CHAPTER 3

RESULTS

3.1 Physical properties of the plant extracts

The plant extracts were prepared and supplied by S&J International Enterprise Pubic Company Limited. The 70% hydroglycol was used as solvent for *T. chebula* Retz. and *T. bellerica* extraction, whereas 50% hydroglycol was used as solvent for *E. elatior* (Jack) R.M. Smith, *R. damascena* and *R. kerrii* Meijer extraction. The extracts were investigated their physical characteristics such as color and the precipitation of plant extracts. It was found that the solutions of five kinds of plant extracts were suspended liquid and contained small amount of precipitation in hydroglycol and the colors were ranged from light brown to dark brown. The results were shown Figure 3.1 and Table 3.1. The colors of *T. chebula* Retz. and *T. bellerica* extracts were dark brown whereas the colors of *E. elatior* (Jack) R.M. Smith, *R. damascena* and *R. kerrii* Meijer extracts were light brown. Then, the plant extracts were examined by using Ultraviolet-visible spectrophotometer, IR and NMR.

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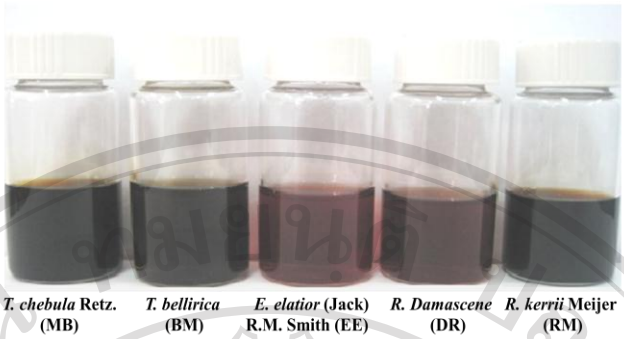

Figure 3.1 Color of the extracts: *T. chebula* Retz. (MB), *T. bellerica* (BM), *E. elatior* (Jack) R.M. Smith (EE), *R. damascena* (DR) and *R. kerrii* Meijer (RM)

Table 3.1 Physical appearance of the plant extracts

Plant extracts	Code	Solvent	Appearance of liquid
T. chebula Retz.	MB	70% hydroglycol	dark brown liquid
T. bellerica	BM	70% hydroglycol	dark brown liquid
E. elatior (Jack) R.M. Smith	EE	50% hydroglycol	brown liquid
R. damascena	DR	50% hydroglycol	brown liquid
R. kerrii Meijer	RM	50% hydroglycol	brown liquid

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3.2 Characterization of plant extracts by Spectroscopy

All of the extracts were examined for the constituents using ultraviolet-visible spectrophotometer (UV-Visible), infrared spectrometer (IR) and nuclear magnetic resonance (NMR).

3.2.1 Characterization of plant extracts by Ultraviolet-Visible spectrophotometer (UV-Visible spectrophotometer)

In this study, the plant extracts were examined for certain organic compounds by using UV-visible spectrophotometer. The spectra of the 0.30 mg/ml extracts were scanned at wavelength between 200 and 500 nm. The extracts of *T. chebula* Retz., *T. bellerica*, *E. elatior* (Jack) R.M. Smith, *R. damascena* and *R. kerrii* Meijer showed medium to strong absorbance between 250 and 300 nm as shown in Figure 3.2 A-F when using 50% hydroglycal and 70% hydroglycol as blank controls. The extracts of *T. chebula* Retz. and *T. bellerica* showed maximum absorbance (λ_{max}) at 260 and 275 nm, respectively (Figure 3.2 A and B). Whereas, the extracts of *E. elatior* (Jack) R.M. Smith, *R. damascena* and *R. kerrii* Meijer showed maximum absorbance (λ_{max}) at 279, 263 and 271 nm, respectively as shown in Figure 3.2 (C-E).

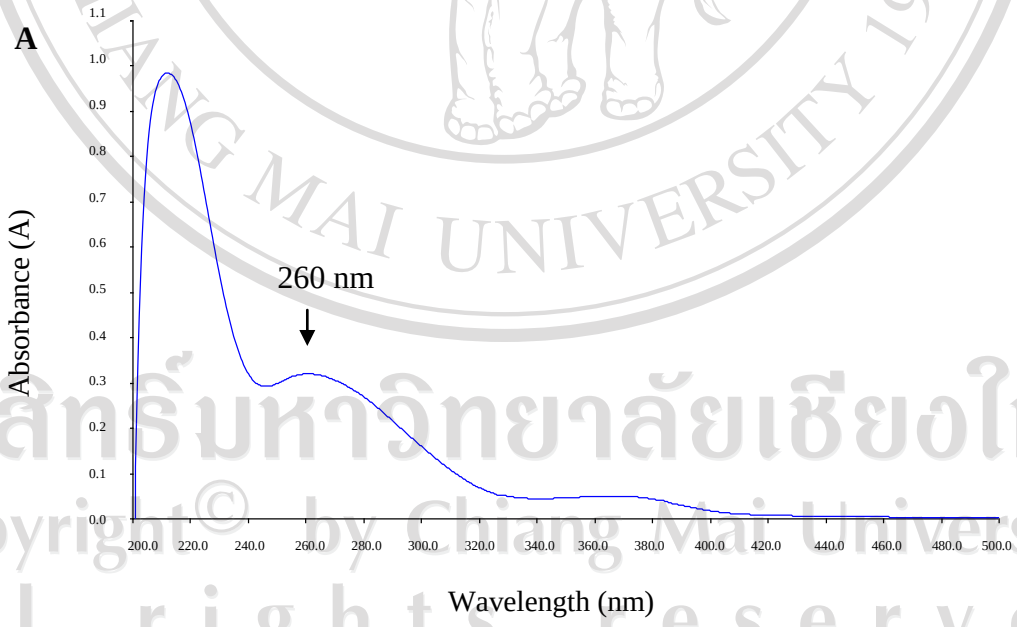

Figure 3.2 UV-Visible spectrum of *T. chebula* Retz. (A), *T. bellerica* (B), *E. elatior* (Jack) R.M. Smith (C), *R. damascena* (D) and *R. kerrii* Meijer (E) at wavelength between 200 and 500 nm.

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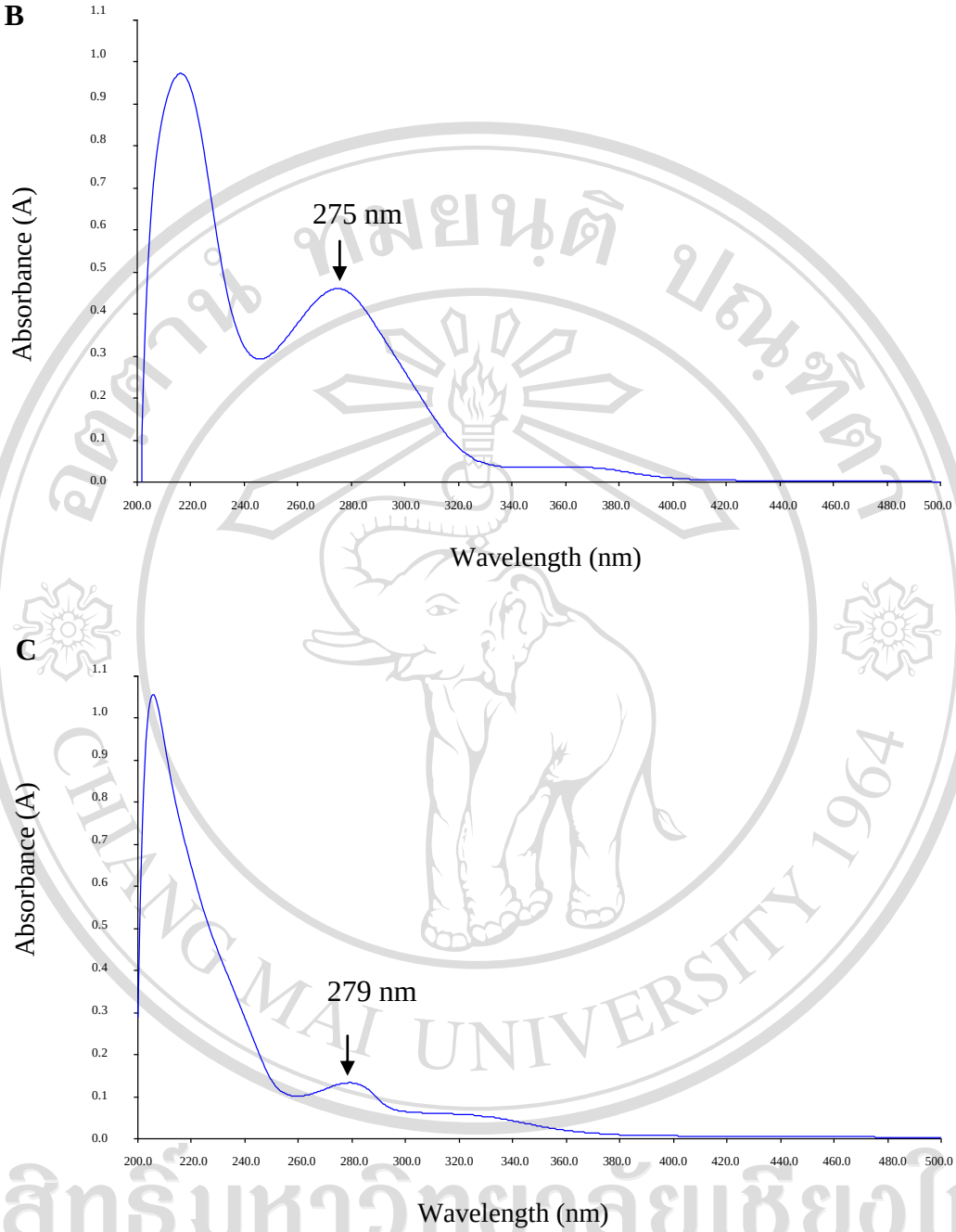

Figure 3.2 UV-Visible spectrum of *T. chebula* Retz. (A), *T. bellerica* (B), *E. elatior* (Jack) R.M. Smith (C), *R. damascena* (D) and *R. kerrii* Meijer (E) at wavelength between 200 and 500 nm (continued).

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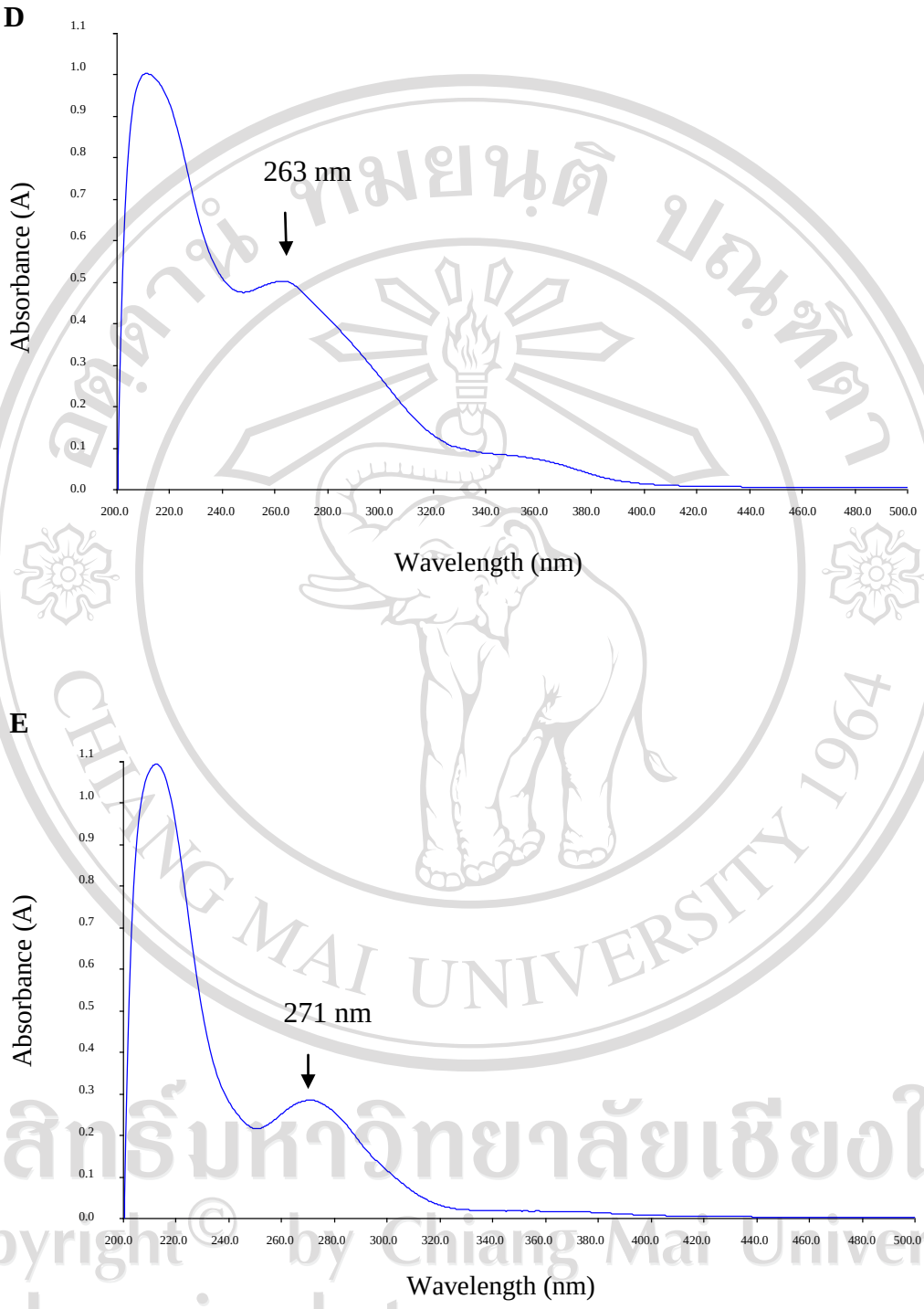

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Figure 3.2 UV-Visible spectrum of *T. chebula* Retz. (A), *T. bellerica* (B), *E. elatior* (Jack) R.M. Smith (C), *R. damascena* (D) and *R. kerrii* Meijer (E) at wavelength between 200 and 500 nm (continued).

3.2.2 Characterization of plant extracts by Infrared spectroscopy (IR)

The properties of the plant extracts were examined by IR. The results were shown in Figure 3.3 A-E. The spectrum of five plant extracts showed the strong absorption of O-H stretching of solvent and aromatic compounds. From the results, the *T. chebula* Retz. extract in the solvent 70% hydroglycol showed the similar IR spectrum as the *T. bellerica* extract (Figure 3.3 A-B).

The extract of *T. chebula* Retz. showed the absorption regions for O-H group of solvent (3391 cm^{-1}), C=C group of aromatic compound (1651-1654 cm^{-1}) and C-O group (1044-1136 cm^{-1}) as expected. The extract may contain hydrolysable tannins such as chebulagic acid and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (PGG) which are major compound in *T.chebula* Retz. (Reddy *et al.,* 2009 and Kim *et al.,* 2010). Whereas, the extract of *T. bellerica* showed the absorption regions for O-H group of solvent (3391 cm^{-1}), C=C group of aromatic compound (1654 cm^{-1}) and C-O group (1044-1137 cm^{-1}). There IR absorptions may cause by major compound such as gallic acid and gallate esters which is highly abundance in the fruit of *T. bellerica* (Pfundanstein *et al.,* 2009).

The plant extracts in the solvent 50% hydroglycol including *E. elatior* (Jack) R.M. Smith, *R. damascena* and *R. kerrii* Meijer showed the similar IR spectrum (Figure 3.3 C-E). The extract of *E. elatior* (Jack) R.M. Smith showed the absorption regions for O-H group of solvent (3400 cm^{-1}), C=C group of aromatic compound (1651cm^{-1}) and C-O group (1043-1136 cm^{-1}) as expected. The *E. elatior* (Jack) R.M. Smith extract may contain caffeoylquinic acids and chlorogenic acid as showed in previously report (Chan *et al.,* 2009). The extract of *R. damascena* showed the absorption regions for O-H group of solvent (3400 cm^{-1}), C=C group of aromatic

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compound (1641cm^{-1}) and C-O group (1045-1133 cm^{-1}) due to highly contents of polyphenolic compounds such as quercetine-3-*O*-glucoside, kaemp-ferol-3-*O*-rhamnoside and kaempferol-3-*O*-arabinoside (Yassa *et al.*, 2009). The extract of *R. kerrii* Meijer showed the absorption regions for O-H group of solvent (3401 cm^{-1}), C=C group of aromatic compound (1652 cm^{-1}) and C-O group (1042-1136 cm^{-1}) as expected. These may occur due to the compositions of 1,2,4,6- tetra-*O*-galloyl-β-D-glucopyranoside and syringing in the *R. kerrii* Meijer extract (Kanchanapoom *et al.*, 2007).

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A

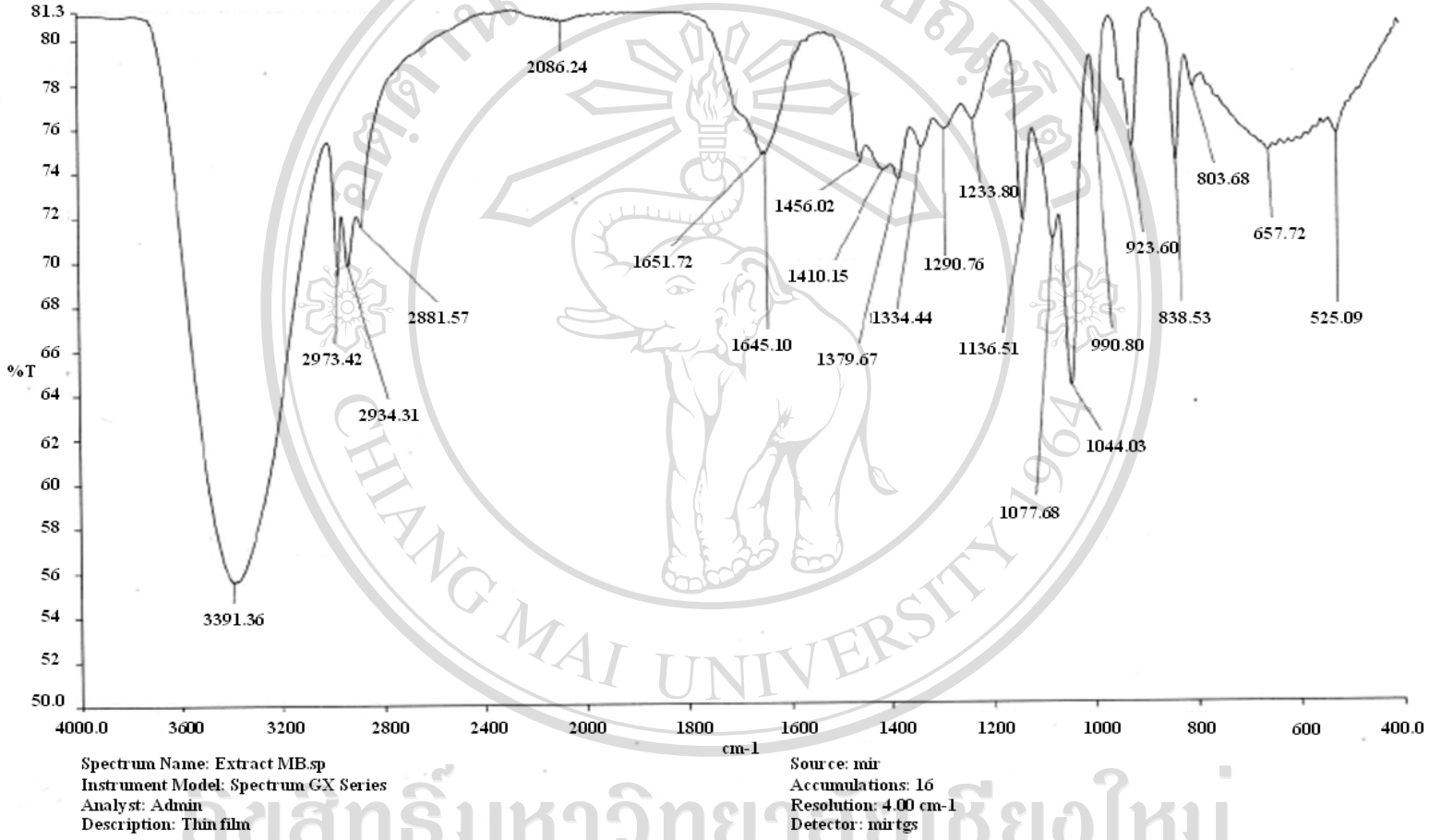

Figure 3.3 IR spectrum of *T. chebula* Retz. (A), *T. bellerica* (B), *E. elatior* (Jack) R.M. Smith (C), *R.damascena* (D) and *R. kerrii* Meijer (E)

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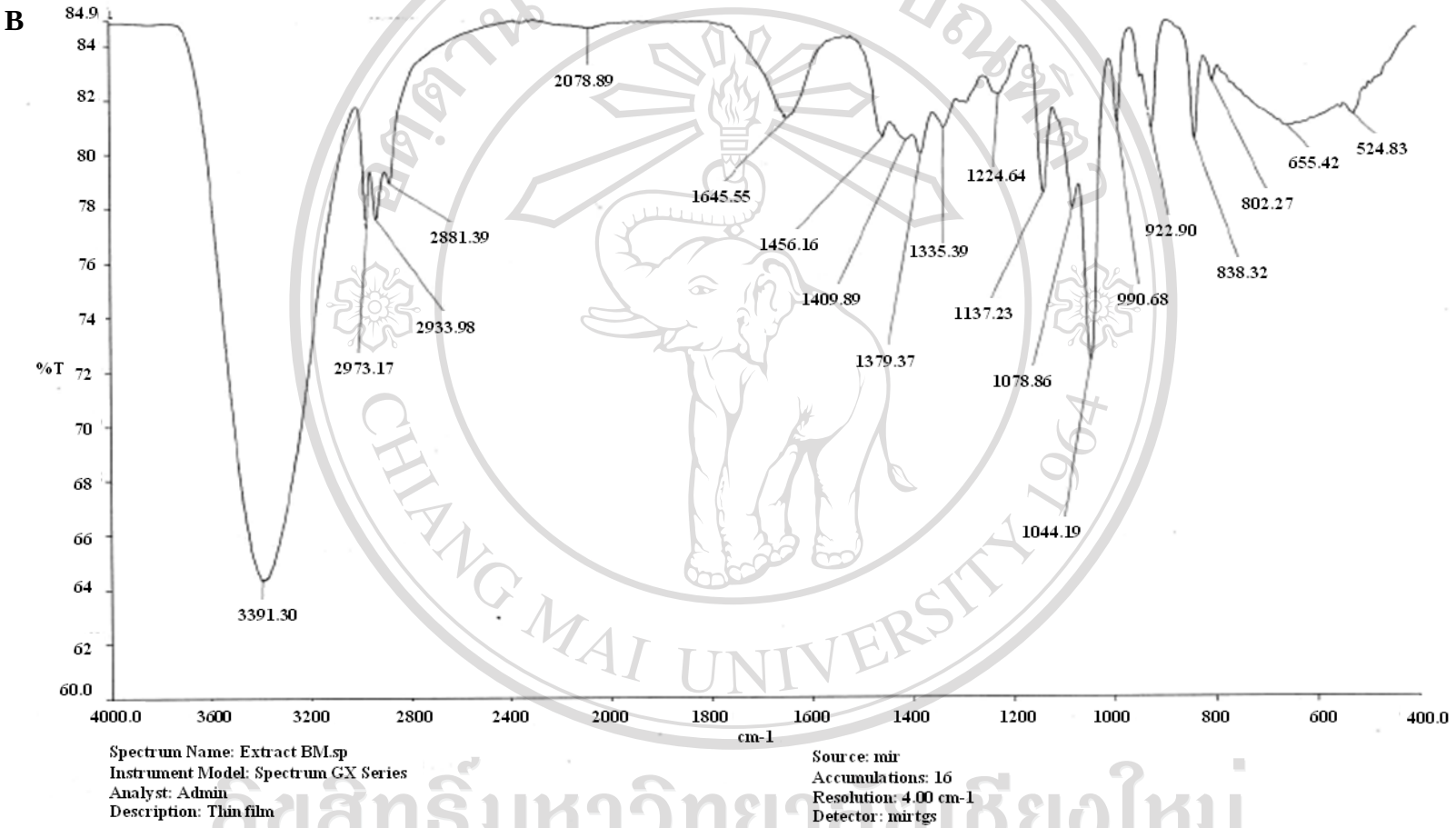

Figure 3.3 IR spectrum of *T. chebula* Retz. (A), *T. bellerica* (B), *E. elatior* (Jack) R.M. Smith (C), *R. damascena* (D) and *R. kerrii* Meijer (E) (continued).

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C

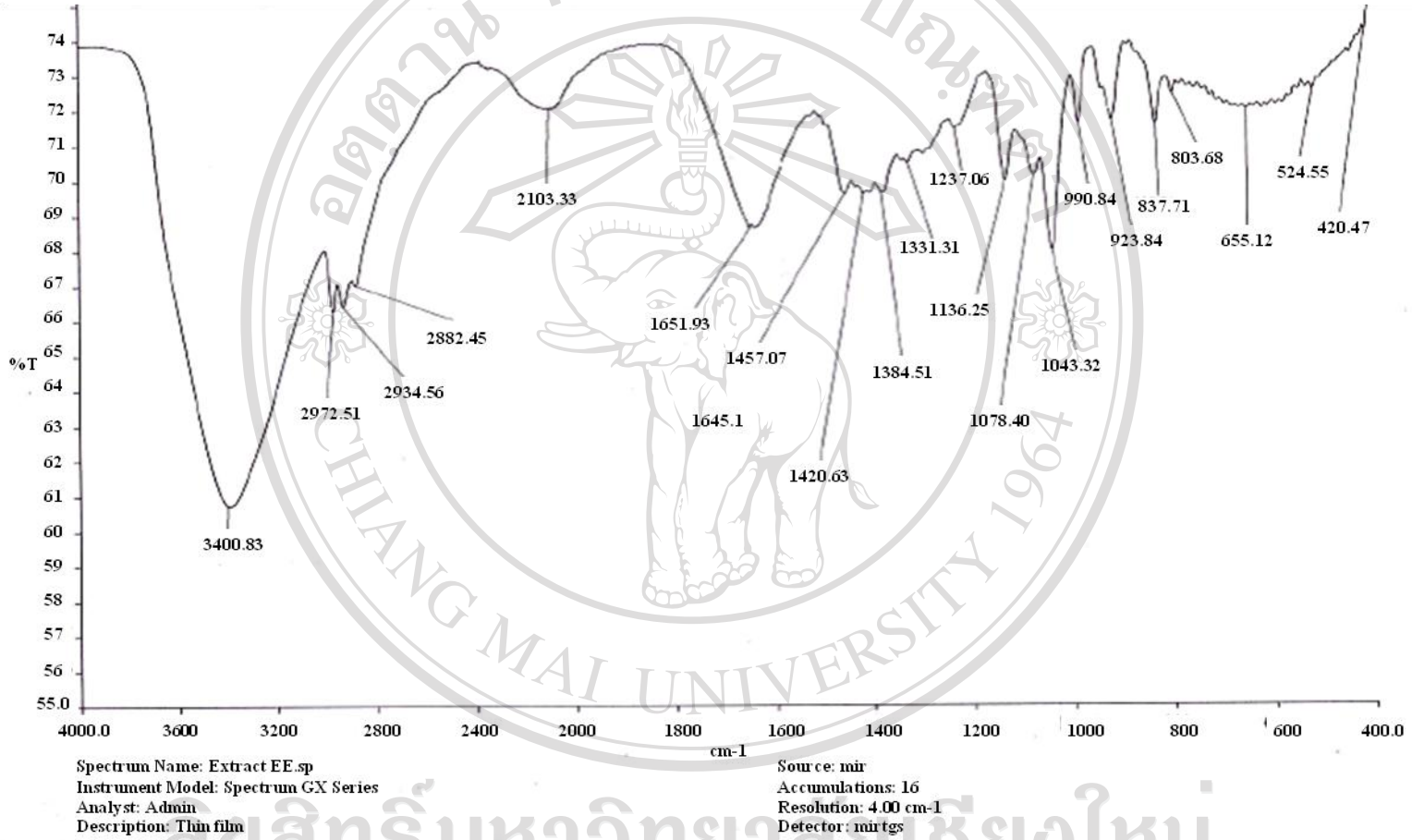

Figure 3.3 IR spectrum of *T. chebula* Retz. (A), *T. bellerica* (B), *E. elatior* (Jack) R.M. Smith (C), *R. damascena* (D) and *R. kerrii* Meijer (E) (continued).

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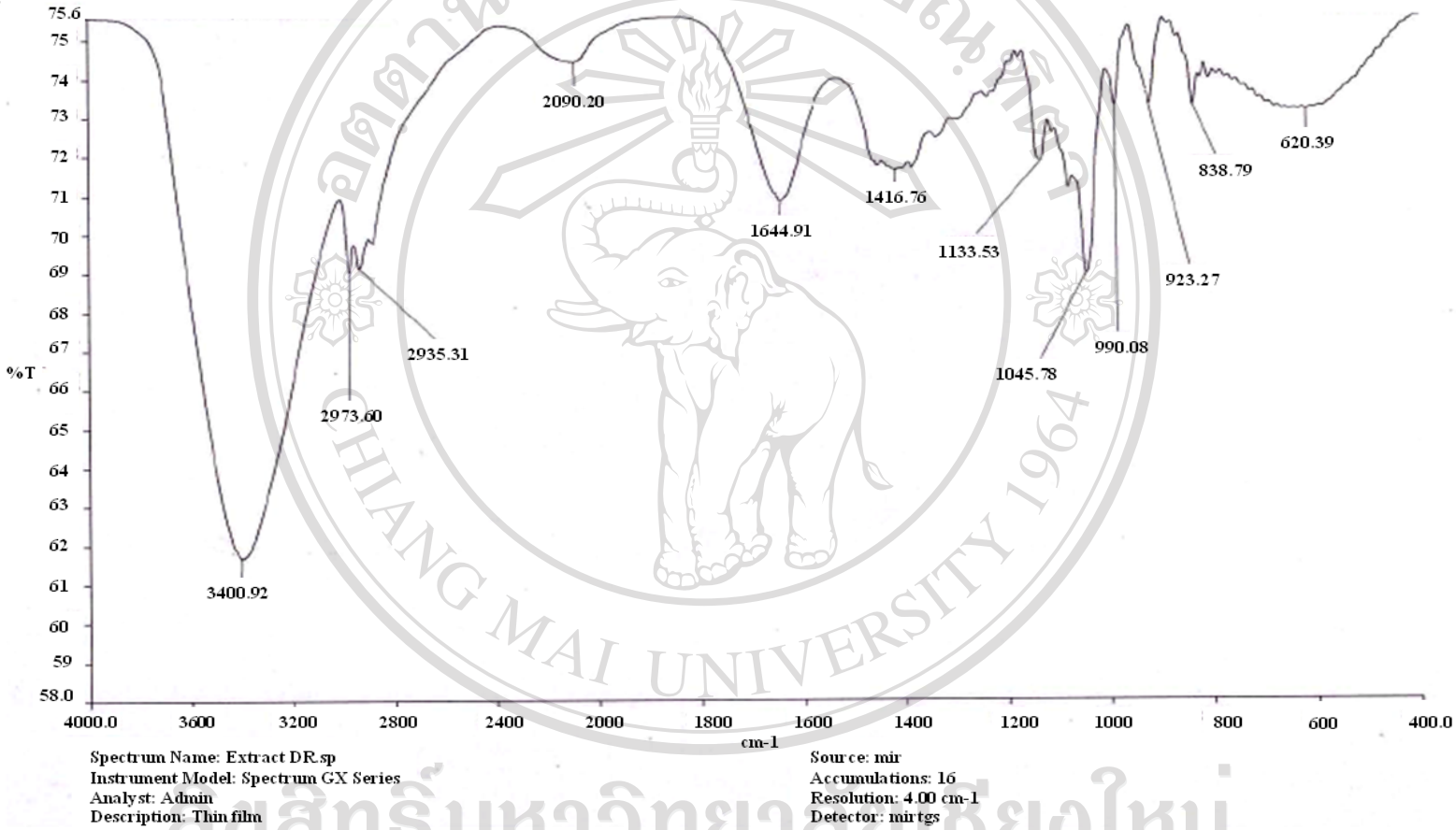

Figure 3.3 IR spectrum of *T. chebula* Retz. (A), *T. bellerica* (B), *E. elatior* (Jack) R.M. Smith (C), *R. damascena* (D) and *R. kerrii* Meijer (E) (continued).

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E

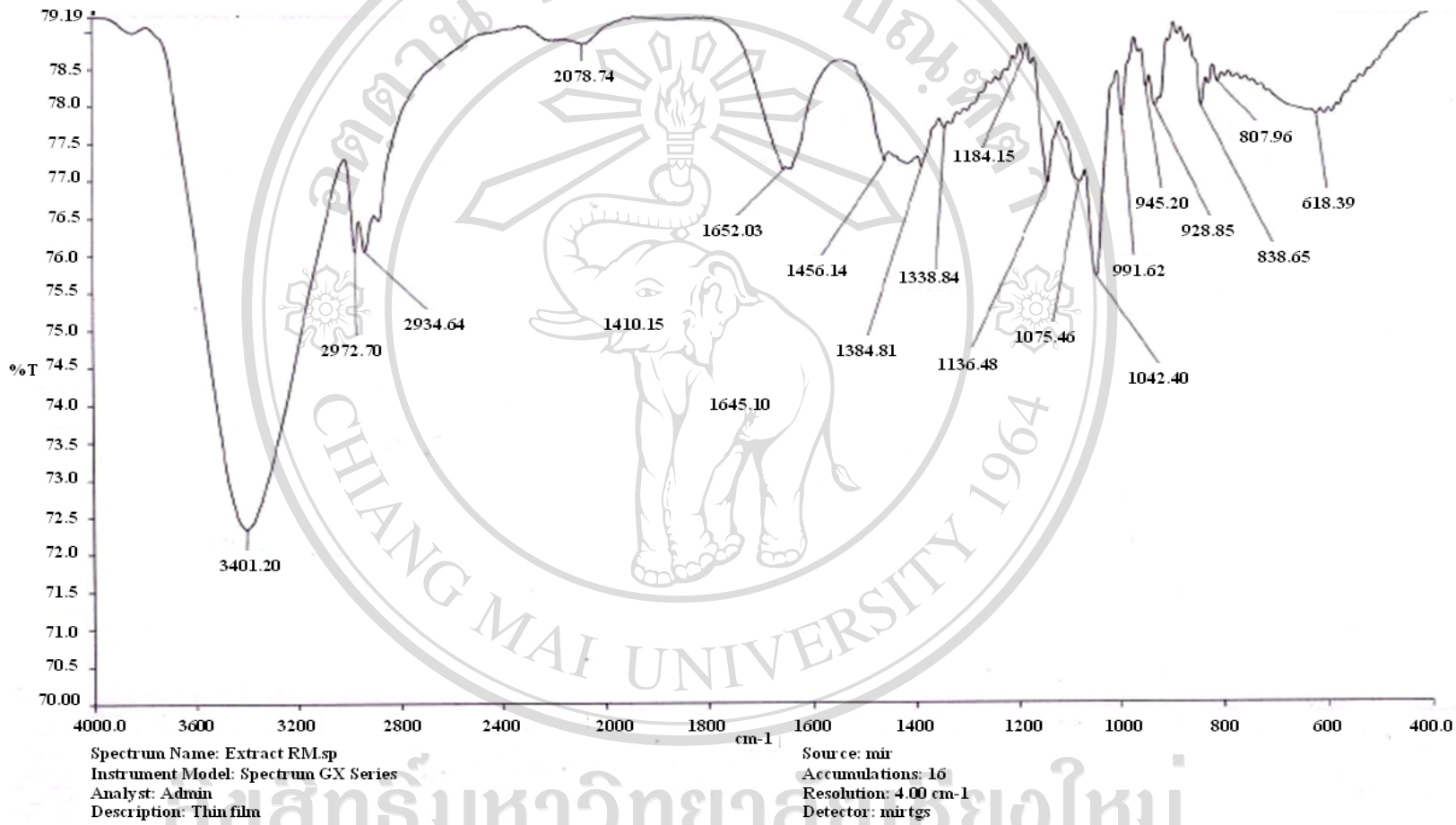

Figure 3.3 IR spectrum of *T. chebula* Retz. (A), *T. bellerica* (B), *E. elatior* (Jack) R.M. Smith (C), *R. damascena* (D) and *R. kerrii* Meijer (E) (continued).

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3.2.3 Characterization of plant extracts by nuclear magnetic resonance (NMR)

The properties of the plant extracts were examined by nuclear magnetic resonance (NMR). The results were showed in Figure 3.4 A-F. From the Figure 3.4 (A), the solvent of the extracts showed the signal proton at δ 3.0-5.0 ppm. All the extracts contained unsaturated proton at δ 6.5-7.0 ppm and glycoside at 4.0-5.0 ppm. The extract of *T. chebula* Retz. showed unsaturated proton at δ 6.5-7.0 ppm and glycoside at 4.0-5.0 ppm as epected. The extract may contain hydrolysable tannins such as chebulagic acid (^{1}H NMR: δ 2.1-7.4 ppm of chebuloyl, δ 6.6-7.0 ppm of galloyl and δ 4.3-6.5 ppm of glucose) and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (PGG, ^{1}H NMR: δ 7.1-6.9 ppm of galloyl and δ 6.3-4.3 ppm of glucose) which are major compound in *T.chebula* Retz. (Reddy *et al.*, 2009, Kim *et al.*, 2010, Pfundanstein *et al.*, 2009). The extract of *T. bellerica* contained unsaturated proton at δ 6.3-7.2 ppm and glycoside at 4.3-5.4 ppm due to highly content of polyphenolic compound such as gallic acid, methyl gallate and 1,3,4,6-penta-*O*-galloyl-β-D-glucose (^{1}H NMR: δ 7.1-6.9 ppm of galloyl and δ 6.1-3.9 ppm of glucose) (Pfundanstein *et al.*, 2009).

The extract of *E. elatior* (Jack) R.M. showed unsaturated proton at δ 6.8 ppm, aromatic proton at δ 8.3 ppm and and glycoside at 4.4-5.1 ppm as expected. The extract may contain caffeoylquinic acids and chlorogenic acid (^{1}H NMR: δ 6.7, 6.9 and 7.0 ppm of caffeoyl group) as showed in previously report (Chan *et al.*, 2009). The extracts of *R. damascena* showed unsaturated proton at δ 6.8 ppm and glycoside at 4.2-5.2 ppm as expected. This may be due to the compositions of kaempferol and quercetin such as quercetine-3-*O*-glucoside (^{1}H NMR: δ 6.2-6.3 ppm of quercetine, δ 6.8-7.5 ppm of aromatic proton and δ 5.2, 3.2-3.4 ppm of glucose), kaempferol-3-*O*-

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rhamnoside (^{1}H NMR: δ 6.2-6.4 ppm of kaempferol, δ 6.9-7.7 ppm of aromatic proton and δ 5.3, 3.4-3.8 ppm of L-rhamnose) (Yassa *et al.*, 2009, Lim *et al.*, 2007 and Soliman *et al.*, 2002). Whereas, the extract of *R. kerrii* Meijer showed unsaturated proton at δ 6.8 ppm and signal proton of solvent at δ 3.0-6.0 ppm when compared the spectum of solvent in Figture 3.4 (A). The extract contained of hydrolyzable tannins such as 1,2,4,6- tetra-*O*-galloyl-β-D-glucopyranoside and syringin in the *R. kerrii* Meijer extract (Kanchanapoom *et al.*, 2007). The result suggested that the five plant extracts contained the aromatic compounds which were phenolic compounds and glycoside that occured in natural products (Ersöz *et. al.*, 2002). Then, the biological activities of plant extracts were investigated for cytotoxicity, mutagenicity, antimutagenicity and morphological changes after induced by UV radiation.

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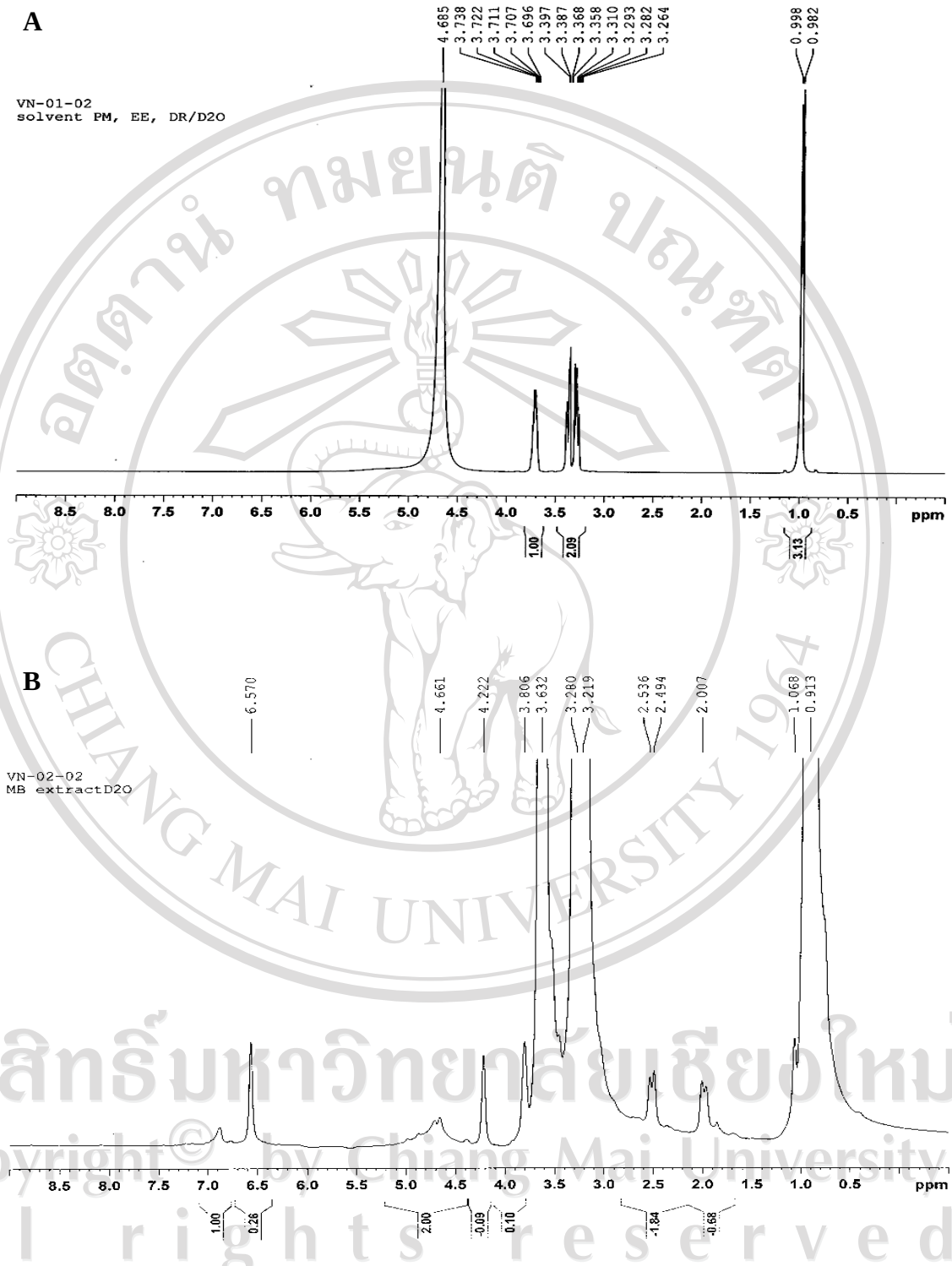

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Figure 3.4 [1]HNMR spectrum of hydroglycol (A), *T. chebula* Retz. (B), *T. bellerica* (C), *E. elatior* (Jack) R.M. Smith (D), *R. damascena* (E) and *R. kerrii* Meijer (F)

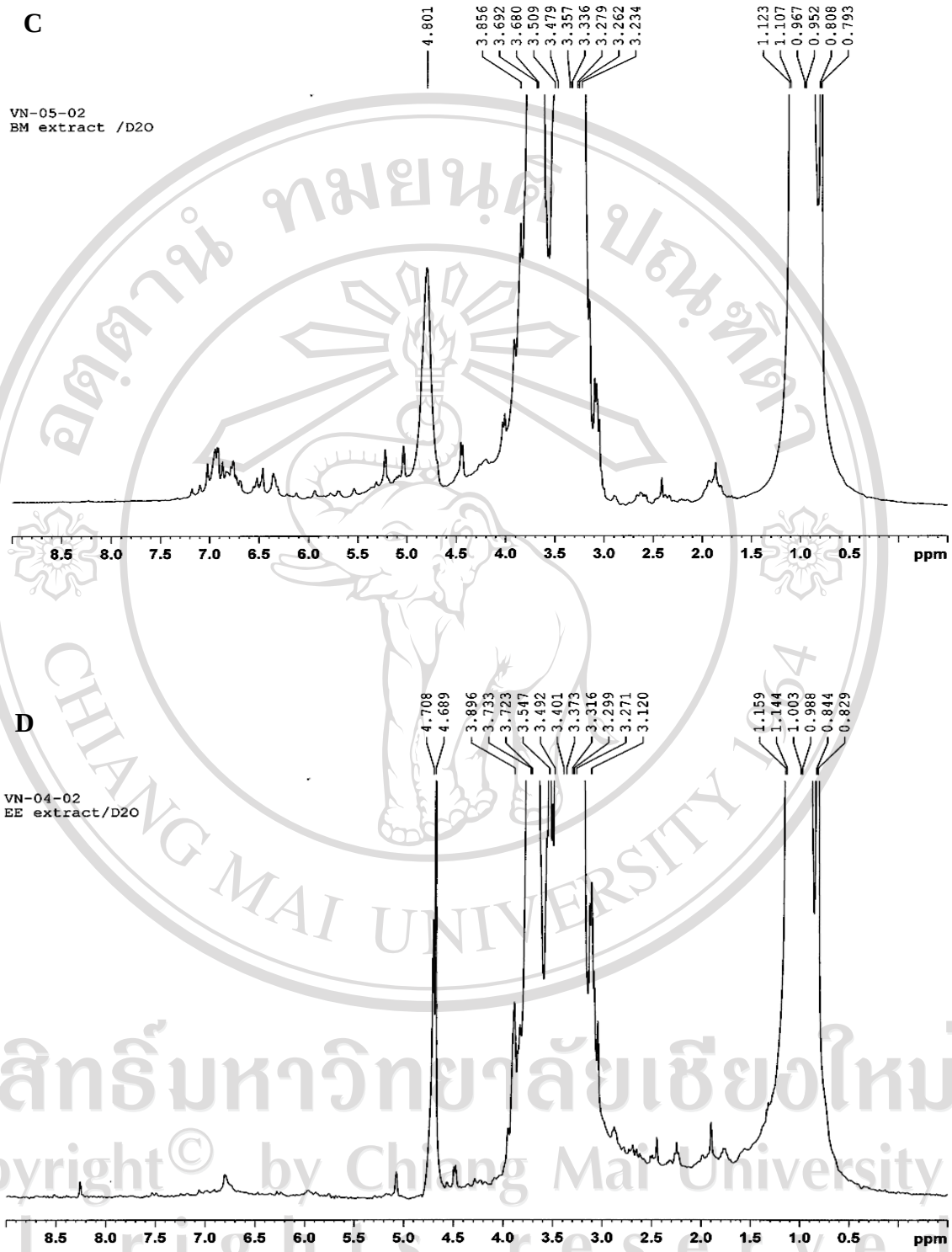

Figure 3.4 ^{1}HNMR spectrum of hydroglycol (A), *T. chebula* Retz. (B), *T. bellerica* (C), *E. elatior* (Jack) R.M. Smith (D), *R. damascena* (E) and *R. kerrii* Meijer (F) (continued).

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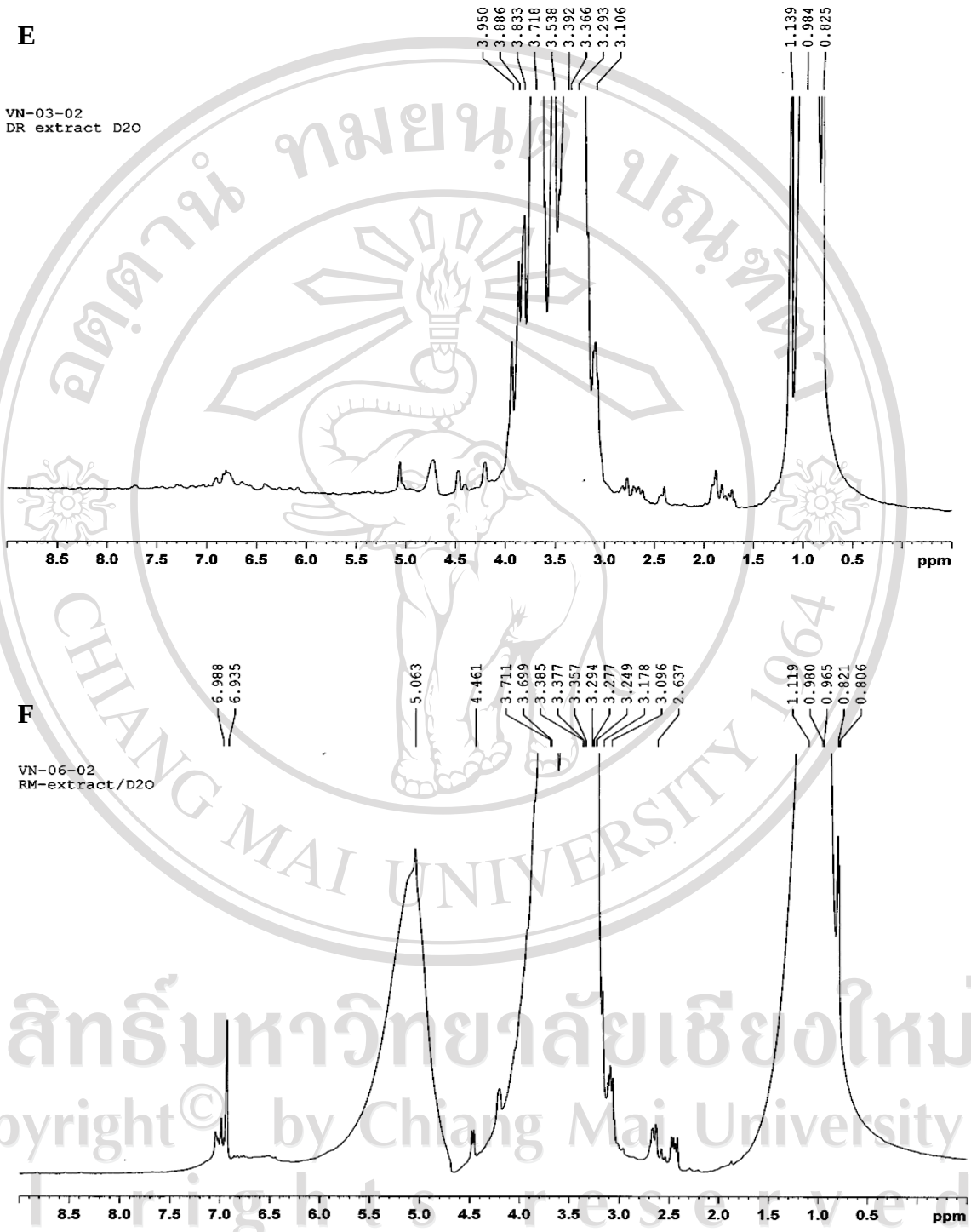

Figure 3.4 [1]HNMR spectrum of hydroglycol (A), *T. chebula* Retz. (B), *T. bellerica* (C), *E. elatior* (Jack) R.M. Smith (D), *R. damascena* (E) and *R. kerrii* Meijer (F) (continued).

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3.3 Determination of total phenolic contents in plant extracts

The total phenolic contents (TPCs) of the five plant extracts were determined by the Folin-Ciocalteu method to examine total phenolic levels in the extracts. Folin-Ciocalteu method is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. This method was relied on the transfer of electrons in alkaline medium from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes to form blue product complexes. The products of the metal oxide are reduced to give a blue complex that exhibits a broad light absorption with a maximum at 765 nm (Singleton and Rossi *et al.*, 1965). The advantages of Folin-Ciocalteu method are commonly accepted assay and long-wavelength absorption of the chromophore minimizes interference from the sample matrix, which is often colored (Huang *et al*., 2005). The colors of five plant extracts were showed the UV absorption at 260-275 nm as shown in Figure 3.2 (C-E). This method is suitable for total phenolic contents determination in all plant extracts due to the colors of all extracts do not interfere with wavelength of blue produce complexes.

In this experiment, the total phenolic contents were estimated as gallic acid equivalents**.** The gallic acid standard cuvre was shown in Appendix D. The standard concentrations of gallic acid were used at concentrations between 0.005 and 0.070 mg/ml ($Y = 23.85x$, $R^2 = 0.997$). The plant extracts showed the total phenolic contents from 14.90 ± 0.02 to 112.40 ± 0.08 mg GAE/g of wet weight as shown in Table 3.2. The extract of *R. kerrii* Meijer showed the highest total phenolic content at 112.40 ± 0.08 mg GAE/g of wet weight follow by *T. chebula* Retz*., T. bellerica, R. damascena* and *E. elatior* (Jack) R.M. Smith which gave the total phenolic contents at 101.18 ± 0.07, 94.37 ± 0.03, 34.23 ± 0.01 and 14.90 ± 0.02 mg GAE/g of wet weight,

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respectively. The results were shown in Figure 3.5. Next, the plant extracts were evaluated for the mutagenicity and antimutagenicity in *Salmonella typhimurium* TA98 and TA100 strains by Ames test.

Table 3.2 The total phenolic contents (TPCs) of the five plant extracts after examined by Folin-Ciocalteu method

Plant extracts	**Total phenolic contents (mg GAE/g of wet weight)**
T. chebula Retz.	101.18 ± 0.07
T. bellerica	94.37 ± 0.03
E. elatior (Jack) R.M. Smith	14.90 ± 0.02
R. damascena	34.23 ± 0.01
R. kerrii Meijer	112.40 ± 0.08

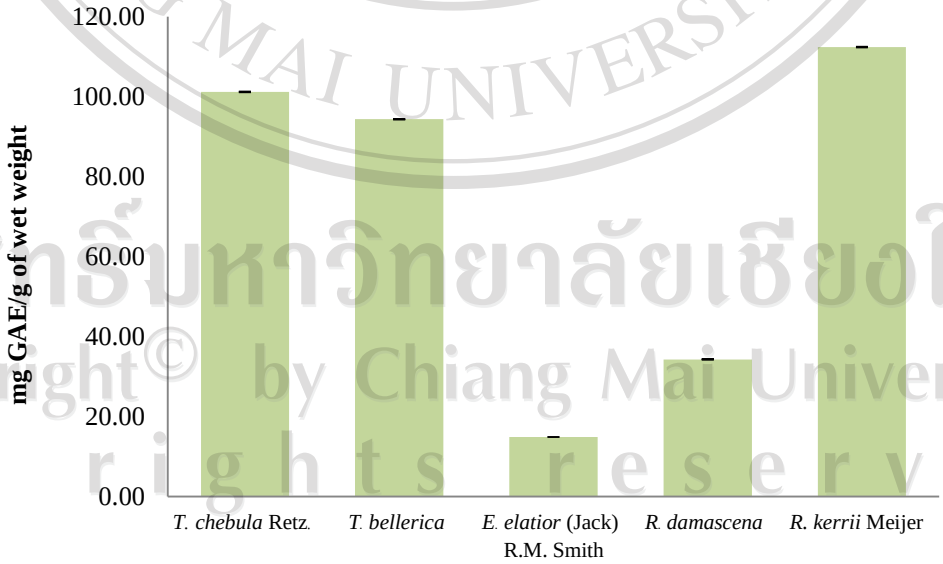

Figure 3.5 Comparison of the total phenolic contents of the five plants extracts at mg GAE/g of wet weight which were estimated as gallic acid equivalents**.**

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3.4 Cytotoxicity of the plant extracts

3.4.1 Cytotoxicity of the plant extracts in normal and melanoma mouse fibroblast cell lines using MTT assay

To evaluate the safety of the plant extracts, MTT assay was performed to investigate the cytotoxic effect of the extracts by normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines. The results were shown in Table 3.3. Cell viability was calculated for the 50% cytotoxic dose (CD_{50}) which was the concentration of the extract at 50% of the viable cells.

The result revealed that all extracts showed low cytotoxic effect on both fibroblast cell lines with 50% cytotoxicity (CD_{50}) value at ranges between 5.43 ± 0.18 and 39.39 ± 0.21 mg/ml for normal fibroblast cell lines. Among of five plant extracts, *T. bellerica* showed the highest cytotoxicity with CD_{50} value at 5.43 ± 0.18 mg/ml following by *T. chebula* Retz., *R. damascena*, *E. elatior* (Jack) R.M. Smith, respectively. Whereas, *R. kerrii* Meijer showed lowest cytotoxicity with CD_{50} value at 39.39 ± 0.21 mg/ml for normal fibroblast cell line. In mouse melanoma fibroblast cell line, all of the extracts also showed the low cytotoxicity with CD_{50} values at ranges between 2.00 ± 0.18 and 58.23 ± 0.18 mg/ml. It was found that *T. bellerica* extract showed the highest cytotoxic effect with CD_{50} value at 2.00 ± 0.18 mg/ml following by *T. chebula* Retz., *R. damascena* and *R. kerrii* Meijer. Moreover, *E. elatior* (Jack) R.M. Smith showed the lowest cytotoxic effect with CD_{50} at 58.23 ± 0.18 mg/ml. Interestingly, *T. bellerica* extract showed the highest cytotoxicity only on mouse melanoma B16F10 cell line with CD_{50} value at 2.00 ± 0.18 mg/ml. This indicated that the biological compounds *T. bellerica* might affect only on melanoma cell line.

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Table 3.3 Cytotoxicity of plant extracts on normal mouse fibroblast L929 and melanoma fibroblast B16F10 cell lines expressed as 50% cytotoxicity dose (CD_{50}) values which were obtained in MTT assay for 48 h

Plant extracts	**50% cytotoxic dose (mg/ml)**	
	mouse fibroblast L929 cell line	**mouse melanoma B16F10 cell line**
T. chebula Retz.	11.96 ± 0.80	4.35 ± 0.33
T. bellerica	5.43 ± 0.18	2.00 ± 0.18
E. elatior (Jack) R.M. Smith	19.85 ± 0.65	58.23 ± 0.18
R. damascena	17.13 ± 0.39	10.04 ± 0.24
R. kerrii Meijer	39.39 ± 0.21	26.47 ± 0.47

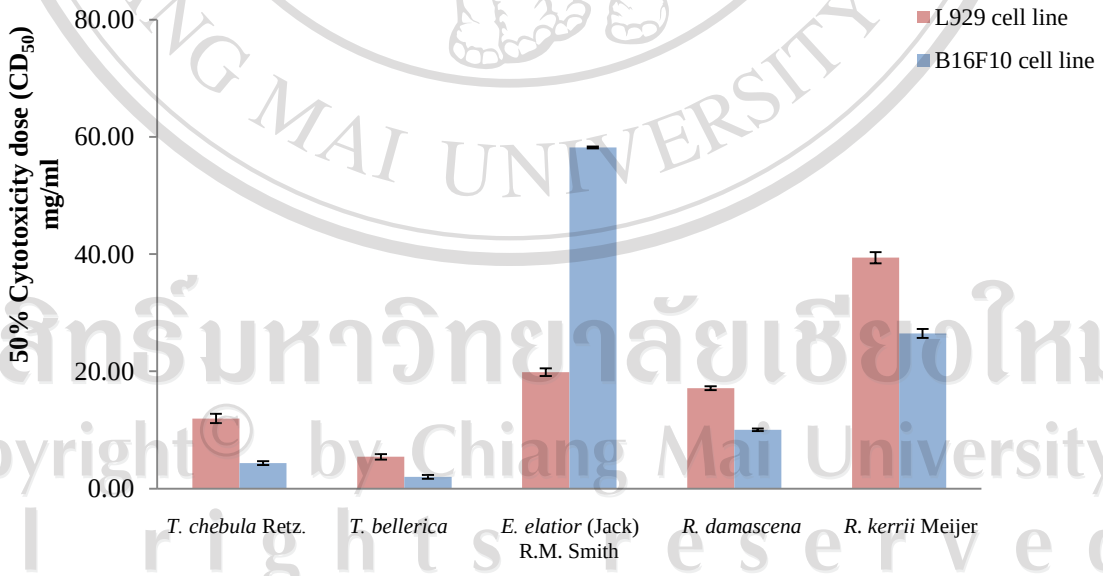

Figure 3.6 50% cytotoxicity dose (CD_{50}) of the extracts for mouse normal skin fibroblast L929 and mouse melanoma fibroblast B16F10 cell lines by MTT assay.

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MTT assay was performed to determine the mitochondrial reductase activity in active cell lines after treated with the extracts. In order to confirm the results of the cytotoxicity of plant extracts, dye exclusion method was also performed to determine the number of viable cells which present in a cell suspension.

3.4.2 Cytotoxicity of the plant extracts in normal and melanoma mouse fibroblast cell lines by dye exclusion method

In this study, the cytotoxicity of plant extracts were performed in normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines by using dye exclusion method. Dye exclusion was used to determine the number of viable cells present in a cell suspension using trypan blue staining. The results were shown in Table 3.4. The extracts of *T. bellerica, E. elatior* (Jack) R.M. Smith and *R. damascena* showed low cytotoxic effect with CD_{50} values between 8.73 ± 1.40 – 22.93 ± 0.55 mg/ml for normal fibroblast cell lines. On the other hand, the cytotoxicity of *T. chebula* Retz. and *R. kerrii* Meijer extracts could not be obtained because normal mouse fibroblast cell lines were extremely fragile and lysis which leaded to cell debris after adding both extracts into the cells. In melanoma fibroblast cell lines, the extracts of *T. chebula* Retz., *T. bellerica, E. elatior* (Jack) R.M. Smith and *R. damascena* showed cytotoxicity with CD_{50} values between 1.75 ± 0.63 – 43.17 ± 0.14 mg/ml. While, the extract of *R. kerrii* Meijer caused melanoma fibroblast cell lines lysis and also leaded to cell debris.

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Table 3.4 Cytotoxicity of plant extracts on normal mouse fibroblast L929 and melanoma fibroblast B16F10 cell lines expressed as 50% cytotoxic dose (CD_{50}) values which were obtained in dye exclusion for 24 h

Plant extracts	**50% cytotoxic dose (mg/ml)**	
	mouse fibroblast L929 cell line	**mouse melanoma B16F10 cell line**
T. chebula Retz.	ND	1.75 ± 0.63
T. bellerica	8.73 ± 1.40	8.73 ± 1.56
E. elatior (Jack) R.M. Smith	10.92 ± 3.15	10.92 ± 4.20
R. damascena	22.93 ± 0.50	43.17 ± 0.14
R. kerrii Meijer	ND	ND

ND: not detectable

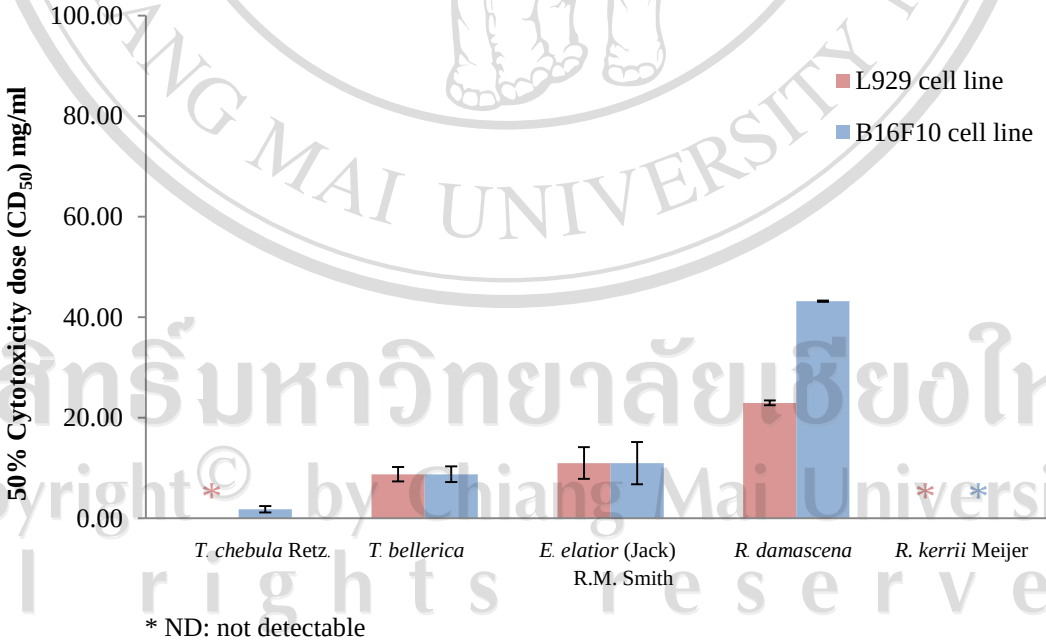

Figure 3.7 50% cytotoxicity dose (CD_{50}) of the extracts for mouse normal skin fibroblast L929 and melanoma skin fibroblast B16F10 cell lines by dye exclusion.

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3.5 Mutagenicity of plant extracts

To evaluate the mutagenicity of plant extracts, Ames test was performed using *his*-dependent bacterial strains of *S. typhimurium* TA98 and TA100 strains in the presence and absence of metabolic activation (S9 microsomal fraction). The numbers of revertant colonies were counted and compared with 50% hydroglycol, 70% hydroglycol and DMSO which were used as negative controls. In positive controls, 2-aminoanthracene (2-AA) and 2-(2-furyl)-3-5-nitro-2-furyl) acrylamide (AF-2) were used for metabolic pathway activation and non metabolic pathway activation, respectively. In this study, the concentrations of five plant extracts between 0.088 and 87.34 mg/0.1 ml were added to the bacterial culture plates.

T. chebula Retz. at the concentrations between 0.088 and 87.34 mg/0.1 ml were added to the culture then plated on the agar and incubated at 37°C for 48 h. The result was shown in Table 3.5. In *S. typhimurium* TA98 strain, the extract of *T. chebula* Retz. showed the number of revertant colonies between 51 ± 8 and 62 ± 3 colonies/plate, and between 105 ± 5 and 133 ± 5 colonies/plate for *S. typhimurium* TA100 strain in the presence and absence of metabolic activation. 70% hydroglycol and DMSO showed the number of revertant colonies between 39 ± 5 and 68 ± 4 colonies/plate in strain TA98.

While, in *S. typhimurium* TA100, the negative controls showed the number of revertant colonies between 122 ± 8 and 150 ± 4 colonies/plate in the presence and absence of metabolic activation. After adding 2-AA as positive controls for metabolic pathway activation, the numbers of revertant colonies were observed between 461 ± 34 and 1,290 ± 8 colonies/plate in strains TA98 and TA100, respectively. Whereas, in

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non metabolic pathway activation, the numbers of revertant colonies between 360 ± 33 and 638 ± 51 colonies/plate in *S. typhimurium* TA98 and TA100 strains were observed after adding AF-2 as positive controls. The results revealed that the extract of *T. chebula* Retz. showed no mutagenicity in both *S. typhimurium* TA98 and TA100 strains in both the presence and absence of metabolic activation. Moreover, after adding the *T. chebula* Retz. extract, the numbers of revertant colonies were not increased less than two and ten-fold when compared with the negative control (70% hydroglycol and DMSO) and positive control (2-AA and AF-2), respectively.

Table 3.5 Mutagenicity of 70% hydroglycol extract of *T. chebula* Retz. at concentrations between 0.088 and 87.34 mg/0.1 ml in *S. typhimurium* TA98 and TA100 strains

Dose Level (mg/0.1 ml)	Revertant colonies/plate (mean 4n = 2 ± S.D.)			
	TA98		TA100	
	-S9	+S9	-S9	+S9
NC (DMSO)	39 ± 5	42 ± 5	122 ± 8	148 ± 11
NC (70% hydroglycol)	58 ± 8	68 ± 4	124 ± 7	150 ± 4
0.088	62 ± 3	62 ± 3	105 ± 5	115 ± 6
0.874	58 ± 6	59 ± 1	119 ± 7	132 ± 8
8.734	51 ± 8	56 ± 1	110 ± 10	131 ± 9
87.34	57 ± 8	61 ± 4	125 ± 12	133 ± 5
PC AF-2 (0.10 μg/0.1 ml)	360 ± 33	NA	638 ± 51	NA
PC 2-AA (1.0 μg/0.1 ml)	NA	461 ± 34	NA	1290 ± 8

NA: not applicable, n: No. of replicates

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The mutagenicity of *T. bellerica* was shown in Table 3.6. It was found that the extract of *T. bellerica* showed no mutagenicity in both bacterial strains in the presence and absence of metabolic activation. In *S. typhimurium* TA98 strain, the numbers of revertant colonies were observed between 51 ± 8 and 69 ± 5 colonies/plate, and 112 ± 5 and 136 ± 8 colonies/plate were found in for *S. typhimurium* TA100 strain after treated with *T. bellerica* extract.

Table 3.6 Mutagenicity of 70% hydroglycol extract of *T. bellerica* at concentrations between 0.088 and 87.34 mg/0.1 ml in *S. typhimurium* TA98 and TA100 strains

Dose Level (mg/0.1 ml)	Revertant colonies/plate (mean 4n = 2 ± S.D.)			
	TA98		TA100	
	-S9	+S9	-S9	+S9
NC (DMSO)	39 ± 5	42 ± 5	122 ± 8	148 ± 11
NC (70% hydroglycol)	58 ± 8	68 ± 4	124 ± 7	150 ± 4
0.088	51 ± 8	66 ± 4	114 ± 3	112 ± 5
0.874	50 ± 4	58 ± 7	113 ± 11	113 ± 4
8.734	55 ± 10	64 ± 5	119 ± 7	123 ± 6
87.34	59 ± 9	69 ± 5	112 ± 6	136 ± 8
PC AF-2 (0.10 μg/0.1 ml)	360 ± 33	NA	638 ± 51	NA
PC 2-AA (1.0 μg/0.1 ml)	NA	461 ± 34	NA	1290 ± 8

NA: not applicable, n: No. of replicates

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From the results in Table 3.7, the extract of *E. elatior* (Jack) R.M. Smith also showed no mutagenic activity with the number of revertant colonies in *S. typhimurium* TA100 between 129 ± 5 and 149 ± 5 colonies/plate, and 33 ± 3 and 54 ± 3 colonies/plate for TA98 in presence and absence of metabolic pathway activation. In *S. typhimurium* TA98 strain, less than 10-fold of revertant colonies were observed after treated with 2AA and AF-2 mutagens in the presence and absence of metabolic activation. In contrast, the revertant colonies in *S. typhimurium* TA100 strain were observed between 5 and 10-fold when treated with AF-2 and 2-AA mutagens, respectively.

Table 3.7 Mutagenicity of 50% hydroglycol extract of *E. elatior* (Jack) R.M. Smith at concentrations between 0.088 and 87.34 mg/0.1 ml in *S. typhimurium* TA98 and TA100 strains

Dose Level (mg/0.1 ml)	Revertant colonies/plate (mean 4n = 2 ± S.D.)			
	TA98		TA100	
	-S9	+S9	-S9	+S9
NC (DMSO)	39 ± 5	42 ± 5	122 ± 8	148 ± 11
NC (50% hydroglycol)	28 ± 8	43 ± 2	122 ± 18	146 ± 9
0.088	42 ± 2	52 ± 1	133 ± 3	143 ± 3
0.874	34 ± 2	54 ± 3	131 ± 7	135 ± 4
8.734	33 ± 3	51 ± 6	129 ± 5	146 ± 1
87.34	51 ± 4	48 ± 5	137 ± 10	149 ± 5
PC AF-2 (0.10 μg/0.1 ml)	360 ± 33	NA	638 ± 51	NA
PC 2-AA (1.0 μg/0.1 ml)	NA	461 ± 34	NA	1290 ± 8

NA: not applicable, n: No. of replicates

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Determinations of mutagenicity of *R. damascena* in both *S. typhimurium* were also performed using the same protocol as above the result was shown in Table 3.8. The extract of *R. damascena* was found to be non-mutagenic to both *S. typhimurium* in the presence and absence of metabolic activation as compared to negative controls (50% hydroglycol and DMSO) and positive control (2-AA and AF-2). In *S. typhimurium* TA98 strain showed the number of revertant colonies between 33 ± 3 and 59 ± 4 colonies/plate, and 124 ± 3 and 152 ± 2 colonies/plate for *S. typhimurium* TA100 strain.

Table 3.8 Mutagenicity of 50% hydroglycol extract of *R. damascena* at concentrations between 0.088 and 87.34 mg/0.1 ml in *S. typhimurium* TA98 and TA100 strains

Dose Level (mg/0.1 ml)	Revertant colonies/plate (mean 4n = 2 ± S.D.)			
	TA98		TA100	
	-S9	+S9	-S9	+S9
NC (DMSO)	39 ± 5	42 ± 5	122 ± 8	148 ± 11
NC (50% hydroglycol)	28 ± 8	43 ± 2	122 ± 18	146 ± 9
0.088	33 ± 3	41 ± 6	124 ± 3	128 ± 6
0.874	33 ± 7	45 ± 2	132 ± 6	136 ± 6
8.734	30 ± 2	42 ± 5	130 ± 3	132 ± 1
87.34	38 ± 3	59 ± 4	130 ± 9	152 ± 2
PC AF-2 (0.10 μg/0.1 ml)	360 ± 33	NA	638 ± 51	NA
PC 2-AA (1.0 μg/0.1 ml)	NA	461 ± 34	NA	1290 ± 8

NA: not applicable, n: No. of replicates

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Both strains of *S. typhimurium* TA98 and TA100 which were treated with different concentrations of *R. kerrii* Meijer did not show mutagenic activity as compared with the negative and positive controls as shown in Table 3.9. In *S. typhimurium* TA98 strain showed the number of revertant colonies between 34 ± 1 and 55 ± 3 colonies/plate. On the other hand, *S. typhimurium* TA100 strain showed the number of revertant colonies between 112 ± 5 and 136 ± 4 colonies/plate.

Table 3.9 Mutagenicity of 50% hydroglycol extract of *R. kerrii* Meijer at concentrations between 0.088 and 87.34 mg/0.1 ml in *S. typhimurium* TA98 and TA100 strains

Dose Level (mg/0.1 ml)	Revertant colonies/plate (mean 4n = 2 ± S.D.)			
	TA98		TA100	
	-S9	+S9	-S9	+S9
NC (DMSO)	39 ± 5	42 ± 5	122 ± 8	148 ± 11
NC (50% hydroglycol)	28 ± 8	43 ± 2	122 ± 18	146 ± 9
0.088	36 ± 1	42 ± 1	120 ± 8	136 ± 4
0.874	35 ± 4	47 ± 1	119 ± 10	131 ± 8
8.734	34 ± 1	48 ± 4	112 ± 5	128 ± 7
87.34	44 ± 3	55 ± 3	112 ± 5	133 ± 6
PC AF-2 (0.10 μg/0.1 ml)	360 ± 33	NA	638 ± 51	NA
PC 2-AA (1.0 μg/0.1 ml)	NA	461 ± 34	NA	1290 ± 8

NA: not applicable, n: No. of replicates

Interestingly, the results suggested that all five plant extracts showed no mutagenicity up to 87.34 mg/plate on *S. typhimurium* strain TA98 and TA100 in the

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presence or absence of metabolic activation. The results revealed that the mean of number of *his*+ revertant colonies in the treatment groups were the same as the mean of number of revertant colonies in the negative control groups (DMSO, 50% hydroglycol and 70% hydroglycol) and less than two-fold when compared to as positive control (2-AA and AF-2) in both bacterial strains. The results indicated that the extracts can be used in cosmetic application; however the antimutagenic properties of the plant extracts were also investigated for their advantages in cosmetic application.

3.6 Antimutagenicity of plant extracts

The antimutagenic substances are usually found in fruits, vegetables and some medicinal plants. The antimutagenic compounds such as cinnamaldehyde, coumarin, vanniline, epigallocatechin gallate and tannic acids which were isolated from plants have been investigated. Since, there are no evidences of antimugenicity data available for plant extracts from *E.elatior* (Jack) R.M. Smith, *R. damscene* and *R. kerrii* Meijer, hence we have investigated the antimutagenicity of these extracts.

According to the investigation of mutagenic properties in the plant extracts by Ames assay, it was revealed that all the plant extracts showed no mutagenic effects on both *S. typhimurium* strain TA98 and TA100 in the presence or absence of metabolic activation. Then, the plant extracts were further tested for antimutagenicity properties using modified Ames test by adding mutagens, either 2-AA or AF-2, into the mixture. The numbers of revertant colonies were counted and compared with positive controls. The results were shown as % inhibition of mutagenesis. In this experiment, all of plant extracts were tested for their antimutagenic properties at five different

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concentrations between 10 and 80 mg/ 0.1 ml. The extract of *T. chebula* Retz. was tested at different concentrations and stimulated with mutagens either 2-AA for metabolic activation or AF-2 for non metabolic activation for 48 h. It was found that *T. chebula* Retz extract showed significantly antimutagenic properties on *S. typhimurium* strain TA98 in the presence or absence of metabolic activation as shown in Table 3.10. The concentration of the extract up to 80 mg/plate showed the highest percentage inhibition of mutagenesis in *S. typhimurium* strain TA98 at 99.62% in presence metabolic activation. In contrast at the same concentration, only 24.13% inhibition of mutagenesis was observed in the absence metabolic activation.

Table 3.10 Inhibition of mutagenicity by *T. chebula* Retz. in *S. typhimurium* TA98 assay system

Dose Level (mg/0.1 ml)	*His* + Revertant colonies/ plate (mean 2n = 2 ± S.D.)			
	Present of S9 Mix	% inhibition of mutagenesis	Absence of S9 Mix	% inhibition of mutagenesis
NC (DMSO)	38 ± 2	-	26 ± 1	-
NC (70% hydroglycol)	29 ± 1	-	28 ± 5	-
10	471 ± 11	16.13	331 ± 40	3.81
20	287 ± 24	51.04	314 ± 13	9.21
40	54 ± 11	95.26	277 ± 10	20.95
60	40 ± 1	97.91	270 ± 13	23.17
80	31 ± 1	99.62	267 ± 1	24.13
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

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Besides, the extract of *T. chebula* Retz. could decrease the mutagenicity after induced by 2-AA and AF-2 of *S. typhimurium* strain TA100 as shown in Table 3.11. The concentration of the extract up to 80mg/0.1 ml showed the highest percentage inhibition of mutagenesis at 95.83% in presence metabolic activation and 29.56% in absence metabolic activation, respectively. These results revealed that the extract of *T. chebula* Retz. showed antimutagenicity in both *S. typhimurium* strains in the presence or absence of metabolic activation. Moreover, the number *his*$^+$ revertant colonies of both *S. typhimurium* strain after induced by AF-2 and 2-AA mutagens were decreased in dose-dependent manner when the concentrations of the extract were increased.

Table 3.11 Inhibition of mutagenicity by *T. chebula* Retz. in *S. typhimurium* TA100 assay system

Dose Level (mg/0.1 ml)	*His* + Revertant colonies/ plate (mean 2n = 2 ± S.D.)			
	Present of S9 Mix	% inhibition of mutagenesis	Absence of S9 Mix	% inhibition of mutagenesis
NC (DMSO)	93 ± 4	-	85 ± 2	-
NC (70% hydroglycol)	85 ± 14	-	89 ± 1	-
10	397 ± 12	35.00	512 ± 8	6.00
20	206 ± 29	74.79	460 ± 4	17.56
40	125 ± 1	91.67	454 ± 8	18.89
60	119 ± 8	92.92	405 ± 14	29.78
80	105 ± 5	95.83	406 ± 6	29.56
PC AF-2 (0.02 μg/0.1 ml)	NA	-	535 ± 8	-
PC 2-AA (0.50 μg/0.1 ml)	573 ± 14	-	NA	-

NA: not applicable, n: No. of replicates

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The inhibition of mutagenicity by *T. bellerica* in *S. typhimurium* TA98 under the presence or absence of metabolic activation was shown in Table 3.12. In presence metabolic activation after stimulated by 2-AA, the concentration of the extract up to 80 mg/0.1 ml showed the highest percentage inhibition of mutagenesis at 99.05%, while the extract showed the highest percentage inhibition of mutagenesis at 37.46% in absence of metabolic activation after stimulated with AF-2.

Table 3.12 Inhibition of mutagenicity by *T. bellerica* in *S. typhimurium* TA98 assay system

	His + Revertant colonies/ plate (mean 2n = 2 ± S.D.)			
Dose Level (mg/0.1 ml)	Present of S9 Mix	% inhibition of mutagenesis	Absence of S9 Mix	% inhibition of mutagenesis
NC (DMSO)	38 ± 2	-	26 ± 1	-
NC (70% hydroglycol)	29 ± 1	-	28 ± 5	-
10	552 ± 9	0.76	280 ± 15	20.00
20	499 ± 2	10.38	274 ± 6	21.90
40	123 ± 8	82.16	289 ± 8	17.14
60	53 ± 1	95.45	274 ± 1	21.90
80	34 ± 1	99.05	225 ± 16	37.46
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

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On the other hand, the extract of *T. bellerica* also was tested for antimutagenicity in *S. typhimurium* TA100 under the presence or absence of metabolic activation. The results were shown in Table 3.13. The extract of *T. bellerica* at concentration of 80 mg/0.1 ml showed the highest percentage inhibition of mutagenesis at 96.04% and 45.11% in the presence or absence of metabolic activation, respectively. The extract of *T. bellerica* had also showed antimutagenicity on both *S. typhimurium* strains in the presence or absence of metabolic activation in does dependent manner.

Table 3.13 Inhibition of mutagenicity by *T. bellerica* in *S. typhimurium* TA100 assay system

Dose Level (mg/0.1ml)	*His* + Revertant colonies/ plate (mean 2n = 2 ± S.D.)			
	Present of S9 Mix	% inhibition of mutagenesis	Absence of S9 Mix	% inhibition of mutagenesis
NC (DMSO)	93 ± 4	-	85 ± 2	-
NC (70% hydroglycol)	85 ± 14	-	89 ± 1	-
10	413 ± 2	27.92	520 ± 8	4.22
20	343 ± 14	46.25	413 ± 17	28.00
40	144 ± 6	87.71	441 ± 13	21.78
60	143 ± 6	87.92	420 ± 6	26.44
80	104 ± 2	96.04	336 ± 21	45.11
PC AF-2 (0.02 μg/0.1 ml)	NA	-	535 ± 8	-
PC 2-AA (0.50 μg/0.1 ml)	573 ± 14	-	NA	-

NA: not applicable, n: No. of replicates

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The antimutagenic activity assay of *E. elatior* (Jack) R.M. Smith in *S. typhimurium* strain TA 98 under the presence or absence of metabolic activation were shown in Table 3.14. Interestingly, *E. elatior* (Jack) R.M. Smith extract has no antimutagenic activity against 2-AA and AF-2 mutagen in both presence and absence of metabolic pathways when compared the number of revertant colonies with positive control groups. The number of revertant colonies at 1183 ± 25 colonies/plate were observed in the presence of metabolic activation after stimulated by 2-AA and treated with the extract at concentration of 80 mg/ plate which higher than the number of revertant colonies at 565 ± 18 colonies/ plate in positive control group. The similar result was also observed in absence of metabolic activation which stimulated with AF-2 and treated with the extract. The numbers of revertant colonies at 401 ± 6 colonies/plate were determined which were higher than positive control group. From these results, the percentage inhibition of mutagenesis was calculated. The percentages of inhibition of mutagenesis of the extract were determined at -118.41% and -17.46% in presence and absence of metabolic pathway, respectively. These indicated that the *E. elatior* (Jack) R.M. Smith extract showed co-mutagen properties with both mutagens.

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Table 3.14 Inhibition of mutagenicity by *E. elatior* (Jack) R.M. Smith in *S. typhimurium* TA98 assay system

Dose Level (mg/0.1 ml)	*His* + Revertant colonies/ plate (mean 2n = 2± S.D.)			
	Present of S9 Mix	% inhibition of mutagenesis	Absence of S9 Mix	% inhibition of mutagenesis
NC (DMSO)	38 ± 2	-	26 ± 1	-
NC (50% hydroglycol)	32 ± 2	-	31 ± 1	-
10	931 ± 16	- 70.59	259 ± 20	27.62
20	993 ± 6	- 82.35	361 ± 8	- 4.76
40	1108 ± 15	- 104.17	318 ± 13	8.89
60	1124 ± 16	- 107.21	363 ± 12	- 5.40
80	1183 ± 25	- 118.41	401 ± 6	- 17.46
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

The inhibition of mutagenicity of *E. elatior* (Jack) R.M. Smith in *S. typhimurium* strain TA100 under the presence and absence of metabolic activation was shown in Table 3.15. It was found that the extract has also showed no antimutagenic activity in both presence and absence of metabolic pathway which was similar to inhibition of mutagenicity in *S. typhimurium* strain TA98. The extract exhibited the percentage inhibition of mutagenesis at -47.92% and -44.89% in presence and absence of metabolic pathway, respectively. These results suggested that the extract of *E. elatior* (Jack) R.M. Smith might contain the active compounds which act as co-mutagen together with 2-AA and AF-2 mutagens in both bacterial strains. In

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order to confirm the co-mutagen activity of the extract, the Ames test in higher eukaryotic cells such as mammalian cells will be further investigated.

Table 3.15 Inhibition of mutagenicity by *E. elatior* (Jack) R.M. Smith in *S. typhimurium* TA100 assay system

	His + Revertant colonies/ plate (mean 2n = 2± S.D.)			
Dose Level (mg/0.1 ml)	Present of S9 Mix	% inhibition of mutagenesis	Absence of S9 Mix	% inhibition of mutagenesis
NC (DMSO)	93 ± 4	-	85 ± 2	-
NC (50% hydroglycol)	83 ± 18	-	85 ± 7	-
10	705 ± 10	- 29.58	543 ± 26	- 1.78
20	762 ± 18	- 41.46	527 ± 27	1.78
40	761 ± 24	- 41.25	508 ± 13	6.00
60	764 ± 16	- 41.88	609 ± 25	- 16.44
80	793 ± 7	- 47.92	737 ± 6	- 44.89
PC AF-2 (0.02 μg/0.1 ml)	NA	-	535 ± 8	-
PC 2-AA (0.50 μg/0.1 ml)	573 ± 14	-	NA	-

NA: not applicable, n: No. of replicates

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The extract from *R. damascena* was also investigated for antimutagenic activity in *S. typhimurium* strain TA98 in the presence and absence of metabolic activation. The results were shown in Table 3.16. It was found that the extract has no antimutagenic activity against 2-AA and AF-2 mutagen in both presence and absence of metabolic pathways. The extract at the concentrations between 10 - 40 mg/0.1 ml showed co-mutagenic properties in the presence of 2-AA. In contrast, after increasing

the concentration of extract up to 80 mg/0.1 ml the percentage inhibition of mutagenesis was observed at 86.15% in the present of metabolic activation. Whereas, in the absence of metabolic pathway, the percentage inhibition of mutagenesis at 13.65% was determined after treated with the extract at the concentration of 80 mg/ 0.1 ml

Table 3.16 Inhibition of mutagenicity by *R. damascena* in *S. typhimurium* TA98 assay system

Dose Level (mg/0.1 ml)	*His* + Revertant colonies/ plate (mean 2n = 2 ± S.D.)			
	Present of S9 Mix	% inhibition of mutagenesis	Absence of S9 Mix	% inhibition of mutagenesis
NC (DMSO)	38 ± 2	-	26 ± 1	-
NC (50% hydroglycol)	32 ± 2	-	31 ± 1	-
10	1136 ± 20	- 109.49	374 ± 0	- 8.89
20	1030 ± 14	- 89.37	362 ± 21	- 5.08
40	594 ± 23	- 6.64	335 ± 5	-3.49
60	411 ± 21	28.08	306 ± 18	12.70
80	105 ± 1	86.15	303 ± 9	13.65
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

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The antimutagenic activities of *R. damascena* on *S. typhimurium* strain TA100 were shown in Table 3.17. The extract has no antimutagenic activity against both 2-AA and AF-2 mutagens in presence and absence of metabolic pathway. These results

were similar to the antimutagenic activity of *R. damascena* in *S. typhimurium* strain TA98. The concentrations between 40 and 80 mg/0.1 ml of the extract showed the percentage of inhibition of mutagenesis at 87.08% and 31.11% in presence and absence of metabolic pathway, respectively. While, the concentrations of extract were decreased less than 40 mg/0.1 ml, the extract has no antimutagenic activity against 2-AA and AF-2 mutagens in both presence and absence of metabolic pathway, respectively. These result indicated that *R. damascena* extract at the concentration lower than 40 mg/0.1 ml showed co-mutagen properties. However, increasing of the concentration of the extract also showed antimutagenic activity.

Table 3.17 Inhibition of mutagenicity by *R. damascena* in *S. typhimurium* TA100 assay system

Dose Level (mg/0.1 ml)	*His* + Revertant colonies/ plate (mean 2n = 2 ± S.D.)			
	Present of S9 Mix	% inhibition of mutagenesis	Absence of S9 Mix	% inhibition of mutagenesis
NC (DMSO)	93 ± 4	-	85 ± 2	-
NC (50% hydroglycol)	83 ± 18	-	85 ± 7	-
10	748 ± 15	- 38.54	737 ± 18	- 44.89
20	989 ± 10	- 88.75	595 ± 35	- 13.33
40	413 ± 6	31.25	506 ± 25	6.44
60	240 ± 10	67.29	392 ± 18	31.78
80	145 ± 4	87.08	395 ± 8	31.11
PC AF-2 (0.02 μg/0.1 ml)	NA	-	535 ± 8	-
PC 2-AA (0.25 μg/0.1 ml)	573 ± 14	-	NA	-

NA: not applicable, n: No. of replicates

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Next, the extract of *R. kerrii* Meijer was also tested for antimutagenic activity in *S. typhimurium* strain TA 98. The result was shown in Table 3.18. The extract showed highly antimutagennicity in *S. typhimurium* strain TA98 under both the presence and absence of metabolic activation. The concentration of the extract at 80 mg/0.1 ml showed the highest percentage of inhibition of mutagenesis in *S. typhimurium* strain TA98 at 99.05% in presence metabolic activation and 39.37% in absence metabolic activation. Moreover, *R. kerrii* Meijer has also showed antimutagenic activity in dose-dependent manner in *S. typhimurium* strain TA 98 in the presence or absence of metabolic activation when treated with AF-2 and 2-AA mutagens, respectively.

Table 3.18 Inhibition of mutagenicity by *R. kerrii* Meijer in *S. typhimurium* TA98 assay system

Dose Level (mg/0.1 ml)	*His* + Revertant colonies/ plate (mean 2n = 2 ± S.D.)			
	Present of S9 Mix	% inhibition of mutagenesis	Absence of S9 Mix	% inhibition of mutagenesis
NC (DMSO)	38 ± 2	-	26 ± 1	-
NC (50% hydroglycol)	32 ± 2	-	31 ± 1	-
10	341 ± 15	41.46	338 ± 4	2.54
20	109 ± 8	85.39	325 ± 3	6.67
40	56 ± 6	95.45	308 ± 2	12.22
60	47 ± 1	97.15	231 ± 5	36.67
80	37 ± 1	99.05	222 ± 6	39.37
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

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The antimutagenic activity assay of *R. kerrii* Meijer in *S. typhimurium* strain TA100 under the presence or absence of metabolic activation was also investigated. The result was shown in Table 3.19. The antimutagenic activity of the extract in *S. typhimurium* strain TA100 was observed which was similar to the antimutagenic activity in *S. typhimurium* strain TA98. The extract showed the highest percentage inhibition of mutagenesis at 98.23% and 15.78% in presence and absence of metabolic pathway, respectively. These results suggested that the extract of *R. kerrii* Meijer has highly antimugenicity against 2AA and AF-2 in both bacterial strains.

Table 3.19 Inhibition of mutagenicity by *R. kerrii* Meijer in *S. typhimurium* TA100 assay system

Dose Level (mg/0.1 ml)	*His* + Revertant colonies/ plate (mean 2n = 2 ± S.D.)			
	Present of S9 Mix	% inhibition of mutagenesis	Absence of S9 Mix	% inhibition of mutagenesis
NC (DMSO)	93 ± 4	-	85 ± 2	-
NC (50% hydroglycol)	83 ± 18	-	85 ± 7	-
10	449 ± 19	23.85	513 ± 13	4.89
20	138 ± 20	88.54	511 ± 10	5.33
40	107 ± 8	95.00	496 ± 15	8.33
60	101 ± 13	96.25	477 ± 8	13.00
80	92 ± 18	98.23	464 ± 11	15.78
PC AF-2 (0.10 μg/0.1 ml)	NA	-	535 ± 8	-
PC 2-AA (1.0 μg/0.1 ml)	573 ± 14	-	NA	-

NA: not applicable, n: No. of replicates

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The antimutagenic activities of five plant extracts in both bacterial *S. typhimurium* TA98 and TA100 in presence or absence of metabolic activation were shown in Figure 3.8.

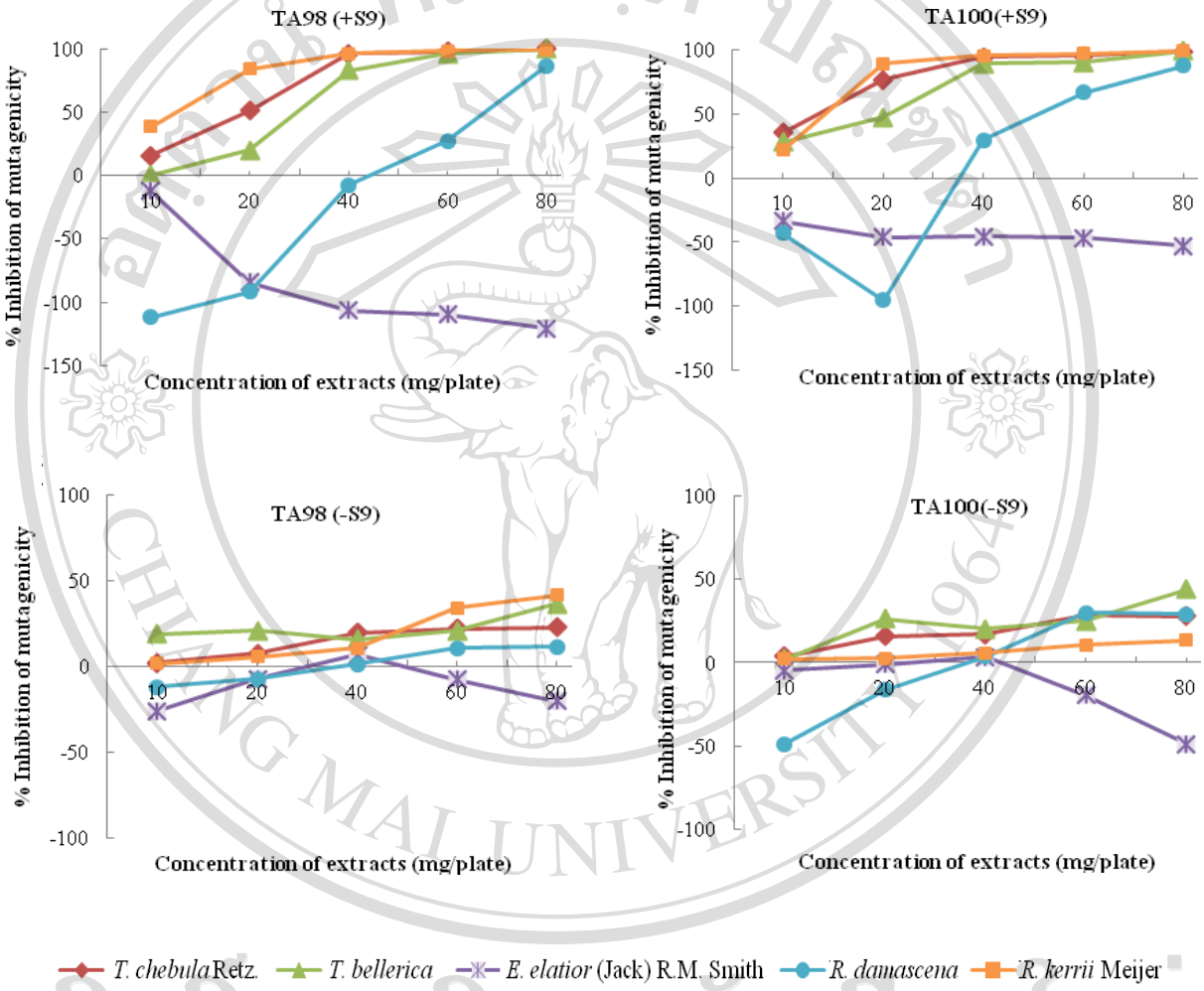

Figure 3.8 Antimutagenic activities of five plant extracts in both *S. typhimurium* after treated with 2-AA and AF-2 mutagens in the presence and absence of metabolic activation, respectively.

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The 50% inhibitory concentrations of all extracts were obtained from the relationship between the % inhibition of mutagenicity and the concentrations of the extracts as shown in Table 3.20. In the presence of metabolic activation, the extracts of *T. chebula* Retz., *T. bellerica*, *R. damascena* and *R. kerrii* Meijer showed the strong antimutagenic activity on both *S. typhimurium* strains. In *S. typhimurium* TA98 the extracts showed the IC_{50} values at between 12 - 68 mg/plate. In *S. typhimurium* TA100, the extracts showed the IC_{50} values at between 12 – 47 mg/plate. In contrast, all the extracts showed lower antimutagenic activities on both bacterial *S. typhimurium* strains in the absence of metabolic activation as shown in Table 3.21

The extract of *R. damascena* at the concentrations up to 45 and 35 mg/plate showed strongly antimutagenic activity in *S. typhimurium* TA98 and TA100 in the presence of 2-AA, respectively. When the concentrations of the extract was decreased, the % inhibition of mutagenicity was also decreased. In previous reported, the extract of *R. damascena* has been observed to show antibacterial activities such as *S. typhimurium* (Özkan *et al*., 2004). However, the extract of *E. elatior* (Jack) R.M. Smith did not showed the antimutagenic activity on both bacterial *S. typhimurium* strains in the presence and absence of metabolic activation. These results indicated that the extract of *E. elatior* (Jack) R.M. Smith might contain the active compounds which act as co-mutagen with 2-AA and AF-2 mutagens in both bacterial strains.

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Table 3.20 50% inhibition of mutagenicity (IC_{50}) by five plant extracts on both *S. typhimurium* strains in presence of metabolic activation.

Plant extract	IC_{50}(mg/plate) in presence of S9 Mix	
	TA98	**TA100**
T. chebula Retz.	20	12
T. bellerica	29	22
E. elatior (Jack) R.M. Smith	< 10	< 10
R. damascena	68	47
R. kerrii Meijer	12	13

Table 3.21 50% inhibition of mutagenicity (IC_{50}) by five plant extracts on both *S. typhimurium* strains in absence of metabolic activation.

Plant extract	IC_{50}(mg/plate) in absence of S9 Mix	
	TA98	**TA100**
T. chebula Retz.	> 80	> 80
T. bellerica	> 80	> 80
E. elatior (Jack) R.M. Smith	< 10	< 10
R. damascena	> 80	> 80
R. kerrii Meijer	> 80	> 80

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3.7 Antityrosinase activity of the plant extracts

All plant extracts were further investigated for tyrosinase inhibitory activity by using the Dopachrome method. In this experiment, 3,4-dihydroxy-L-phenylalanine (L-Dopa) was used as a substrate for tyrosinase assay. The absorbance at 475 nm was measured by using ultraviolet–visible spectrophotometer to determine tyrosinase activity. Ascorbic acid and kojic acid were used as positive controls for tyrosinase inhibitors. For negative controls, 50% and 70% hydroglycol were used. The experiments were performed in triplicates. The % inhibition tyrosinase inhibitory activity and 50% inhibitory concentration values of the plant extracts (IC_{50}) were calculated. The results were shown in Table 3.22 and Figure 3.9.

All of the plant extracts showed tyrosinase inhibitory activity. Interestingly, the extract of *R. kerrii* Meijer showed the greatest % inhibition tyrosinase activity value at 94.46 ± 0.01% followed by the extracts of *T. chebula* Retz., *T. bellerica*, *E. elatior* (Jack) R.M. Smith and *R. damascena* with % inhibition tyrosinase activity values at 77.04 ± 0.04, 74.09 ± 0.04, 73.20 ± 0.01 and 67.69 ± 0.02%, respectively. The % tyrosinase inhibitory activities were calculated to the IC_{50} values of plant extracts as shown in Figure 3.9. It was found that the extract of *R. kerrii* Meijer showed the greatest IC_{50} value at 1.27 ± 0.49 mg/ml following by *R. damascena*, *T. bellerica, E. elatior* (Jack) R.M. Smith and *T. chebula* Retz. with IC_{50} values at 13.00 ± 0.27, 20.07 ± 0.34, 20.00 ± 0.44 and 39.96 ± 0.21 mg/ml, respectively.

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Table 3.22 Tyrosinase inhibitory activity of the five plant extracts on mushroom tyrosinase

Plant extracts and tyrosinase inhibitor	% Tyrosinase inhibitory activity	IC_{50} (mg/ml)
T. chebula Retz.	77.04 ± 0.04	39.96 ± 0.21
T. bellerica	74.90 ± 0.04	20.07 ± 0.34
E. elatior (Jack) R.M. Smith	73.20 ± 0.01	20.00 ± 0.44
R. damascena	67.69 ± 0.02	13.00 ± 0.27
R. kerrii Meijer	94.46 ± 0.01	1.27 ± 0.49
Ascorbic acid	98.95 ± 0.01	0.031 ± 0.40
Kojic acid	98.66 ± 0.01	0.020 ± 0.09

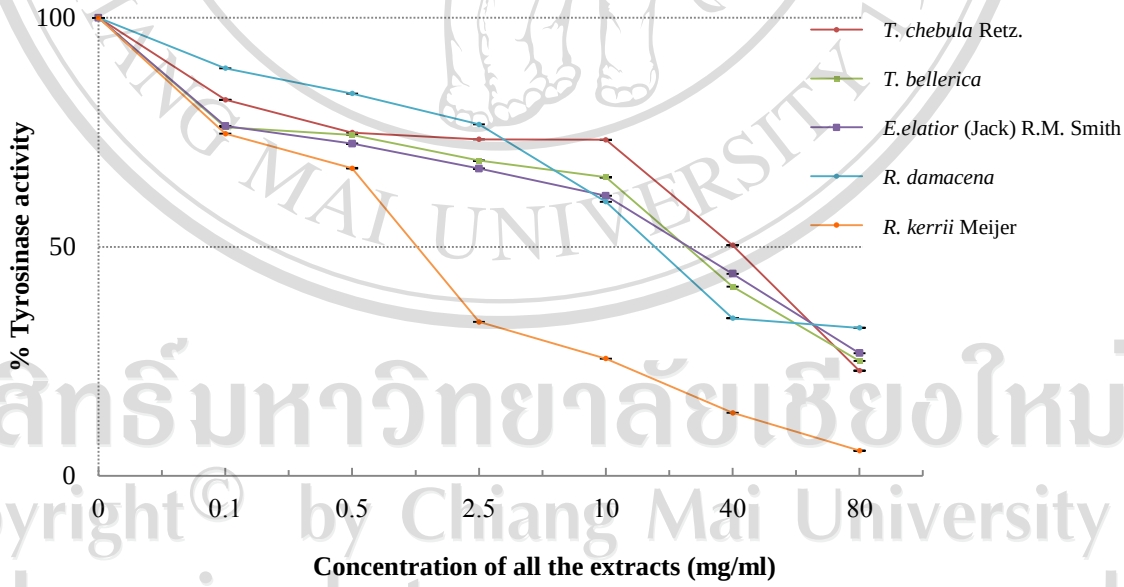

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Figure 3.9 Tyrosinase inhibitory activity and 50% inhibitory concentration values of the plant extracts (IC_{50}) on mushroom tyrosinase

3.8 Morphological changes of normal and melanoma mouse fibroblast cell lines after induced by UVA radiation

The investigation of cellular morphological changes were studied for the possibility of the plant extracts to recover the fibroblast cell morphology after exposed to UV radiation *in vitro* assay. The experiments were performed in normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines. In the experiment, the both cell lines were exposed to UVA radiation with 0.30 J/cm^2 for 22 min 44 sec. Then, the different concentrations of the plant extracts at $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$ were added to UVA treated cells. The morphological changes of cells after exposured to UVA and treated with the extract were investigated under a light microscope.

The results of cell morphology of both cell lines after induced UVA radiation and followed by adding the *T. chebula* Retz. at different concentrations were shown in Figure 3.10. From the results, the cell morphology of treated controls in both cell lines did not change upon treatment with UVA when compared with untreated UVA control group. The possible explanation of this is that the limitation of the UVA power of light source and the condition in UV irradiation equipment was not suitable for cell cultivation system such as the level of carbon dioxide (CO_2). It was noticed that the 70 % hydroglycol solvent also caused morphological changes of mouse melanoma B16F10 cell line; whereas no effect was observed in normal mouse fibroblast L929 cell line. The concentrations of *T. chebula* Retz. at $CD_{3.13}$ 0.75 mg/ml has no effect on morphology changes for normal mouse fibroblast L929 cell line. After increasing the concentrations of the extract at $CD_{6.25}$ (1.50 mg/ml) and $CD_{12.5}$ (3.00 mg/ml), the change of cell morphology of normal mouse fibroblast L929 cell line were observed. The cell lines were shrinked and detached when compared to the

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UVA treated cells with addition of 70% hydroglycol. Whereas, *T. chebula* Retz. extract caused morphological change of mouse melanoma B16F10 cell line when increased concentrations of plant extract from the concentrations of $CD_{3.13}$ (0.27 mg/ml) to $CD_{12.5}$ (1.08 mg/ml). From the result, the extract has affected on morphology of mouse melanoma B16F10 cell line which could be due to the active compounds that present in the extract such as chebulagic acid that showed cytotoxic effects on cancer cell lines such as malignant melanoma and murine melanoma (Kashiwada *et al.*, 1992 and Manosroi *et al.*, 2010).

The morphological changes study of normal mouse fibroblast L929 and melanoma B16F10 cell lines after induction of UVA and added the extract of *T. bellerica* at different concentrations were performed as shown Figure 3.11. The concentrations of *T. bellerica* at $CD_{3.13}$ (0.33 mg/ml) and $CD_{6.25}$ (0.66 mg/ml) have no effect on morphology changed for normal mouse fibroblast L929 cell line. Whereas, the concentration of the extract at $CD_{12.5}$ (1.32 mg/ml) has affected on morphological changes of normal cell line. The cell lines were shrinked and detached when compared to the UVA treated cells with addition of 70% hydroglycol. On the other hand, the concentrations the extract of *T. bellerica* at $CD_{3.13}$ (0.13 mg/ml), $CD_{6.25}$ (0.26 mg/ml) and $CD_{12.5}$ (0.52 mg/ml) have also affected on morphological changed of melanoma mouse fibroblast B16F10 cell line. From the result, the extract has affected on morphology of mouse melanoma B16F10 cell line rather than normal mouse fibroblast L929 cell line. From the result, the extract has affected on morphology of mouse melanoma B16F10 cell line which could be due to the active compounds that presented in the extract such as gallic acid, the major compounds often found in this plant which exhibited anti-proliferative effects in several cancer

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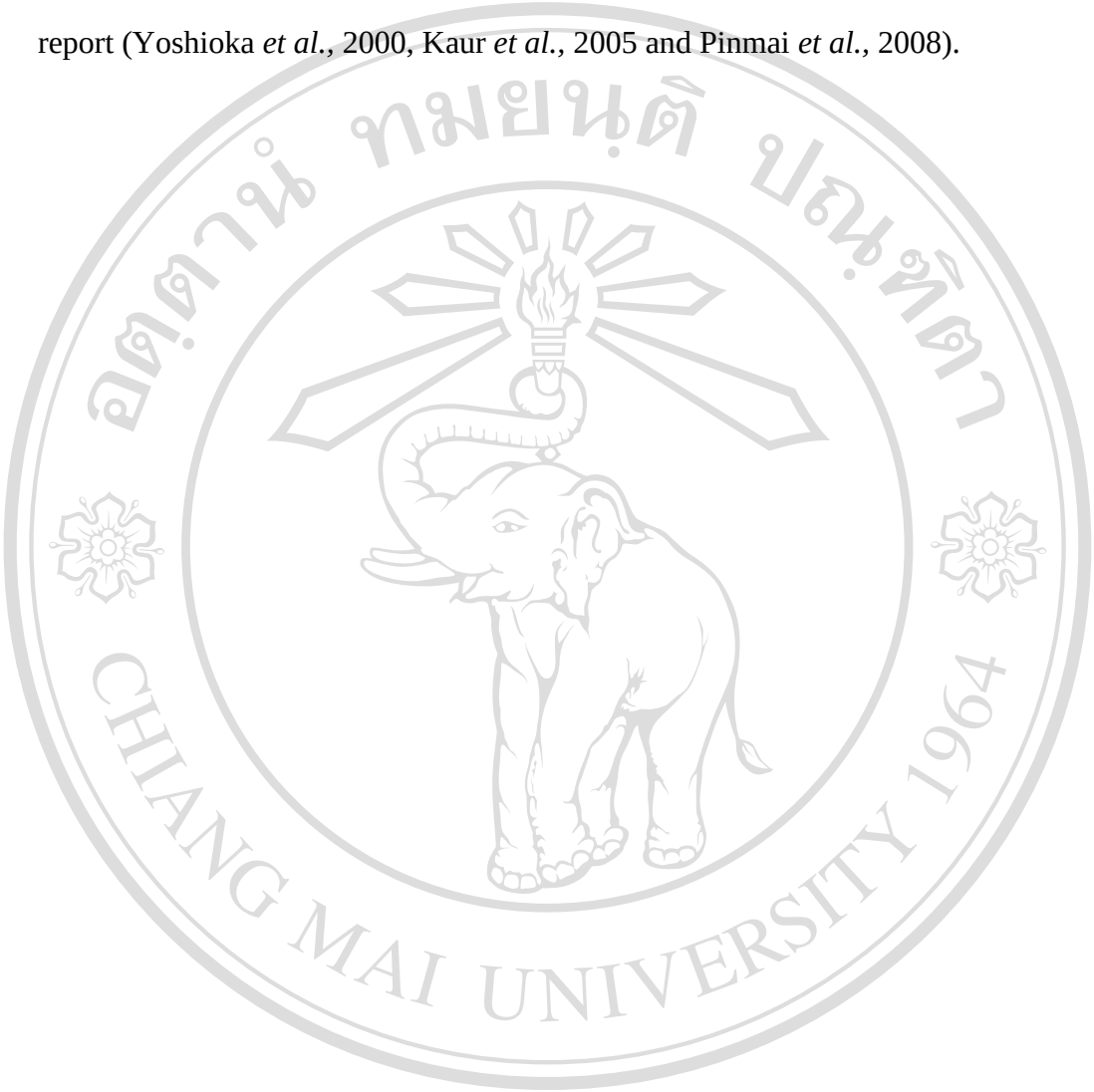
cell lines including human hepatocellular carcinoma (HepG2), human stomach cancer (Kato III) and human colon adenocarcinoma (COLO 205) as showed in previously report (Yoshioka *et al.*, 2000, Kaur *et al.*, 2005 and Pinmai *et al.*, 2008).



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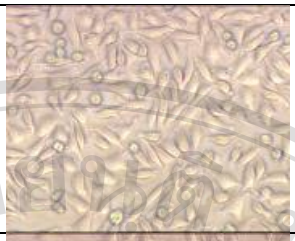
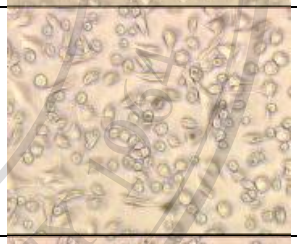
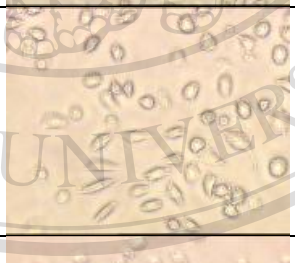
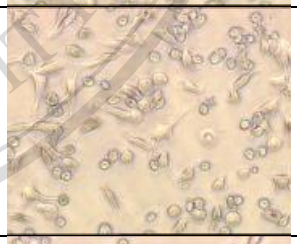
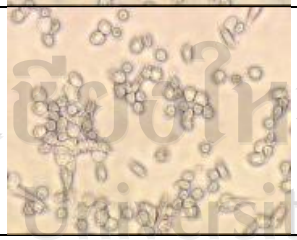
	L929	B16F10
Control without UVA treated		
Control with UVA treated		
70% (v/v) hydroglycol		
T. chebula Retz. $CD_{3.13}$ = 0.75 mg/ml (L929) $CD_{3.13}$ = 0.27 mg/ml (B16F10)		
$CD_{6.25}$ = 1.50 mg/ml (L929) $CD_{6.25}$ = 0.54 mg/ml (B16F10)		
$CD_{12.5}$ = 3.00 mg/ml (L929) $CD_{12.5}$ = 1.08 mg/ml (B16F10)		

Figure 3.10 Morphology of normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines after induced by UVA radiation and then treated with *T. chebula* Retz. extract at the concentrations of $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$.

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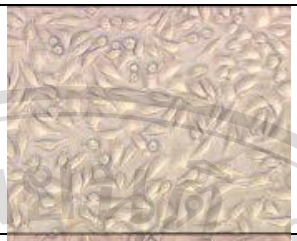
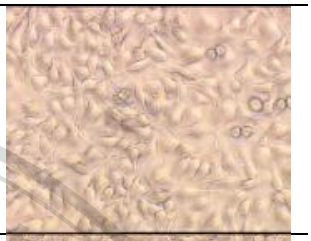
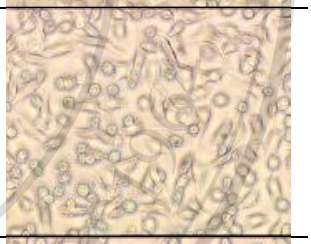
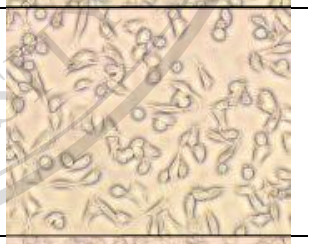
	L929	B16F10
Control without UVA treated		
Control with plus UVA treated		
70% (v/v) hydroglycol		
T. bellerica $CD_{3.13}$ = 0.33 mg/ml (L929) $CD_{3.13}$ = 0.13 mg/ml (B16F10)		
$CD_{6.25}$ = 0.66 mg/ml (L929) $CD_{6.25}$ = 0.26 mg/ml (B16F10)		
$CD_{12.5}$ = 1.32 mg/ml (L929) $CD_{12.5}$ = 0.52 mg/ml (B16F10)		

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Figure 3.11 Morphology of normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines after induced by UVA radiation and treated with *T. bellerica* extract at the concentrations of $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$.

The effect of *E. elatior* (Jack) R.M. Smith on morphological changes of cell lines was performed as shown in Figure 3.12. It was found that the 50% hydroglycol has caused morphological changed only in normal fibroblast cell lines. When adding the extract of *E. elatior* (Jack) R.M. Smith at the concentration $CD_{3.13}$ (1.24 mg/ml) and $CD_{6.25}$ (2.48 mg/ml) in normal mouse fibroblast L929, the morphology changed of cell line had not been observed. In contrast, at the concentrations of the extract at $CD_{12.5}$ (4.96 mg/ml), the morphological changes of normal cell line was observed. However, in mouse melanoma B16F10 cell line, the extract of *E. elatior* (Jack) R.M. Smith at the concentrations $CD_{3.13}$ (3.65 mg/ml) and $CD_{6.25}$ (7.30 mg/ml) had no effect on morphological changes. The morphological change of melanoma cell line was observed when increasing the concentration of the extract at $CD_{12.5}$ (14.60 mg/ml). From the result, the extract has affected on morphology of both cell lines which could be due to the active compounds that present in the extract and it showed inhibitory effect on the cell growth of human cervical carcinoma as showed in previously report (Meckeen *et al.*, 1997).

The morphological changes results of normal mouse fibroblast L929 and melanoma B16F10 cell lines after induced by UVA and added *R. damascena* extract at different concentrations were shown in Figure 3.13. After adding the extract of *R. damascena*, it was revealed that the concentration of the extract at $CD_{3.13}$ (1.07 mg/ml) has affected on morphological changes of normal mouse fibroblast L929 cell line. When the concentrations of the extract were increased, almost all morphology of normal mouse fibroblast L929 cells were changed. The cell line was shrinked and detached from the 24-well plates. While, the concentrations of the extract at $CD_{3.13}$ (0.63 mg/ml) and $CD_{6.25}$ (1.24 mg/ml) have no effect on morphological changes in

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melanoma B16F10 cell line. In contrast, the concentration of the extract at $CD_{12.5}$ (2.52 mg/ml) has shown effect on morphology changes of cell line. The extract of *R. damascena* may contain the phenolic compounds such as quercetine-3-*O*-glucoside, kaempferol-3-*O*-rhamnoside or active compound in essential oil which exhibited the growth of cancer cell lines such as human lung carcinoma (A549), human prostate carcinoma (PC-3) and human breast cancer cell lines (MCF-7) as report (Zu *et al.*, 2010, Ulusoy *et al.*, 2009 and Yassa *et al.*, 2009).

The result of morphological changes on normal mouse fibroblast L929 and melanoma fibroblast B16F10 cell lines after induced of UVA and added *R. kerrii* Meijer extract at different concentrations were shown in Figure 3.14. From the result, the 50% hydroglycol caused morphological changes in normal mouse fibroblast L929 cell line. No morphological changes in normal mouse fibroblast L929 cell line was observed when adding the extract at the concentrations $CD_{3.13}$ (2.47 mg/ml), $CD_{6.25}$ (4.94 mg/ml) and $CD_{12.5}$ (9.88 mg/ml). In contrast, the extract has affected on morphological changes in melanoma fibroblast B16F10 cell line when the concentrations of the extract were increased. From the result, the extract has affected on morphology of mouse melanoma B16F10 cell line which could be due to the active compounds that present in the extract such as 1,2,4,6-tetra-*O*-galloyl-β-D-glucopyranoside, 1,2,6-tri-*O*-galloyl-β-D-glucopyranoside, 1,4,6-tri-*O*-galloyl-β-D-glucopyranoside, 1,2,4-tri-*O*-galloyl-β-D-glucopyranoside and syringin in the *R. kerrii* Meijer extract that exhibited of growth cancer cell line (Kanchanapoom *et al.*, 2007).

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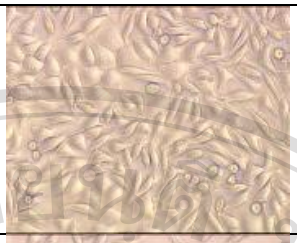
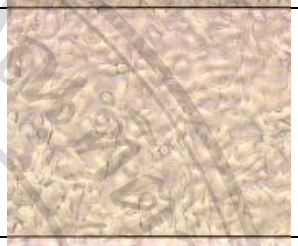
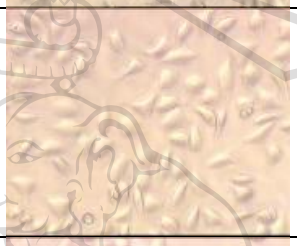
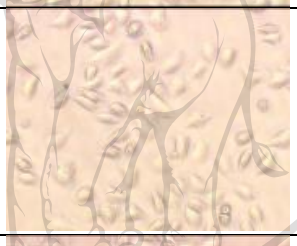
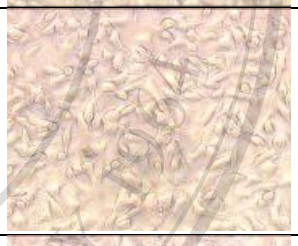
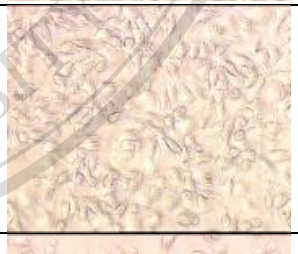
	L929	B16F10
Control without UVA treated		
Control with UVA treated		
50% (v/v) hydroglycol		
E. elatior (Jack) R.M. Smith $CD_{3.13}$ = 1.24 mg/ml (L929) $CD_{3.13}$ = 3.65 mg/ml (B16F10)		
$CD_{6.25}$ = 2.48 mg/ml (L929) $CD_{6.25}$ = 7.30 mg/ml (B16F10)		
$CD_{12.5}$ = 4.96 mg/ml (L929) $CD_{12.5}$ = 14.60 mg/ml (B16F10)		

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Figure 3.12 Morphology of normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines after induced by UVA radiation and treated with *E. elatior* (Jack) R.M. Smith extract at the concentrations of $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$.

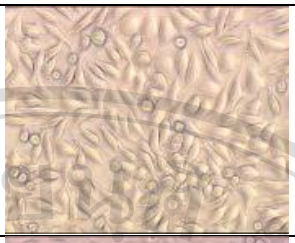
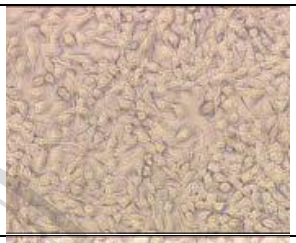
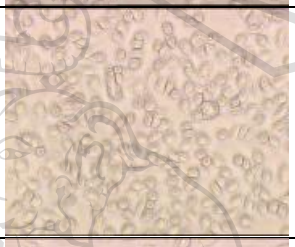
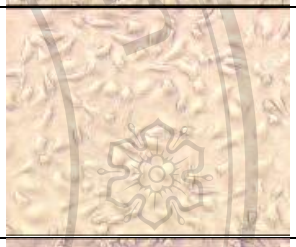
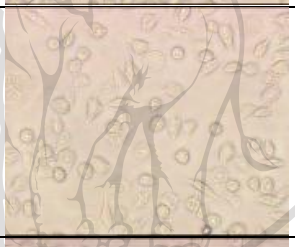
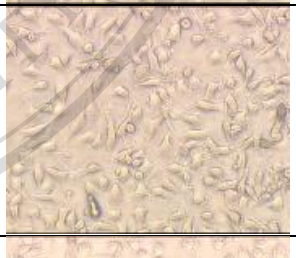
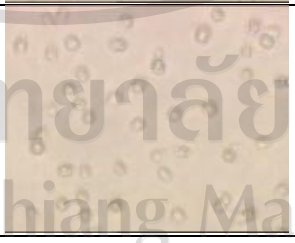
	L929	B16F10
Control without UVA treated		
Control with UVA treated		
50% (v/v) hydroglycol		
R. damascena $CD_{3.13}$ = 1.07 mg/ml (L929) $CD_{3.13}$ = 0.63 mg/ml (B16F10)		
$CD_{6.25}$ = 2.14 mg/ml (L929) $CD_{6.25}$ = 1.26 mg/ml (B16F10)		
$CD_{12.5}$ = 4.28 mg/ml (L929) $CD_{12.5}$ = 2.52 mg/ml (B16F10)		

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Figure 3.13 Morphology of normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines after induced by UVA radiation and treated with *R. damascena* extract at the concentrations of $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$.

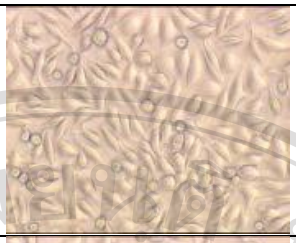
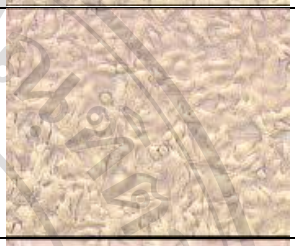
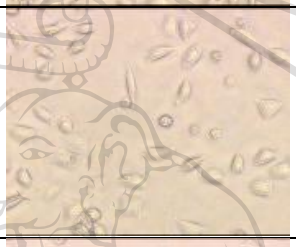
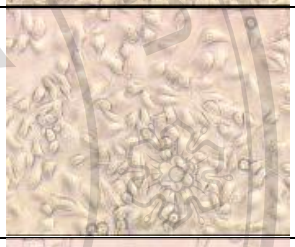
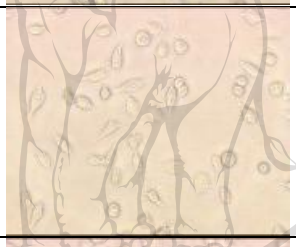
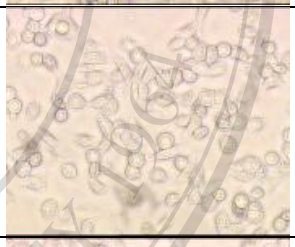
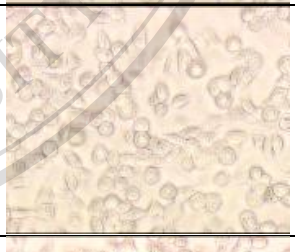
	L929	B16F10
Control without UVA treated		
Control with UVA treated		
50% (v/v) hydroglycol		
R. kerrii Meijer $CD_{3.13}$= 2.47 mg/ml (L929) $CD_{3.13}$ = 1.59 mg/ml (B16F10)		
$CD_{6.25}$= 4.94 mg/ml (L929) $CD_{6.25}$= 3.18 mg/ml (B16F10)		
$CD_{12.5}$= 9.88 mg/ml (L929) $CD_{12.5}$= 6.36 mg/ml (B16F10)		

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Figure 3.14 Morphology of normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines after induced by UVA radiation and treated with *R. kerrii* Meijer extract at the concentrations of $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$.

The morphological changes of both cell lines after exposured to UVA and treated with the extracts were concluded in (Table 3.23). The results suggested that the concentrations of *T. bellerica* and *E. elatior* (Jack) R.M. Smith extracts at $CD_{3.13}$ and $CD_{6.25}$ had no effect on morphological changes in normal mouse fibroblast L929 cell line. When the concentrations of the extracts were increased at $CD_{12.5}$, the morphology of normal cell lines were changed. The concentrations of *T. chebula* Retz. extract at $CD_{6.25}$ and $CD_{12.50}$ had affected on morphological changes. All the concentrations of *R. damascena* extracts affected on morphological changes of normal mouse fibroblast L929 cell line. While, the concentrations of *R. kerrii* Meijer extract at $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$ showed no effect on morphological changes in normal mouse fibroblast L929 cell line. In addition, the 50% hydroglycol has also affected on morphological changes of normal fibroblast cell lines. Whereas, the 70% hydroglycol has affected on morphological changes of normal fibroblast cell lines.

In mouse melanoma B16F10 cell line, after induced by UVA radiation and treated the extracts, it was revealed that the concentrations of *T. chebula* Retz., *T. bellerica*, and *R. kerrii* Meijer extracts at $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$ affected on morphological changed. The concentrations of the *E. elatior* (Jack) R.M. Smith and *R. damascena* at $CD_{12.5}$ affected on morphology changes of mouse melanoma B16F10 cell line. From the result, the five plant extracts affected on morphology of mouse melanoma B16F10 cell line rather than normal mouse fibroblast L929 cell line. These observations suggest a possible application of the five plant extracts to the treatment of skin cancer.

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Table 3.23 The morphological changes of normal mouse fibroblast L929 and mouse melanoma B16F10 cell line after exposed to UVA and treated with the five plant extracts at different concentrations**.**

Extracts (mg/ml)	Morphological changes			
	Without UVA		With UVA	
	L929	B16F10	L929	B16F10
50% hydroglycol	-	-	+	-
70% hydroglycol	-	-	-	+
T. chebula Retz.				
$CD_{3.13}$	ND	ND	-	+
$CD_{6.25}$	ND	ND	+	+
$CD_{12.50}$	-	-	++	++
T. bellerica				
$CD_{3.13}$	ND	ND	-	+
$CD_{6.25}$	ND	ND	-	+
$CD_{12.50}$	-	-	++	++
E. elatior (Jack) R.M. Smith				
$CD_{3.13}$	ND	ND	-	-
$CD_{6.25}$	ND	ND	-	-
$CD_{12.50}$	++	++	+	+
R. damascena				
$CD_{3.13}$	ND	ND	+	-
$CD_{6.25}$	ND	ND	+	-
$CD_{12.50}$	-	-	++	+
R. kerrii Meijer				
$CD_{3.13}$	ND	ND	-	+
$CD_{6.25}$	ND	ND	-	+
$CD_{12.50}$	-	+	-	++

(-) = the extract has no effect on morphological changes of cell line

(+) = the extract has slightly effect on morphological changes of cell line

(++) = the extract has greater effect on morphological changes of cell line

ND = not determined

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3.9 Morphological changes of normal and melanoma mouse fibroblast cell lines after induced by UVB radiation

In this experiment, both cell lines were exposured to UVB radiation with 30 mJ/cm^2 for 6 min 45 sec. Then, the plant extracts at different concentrations (the extracts concentrations were used in the range of $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$ from cytotoxicity assay) were added to the UVB treated cells. The morphological changes of cells after exposured to UVB were investigated under a light microscope.

The result of morphological changes of normal mouse fibroblast L929 and melanoma fibroblast B16F10 cell lines after induced by UVB and treated with of *T. chebula* Retz. extract at different concentrations were shown in Figure 3.15. From the results, the cell morphology of UVB treated controls in both cell lines did not change upon treatment with UVB when compared with untreated UVB control group. The possible explanation of this was that the limitation of the UVB power of light source and the condition in UV irradiation equipment was not suitable for cell cultivation system such as the level of carbon dioxide (CO_2) that was the same problem as found in UVA treated experiment (chepter 3.8). The 70 % hydroglycol the had no effect on morphology changes in both cell lines. After adding the extract of *T. chebula* Retz. at different concentrations, it was found that all of the concentrations of the extract has no effect on morphological changes of normal mouse fibroblast L929 cell line. In melanoma fibroblast B16F10 cell line, the concentrations of the extract at $CD_{3.13}$ (0.17 mg/ml), $CD_{6.25}$ (0.54 mg/ml) and $CD_{12.5}$ (1.08 mg/ml) have affected on morphological changes in dose dependent manner.

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Next, the extract of *T. bellerica* was tested at different concentrations and exposured to UVB radiation. It was found that the concentrations of the extract at $CD_{3.13}$ (0.33 mg/ml), $CD_{6.25}$ (0.66 mg/ml) and $CD_{12.5}$ (1.32 mg/ml) have no effect on morphological changes of normal mouse fibroblast L929 cell line (Figure 3.16). While, the concentrations of the *T. bellerica* extract at $CD_{3.13}$ (0.13 mg/ml), $CD_{6.25}$ (0.26 mg/ml) and $CD_{12.5}$ (0.52 mg/ml) have leaded to morphological changes in melanoma fibroblast B16F10 cell line as expected. The same result was also found in UVA irradiation. From the result, the extract has affected on morphology of mouse melanoma B16F10 cell line which could be due to the active compounds that present in the extract such as gallic acid that exhibited anti-proliferative and inducred apoptotic death in various cancer cell lines in previously report (Yoshioka *et al.*, 2000, Kaur *et al.*, 2005 and Pinmai *et al.*, 2008)

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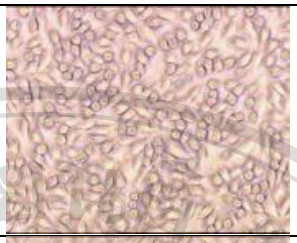
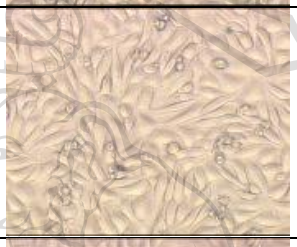
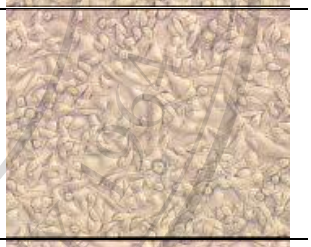
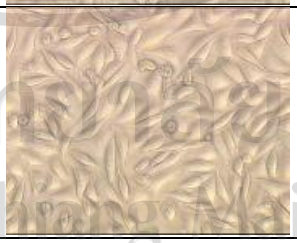
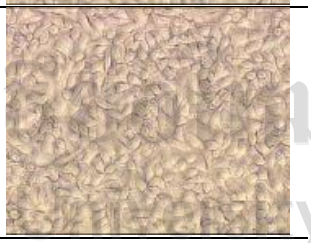
	L929	B16F10
Control without UVB treated		
Control with UVB treated		
70% (v/v) hydroglycol		
T. chebula Retz. $CD_{3.13}$ = 0.75 mg/ml (L929) $CD_{3.13}$ = 0.27 mg/ml (B16F10)		
$CD_{6.25}$ = 1.50 mg/ml (L929) $CD_{6.25}$ = 0.54 mg/ml (B16F10)		
$CD_{12.5}$ = 3.00 mg/ml (L929) $CD_{12.5}$ = 1.08 mg/ml (B16F10)		

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Figure 3.15 Morphology of normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines after induced by UVB radiation and treated with *T. chebula* Retz. extract at the concentrations of $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$.

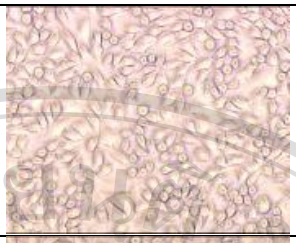
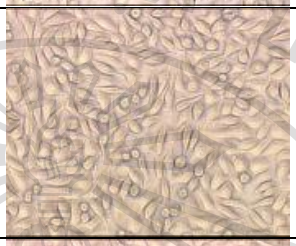
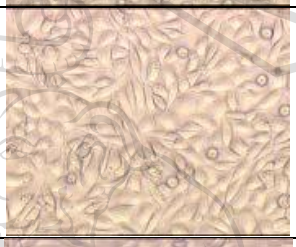
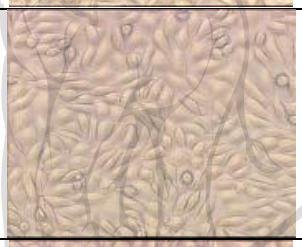
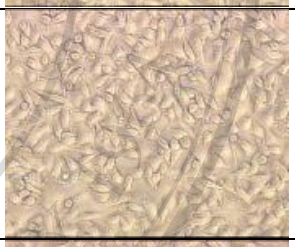
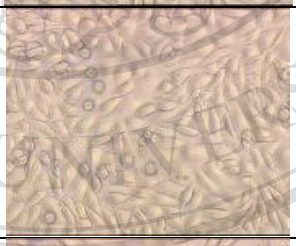
	L929	B16F10
Control without UVB treated		
Control with UVB treated		
70% (v/v) hydroglycol		
T. bellerica $CD_{3.13}$ = 0.33 mg/ml (L929) $CD_{3.13}$ = 0.13 mg/ml (B16F10)		
$CD_{6.25}$ = 0.66 mg/ml (L929) $CD_{6.25}$ = 0.26 mg/ml (B16F10)		
$CD_{12.5}$ = 1.32 mg/ml (L929) $CD_{12.5}$ = 0.52 mg/ml (B16F10)		

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Figure 3.16 Morphology of normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines after induced by UVB radiation and treated with *T. bellerica* extract at the concentrations of $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$.

The effect of *E. elatior* (Jack) R.M. Smith extract on morphological changes was shown in Figure 3.17. As a negative control, the 50% hydroglycol had no effect on morphological changes in both normal mouse fibroblast L929 and melanoma B16F10 cell lines. The concentrations of the *E. elatior* (Jack) R.M. Smith extract at $CD_{3.13}$ (1.24 mg/ml) and $CD_{6.25}$ (2.48 mg/ml) also showed no effect on morphological changes of normal mouse fibroblast L929 cell line. When the concentration of the extract was increased to $CD_{12.5}$ (4.96 mg/ml), the cell morphology was changed. In melanoma B16F10 cell line, all of the concentrations of the *E. elatior* (Jack) R.M. Smith extract had affected on morphological changes of cancer cell line.

The morphological changes in both cell lines after induced UVB radiation and followed by adding *R. damascena* extract at different concentrations were shown in Figure 3.18. It was found that all the concentrations of the *R. damascena* extract have no effect on morphological changes in normal mouse fibroblast L929 cell line. In melanoma B16F10 cell line, it was found that all the concentrations of the *R. damascena* extract have no effect on morphological changes of cell line.

The investigation of morphological changes of normal mouse fibroblast L929 and melanoma B16F10 cell lines after induced UVB radiation and added the *R. kerrii* Meijer at different concentrations were also performed (Figure 3.19). In normal mouse fibroblast L929 cell line, it was found that all of the concentrations of the extract have no effect on morphological changes. On the other hand, it was found that the concentrations of *R. kerrii* Meijer extract at $CD_{3.13}$ (1.59 mg/ml), $CD_{6.25}$ (3.18 mg/ml) and $CD_{12.5}$ (6.36 mg/ml) were also caused morphological changes of melanoma B16F10 cell line.

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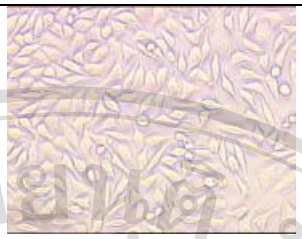
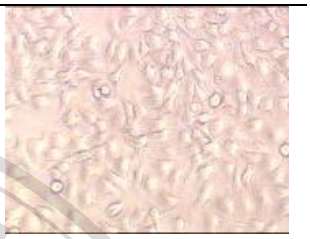
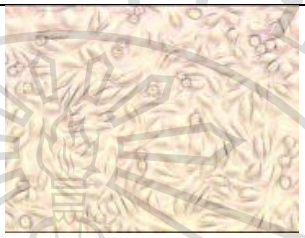
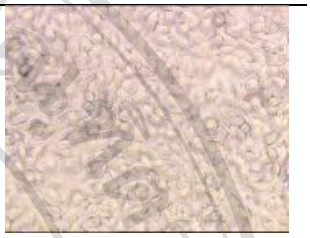
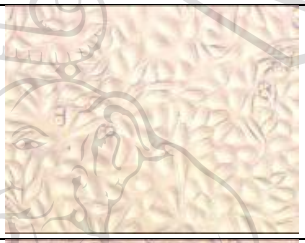
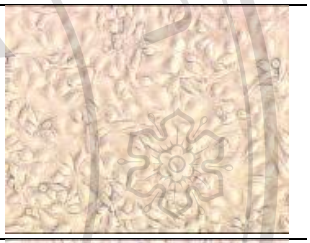
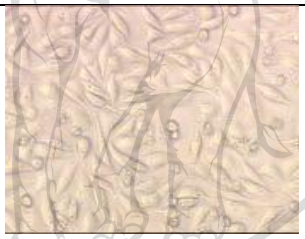
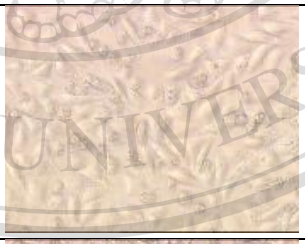
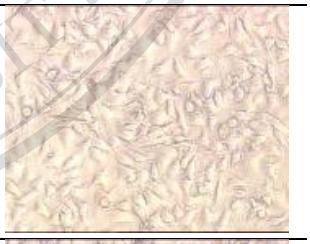
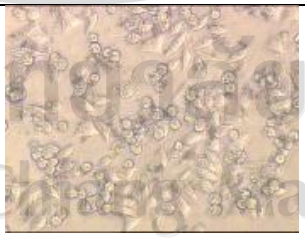
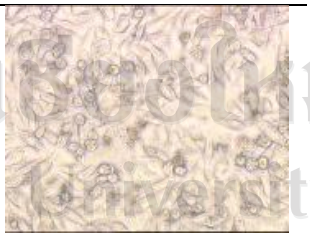
	L929	B16F10
Control without UVB treated		
Control with UVB treated		
50% (v/v) hydroglycol		
E. elatior (Jack) R.M. Smith $CD_{3.13}$ = 1.24 mg/ml (L929) $CD_{3.13}$ = 3.65 mg/ml (B16F10)		
$CD_{6.25}$ = 2.48 mg/ml (L929) $CD_{6.25}$ = 7.30 mg/ml (B16F10)		
$CD_{12.5}$ = 4.96 mg/ml (L929) $CD_{12.5}$= 14.60 mg/ml (B16F10)		

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Figure 3.17 Morphology of normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines after induced by UVB radiation and treated with *E. elatior* (Jack) R.M. Smith extract at the concentrations of $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$.

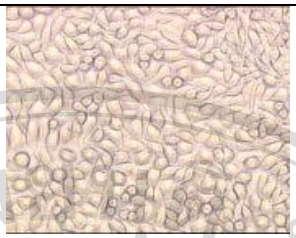
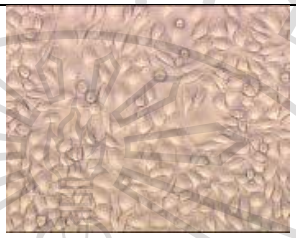
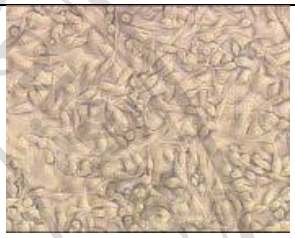
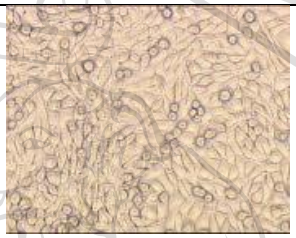
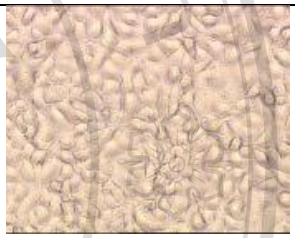
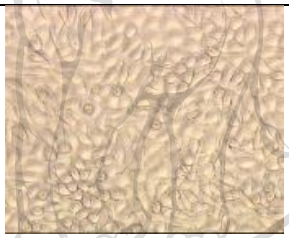
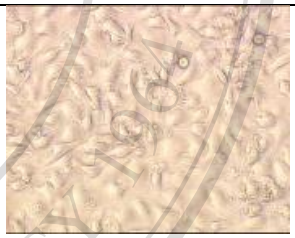
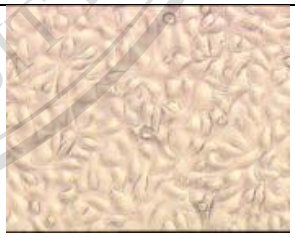
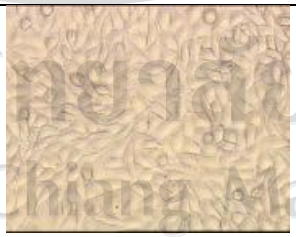
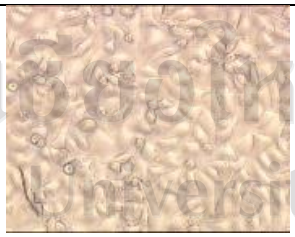
	L929	B16F10
Control without UVB treated		
Control with UVB treated		
50% (v/v) hydroglycol		
R. damascena $CD_{3.13}$ = 1.07 mg/ml (L929) $CD_{3.13}$ = 0.63 mg/ml (B16F10)		
$CD_{6.25}$ = 2.14 mg/ml (L929) $CD_{6.25}$ = 1.26 mg/ml (B16F10)		
$CD_{12.5}$ = 4.28 mg/ml (L929) $CD_{12.5}$ = 2.52 mg/ml (B16F10)		

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Figure 3.18 Morphology of normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines after induced by UVB radiation and treated with *R. damascena* extract at the concentrations of $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$.

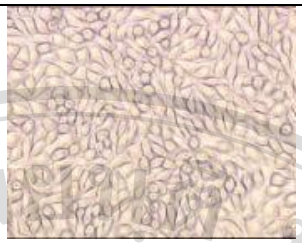
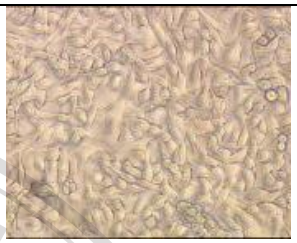
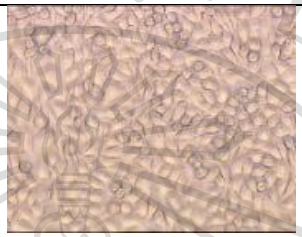
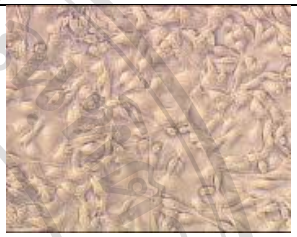
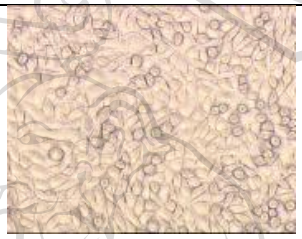
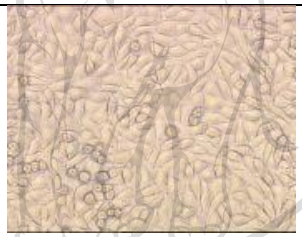
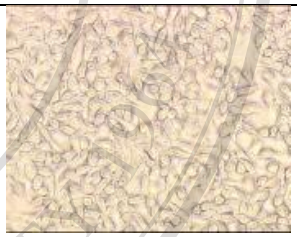
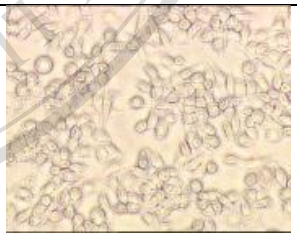
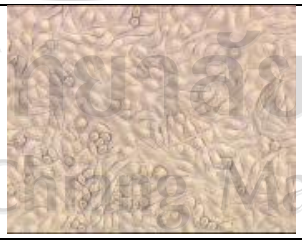
	L929	B16F10
Control without UVB treated		
Control with UVB treated		
50% (v/v) hydroglycol		
R. kerrii Meijer $CD_{3.13}$= 2.47 mg/ml (L929) $CD_{3.13}$ = 1.59 mg/ml (B16F10)		
$CD_{6.25}$= 4.94 mg/ml (L929) $CD_{6.25}$= 3.18 mg/ml (B16F10)		
$CD_{12.5}$= 9.88 mg/ml (L929) $CD_{12.5}$= 6.36 mg/ml (B16F10)		

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Figure 3.19 Morphology of normal mouse fibroblast L929 and mouse melanoma B16F10 cell lines after induced by UVB radiation and treated with *R. kerrii* Meijer extract at the concentrations of $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$**.**

The morphological changes of cells after exposured to UVB and treated with the extracts were concluded as in Table 3.24. The results suggested that the concentrations of *T. chebula* Retz., *T. bellerica*, *R. damascena* and *R. kerrii* Meijer extracts at $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$ had no effect on morphological changes in normal mouse fibroblast L929 cell line. In contrast, the concentrations of *E. elatior* (Jack) R.M. Smith extract at $CD_{12.5}$ showed effect on morphological changes in normal mouse fibroblast L929 cell line. In the mouse melanoma B16F10 cell line, all the concentrations of *T. chebula* Retz., *T. bellerica*, *E. elatior* (Jack) R.M. Smith and *R. kerrii* Meijer extracts have affected on morphological changes. Whereas, the concentrations of *R. damascena* extract at $CD_{3.13}$, $CD_{6.25}$ and $CD_{12.5}$ has no effect on morphological changes in the mouse melanoma B16F10 cell line. From the result, the five plant extracts have affected on morphology of mouse melanoma B16F10 cell line rather than normal mouse fibroblast L929 cell line. These observations suggest a possible application of the five plant extracts to the treatment of skin cancer.

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Table 3.24 The morphological changes of normal mouse fibroblast L929 and mouse melanoma B16F10 cell line after exposed to UVB and treated with the five plant extracts at different concentrations.

Extracts (mg/ml)	Morphological changes			
	Without UVB		With UVB	
	L929	B16F10	L929	B16F10
50% hydroglycol	-	-	-	-
70% hydroglycol	-	-	-	-
T. chebula Retz.				
$CD_{3.13}$	ND	ND	-	+
$CD_{6.25}$	ND	ND	-	+
$CD_{12.50}$	-	-	-	+
T. bellerica				
$CD_{3.13}$	ND	ND	-	+
$CD_{6.25}$	ND	ND	-	+
$CD_{12.50}$	-	-	-	+
E. elatior (Jack) R.M. Smith				
$CD_{3.13}$	ND	ND	-	+
$CD_{6.25}$	ND	ND	-	+
$CD_{12.50}$	++	++	++	++
R. damascena				
$CD_{3.13}$	ND	ND	-	-
$CD_{6.25}$	ND	ND	-	-
$CD_{12.50}$	-	-	-	-
R. kerrii Meijer				
$CD_{3.13}$	ND	ND	-	+
$CD_{6.25}$	ND	ND	-	++
$CD_{12.50}$	-	-	-	++

(-) = the extract has no effect on morphological changes of cell line

(+) = the extract has slightly effect on morphological changes of cell line

(++) = the extract has greater effect on morphological changes of cell line

ND = not determined

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