CHAPTER 3

RESULTS

3.1 Anti-gastric ulcer activity

The anti-gastric ulcer activity of the aqueous extract of *C. racemosa* var. *cylindracea* (AqCR) was investigated in rats using various experiment models. The gastric lesions in rats were induced by: 1) restraint water immersion stress 2) HCl/Ethanol 3) indomethacin

3.1.1) Restraint water immersion stress-induced gastric ulcer in rats

Multiple mucosal lesions of different length were produced in the glandular part of the stomach in response to stress induced by restraint and exposure to cool. Table 1 and Figure 11 illustrate the inhibitory effects of the AqCR and cimetidine on stress-induced gastric lesions. The rats in the control group showed an ulcer index of 10.08±1.23 mm. The AqCR caused a dose-dependent reduction of the gastric lesions. The AqCR at the doses of 100 and 500 mg/kg showed ulcer indexes of 4.13±0.85, 0.23±0.09 mm, which were significantly less than that of the control, and % inhibition of 59.03% and 97.72%, respectively. In the case of cimetidine (100 mg/kg), the ulcer index was 0.83±0.05 mm which was significantly less than that of the control and the percent inhibition was 99.21%.

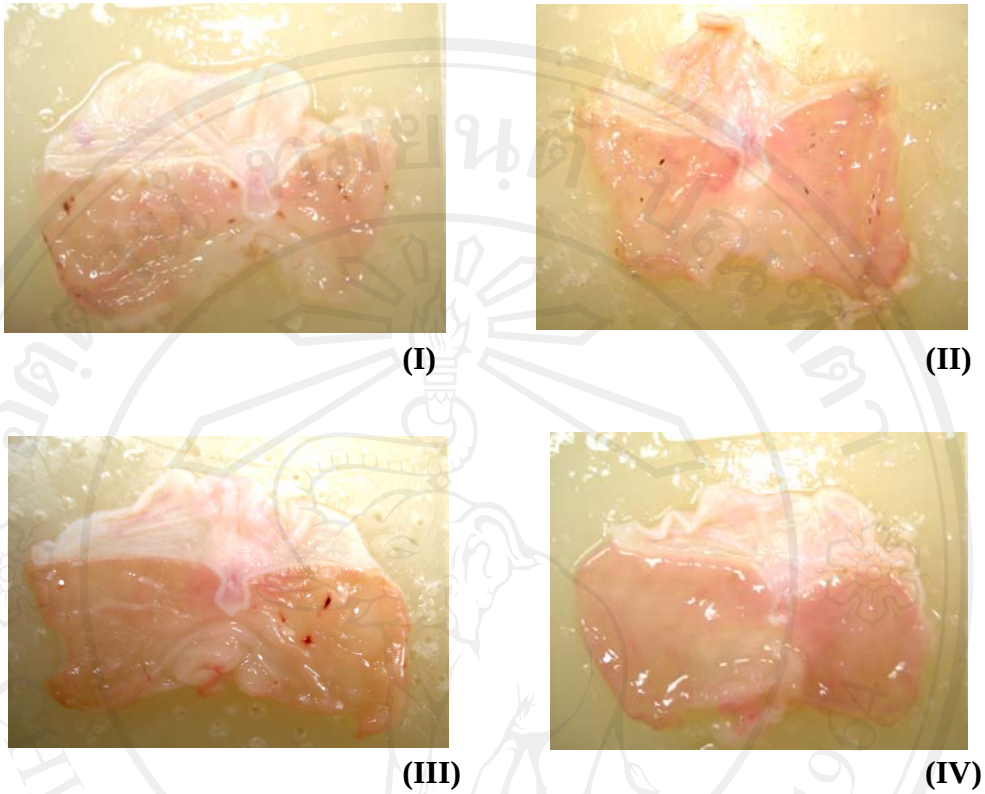


Figure 11 Gastric ulcers induced in rats by restraint water immersion stress: I—control; II—cimetidine (100 mg/kg); III—AqCR (100 mg/kg); IV—AqCR (500 mg/kg)

Table 1 Effect of the aqueous extract of *C. racemosa* (AqCR) on restraint water-immersion stress-induced gastric ulcer in rats.

Groups	Ulcer index (mm)	% Inhibition
Control	10.08±1.23	-
Cimetidine 100 mg/kg	0.83±0.05*	99.21
AqCR 100 mg/kg	4.13±0.85*	59.03
AqCR 500 mg/kg	0.23±0.09*	97.72

Values were expressed as mean± S.E.M. (n=6)

Significantly different from the control group: *$p<0.001$

Cimetidine or the aqueous extract of *C. racemosa* (AqCR) was given orally 1 h before performing restraint water immersion.

3.1.2) HCl/Ethanol-induced gastric ulcer in rats.

An oral administration of HCl/Ethanol caused a direct damage to gastric mucosa, resulted in characteristic striated lesions presence in the glandular portion of the stomach. The protective effect of the AqCR against HCl/Ethanol induced gastric mucosal lesions is shown in Figure 12 and Table 2. The administration of the 2 doses: 100 and 500 mg/kg of the AqCR significantly inhibited the ulcer lesions. The ulcer index of the control group was 151.82±7.36 mm whereas those of the AqCR at the doses of 100 and 500 mg/kg were 27.40±5.16 and 1.38±0.79 mm, with present inhibition of 81.95 and 99.09 respectively. Cimetidine significantly inhibited the ulcer formation, showing an ulcer index of 78.85±16.66 mm and percent inhibition of 48.06

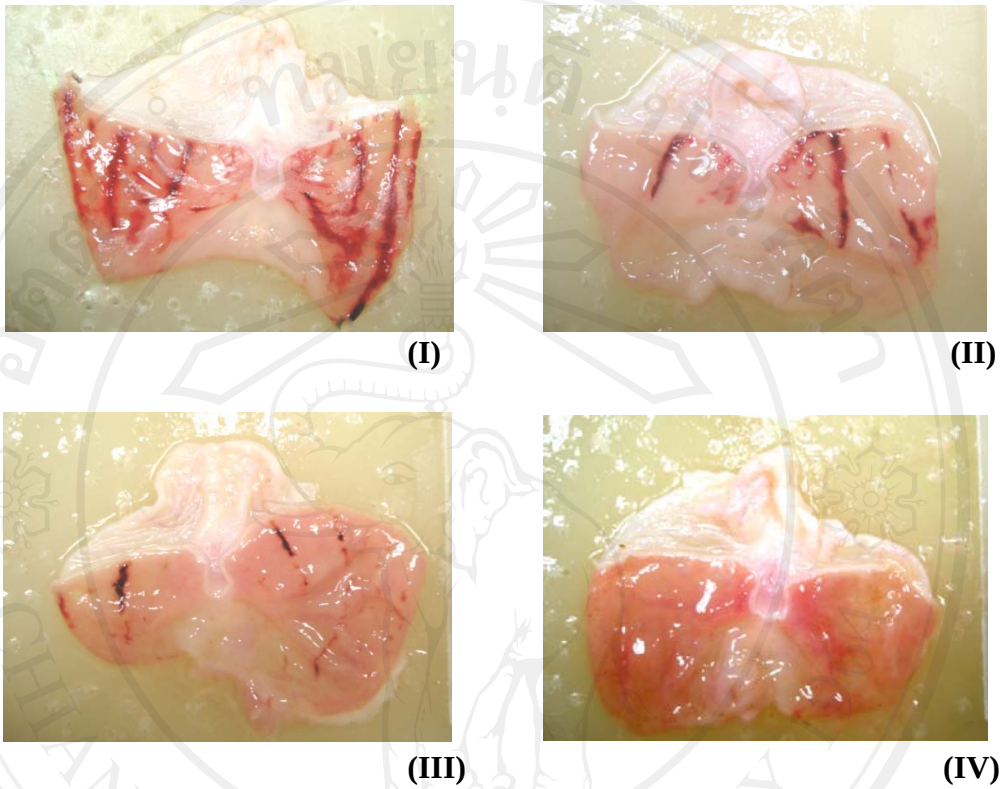


Figure 12 Gastric ulcers induced in rats by HCl/EtOH: I—control; II—cimetidine (100 mg/kg); III—AqCR (100 mg/kg); IV—AqCR (500 mg/kg)

Table 2 Effect of the aqueous extract of *C. racemosa* (AqCR) on HCl/Ethanol-induced gastric ulcer in rats.

Groups	Ulcer index (mm)	% Inhibition
Control	151.82±7.36	-
Cimetidine 100 mg/kg	78.85±16.66*	48.06
AqCR 100 mg/kg	27.40±5.16*	81.95
AqCR 500 mg/kg	1.38±0.79*	99.09

Values are expressed as mean± S.E.M. (n=6)

Significantly different from the control group: *$p<0.001$

Cimetidine or the aqueous extract of *C. racemosa* (AqCR) was given orally 1 h before administration of HCl/Ethanol.

3.1.3) Indomethacin-induced gastric ulcers in rats

An intraperitonal injection of indomethacin produced a lot of small lesions or peticheal ulcers in the stomach. Data illustrated in Table 3 and Figure 13 demonstrate the preventive effects of the AqCR and cimetidine against indomethacin-induced gastric ulcers. An ulcer index of 4.85±0.52 mm was observed in the control group. The AqCR significantly decreased the ulcer formation and the doses of 100 and 500 mg/kg showed ulcer indexes of 0.67±0.33 and 0.05±0.34 mm and with percent inhibition of 86.19 and 98.97 respectively. Significant decrease of an ulcer index was found with the administration of cimetidine. The ulcer indexes of 0.12±0.54 mm and % inhibition of 97.53 were observed.

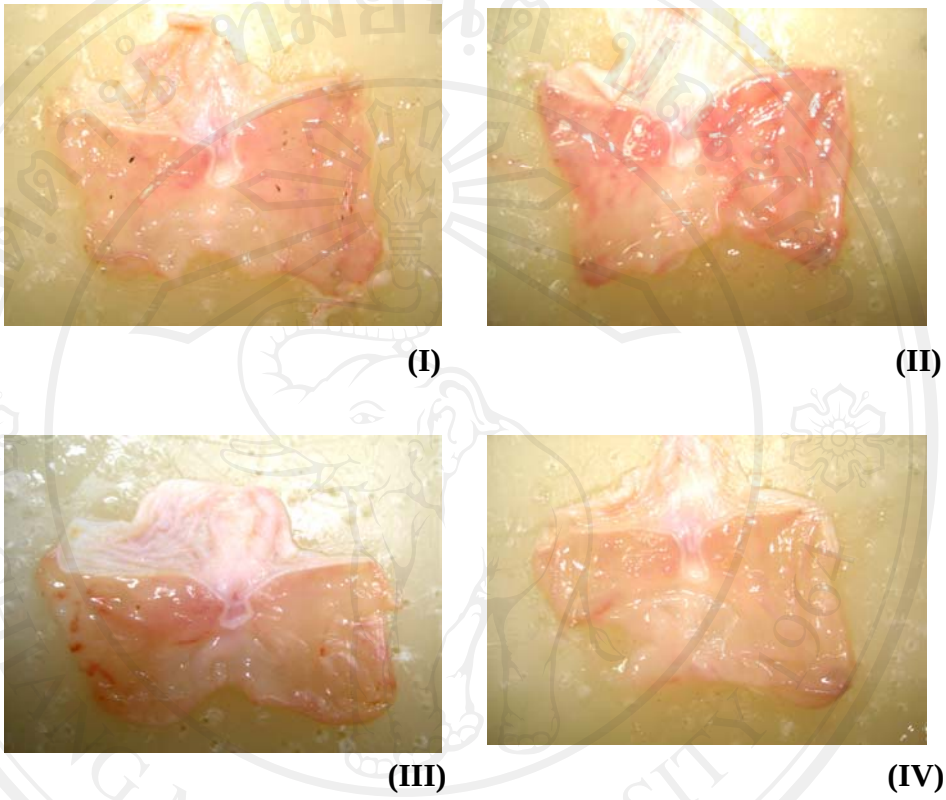


Figure 13 Gastric ulcers induced in rats by indomethacin: I—control; II—cimetidine (100 mg/kg); III—AqCR (100 mg/kg); IV—AqCR (500 mg/kg)

Table 3 Effect of the aqueous extract of *C. racemosa* (AqCR) on indomethacin-induced gastric ulcer in rats

Groups	Ulcer index (mm)	% Inhibition
Control	4.85±0.52	-
Cimetidine 100 mg/kg	0.12±0.54*	97.53
AqCR 100 mg/kg	0.67±0.33*	86.19
AqCR 500 mg/kg	0.05±0.34*	98.97

Values are expressed as mean± S.E.M. (n=6)

Significantly different from the control group: *p<0.001

Cimetidine or the aqueous extract of *C. racemosa* (AqCR) was given orally 1 h before an intraperitoneal injection of indomethacin (30 mg/kg)

Comparison of anti-gastric activity of AqCR in 3 models of gastric ulceration

Figure 14 depicts the anti-gastric activity of the AqCR at the dose of 100 mg/kg in 3 models of gastric ulceration. Percent inhibitions of gastric ulcer were 86.19, 81.95 and 59.03 in indomethacin-, HCl/Ethanol- and restraint water immersion stress-induced gastric ulcer, respectively.

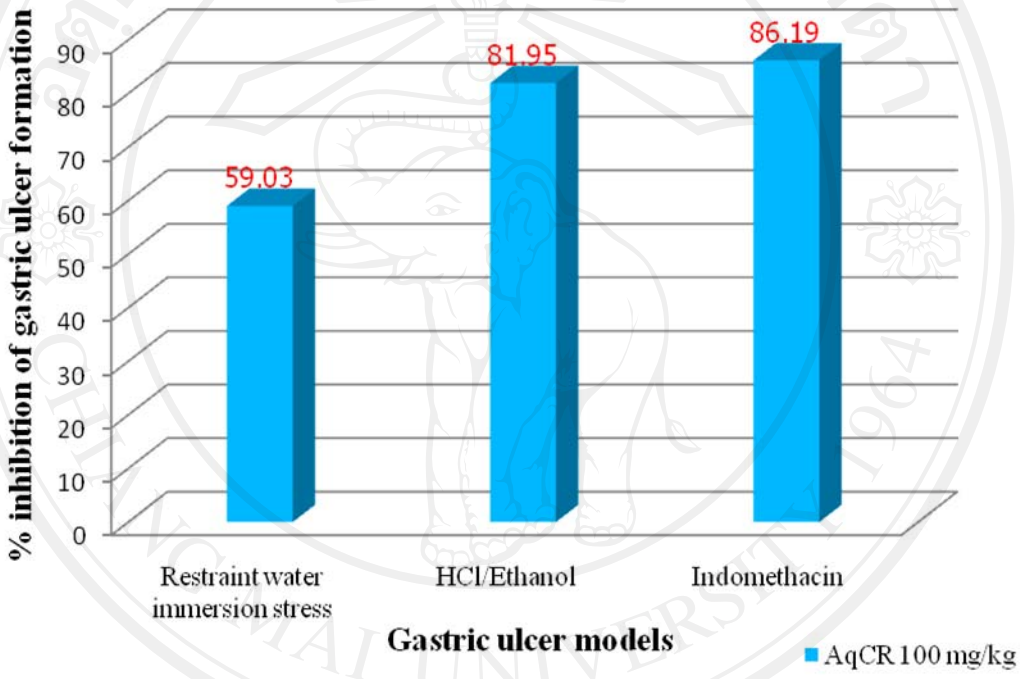


Figure 14 Anti-gastric ulcer activity (% inhibition of gastric ulcer formation) of the AqCR at the dose of 100 mg/kg in different gastric ulceration models: 1) restraint water immersion stress, 2) HCl/Ethanol and 3) indomethacin-induced gastric ulcer in rats.

3.2 Hepatropotective activity of the aqueous extract of *C. racemosa* (AqCR)

The AqCR was tested for hepatoprotective activity in rats with CCl_4-induced hepatic damage. Levels of ALT and AST enzymes and histopathological examination of livers were determined.

3.2.1) Levels of ALT and AST enzymes

The levels of ALT and AST were increased in rats with CCl_4-induced hepatic damage. As shown in Table 4, the levels of ALT and AST of normal rats were found to be 59.83±3.83 and 185.83±17.64 IU/L, respectively, whereas those of the CCl_4 treated rats were 117.33±21.36 and 272.83±12.84 IU/L respectively. Pretreatment with 100 or 500 mg/kg of the AqCR or 100 mg/kg silymarin in CCl_4-induced hepatic damage could significantly lessen the increased enzymes caused by CCl_4 administration. The enzyme levels of groups pretreatment with 100 and 500 mg/kg of the AqCR, and silymarin in CCl_4-induced hepatic damage were 55.33±5.96, 85.33±6.61 and 49.17±1.72 IU/L (ALT) and 148.00±10.08, 187.83±5.55 and 117.33±4.37IU/L (AST), respectively. The ALT and AST levels of the group with 500 mg/kg the AqCR treatment (for 7days) were 53.67±2.33 and 131.67±7.89 IU/L, respectively. Only the ALT level was not significant difference from that of normal rats.

Table 4 Effect of the aqueous extract of *C. racemosa* (AqCR) on serum ALT and AST in rats with CCl_4-induced hepatic damage

Groups	ALT (IU/L)	AST (IU/L)
Normal	59.83±3.83	185.83±17.64
AqCR 500 mg/kg (7 days)	53.67±2.33	131.67±7.89*
CCl_4 -induced hepatic damage €		
- Control (distilled water)	117.33±21.36*	272.83±12.84*
- Silymarin 100 mg/kg	49.17±1.72#	117.33±4.37#
- AqCR 100 mg/kg	55.33±5.96#	148.00±10.08#
- AqCR 500 mg/kg	85.33±6.61#	187.83±5.55#

Values are expressed as mean± S.E.M. (n=6)

Significantly different from normal group: $^{*}p < 0.05$

Significantly different from control group (CCl_4): $^{\#}p < 0.05$

€Distilled water, Silymarin and, the AqCR (at the doses of 100, 500 mg/kg) were given orally for 7 days, and CCl_4 (2ml/kg) was intraperitoneally injected 1 h later.

3.2.2) Histopathologic examination of the livers

Typical sections of the liver specimens of the normal group rats, the AqCR at dose 500 mg/kg (for 7days), the AqCR (100 and 500 mg/kg), silymarin (100 mg/kg) are shown in Figure 15 and 16.

The section from the normal group showed a normal hepatic architecture with distinct hepatic cells, sinusoidal spaces and central vein (Figure 15, I and Figure 16, I). Treatment with the AqCR at dose 500 mg/kg (for 7days) did not reveal any degenerative signs (Figure 15, II). Treatment with CCl_4 caused hepatic damage which was seen as the presences of mild necrosis, fatty changes and degenerative changes such as cytoplasmic vacuolation and pycnotic nuclei in the parenchymal cells in the centrilobular areas (Figure 16, II). The liver section of the silymarin group showed almost normal of hepatocytes, minimal inflammatory cells infiltration (Figure 16, III). Pretreatment with the AqCR at dose 100 mg/kg in rats with CCl_4-induced hepatic damage caused minimal fatty changes and mild necrosis (Figure 16, IV), whereas only minimal necrosis was seen with the dose of 500 mg/kg (Figure 16, V).

Table 5 summarized the histopathologic findings of the livers of rats of each groups. Edema was observed as +1(1/5), +2(4/5) in control, +1(2/5) in the AqCR 500 mg/kg (for 7days), +1(1/5), +2(1/5) in silymarin pretreatment, +1(1/5) in the AqCR 100 mg/kg pretreatment, +1(2/5) in the AqCR 500 mg/kg pretreatment groups.

Cytoplasmic vascuolation was observed +1(5/5) in control, negative in the AqCR 500 mg/kg (for 7days), +1(3/5) in silymarin pretreatment, +1(1/5) in the AqCR 100 mg/kg pretreatment, +1(1/5) in the AqCR 500 mg/kg pretreatment groups.

Fattay change was observed +1(3/5), +2(2/5) in control, negative in the AqCR 500 mg/kg (for 7days), negative, +1(3/5), +2(1/5) in silymarin pretreatment, +2(5/5) in the AqCR 100 mg/kg pretreatment, +1(2/5) in the AqCR 500 mg/kg pretreatment groups.

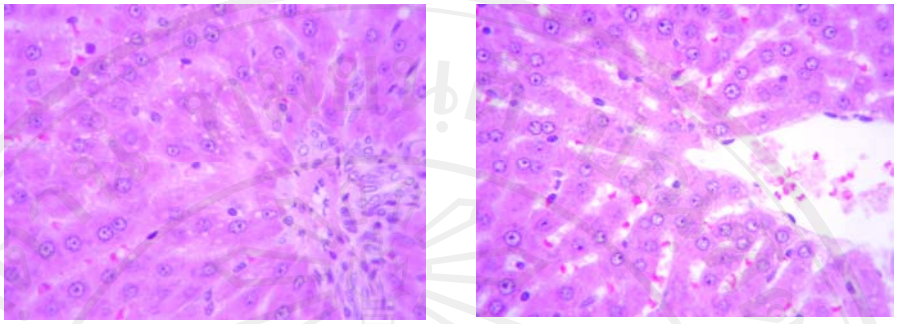
Necrosis was observed mild, scattered (5/5) in control, negative (5/5) in the AqCR 500 mg/kg (for 7days), mild, scattered (5/5) in silymarin pretreatment, minimal, scattered (2/5) in the AqCR 100 mg/kg pretreatment, minimal, scattered (2/5) in the AqCR 500 mg/kg pretreatment groups.

Table 5 Hepatoprotective activity of the aqueous extract of *C. racemosa* (AqCR) against CCl_4-induced hepatic damage in rats: histopathological findings

Groups	Histopathological changes of livers			
	Edema	**Cytoplasmic vacuolation**	**Fatty change**	**Necrosis**
Normal	----------No pathologic conditions----------			
AqCR 500 mg/kg alone	1+[2/5]	Neg	Neg	Neg
CCl_4 -induced hepatic damage [€]				
- Control (distilled water)	1+[1/5] 2+[4/5]	1+[5/5*]	1+[3/5] 2+[2/5]	mild, [5/5] scattered
- Silymarin 100 mg/kg	1+[1/5] 2+[1/5]	1+[3/5]	Neg [1/5] 1+[3/5] 2+[1/5]	mild, [5/5] scattered
- AqCR 100 mg/kg	1+[1/5]	1+[1/5*]	2+[5/5*]	minimal,[2/5] scattered
- AqCR 500 mg/kg	1+[2/5]	1+[1/5*]	1+[2/5*]	minimal,[2/5] scattered

[€]Distilled water, silymarin and, the AqCR (at the doses of 100, 500 mg/kg) were given orally for 7 days, and CCl_4 (2ml/kg) was intraperitoneally injected 1 h later.

*Note: Neg = Negative, * Centro lobular area*



(I) **(II)**

Figure 15 Histopathological sections of the rat liver (Hematoxylin & Eosin staining) Magnification, X400: Normal, 500 mg/kg the AqCR for day 7

(I) : Normal (distilled water for 7 day)

(II) : 500 mg/kg of the aqueous extract of *C. racemosa* (AqCR) for 7 day

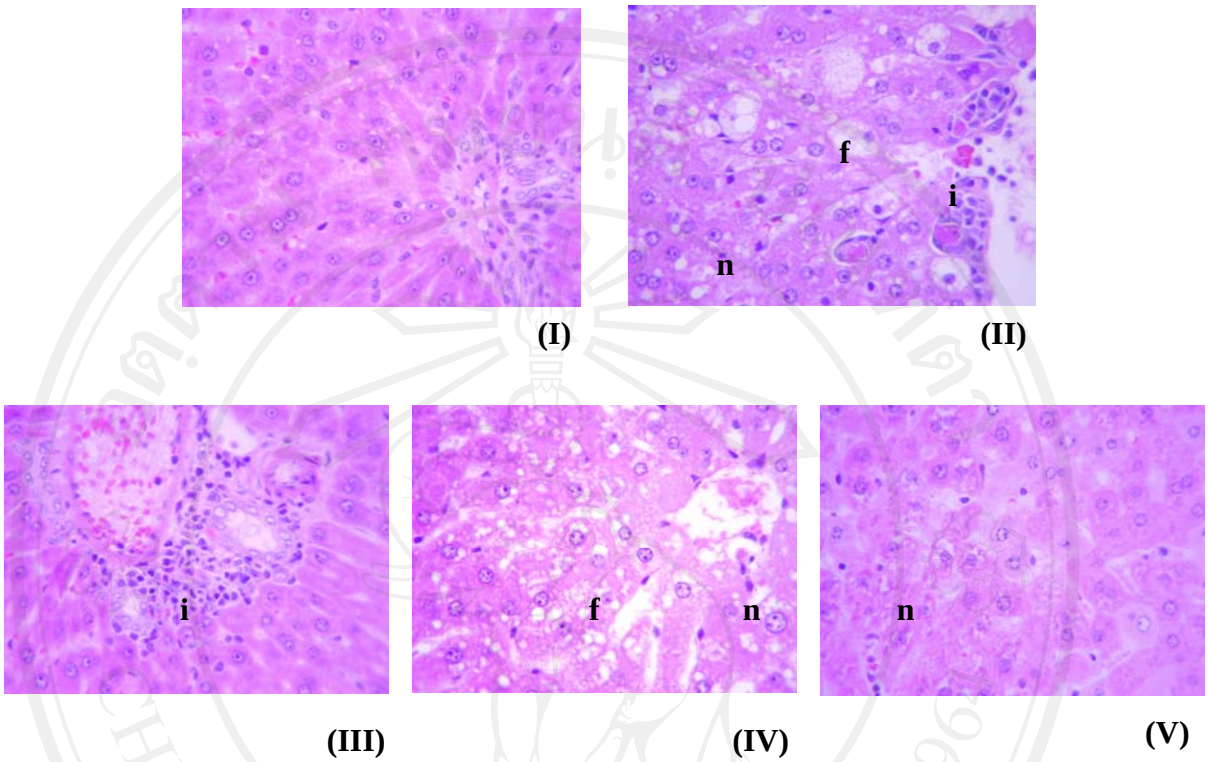


Figure 16 Histopathological sections of the rat liver (Hematoxylin & Eosin staining) Magnification, X400: Normal, the pretreatments with CCl_4 -induced hepatic damage

Normal

(I) : distilled water

CCl_4 -induced hepatic damage

(II) : distilled water

(III) : 100mg/kg of silymarin

(IV) : 100 mg/kg of the aqueous extract of *C. racemosa* (AqCR)

(V) : 500 mg/kg of the aqueous extract of *C. racemosa* (AqCR)

Note: f = fatty change; i = inflammatory cells infiltration; n = necrosis

3.3 Antioxidant activity

3.3.1) DPPH radical scavenging assay

The DPPH radical scavenging activity of the AqCR is presented in Figure 17. The potency and maximal effect of the AqCR were lower than those of gallic acid. The EC50 value of DPPH radical scavenging activity was determined from the linear regression analysis. The EC50 value of the AqCR was 15.09±0.30 μg/ml and that of gallic acid was 8.0429±0.056 μg/ml. The gallic acid equivalent (GAE) value of the AqCR was 1,876.19±37.24mg sample/ 1mg gallic acid.

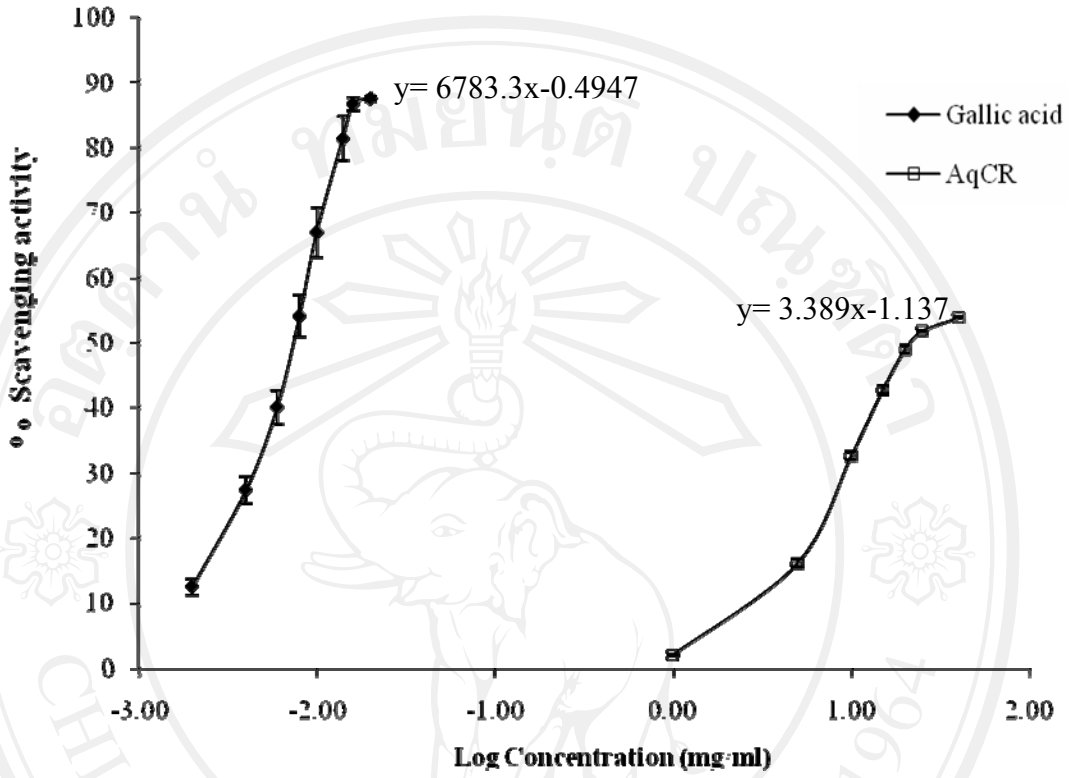


Figure 17 Concentration-response (DPPH radical-scavenging activity) curves of the aqueous extract of *C. racemosa* (AqCR) and gallic acid

Gallic acid: EC50= 8.0429±0.056 µg/ml

AqCR: EC50= 15.09±0.30 mg/ml

Gallic acid equivalent (GAE) = 1,876.19±37.24 mg sample/ 1mg gallic acid

3.3.2) Anti-lipid peroxidation activity

The anti-lipid peroxidation activity of the AqCR is presented in Figure 18. The potency and maximal effect of the AqCR were lower than those of trolox. The concentration which produced 50% of inhibition of lipid peroxidation (EC50) was determined from the linear regression analysis. The EC50 value of the AqCR was 255.364±30.71 mg/ml and that of trolox was 0.107±0.001 mg/ml. Trolox equivalent antioxidant capacity (TEAC) value of the AqCR was 2,386.579±0.22mg sample/ 1 mg trolox

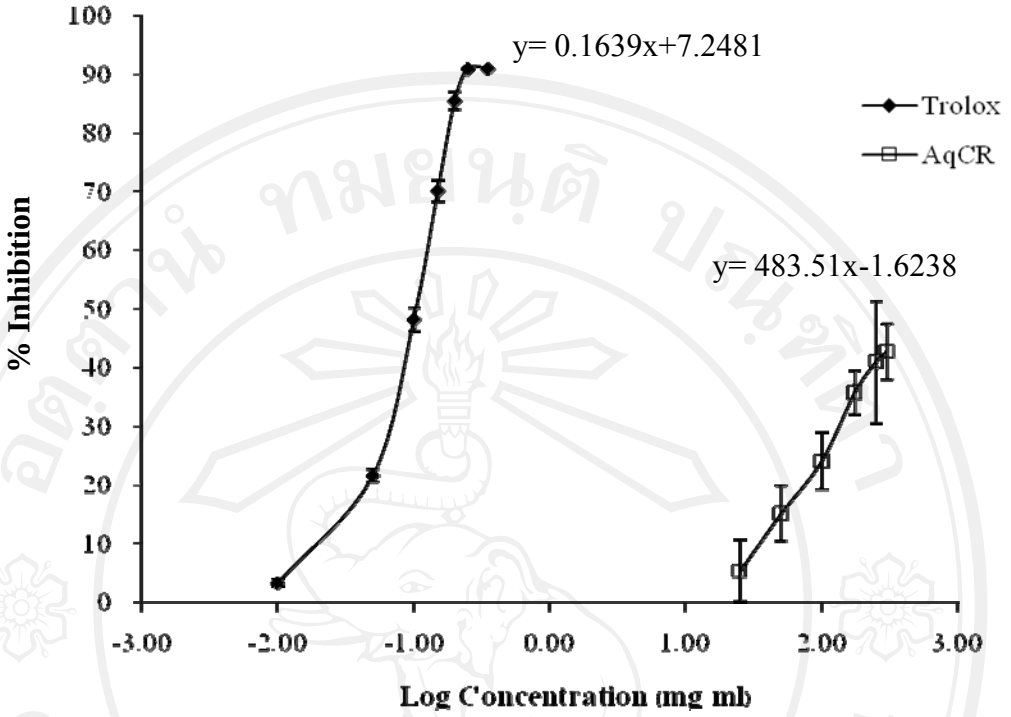


Figure 18 Concentration-response (anti-lipid peroxidation effect) curves of the aqueous extract of *C. racemosa* (AqCR) and trolox

Trolox: EC50= 0.107±0.001 mg/ml

AqCR: EC50= 255.364±30.71 mg/ml

Trolox equivalent antioxidant capacity (TEAC) = 2,386.58±0.22mg sample / 1 mg trolox

3.4 Total phenolic contents

Figure 19 illustrates the graphs represented the amount of phenolic compounds present in the AqCR. The concentrations of the AqCR and gallic acid which showed equal absorbance were determined from the linear regression analysis. The gallic acid equivalent (GAE) value of the AqCR was found to be 2,201.68±111.14 mg sample/1mg gallic acid.

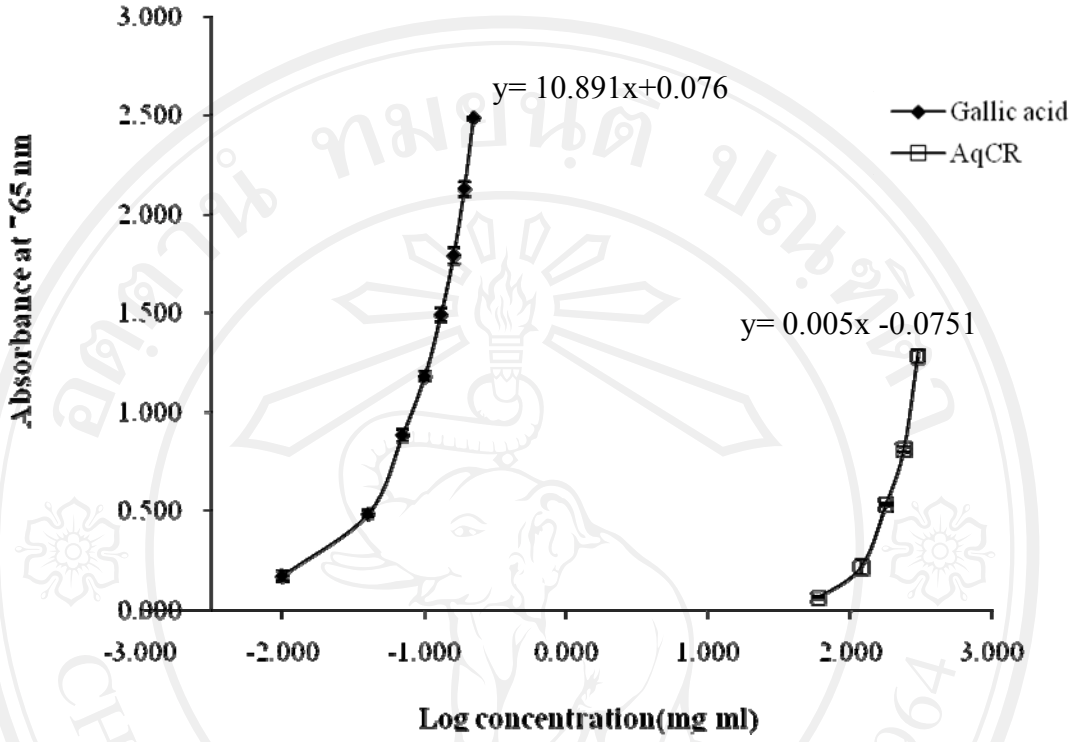


Figure 19 Concentration-response (absorbance at 765 nm) curves of the aqueous extract of *C. racemosa* (AqCR) and gallic acid

Phenolic content expressed as gallic acid equivalent (GAE) = 2,201.68±111.14 mg sample/1mg gallic acid