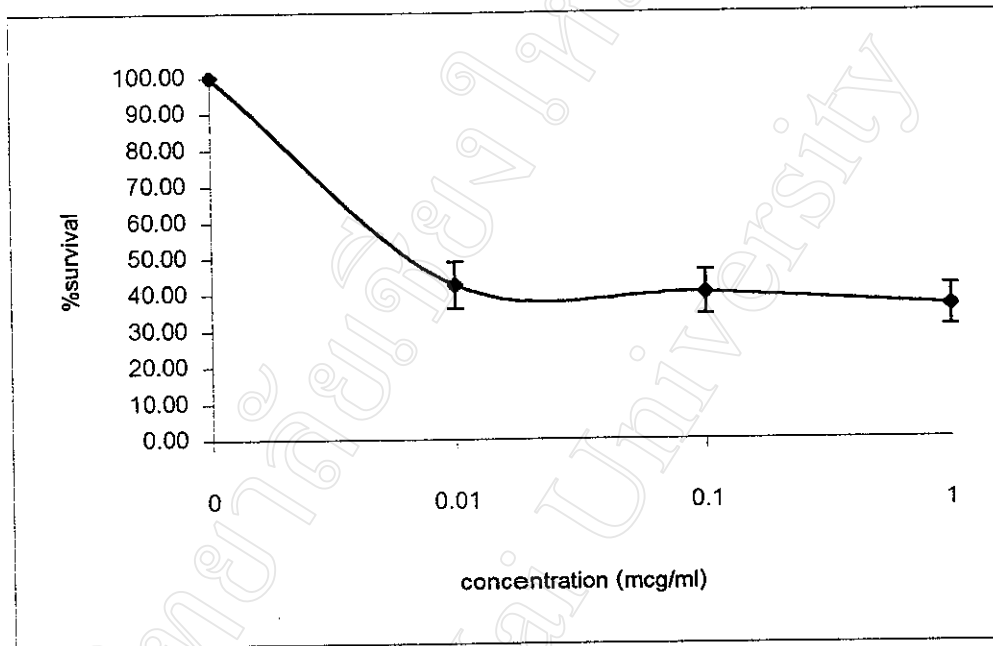


Figure 30. Concentration of vinblastine (positive control) and percent survival of KB-3-1 cell lines (n=6); IC_{50} (average) 7.4 ± 0.8 ng/ml

Appendix D

Pictures of Samples Extracts and MTT Assay



Figure 31. Crude ethanol extract of plant selected in screening process



Figure 32. Crude ethanol extract of *Gardenia obtusifolia*



Figure 33. Chloroform extract of *G. obtusifolia* after partition



Figure 34. Chloroform : ethyl acetate (1:1) fraction of *G. obtusifolia* from column chromatography



Figure 35. Fraction 2 and Fraction 3 of chloroform : ethyl acetate (1:1) column chromatography



Figure 36. Column chromatography of *G. obtusifolia*

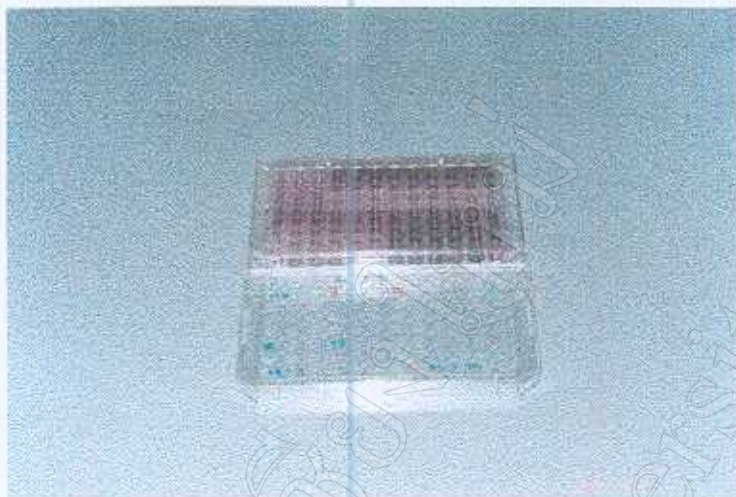


Figure 37. Cytotoxic activity test of Rubiaceae plants extracts with KB-3-1 cells; detected with MTT assay

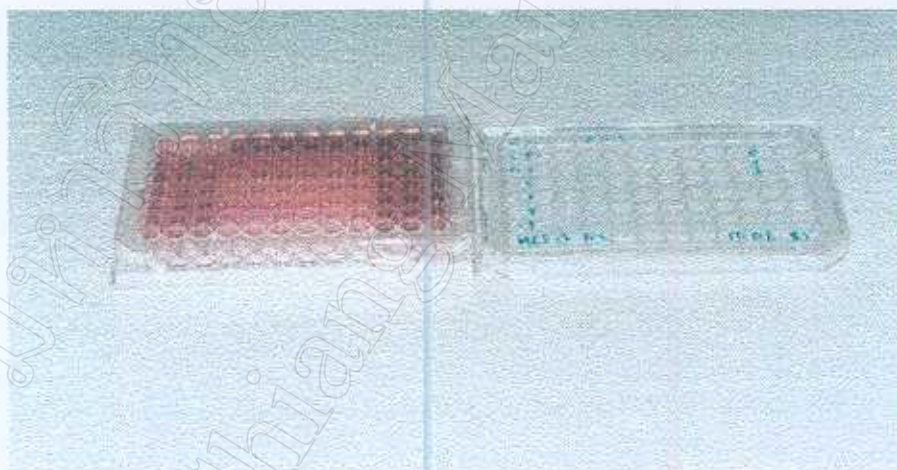


Figure 38. Cytotoxic activity test of fractions from *G. obtusifolia* with MCF-7 cells; detected with MTT assay

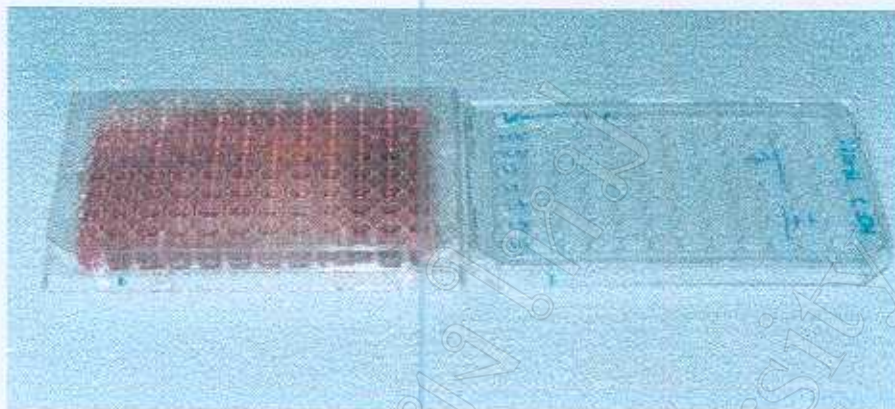


Figure 39. Cytotoxic activity test of fraction 3 from *G. obtusifolia* with MCF-7 cells; detected with MTT assay



Figure 40. Cytotoxic activity test of Chloroform:ethyl acetate (1:1) fractions from *G. obtusifolia* with KB-3-1 cells; detected with MTT assay

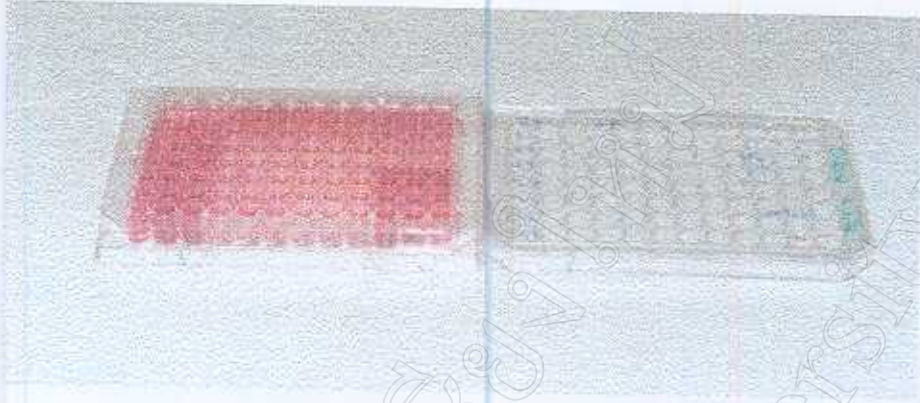


Figure 41. Cytotoxic activity test of subfractions 2 and GO. 1 from *G. obtusifolia* with MCF-7 cells; detected with MTT assay

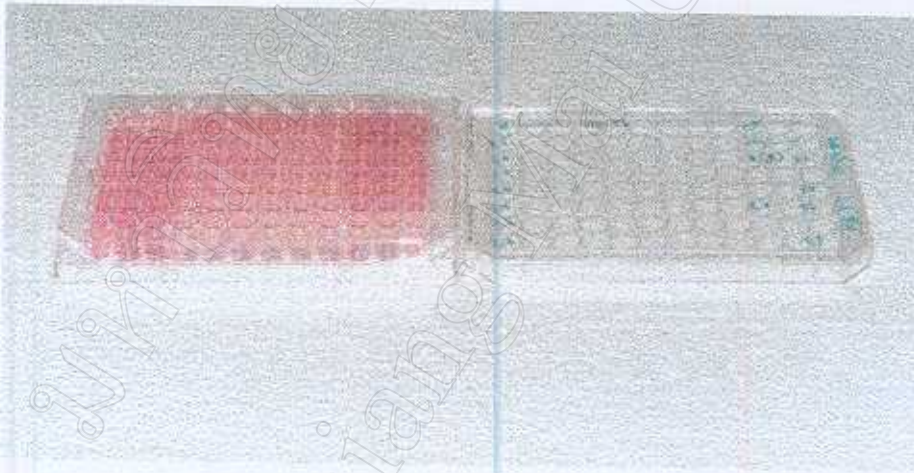


Figure 42. Cytotoxic activity test of subfractions 2 and GO. 1 from *G. obtusifolia* with KB-3-1 cells; detected with MTT assay

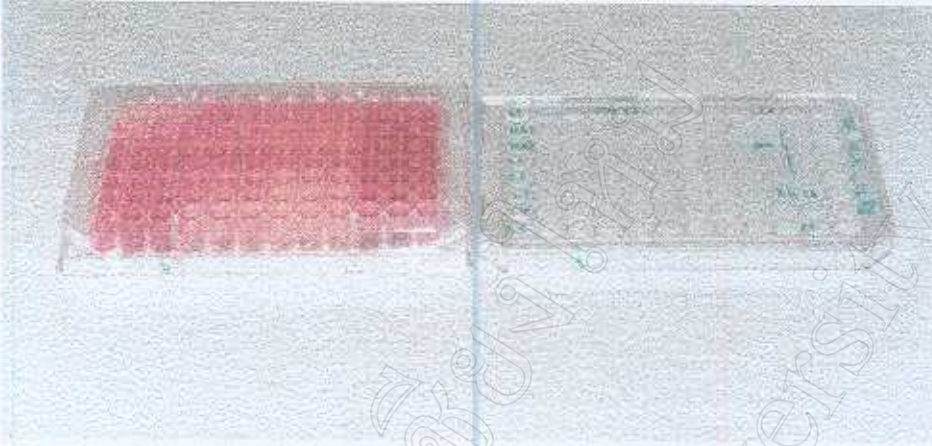


Figure 43. Cytotoxic activity test of subfractions 3 and GO. 2 from *G. obtusifolia* with KB-3-1 cells; detected with MTT assay

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Publication Chantasitipom, J., Chumchit, C., Tanamatayarat, P., Wongtieng, W., Yaipakdee, P., and Poobrasert, O. 2000. Biological Activity of Plant Extracts From Thai Traditional Medicinal Text for Cancer Therapy. *J. Multidisciplinary Res.* 13, 24-28.