APPENDICES

APPENDIX A

Preparation of reagents and buffers

Reagent for Mouse fibroblast cell culture

1. RPMI 1640 medium

RPMI 1640 medium was containing 10% (v/v) fetal bovine serum and penicillin (100 IU/ml) and streptomycin (100 µg/ml). The solution was mixed and sterilized filter and store at 4 °C until used.

2. 0.1 mM Phosphate buffer saline (PBS pH 7.4)

Sodium chloride	8.00 g
Potassium chloride	0.20 g
Sodium dihydrogen phosphate	1.44 g
Potassium dihydrogen phosphate	0.24 g
Deionized water	800 ml

The solution was adjusted pH to 7.4 by using 1 M hydrochloric acid then adjusted volume to 1000 ml and sterilized by autoclaving at 15 psi, 121 °C for 20 min.

3. Trypsin for trypsinization (0.05% trypsin – 0.02% EDTA)

0.25% trypsin (stock solution)

Trypsin	0.250 g
Phosphate buffer saline	100 ml

Filtrate the solution though 0.45 µM microfilter membrane and store at 4 °C until used.

0.025% Ethylene diaminetetraacetic acid (EDTA) (stock solution)

EDTA	0.025 g
Phosphate buffer saline	100 ml

Sterilize by autoclaving at 15 psi, 121°C for 20 min.

0.05% trypsin – 0.02% EDTA (working solution)

0.25% trypsin	20 ml
0.025% EDTA	80 ml

The solution of 0.05% trypsin – 0.02% EDTA (working solution) were prepared by pipetting 20 ml of 0.25% trypsin and 80 ml of 0.025% EDTA and mixed together. Then, the solution was filtrated though a 0.45 µM microfilter membrane and at 4 °C until used.

4. 0.4% Trypan blue stain

trypan blue	0.2 g
Phosphate buffer saline, pH 7.4	50 ml

The solution was filtrated though 0.45 µM microfilter membrane and kept at 4 °C until used.

5. MTT solution (5 mg/ml)

MTT dye	5 mg
Phosphate buffer saline, pH 7.4	1 ml

MTT solution was filtrated though a 0.45 µM microfilter membrane and use immediately.

Reagent for determination of total phenolic contents

1. 20% (w/v) Sodium carbonate

Sodium carbonate	20 g
Deionized water	60 ml

The solution was adjusted volume to 100 ml and kept at 4 °C.

Reagent for mutagenicity and antimutagenicity assay

1. Nutrient broth

Oxoid nutrition broth No.2	2.5 g
Sterilize deionized water	100 ml

Nutrient broth was sterilized by autoclaving at 15 psi, 121 °C for 20 min.

2. Vogel-Bonner medium E (10X)

Magnesium sulphate heptahydrate	2 g
Citric acid monohydrate	20 g
Dipotassium hydrogen phosphate (anhydrous)	100 g
Sodium dihydrogen phosphate (anhydrous)	19.2 g
Sodium hydroxide	6.6 g
Sterilize deionized water	1800 ml

The medium was adjusted pH to 7.0 and then the volume was adjusted to 2 L and sterilized by autoclaving at 15 psi, 121 °C for 20 min.

3. 0.5 mM histidine-biotin

Biotin	124 mg
Histidine	125 mg
Sterilize deionized water	1000 ml

The solution was filtrated though a 0.45 µM microfilter membrane and kept at 4 °C.

4. 0.1 M Sodium phosphate buffer, pH 7.4

0.1 M Sodium dihydrogen phosphate (stock solution)

Sodium dihydrogen phosphate	13.8 g
Sterilize deionized water	1000 ml

0.1 M Disodium hydrogen phosphate (stock solution)

Disodium hydrogen phosphate	14.2 g
Sterilize deionized water	1000 ml

0.1 mM Sodium phosphate buffer (working solution)

0.1 M Sodium dihydrogen phosphate	120 ml
0.1 M Disodium hydrogen phosphate	880 ml

Both stock solutions were mixed and then adjusted pH to 7.4 by using 0.1 M disodium hydrogen phosphate. The solution was sterilized by autoclaving at 15 psi, 121 °C for 20 min.

5. Minimal glucose agar (bottom agar)

Bacto agar	15 g
Glucose anhydrous	20 g
Vogel-Bonner medium E (10X)	100 ml
Sterilize deionized water	900 ml

Fifteen gram of agar was added in a flask size 3 L and then 700 ml sterilize deionized water was added and mixed together. The solution was autoclaved for 20 min at 15 psi, 121 °C. The solution was cool down about 45 min to 65°C. Next, 100 ml of Vogel-Bonner medium E (10X) and 200 ml of sterile glucose (10% v/v)

solution were added and then mixed together. Dispense the agar medium approximately 30 ml/plate and incubated at temperature room. When the agar was solidified, the plate was incubated overnight at 37 °C for 48 h to get rid of excess moisture.

6. Top agar

Bacto agar	0.6 g
Sodium chloride	0.5 g
0.5 mM histidine-biotin	10 ml
Sterilize deionized water	100 ml

0.6 g of agar and 0.5 g of sodium chloride were added in a flask size 250 ml and then 100 ml sterilize deionized water was added and mixed together. The solution was autoclaved for 20 min at 15 psi, 121°C. The solution was cool down for about 55 °C. Then, 10 ml of 0.5 mM histidine-biotin (10% v/v) solution was added then mixed together. Dispense the agar medium approximately 2 ml/plate and incubate at room temperature. When the agar was solidified, the plate was incubated overnight at 37 °C for 48 h.

7. Metabolic activation (S9 mix) in 1 ml

0.1 M sodium phosphate buffer, pH 7.4	0.5 ml
0.4 M $MgCl_2$-1.65 M KCl	0.02 ml
1.0 M Glucose-6-phosphate	0.005 ml
0.1 M NADPH	0.04 ml
0.1 M NADH	0.04 ml
Sterilize deionized water	0.295 ml
S9 fraction	0.1 ml

S9 mix was freshly prepared each day and kept in ice. The volume of 0.5 ml of S9 mix was usually added per plate.

Reagent for investigation of antityrosinase activities

1 .0.1 M potassium phosphate buffer, pH 6.8

1 M Dipotassium hydrogen phosphate (stock solution)

Dipotassium hydrogen phosphate	17.42 g
Sterilize deionized water	100 ml

1 M Potassium dihydrogen phosphate (stock solution)

Potassium dihydrogen phosphate	13.61 g
Sterilize deionized water	100 ml

0.1 M potassium phosphate buffer, pH 6.8 (working solution)

1 M Dipotassium hydrogen phosphate	4.97 ml
1 M Potassium dihydrogen phosphate	5.03 ml

After both potassium solutions were mixed, then the solution was adjusted pH to 7.4 using 1 M dipotassium hydrogen phosphate. Then, volume of solution was adjusted to 100 ml with sterilized water by autoclaving at 15 psi, 121 °C for 20 min.

2. Tyrosinase solution

Tyrosinase mushroom powder	12.7 mg
50 mM potassium phosphate buffer	0.5 ml

The final concentration of tyrosinase mushroom was 25.7 mg/ml which were aliquoted to microcentrifuge tubes for 100 µl and storage at -20^{o}C. The reaction mixture was used the concentration at 0.06 mg/ml.

3. L-3,4-dihydroxyphenylalanine (L-Dopa) solution

L-Dopa	10 mg
50 mM potassium phosphate buffer	6.25 ml

The final concentration of L-Dopa is 8 mM. The reaction mixture was used at the concentration of 2.5 mM. The solutions should be freshly prepared due to L-DOPA is rapidly oxidized by air and may darken on exposure to air and light.

APPENDIX B

Calculation of cytotoxicity by MTT assay

MTT assay was performed to investigate the cytotoxic effect of the plant extracts by using normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines. MTT assay was used as a standard colorimetric assay for measuring cellular proliferation or cell growth. The absorbance at 550 nm and 620 nm were measured by a microplate reader. The % cell viability was calculated as shown in the equation below.

$$\%\ \text{cell viability} = \left[\frac{(\text{absorbance of formazan product}) - (\text{mean absorbance of blank})}{(\text{mean absorbance of positive control})}\right] \times 100$$

Calculation of cytotoxicity by MTT assay of *T. bellerica* extract on melanoma mouse fibroblast B16F10. All samples, positive, negative, and media controls are run in five plicate. First, the absorbance of formazan product (A_{550}) after treated extract will be reduced with color medium (A_{620}). The results were shown in Table B.1.

Next, the absorbance of blank (cell line without extract, the absorbance at A = 0.0303 nm) subtracted with the absorbance extract (formazan product deduced color medium) in Table B.1. The results were shown in Table B.2. Then, the absorbances less than 1.0 nm of the formazan was selected from Table B.2 and were calculated to the % cell viability as shown in the equation. The average of these three values should be used in the equations below for the positive (mean the absorbance at A = 2.1835 nm).

Table B.1. Absorbance of formazan product after treated the cell lines with different concentrations of plant extract (A_{550}) which was deduced from the color of medium (A_{620})

Dilutions factors of plant extracts	**Absorbance (A_{550} - A_{620})**				
	1	**2**	**3**	**4**	**5**
2	0.318	0.353	0.395	0.000	0.000
5	0.399	0.246	0.287	0.000	0.000
10	0.168	0.468	0.421	0.448	0.000
20	0.297	0.136	0.429	0.435	0.492
40	0.438	0.475	0.491	0.483	0.511
80	0.477	0.579	0.543	0.611	0.458
100	0.428	0.545	0.428	0.576	0.420
150	0.127	0.171	0.255	0.237	0.339
300	0.178	0.166	0.156	0.146	0.132
500	0.045	0.061	0.154	0.190	0.118
1000	0.579	0.529	0.640	0.473	0.473
2000	0.561	1.206	1.366	1.380	1.059
5000	1.374	1.060	1.287	1.060	1.379

Table B.2. Absorbance of Table B.1- blank (cell line without extract)

Dilutions factors of plant extracts	**Absorbance ($A_{\text{extract in Table B.1}}$ - A_{blank})**				
	1	**2**	**3**	**4**	**5**
2	0.301	0.336	0.378	-	-
5	0.382	0.229	0.27	-	-
10	0.151	0.451	0.404	0.431	
20	0.28	0.119	0.412	0.418	0.475
40	0.421	0.458	0.474	0.466	0.494
80	0.46	0.562	0.526	0.594	0.441
100	0.411	0.528	0.411	0.559	0.403
150	0.11	0.154	0.238	0.22	0.322
300	0.161	0.149	0.139	0.129	0.115
500	0.028	0.044	0.137	0.173	0.101
1000	0.562	0.512	0.623	0.456	0.456
2000	0.544	1.189	1.349	1.363	1.042
5000	1.357	1.043	1.270	1.043	1.362

Finally, The 50% cytotoxic dose (CD_{50}) or the concentrations of the extracts at 50% of the viable cells were obtained from the relationship between the % cell viability and the concentrations of the extract by using BioDataFit software v1.02 from Chang Biosciences Inc. From the calibration curve in Figure B.1, the extracts of *T. bellerica* showed 50% cytotoxic dose (CD_{50}) value at 2.00 ± 0.18 mg/ml (at dilution factor 437) in melanoma mouse fibroblast B16F10 cell line.

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *T. bellerica* in the melanoma mouse fibroblast B16F10 cell line

The concentration of *T. bellerica* = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 437 = 873.4/437 = 2.00 mg/ml

Table B.3. The % cell viability of *T. bellerica* extract at different concentrations

Dilutions factor of *T. bellerica* extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
2	25.12 ± 2.86	2.00 ± 0.18 mg/ml
5	21.80 ± 5.88	
10	31.82 ± 1.75	
20	32.29 ± 2.58	
40	35.49 ± 1.07	
80	41.62 ± 2.53	
100	37.07 ± 5.79	
2000	88.59 ± 11.40	
5000	98.71 ± 3.84	

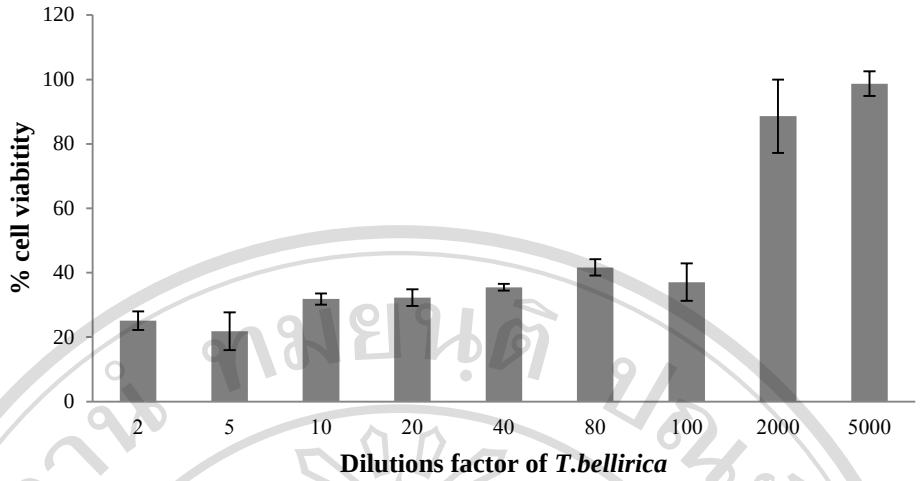


Figure B.1 Calibration curve of *T. bellerica* which was the relationship between % cell viability and the concentrations of *T. bellerica*

The 50% cytotoxic dose (CD_{50}) were obtained from the relationship between the % cell viability and the concentrations of the five plant extract which were shown in Table B.3- B.12 and Figure B.1- B.10. T he results 50% cytotoxic dose (CD_{50}) of five plant extracts were calculation in the same produce and were shown in Table 3.3.

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *T. chebula* Retz. in the normal mouse fibroblast L929 cell line

The concentration of *T. chebula* Retz. = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 73 = 873.4/73 = 11.96 mg/ml

Table B.4. The % cell viability of *T. chebula* Retz. extract at different concentrations

Dilutions factor of *T. chebula* Retz. extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
40	0.00 ± 5.58	11.96 ± 0.80 mg/ml
80	5.65 ± 1.94	
100	34.10 ± 8.64	
150	51.87 ± 6.04	
300	55.56 ± 9.20	
500	72.25 ± 2.64	
1000	91.91 ± 12.92	
2000	90.90 ± 17.92	
10000	77.60 ± 11.33	

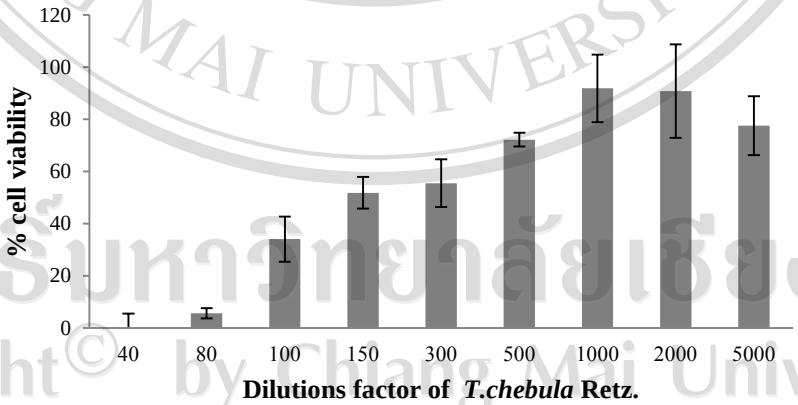


Figure B.2 Calibration curve of *T. chebula* Retz. which was the relationship between % cell viability and the concentrations of *T. chebula* Retz.

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *T. bellerica* in the normal mouse fibroblast L929 cell line

The concentration of *T. bellerica* = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 161 = 873.4/161 = 5.43 mg/ml

Table B.5. The % cell viability of *T. bellerica* extract at different concentrations

Dilutions factor of *T. bellerica* extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
10	17.18 ± 6.00	5.43 ± 0.18 mg/ml
20	22.27 ± 3.59	
40	24.00 ± 3.95	
80	39.58 ± 2.26	
150	48.97 ± 9.30	
300	63.57 ± 6.46	
2000	71.67 ± 16.26	
5000	87.27 ± 10.92	

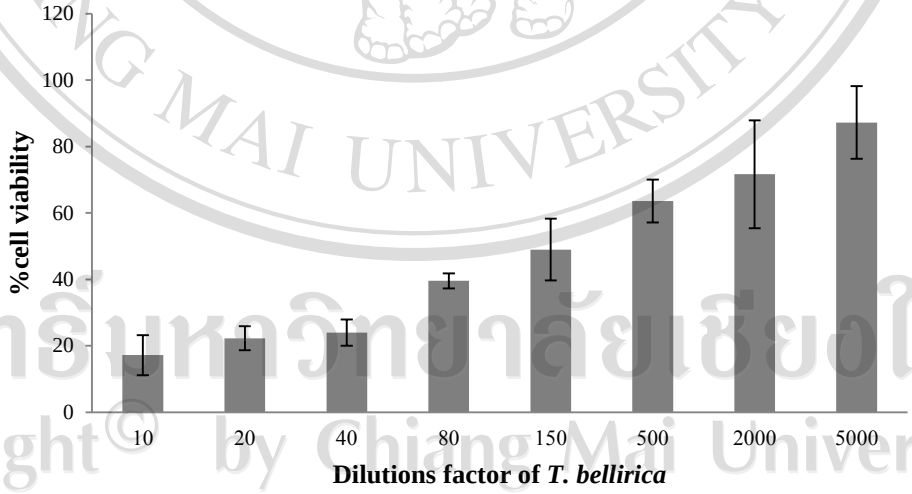


Figure B.3 Calibration curve of *T. bellerica* which was the relationship between % cell viability and the concentrations of *T. bellerica*

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *E. elatior (*Jack) R.M. Smith in the normal mouse fibroblast L929 cell line

The concentration of *E. elatior* (Jack) R.M. Smith= 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 44 = 873.4/44 = 19.85 mg/ml

Table B.6. The % cell viability of *E. elatior (*Jack) R.M. Smith. extract at different concentrations

Dilutions factor of *E. elatior (*Jack) R.M. Smith extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
1	0.00 ± 1.04	19.85 ± 0.65 mg/ml
2	1.27 ± 1.03	
5	4.44 ± 2.18	
10	17.96 ± 15.91	
20	19.76 ± 10.55	
40	56.47 ± 7.33	
80	103.85 ± 4.68	
100	112.98 ± 11.10	
150	135.24 ± 15.59	
300	100.71 ± 8.70	

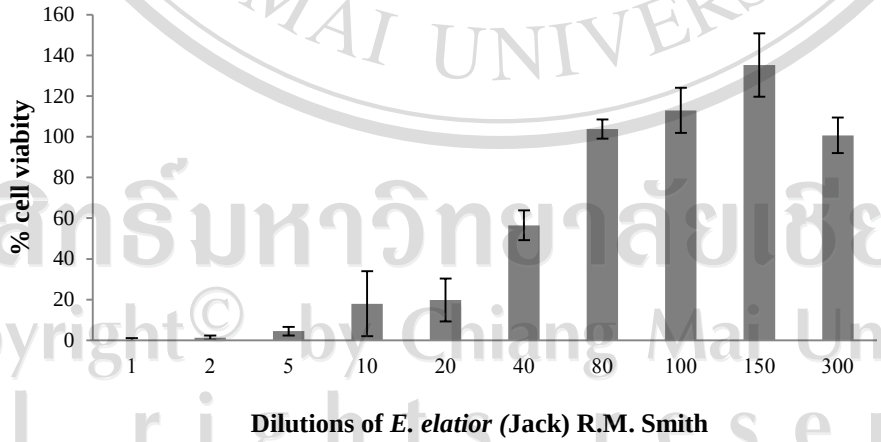


Figure B.4 Calibration curve of *E. elatior* which was the relationship between % cell viability and the concentrations of *E. elatior*

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *R. damacena* in the normal mouse fibroblast L929 cell line

The concentration of *R. damacena* = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 51 = 873.4/51 = 17.13 mg/ml

Table B.7. The % cell viability of *R. damacena* extract at different concentrations

Dilutions factor of *R. damacena* extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
1	4.43 ± 1.09	17.13 ± 0.39 mg/ml
2	7.72 ± 1.00	
5	15.04 ± 4.24	
10	27.64 ± 1.33	
20	30.98 ± 2.46	
40	71.21 ± 1.44	
80	109.80 ± 3.40	
100	118.83 ± 11.88	
150	122.02 ± 22.52	
300	135.31 ± 23.04	

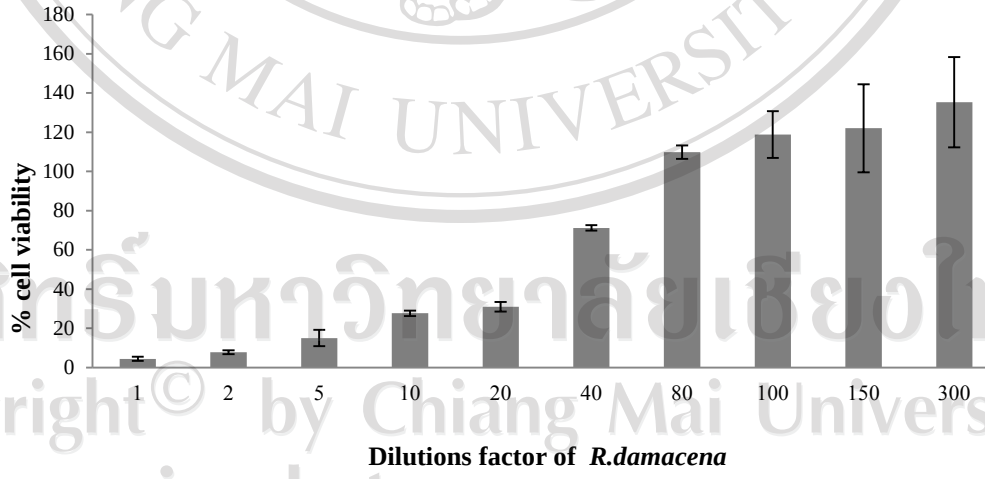


Figure B.5 Calibration curve of *R. damacena* which was the relationship between % cell viability and the concentrations of *R. damacena*

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *R. kerrii* Meijer in the normal mouse fibroblast L929 cell line

The concentration of *R. kerrii* Meijer = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 24 = 873.4/24 =36.39 mg/ml

Table B.8. The % cell viability of *R. kerrii* Meijer extract at different concentrations

Dilutions factor of *R. kerrii* Meijer extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
2	14.75 ± 8.21	36.39 ± 0.21 mg/ml
5	14.49 ± 1.17	
10	34.03 ± 3.66	
40	62.66 ± 1.58	
80	65.93 ± 9.18	
150	77.71 ± 12.80	
1000	81.59 ± 2.46	
5000	87.93 ± 19.86	
10000	98.61± 8.28	

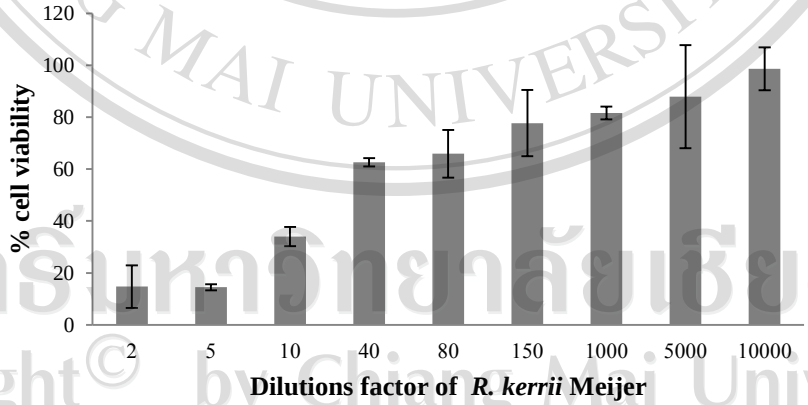


Figure B.6 Calibration curve of *R. kerrii* Meijer which was the relationship between % cell viability and the concentrations of *R. kerrii* Meijer

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *T. chebula* Retz. in the melanoma mouse fibroblast B16F10 cell line

The concentration of *T. chebula* Retz. = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 209 = 873.4/209 = 4.35 mg/ml

Table B.9. The % cell viability of *T. chebula* Retz. extract at different concentrations

Dilutions factor of *T. chebula* Retz. extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
40	0.00 ± 2.29	4.35 ± 0.33 mg/ml
80	0.00 ± 1.43	
100	14.25 ± 5.11	
150	30.00 ± 4.15	
300	48.77 ± 3.00	
500	50.98 ± 0.99	
1000	79.19 ± 6.00	
2000	93.73 ± 5.95	
5000	86.38 ± 10.94	
10000	87.43 ± 9.43	

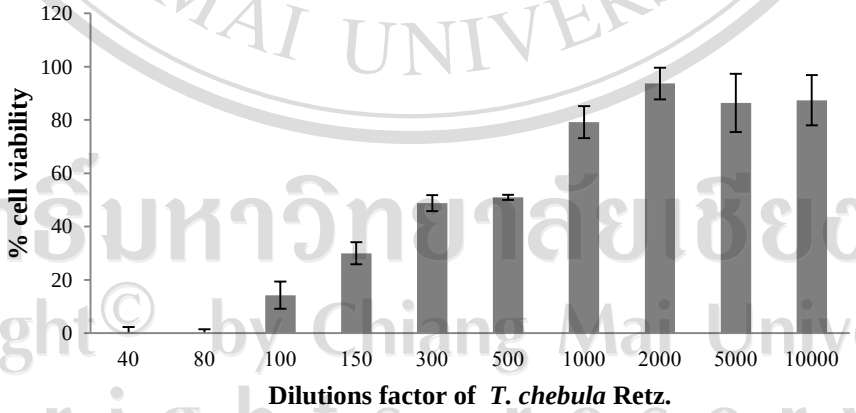


Figure B.7 Calibration curve of *T. chebula* Retz. which was the relationship between % cell viability and the concentrations of *T. chebula* Retz.

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *E. elatior (*Jack) R.M. Smith in the melanoma mouse fibroblast B16F10 cell line

The concentration of *E. elatior* (Jack) R.M. Smith= 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 15 = 873.4/15 = 58.23 mg/ml

Table B.10. The % cell viability of *E. elatior (*Jack) R.M. Smith. extract at different concentrations

Dilutions factor of *E. elatior (*Jack) R.M. Smith extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
1	2.43 ± 1.21	58.23 ± 0.18 mg/ml
2	2.34 ± 0.76	
5	8.11 ± 1.97	
10	14.76 ± 2.46	
20	64.45 ± 9.84	
40	62.15 ± 6.02	
80	55.74 ± 6.84	
100	71.78 ± 15.02	
150	77.18 ± 9.99	
300	83.08 ± 20.44	
500	80.42 ± 8.75	
1000	79.90 ± 4.24	

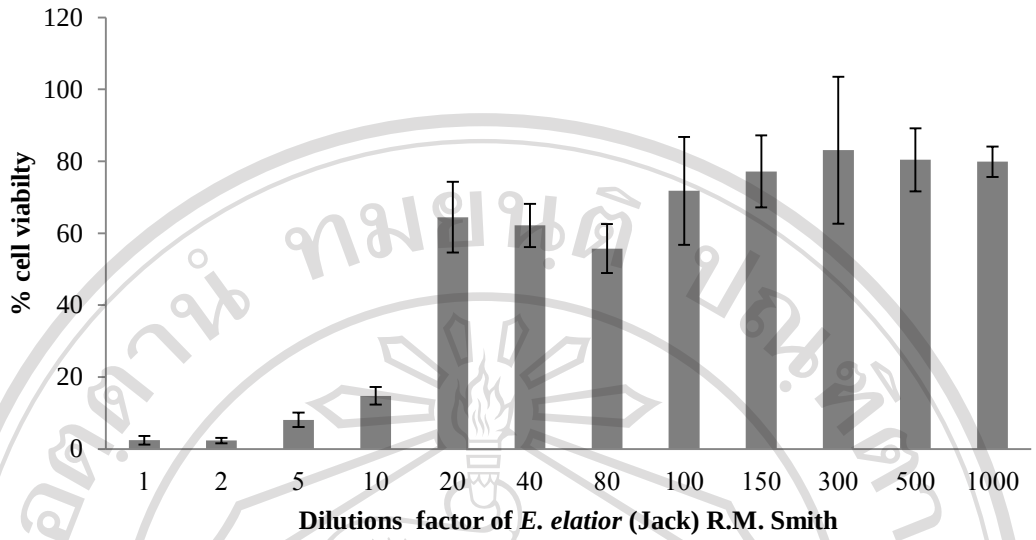


Figure B.8 Calibration curve of *E. elatior* (Jack) R.M. Smith. which was the relationship between % cell viability and the concentrations of *E. elatior* (Jack) R.M. Smith

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *R. damacena* in the melanoma mouse fibroblast B16F10 cell line

The concentration of *R. damacena* = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 87 = 873.4/87 = 10.04 mg/ml

Table B.11. The % cell viability of *R. damacena* extract at different concentrations

Dilutions factor of *R. damacena* extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
1	8.58 ± 1.00	10.04 ± 0.24 mg/ml
2	12.51 ± 2.02	
5	5.09 ± 2.01	
10	19.79 ± 4.27	
20	28.80 ± 3.29	
40	21.73 ± 3.52	
80	47.54 ± 12.04	
100	52.06 ± 13.39	
150	76.67 ± 5.02	
300	82.14 ± 10.73	
500	87.48 ± 21.05	
1000	98.42 ± 14.09	
2000	82.69 ± 4.68	
5000	97.22 ± 15.76	

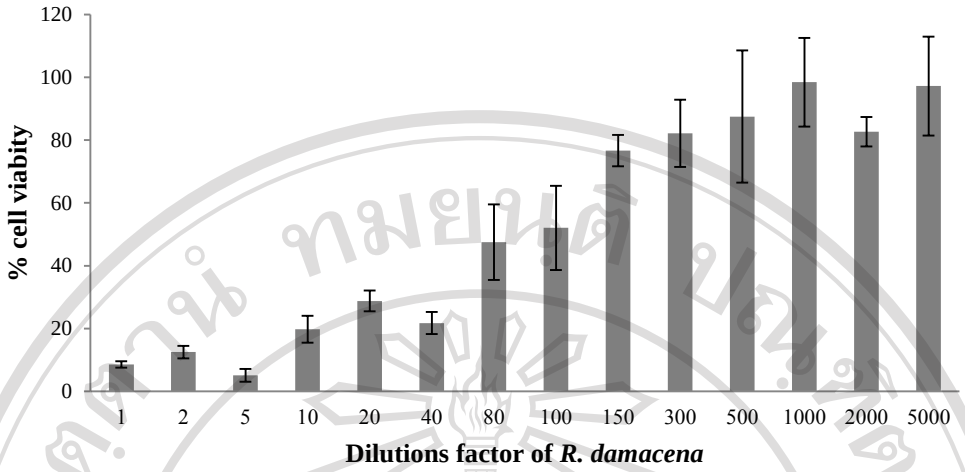


Figure B.9 Calibration curve of *R. damacena* which was the relationship between % cell viability and the concentrations of *R. damacena*

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *R. kerrii* Meijer in the melanoma mouse fibroblast B16F10 cell line

The concentration of *R. kerrii* Meijer = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 33 = 873.4/33 = 26.47 mg/ml

Table B.12. The % cell viability of *R. kerrii* Meijer extract at different concentrations

Dilutions factor of *R. kerrii* Meijer extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
2	31.46 ± 6.25	26.47± 0.47 mg/ml
5	39.26 ± 18.26	
10	39.41 ± 10.01	
20	40.93 ± 18.99	
40	60.91 ± 9.64	
100	63.36 ± 3.28	
150	60.74 ± 5.27	
300	69.94 ± 3.00	
500	54.20 ± 7.99	
2000	73.38 ± 12.56	
5000	80.61 ± 1.40	

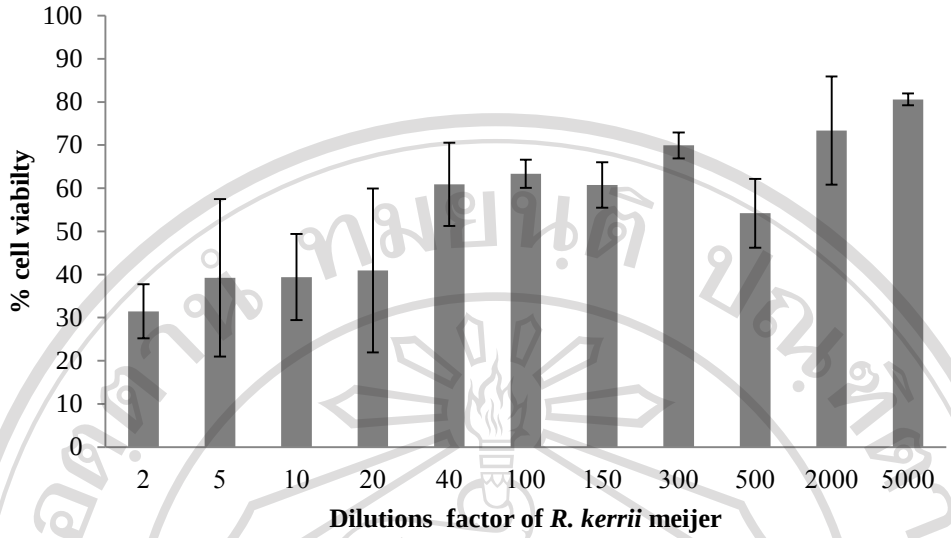


Figure B.10 Calibration curve of *R. kerrii* Meijer which was the relationship between % cell viability and the concentrations of *R. kerrii* Meijer

APPENDIX C

Calculation of cytotoxicity by Dye exclusion method

The cytotoxicity of plant extracts were performed in both normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines**.** Dye exclusion was used to determine the number of viable cells present in a cell suspension using tryphan blue staining. The unstained (viable) and stained (nonviable) cells were counted separately in the hemacytometer in duplicates. The percentages of viable cells were calculated as following equation:

$$\% \text{ cell viability } = \left[\frac{\text{total number of cell viability cell}}{\text{total number of cell}}\right] \times 100$$

The % cell viability of the *T. bellerica* extract in normal mouse fibroblast L929 cell line was calculated as shown in Table C.1. Then, The 50% cytotoxic dose (CD_{50}) or the concentrations of the extracts at 50% of the viable cells were obtained from the relationship between the % cell viability and the concentrations of the extract by using BioDataFit software v 1.02 from Chang Biosciences Inc. From the calibration curve in Figure C.1.

The extracts of *T. bellerica* showed 50% cytotoxic dose (CD_{50}) value at 8.734 ± 0.50 mg/ml (at dilution 100) in the normal mouse fibroblast L929 cell line. The 50% cytotoxic dose (CD_{50}) were obtained from the relationship between the % cell viability and the concentrations of the five plant extract which were shown in Table C.1- C.7 and Figure C.1- C.7. The results 50% cytotoxic dose (CD_{50}) of five plant extracts were calculation in the same produce and were shown in Table 3.4.

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *T. bellerica* in the normal mouse fibroblast L929 cell line

The concentration of *T. bellerica* = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 100 = 873.4/100 = 8.72 mg/ml

Table C.1. The % cell viability of *T. bellerica* extract at different concentrations

Dilutions factor of *T. bellerica* extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
5	0.00 ± 0.00	8.734 ± 1.40 mg/ml
10	4.00 ± 0.00	
20	5.00 ± 1.41	
40	20.00 ± 0.00	
80	27.00 ± 1.41	
100	35.00 ± 1.41	
300	63.00 ± 1.41	
500	80.12 ± 2.12	
1000	74.10 ± 0.17	

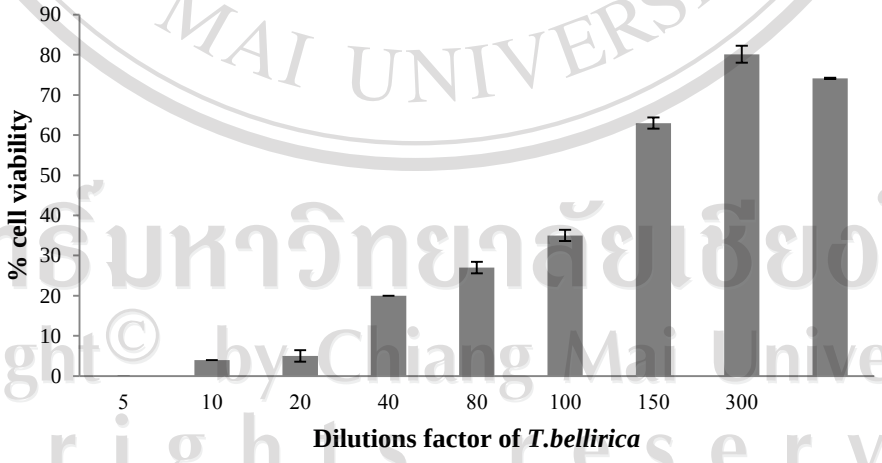


Figure C.1 Calibration curve of *T. bellerica* which was the relationship between % cell viability and the concentrations of *T. bellerica*

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *E. elatior* (Jack) R.M. Smith in the normal mouse fibroblast L929 cell line

The concentration of *E. elatior* (Jack) R.M. Smith = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 80 = 873.4/80 = 10.92 mg/ml

Table C.2. The % cell viability of *E. elatior* (Jack) R.M. Smith extract at different concentrations

Dilutions factor of *E. elatior* (Jack) R.M. Smith extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
20	5.02 ±0.47	10.92 ± 3.15 mg/ml
40	30.58 ±8.75	
80	62.10 ± 3.80	
100	85.29 ± 3.61	
150	92.80 ± 6.14	
300	93.46 ± 0.18	

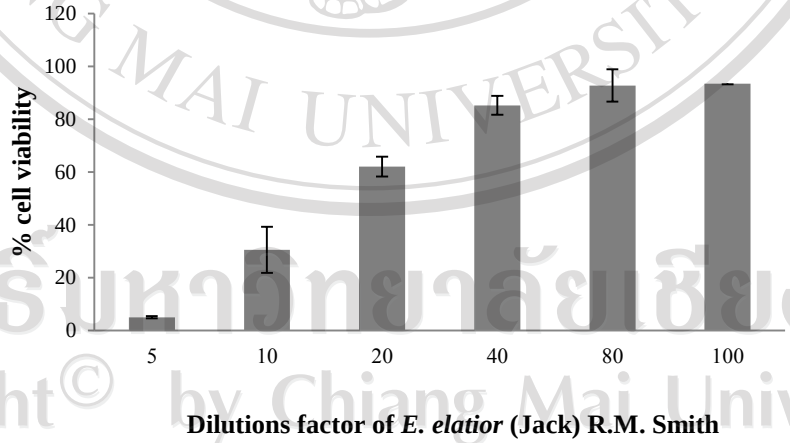


Figure C.2 Calibration curve of *E. elatior* (Jack) R.M. Smith which was the relationship between % cell viability and the concentrations of *E. elatior* (Jack) R.M. Smith

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *R. damacena* in the normal mouse fibroblast L929 cell line

The concentration of *R. damacena* = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 39 = 873.4/39 = 22.93 mg/ml

Table C.3. The % cell viability of *R. damacena* extract at different concentrations

Dilutions factor of *R. damacena* extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
5	23.61 ± 15.38	22.93 ± 0.50 mg/ml
10	9.05 ± 6.06	
20	12.50 ± 0.00	
40	83.75 ± 5.30	
80	97.22 ± 3.93	
100	90.06 ± 2.02	
150	94.66 ± 2.31	
300	100.00 ± 0.00	

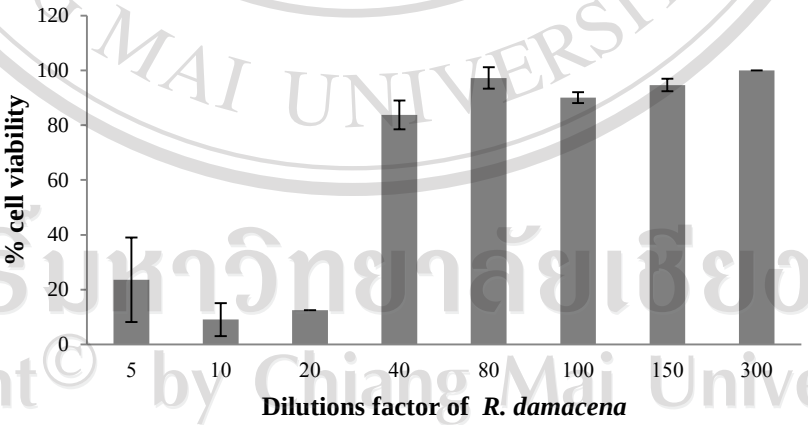


Figure C.3 Calibration curve of *R. damacena* which was the relationship between % cell viability and the concentrations of *R. damacena*

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *T. chebula* Retz. in the melanoma mouse fibroblast B16F10 cell line

The concentration of *T. chebula* Retz. = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 500 = 873.4/500 = 1.75 mg/ml

Table C.4. The % cell viability of *T. chebula* Retz. extract at different concentrations

Dilutions factor of *T. chebula* Retz. extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
80	19.34 ± 10.72	1.75 ± 0.63 mg/ml
100	15.83 ± 10.61	
150	20.89 ± 9.34	
300	43.33 ± 4.71	
500	48.52 ± 7.41	
1000	73.46 ± 4.90	

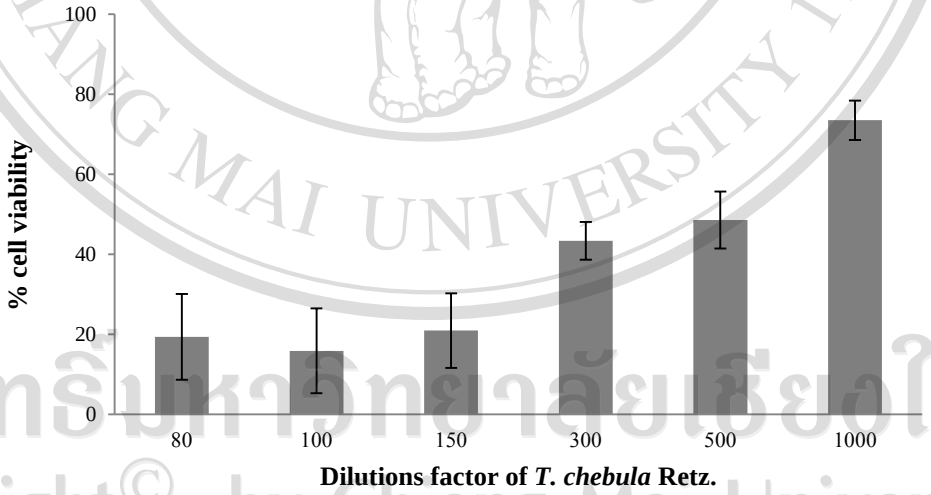


Figure C.4 Calibration curve of *T. chebula* Retz. which was the relationship between % cell viability and the concentrations of *T. chebula* Retz.

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *T. bellerica* in the melanoma mouse fibroblast B16F10 cell line

The concentration of *T. bellerica* = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 100 = 873.4/100 = 8.73 mg/ml

Table C.5. The % cell viability of *T. bellerica* extract at different concentrations

Dilutions factor of *T. bellerica* extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
5	0.00 ± 0.00	8.73 ± 1.56 mg/ml
10	4.00 ± 1.41	
40	20.00 ± 0.00	
100	40.00 ± 14.41	
300	75.18 ± 8.58	
500	71.35 ± 10.66	
1000	82.09 ± 3.50	

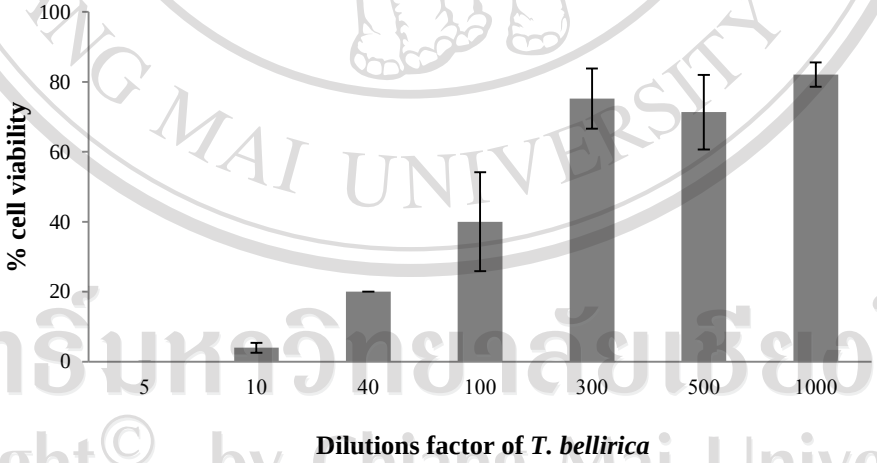


Figure C.5 Calibration curve of *T. bellerica* which was the relationship between % cell viability and the concentrations of *T. bellerica*

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *E. elatior* (Jack) R.M. Smith in the melanoma mouse fibroblast B16F10 cell line

The concentration of *E. elatior* (Jack) R.M. Smith = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 80 = 873.4/80 = 10.92 mg/ml

Table C.6. The % cell viability of *E. elatior* (Jack) R.M. Smith extract at different concentrations

Dilutions factor of *E. elatior* (Jack) R.M. Smith extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
40	25.00 ± 3.93	10.92 ± 4.20 mg/ml
60	53.10 ± 8.10	
80	58.51 ± 6.15	
100	80.04 ± 7.12	
150	61.59 ± 0.84	
300	93.46 ± 0.81	

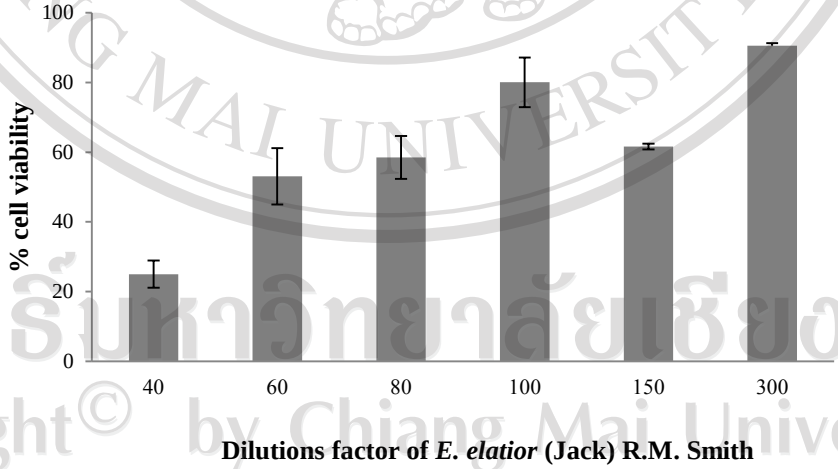


Figure C.6 Calibration curve of *E. elatior* (Jack) R.M. Smith which was the relationship between % cell viability and the concentrations of *E. elatior* (Jack) R.M. Smith

Calculation the concentration of 50% cytotoxic dose (CD_{50}) *R. damacena* in the melanoma mouse fibroblast B16F10 cell line

The concentration of *R. damacena* = 873.4 mg/ml

50% cytotoxic dose (CD_{50}) showed at dilution 20 = 873.4/20 = 43.17 mg/ml

Table C.7. The % cell viability of *R. damacena* extract at different concentrations

Dilutions factor of *R. damacena* extract	**% cell viability (mean ± S.D.)**	**50% cytotoxicity dose (CD_{50})**
20	12.50 ± 0.00	43.17 ± 0.14 mg/ml
40	83.75 ± 5.30	
80	97.22 ± 3.93	
100	90.06 ± 2.02	
300	100.00 ± 0.00	

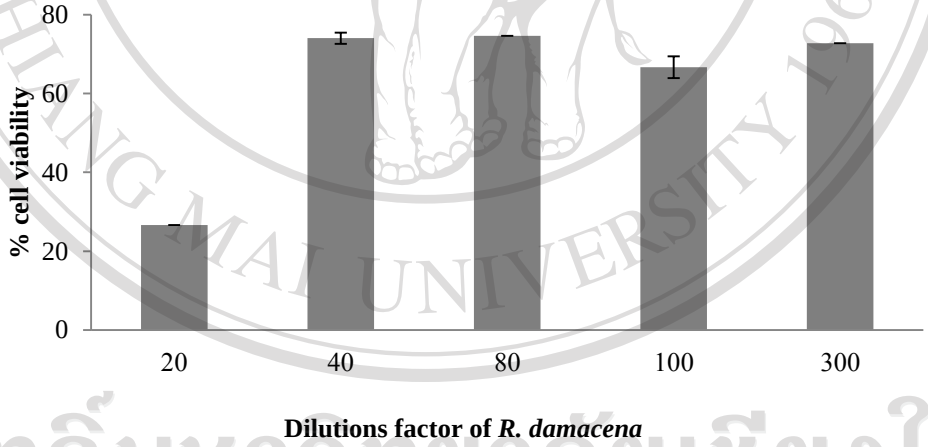


Figure C.7 Calibration curve of *R. damacena* which was the relationship between % cell viability and the concentrations of *R. damacena*

APPENDIX D

Calculation of Total phenolic contents

The total phenolic contents (TPC) of the five plant extracts were determined by Folin-Ciocalteu method. The total phenolic contents were estimated as gallic acid (GAE) equivalents. The reaction mixtures of galic acid were contained with 0.1 ml of the gallic acid at different concentrations (between 0.005 and 0.070 mg/ml). The method was performed the same as procedure as described in Section 2.2.3. The absorbance was measured at 765 nm with Ultraviolet–visible spectrophotometer. The absorbance at A_{765} at different concentrations of gallic acid and the data were plotted as shown in Table D.1 and Figure D.1.

Table D.1 The absorbance at A_{765} at different concentrations of gallic acid

GAE (mg/ml)	0.005	0.010	0.020	0.030	0.040	0.050	0.060	0.070
A_{765}	0.140	0.232	0.497	0.748	0.931	1.207	1.390	1.689

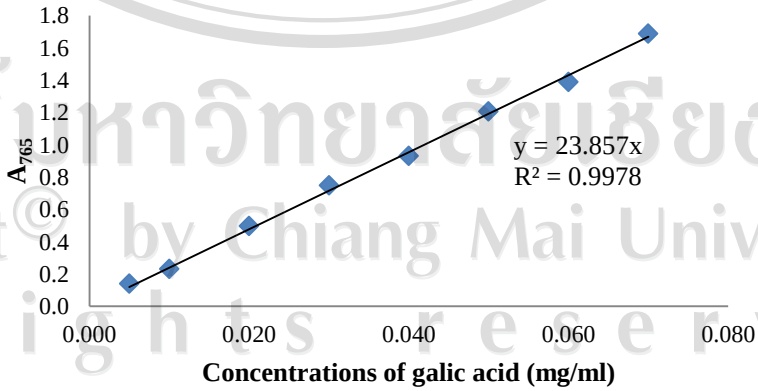


Figure D.1 Calibration curve of gallic acid was presented as the relationship between A_{765} and the concentrations of gallic acid in mg.

For example, calculation the total phenolic contents of *R. kerrii* Meijer extract which were estimated as gallic acid equivalents. The absorbance at 765 nm of *R. kerrii* Meijer extract at different concentrations was shown in Table D.2.

Table D.2 The absorbance at A_{765} of *R. kerrii* Meijer at different concentrations

Concentrations of *R. kerrii* Meijer (mg/wet weight)	A_{765}
0.09	0.025 ± 0.011
0.19	0.070 ± 0.008
0.38	0.161 ± 0.002
0.75	0.346 ± 0.028
1.50	0.804 ± 0.077
3.00	1.608 ± 0.040

Calculation of the total phenolic contents of *R. kerrii* Meijer extract

The equation from calibration curve was $y = 23.85x$, $R^2 = 0.997$

The concentration of *R. kerrii* Meijer extract at 3.0 mg/wet weight has given the absorbance at 765 nm at 1.608 (y) represent to equation

Represented in the equation $y = 23.85x$, $x = 0.0674$ mg/ wet weight

Final volumes of reaction 5 ml

The concentration of of *R. kerrii* Meijer = 0.0674 x 5 = 0.3372 mg of gallic acid

R. kerrii Meijer extract at 3.0 mg/wet weight equivalent to 0.3372 mg of gallic acid

R. kerrii Meijer extract at 1.0 g/wet weight equivalent = (1 g x 0.3372 mg)/ 3.0 mg

= 112.40 mg of gallic acid

The total phenolic contents of five plant extracts were calculated in the same procedure and shown in Table 3.2.

APPENDIX E

Calculation of percentage inhibition of mutagenesis

The plant extracts were also investigated for antimutagenicity at different concentrations. The antimutagenicity was done by modified Ames test in *S. typhimurium* strains TA98 and TA100. The plant extracts were pre-incubated as describe in Section 2.2.6. The number of revertants of *R. kerrii* Meijer in *S. typhimurium* TA98 assay were shown in Table E.1.

Table E.1 Inhibition of mutagenicity by *R. kerrii* Meijer in *S. typhimurium* TA98 assay system

Dose Level (mg/0.1 ml)	Revertant colonies/plate (mean 4n = 2 ± S.D.)	
	Present of S9 Mix	Absence of S9 Mix
NC (DMSO)	38 ± 2	26 ± 1
NC (50% hydroglycol)	32 ± 2	31 ± 1
10	341 ± 15	338 ± 4
20	109 ± 8	325 ± 3
40	56 ± 6	308 ± 2
60	47 ± 1	231 ± 5
80	37 ± 1	222 ± 6
PC AF-2 (0.10 μg/0.1 ml)	NA	340 ± 13
PC 2-AA (1.0 μg/0.1 ml)	565 ± 18	NA

NA: not applicable, n: No. of replicates

From Table E.1, the number of revertants of the *R. kerrii* Meijer extract at 80 mg/0.1 ml in *S. typhimurium* TA98 in presence metabolic activation were used for calculation the % inhibition of mutagenesis as shown in the equation followed:

$$\% \text{ inhibition of mutagenesis} = 100 - \left[\frac{\text{No. of } his+ \text{ revertants from extract - spontaneous reversion (solvent)}}{\text{No. of } his+ \text{ revertants from mutagen - spontaneous reversion (DMSO)}} \times 100\right]$$

Represented in the equation:

$$\% \text{ inhibition of mutagenesis} = 100 - \left[\frac{37 - 32}{565 - 38} \times 100\right]$$

$$= 99.05\ \%$$

The concentration of the *R. kerrii* Meijer extract at 80 mg/0.1 ml showed the percentage inhibition of mutagenesis in *S. typhimurium* strain TA98 at 99.05% in presence metabolic activation. The percentage of inhibition of mutagenesis of five plant extracts were calculated in the same procedure as shown in Table 3.10.-3.19.

APPENDIX F

Calculation of percentage of tyrosinase inhibitory activity

The plant extracts were determined for antityrosinase activity by the dopachrome method. In this experiment, L-3,4-dihydroxyphenylalanine (L-Dopa) was used as a substrate. The absorbance at 475 nm was measured by using ultraviolet–visible spectrophotometer to determine the tyrosinase activity. The absorbance at 475 nm of *R. kerrii* Meijer extract at different concentrations was shown in Table F.1

Table F.1 The absorbance at A_{765} at different concentrations of *R. kerrii* Meijer extract

Concentrations of *R. kerrii* Meijer (mg/wet weight)	**A_{475}**
0.10	0.793 ± 0.040
0.25	0.764 ± 0.022
0.50	0.730 ± 0.007
1.00	0.567 ± 0.009
2.50	0.437 ± 0.010
5.00	0.444 ± 0.018
10.00	0.540 ± 0.007
20.00	0.762 ± 0.027
40.00	1.176 ± 0.092
60.00	1.571 ± 0.015
80.00	1.877 ± 0.010

For example, the percentage of tyrosinase inhibitory activity of the *R. kerrii* Meijer extract at 80 mg/ml was calculated with the following formula:

$$\% \text{ Tyrosinase inhibition} = \left[\frac{(A\text{-}B) - (C\text{-}D)}{(A\text{-}B)}\right] \times 100$$

A is the absorbance of the control (L-Dopa mixed with tyrosinase in buffer, A_{475} = 1.056), B is the absorbance of the blank (L-Dopa in buffer, A_{475} = 0.007), C is the absorbance of the reaction mixture (reaction mixture of the extract at 80 mg/ml, A_{475} = 1.877), D is the absorbance of the blank of C (L-Dopa mixed with test sample without adding tyrosinse in buffer, A_{475} is 1.819 for reaction mixture at 80 mg/ml)

Represented in the equation :

$$\% \text{ Tyrosinase inhibition} = \left[\frac{(1.056 - .0.007) - (1.877\text{-}1.819)}{(1.056\text{-}0.007)}\right] \times 100$$

$$= 94.46\ \%$$

The percentage of tyrosinase inhibitory activity of five plant extracts were calculated in the same procedure as shown in Table 3.22. Then, the % tyrosinase inhibitory activity at different concentrations of five plant extracts were calculated to the IC_{50} values as shown in Figure 3.9.

APPENDIX G

Calculation of the exposed time to UV radiation

The morphological changes of normal mouse fibroblast L929 and mouse melanoma fibroblast B16F10 cell lines were investigated after induced by UVA and UVB radiation. The exposed time with UVA and UVB radiation were calculated with the following formulation (Youn *et al*., 2007) :

$$H_\lambda = t \cdot E_\lambda$$

H_λ is the energy level indicated as exposure per unit area (J/cm^2), t is the exposure time (second), E_λ is the irradiance (W/cm^2), the irradiance is measured by using UV light meter model uv-340.

Calculation of the exposed time to UVA radiation

The cell lines were exposed to UVA radiation with 0.3 J/cm^2

The irradiance of UVA was determined from the UVA lamp = 0.000218 W/cm^2

Represented in the equation: $H_\lambda = t \cdot E_\lambda$

$t = H_\lambda / E_\lambda$

$= 0.3/0.000218 = 22$ min 56 sec

Calculation of the exposed time to UVB radiation

The cell lines were exposed to UVA radiation with 30 mJ/cm^2 or 0.030 J/cm^2

The irradiance of UVA was determined from the UVA lamp = 0.000074 W/cm^2

Represented in the equation: $H_\lambda = t \cdot E_\lambda$

$t = H_\lambda / E_\lambda$

$= 0.030/0.000218 = 6$ min 45 sec

APPENDIX H

Antimutagenicity of plant extracts in *Salmonella typhimurium*

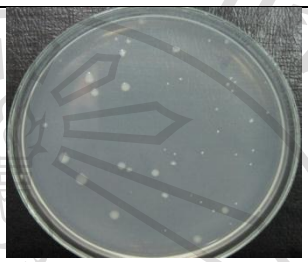
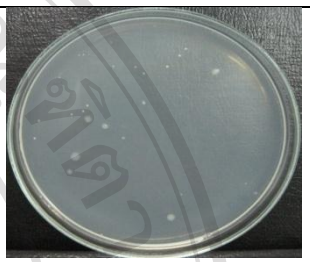
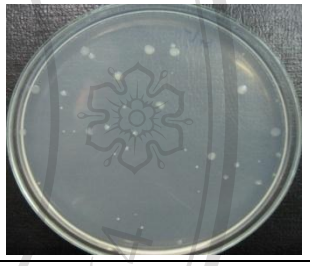
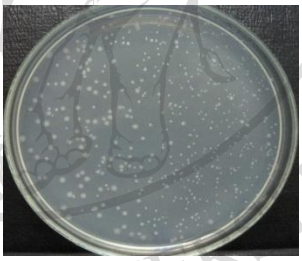
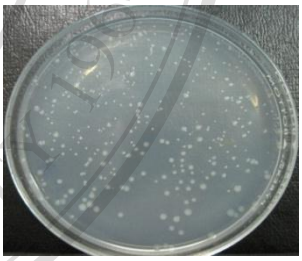
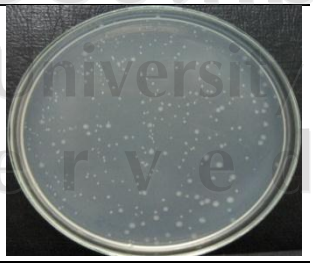
	Presence (+S9)	Absence (-S9)
Negative controls DMSO		
70% hydroglycol		
Positive controls 2-AA mutagen for presence AF-2 mutagen for absence		
***T.chebula* Retz. extract** 2-AA + *T.chebula* Retz. 80 mg/plate AF-2 + *T.chebula* Retz. 80 mg/plate		
***T.chebula* Retz. extract** 2-AA + *T.chebula* Retz. 10 mg/plate AF-2 + *T.chebula* Retz. 10 mg/plate		

Figure H.1 Effect of *T.chebula* Retz. extract against 2-AA and AF-2 mutagens in *S. typhimurium* strain TA98 in present and absence metabolic activation

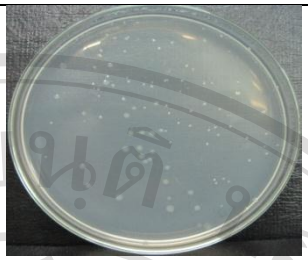
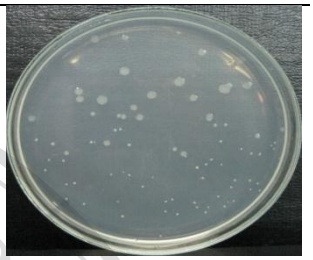
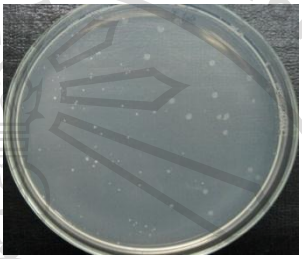
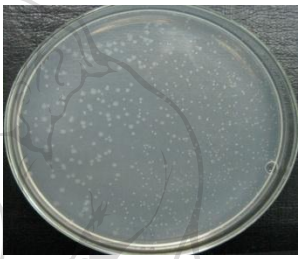
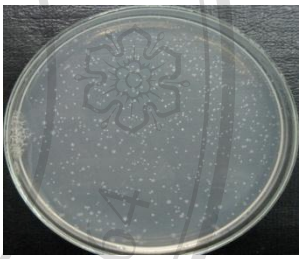
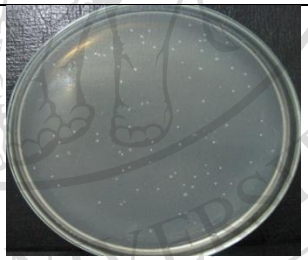
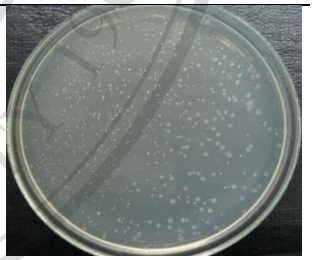
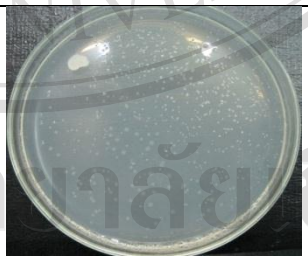
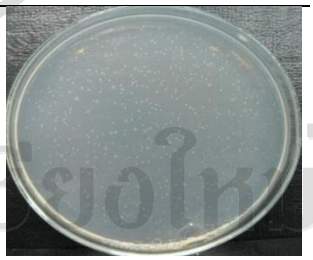
	Presence (+S9)	Absence (-S9)
Negative controls DMSO		
70% hydroglycol		
Positive controls 2-AA mutagen for presence AF-2 mutagen for absence		
***T.chebula* Retz. extract** 2-AA + *T.chebula* Retz. 80 mg/plate AF-2 + *T.chebula* Retz. 80 mg/plate		
***T.chebula* Retz. extract** 2-AA + *T.chebula* Retz. 10 mg/plate AF-2 + *T.chebula* Retz. 10 mg/plate		

Figure H.2 Effect of *T.chebula* Retz. extract against 2-AA and AF-2 mutagens in *S. typhimurium* strain TA100 in present and absence metabolic activation

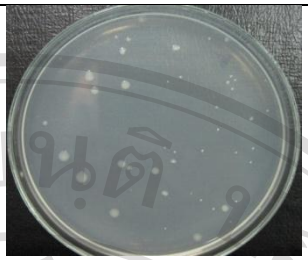
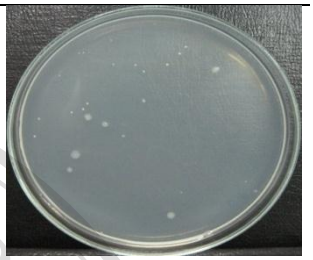
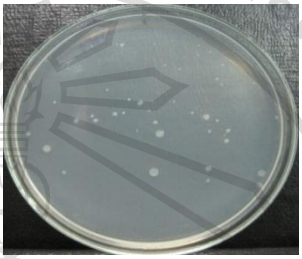
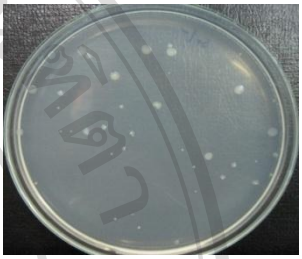
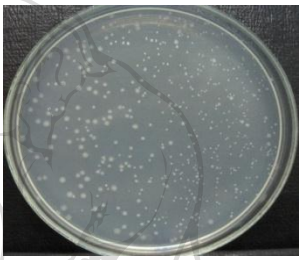
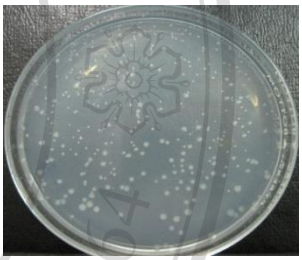
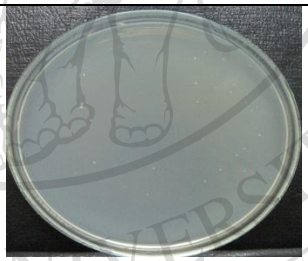
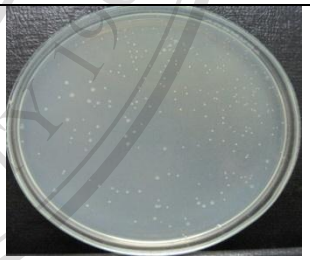
	Presence (+S9)	Absence (-S9)
Negative controls DMSO		
70% hydroglycol		
Positive controls 2-AA mutagen for presence AF-2 mutagen for absence		
***T. bellerica* extract** 2-AA + *T. bellerica* 80 mg/plate AF-2 + *T. bellerica* 80 mg/plate		
***T. bellerica* extract** 2-AA + *T. bellerica* 10 mg/plate AF-2 + *T. bellerica* 10 mg/plate		

Figure H.3 Effect of *T. bellerica* extract against 2-AA and AF-2 mutagens in *S. typhimurium* strain TA98 in present and absence metabolic activation

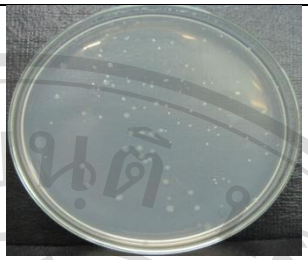
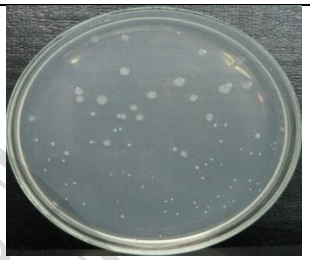
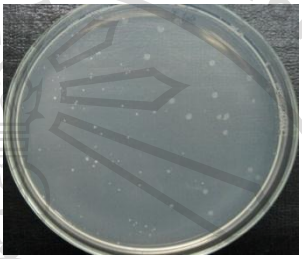
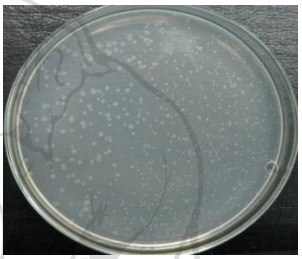
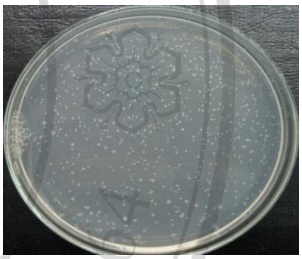
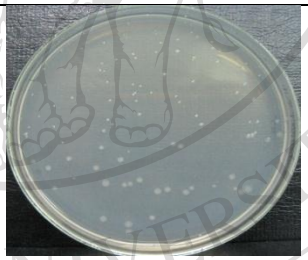
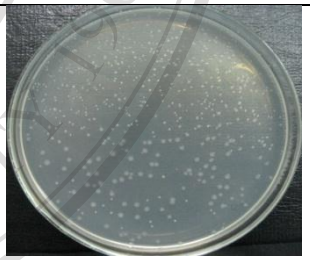
	Presence (+S9)	Absence (-S9)
Negative controls DMSO		
70% hydroglycol		
Positive controls 2-AA mutagen for presence AF-2 mutagen for absence		
***T. bellerica* extract** 2-AA + *T. bellerica* 80 mg/plate AF-2 + *T. bellerica* 80 mg/plate		
***T. bellerica* extract** 2-AA + *T. bellerica* 10 mg/plate AF-2 + *T. bellerica* 10 mg/plate		

Figure H.4 Effect of *T. bellerica* extract against 2-AA and AF-2 mutagens in *S. typhimurium* strain TA100 in present and absence metabolic activation

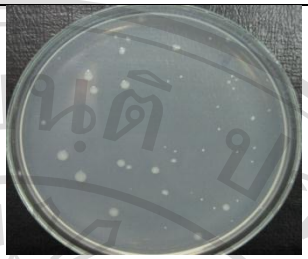
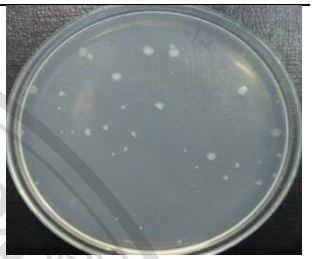
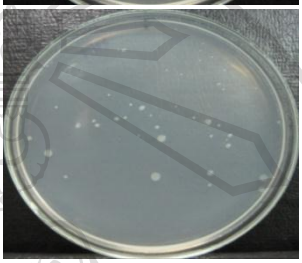
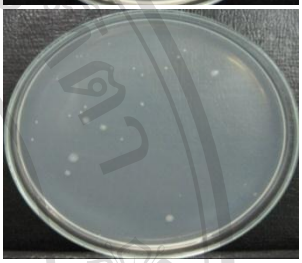
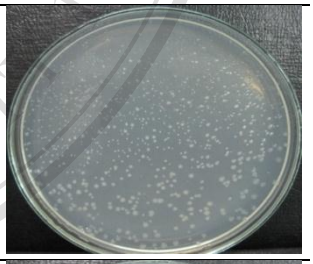
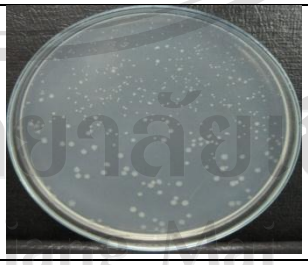
	Presence (+S9)	Absence (-S9)
Negative controls DMSO		
50% hydroglycol		
Positive controls 2-AA mutagen for presence AF-2 mutagen for absence		
***E. elatior* (Jack) R.M. Smith extract** 2-AA + *E. elatior* 80 mg/plate AF-2 + *E. elatior* 80 mg/plate		
***E. elatior* (Jack) R.M. Smith extract** 2-AA + *E. elatior* 10 mg/plate AF-2 + *E. elatior* 10 mg/plate		

Figure H.5 Effect of *E. elatior* (Jack) R.M. Smith extract against 2-AA and AF-2 mutagens in *S. typhimurium* strain TA98 in present and absence metabolic activation

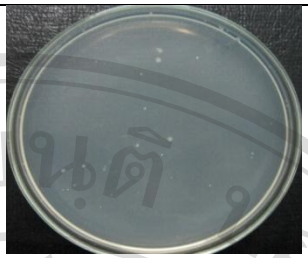
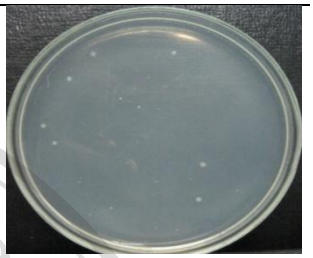
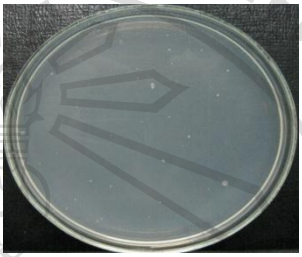
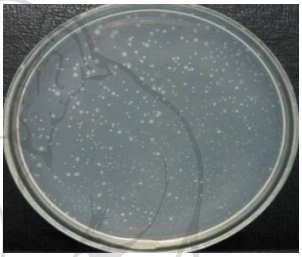
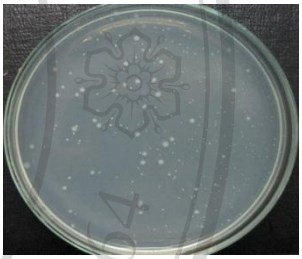
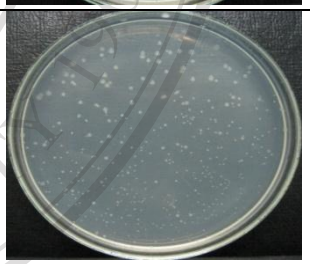
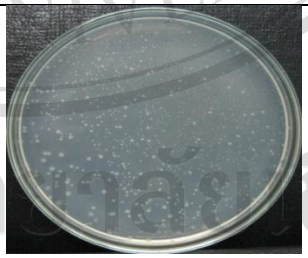
	Presence (+S9)	Absence (-S9)
Negative controls DMSO		
50% hydroglycol		
Positive controls 2-AA mutagen for presence AF-2 mutagen for absence		
***E. elatior* (Jack) R.M. Smith extract** 2-AA + *E. elatior* 80 mg/plate AF-2 + *E. elatior* 80 mg/plate		
***E. elatior* (Jack) R.M. Smith extract** 2-AA + *E. elatior* 10 mg/plate AF-2 + *E. elatior* 10 mg/plate		

Figure H.6 Effect of *E. elatior* (Jack) R.M. Smith extract against 2-AA and AF-2 mutagens in *S. typhimurium* strain TA100 in present and absence metabolic activation

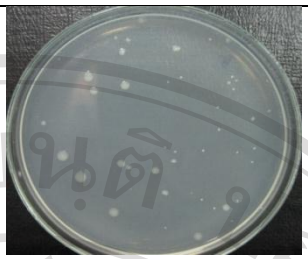
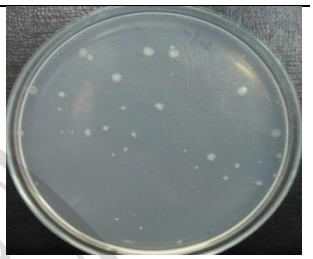
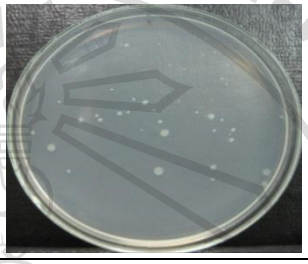
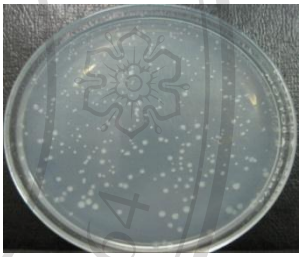
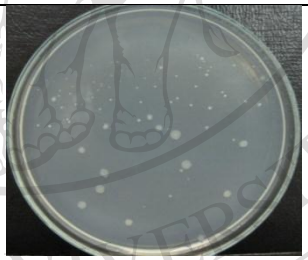
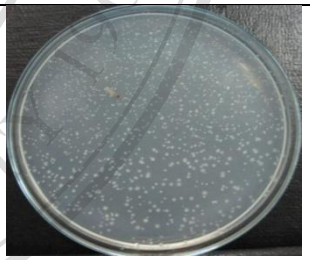
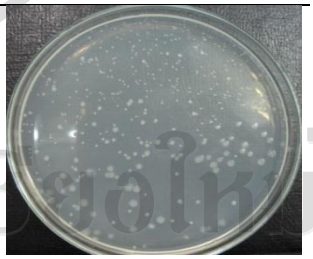
	Presence (+S9)	Absence (-S9)
Negative controls DMSO		
50% hydroglycol		
Positive controls 2-AA mutagen for presence AF-2 mutagen for absence		
***R. damascena* extract** 2-AA + *R. damascena* 80 mg/plate AF-2 + *R. damascena* 80 mg/plate		
***R.damascena* extract** 2-AA + *R. damascena* 80 mg/plate AF-2 + *R. damascena* 80 mg/plate		

Figure H.7 Effect of *R. damascena* extract against 2-AA and AF-2 mutagens in *S. typhimurium* strain TA98 in present and absence metabolic activation

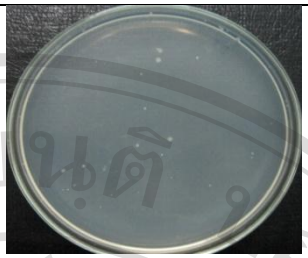
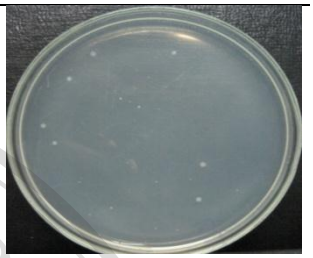
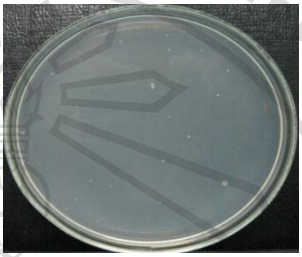
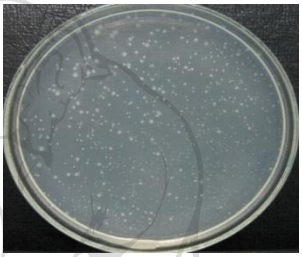
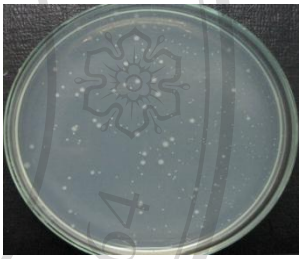
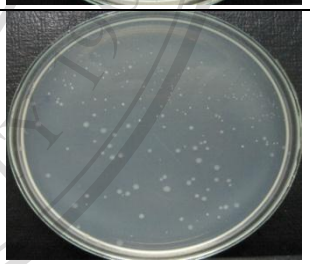
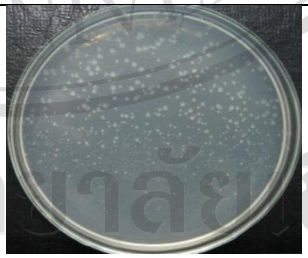
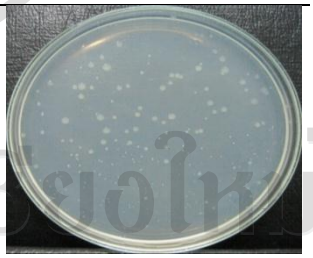
	Presence (+S9)	Absence (-S9)
Negative controls DMSO		
50% hydroglycol		
Positive controls 2-AA mutagen for presence AF-2 mutagen for absence		
***R. damascena* extract** 2-AA + *R. damacena* 80 mg/plate AF-2 + *R. damacena* 80 mg/plate		
***R.damascena* extract** 2-AA + *R. damascena* 10 mg/plate AF-2 + *R. damascena* 10 mg/plate		

Figure H.8 Effect of *R. damascena* extract against 2-AA and AF-2 mutagens in *S. typhimurium* strain TA100 in present and absence metabolic activation

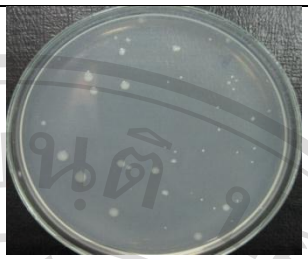
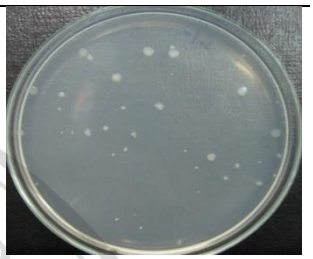
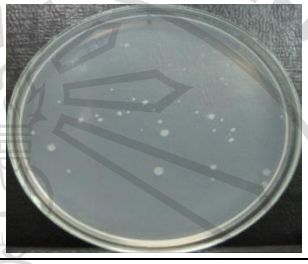
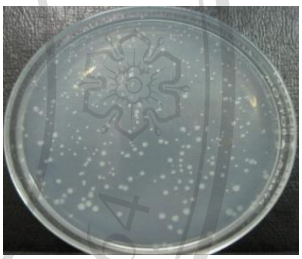
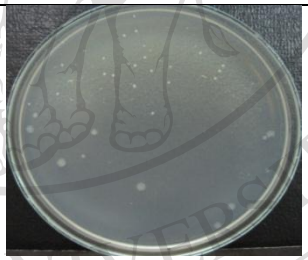
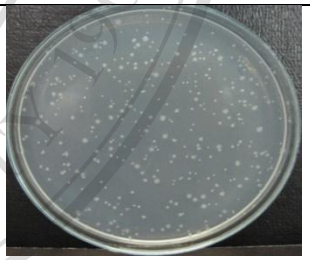
	Presence (+S9)	Absence (-S9)
Negative controls DMSO		
50% hydroglycol		
Positive controls 2-AA mutagen for presence AF-2 mutagen for absence		
***R. kerrii* Meijer extract** 2-AA + *R. kerrii* Meijer 80 mg/plate AF-2 + *R. kerrii* Meijer 80 mg/plate		
***R. kerrii* Meijer extract** 2-AA + *R. kerrii* Meijer 10 mg/plate AF-2 + *R. kerrii* Meijer 10 mg/plate		

Figure H.9 Effect of *R. kerrii* Meijer extract against 2-AA and AF-2 mutagens in *S. typhimurium* strain TA98 in present and absence metabolic activation

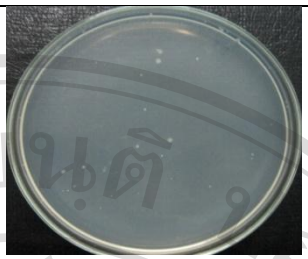
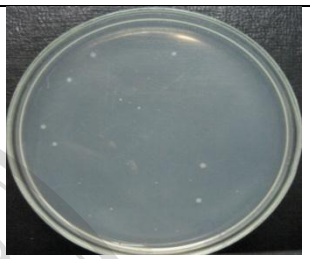
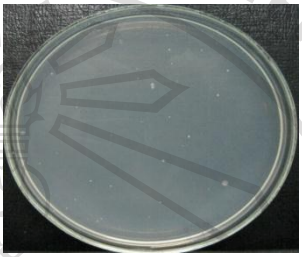
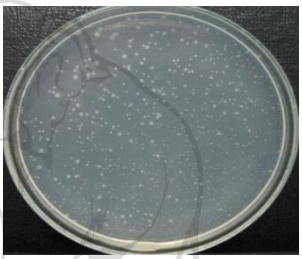
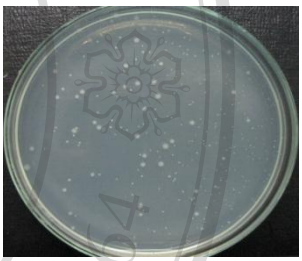
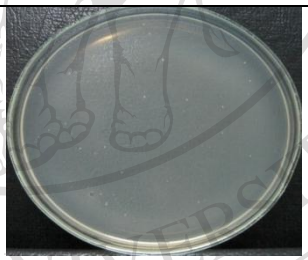
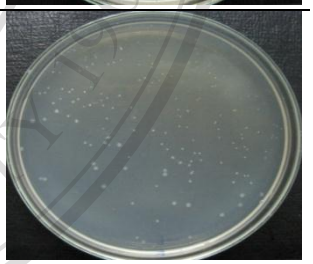
	Presence (+S9)	Absence (-S9)
Negative controls DMSO		
50% hydroglycol		
Positive controls 2-AA mutagen for presence AF-2 mutagen for absence		
***R. kerrii* Meijer extract** 2-AA + *R. kerrii* Meijer 80 mg/plate AF-2 + *R. kerrii* Meijer 80 mg/plate		
***R. kerrii* Meijer extract** 2-AA + *R. kerrii* Meijer 10 mg/plate AF-2 + *R. kerrii* Meijer 10 mg/plate		

Figure H.10 Effect of *R. kerrii* Meijer extract against 2-AA and AF-2 mutagens in *S. typhimurium* strain TA100 in present and absence metabolic activation

APPENDIX I

Morphological changes of both fibroblast cell lines without UV radiation at $CD_{12.5}$ of the extracts

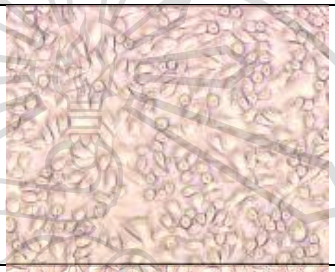
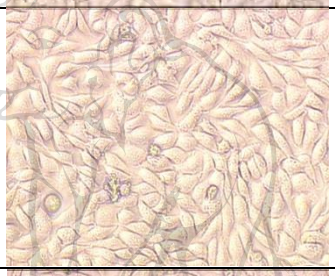
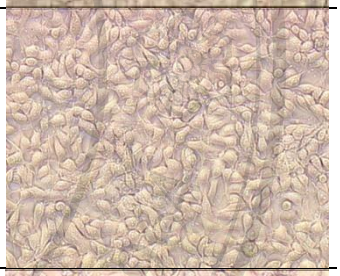
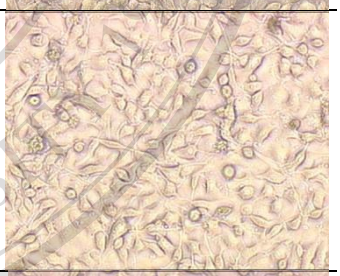
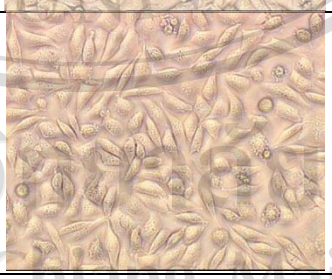
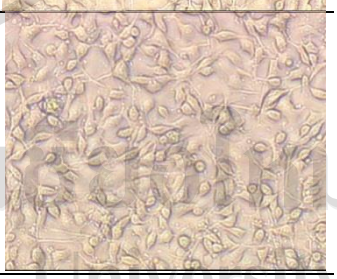
	L929	B16F10
Control without UV treated		
70% (v/v) hydroglycol		
***T. chebula* Retz.** $CD_{12.5}$ = 9.50 μg/L (L929) $CD_{12.5}$ = 3.24 μg/L (B16F10)		
T. bellerica $CD_{12.5}$ = 6.00 μg/L (L929) $CD_{12.5}$ = 0.75 μg/L (B16F10)		

Figure I.1 Morphology of normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines without UV radiation treated with 70% hydroglycol, *T. chebula* Retz. and *T. bellerica* extracts at the concentration $CD_{12.5}$.

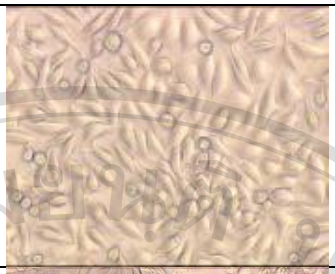
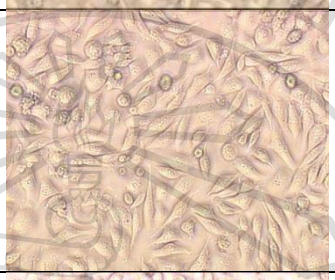
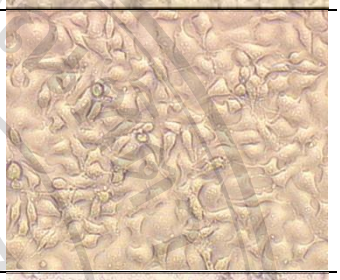
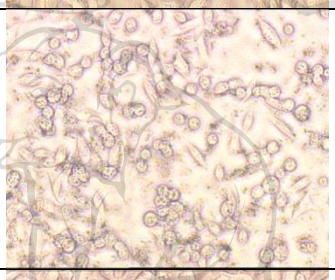
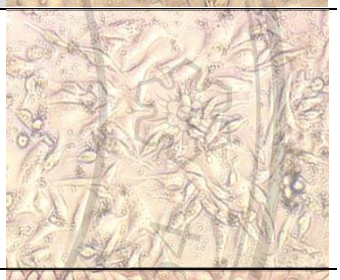
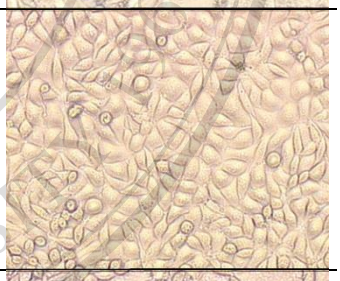
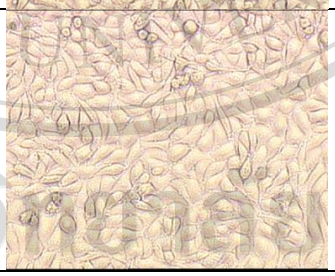
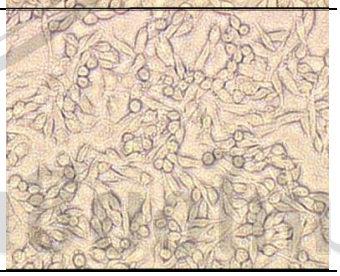
	L929	B16F10
Control without UV treated		
50% (v/v) hydroglycol		
***E. elatior* (Jack) R.M. Smith** $CD_{12.5}$ = 21.03 µg/L (L929) $CD_{12.5}$ = 61.34 µg/L (B16F10)		
R. damascena $CD_{12.5}$ = 13.77 µg/L (L929) $CD_{12.5}$ = 8.01 µg/L (B16F10)		
***R. kerrii* Meijer** $CD_{12.5}$= 55.08 µg/L (L929) $CD_{12.5}$= 41.32 µg/L (B16F10)		

Figure I.2 Morphology of normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines without UV radiation treated with 50% hydroglycol, *E. elatior* (Jack) R.M. Smith, *R. damascena* and *R. kerrii* Meijer extracts at the concentration $CD_{12.5}$.

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Poster Presentations and Proceeding

1. Nittayajaiprom, W., Saovapakhiran, A., Sangthong, P., Chancharunee, S., Wanasawas, P., and Chulasiri, M. (2010). Co-Mutagenic activity of *Etlingera elatior* (Jack) R.M. Smith in *Salmonella typhimurium* TA98. TSB2010: The 22nd Annual Meeting of the Thai Society for Biotechnology, Prince of Songkla University, Trang Campus, Thailand, p.239.

2. Nittayajaiprom, W., Saovapakhiran, A., Sangthong, P., Chancharunee, S., Wanasawas, P., and Chulasiri, M. (2010). Biological activity test of *Terminalia chebula* Retzius, *Terminalia bellirica* and *Rafflesia kerrii* Meijer extracts. ACEM: 2nd Asian Conference on Environmental Mutagens, Pattaya, Thailand, p.131.

3. Nittayajaiprom, W., Saovapakhiran, A., Sangthong, P., Chancharunee, S., Wanasawas, P., and Chulasiri, M. (2011). Biological activity study of *Terminalia chebula* Retzius, *Terminalia bellirica* and *Rafflesia kerrii* Meijer extracts. PERCH-CIC: The 7th International Congress for Innovation Chemistry, Pattaya, Thailand, p.239.

4. Nittayajaiprom, W., Saovapakhiran, A., Sangthong, P., Chancharunee, S., Wanasawas, P., and Chulasiri, M. (2010). Genotoxicity and Anti-mutagenicity studies from *Rafflesia kerrii* Meijer. TSB2010: The 22nd Annual Meeting of the Thai Society for Biotechnology, Prince of Songkla University, Trang Campus, Thailand, p. 1254-1262 (proceeding).