CHAPTER 3

RESULTS

3.1 Antioxidant activity

3.1.1 $ABTS^{+\bullet}$ radical scavenging activity

The scavenging activity of the Aq. *G* and Trolox against $ABTS^{+\bullet}$ radical are shown in Figure 14. The potency and maximal effect of the Aq. *G* were lower than those of Trolox. The maximal scavenging activity of Trolox was 99%. The maximal scavenging activity of Aq. *G* could not be determined owing to its solubility, however, the maximum activity appeared to about 60%. The EC_{50} value of $ABTS^{+\bullet}$ radical scavenging activity was determined from the linear regression lines. The EC_{50} values of the Aq. *G*, and Trolox were found to be 282.08 ± 9.74 and 0.003 ± 0.0001 mg/mL, respectively. Trolox equivalent antioxidant capacity (TEAC) or the capacity of 1 g of the Aq. *G* is equaled to 0.04 ± 0.003 mM of Trolox.

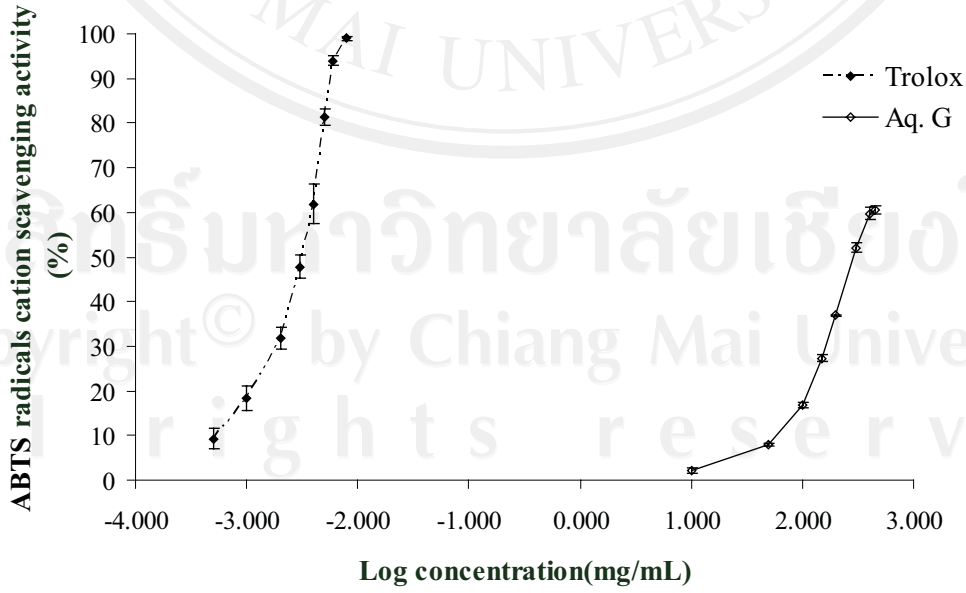

Figure 14 Concentration-response (scavenging activity of $ABTS^{+\bullet}$ radicals) curves of the Aq. *G* and Trolox

3.1.2 Superoxide anion ($O_2^{-\bullet}$) scavenging activity

Figure 15 shows the scavenging activity of the Aq. *G* and gallic acid against superoxide anion. The potency and maximal effect of the Aq. *G* were lower than those of gallic acid. Maximal scavenging activity of the Aq. *G* was 85% whereas that of gallic acid was 95%. According to the linear regression analysis, the EC_{50} values of $O_2^{-\bullet}$ scavenging activity of the Aq. *G* and gallic acid were found to be 3.03 ± 0.23 and 0.23 ± 0.01 mg/mL, respectively. The GAE value of the Aq. *G* was 0.01 ± 0.001, representing that the scavenging activity of the Aq. *G* 0.01 ± 0.001 g equals to 1 mg of gallic acid.

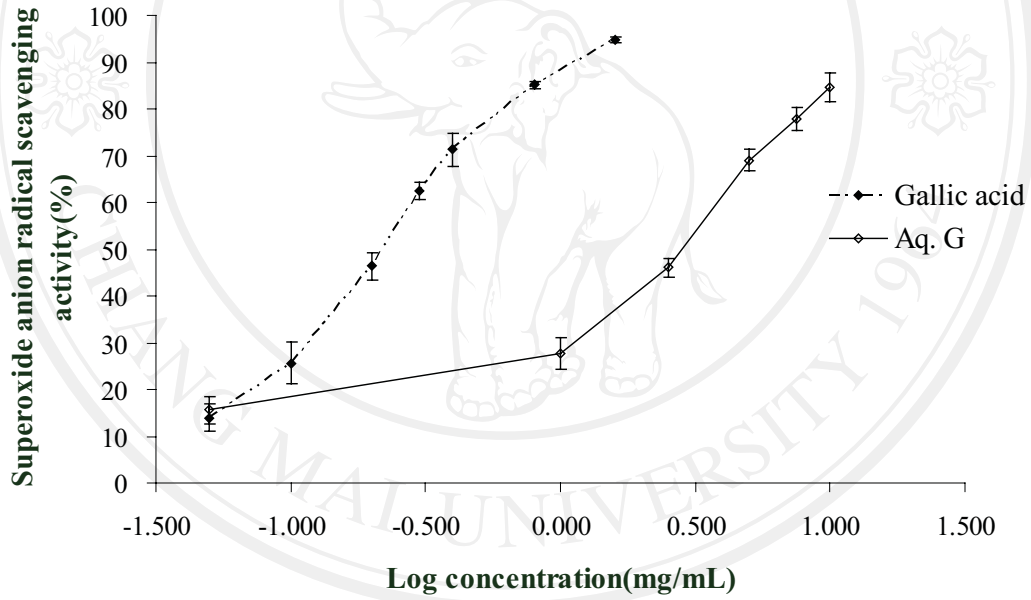

Figure 15 Concentration-response (scavenging activity of superoxide anion radicals) curves of the Aq. *G* and gallic acid

3.1.3 Hydroxyl radical ($^{\bullet}OH$) scavenging activity

The scavenging activity of the Aq. *G* and Trolox against $^{\bullet}OH$ are depicted in Figure 16. The maximal effect of the Aq. *G* was higher than that of Trolox. The Aq. *G* expressed the maximum scavenging activity was 80 % whereas that of Trolox was 58%. The EC_{50} value of $^{\bullet}OH$ scavenging activity was determined from the linear regression analysis. The EC_{50} value of the Aq. *G* was 3.58 ± 0.24 mg/mL and that of Trolox was 1.02 ± 0.24 mg/mL. The TEAC value of the Aq. *G* was 1452.23 ± 393.70.

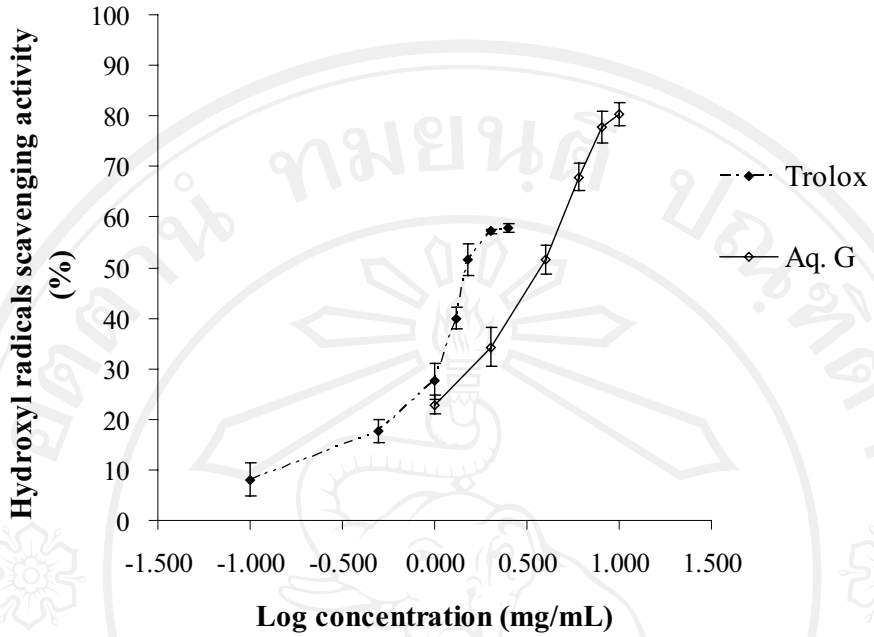

Figure 16 Concentration-response (scavenging activity of hydroxyl radicals) curves of the Aq. *G* and Trolox

3.1.4 Inhibition of lipid peroxidation

Aq. *G* and Trolox could inhibit lipid peroxide formation, as illustrated in Figure 17. Trolox was more potent than the Aq. *G*. The maximal effect of the Aq. *G* was slightly higher than that of Trolox. The Aq. *G* and Trolox showed the maximal inhibition lipid peroxide formation of 93% and 91%, respectively. The EC_{50} values of the Aq. *G* and Trolox determined from the linear regression lines were 0.97 ± 0.01 and 0.11 ± 0.002 mg/mL, respectively. The TEAC value of the Aq. *G* was found to be 464.10 ± 6.66.

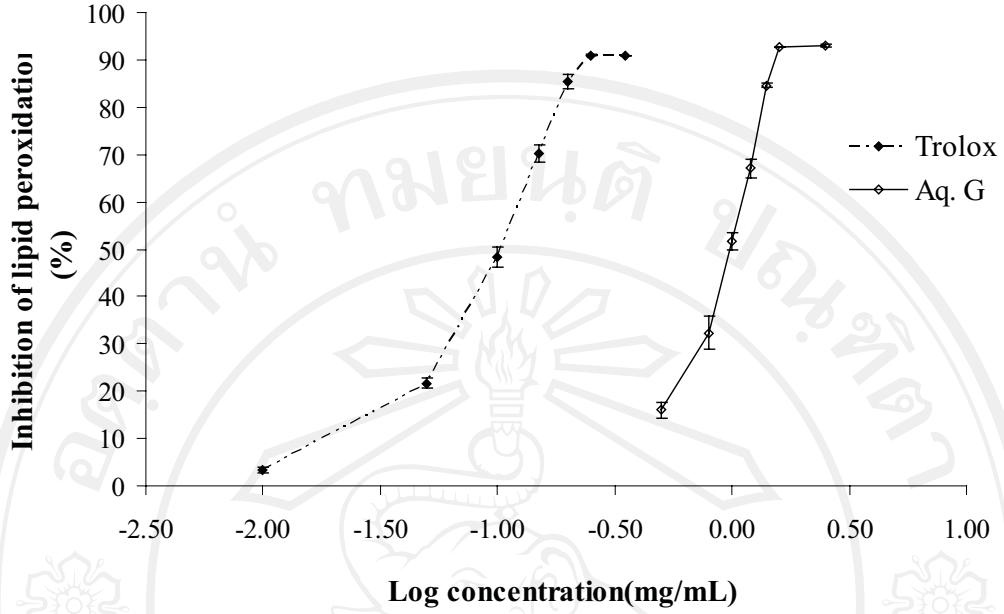

Figure 17 Concentration-response (inhibition of lipid peroxidation) curves of the Aq. *G* and Trolox

3.1.5 Metal chelating activity

Ferrous ion-chelating activity of the Aq. *G* and EDTA are shown in Figure 18. The potency and maximal effect of the Aq. *G* were lower than those of EDTA. The Aq. *G* expressed the maximum chelating activity of 86% whereas that of EDTA was 98%. The EC_{50} value of ferrous ion-chelating activity was determined from the linear regression analysis. The EC_{50} value of the Aq. *G* was 26.69 ± 1.90 mg/mL and that of EDTA was 0.08 ± 0.002 mg/mL. The EDTA equivalent (EDTAE) value of the Aq. *G* was 3047.29 ± 156.606 representing that the activity of 1 g of the Aq. *G* was equaled to 3047.29 ± 156.61 μg of EDTA.

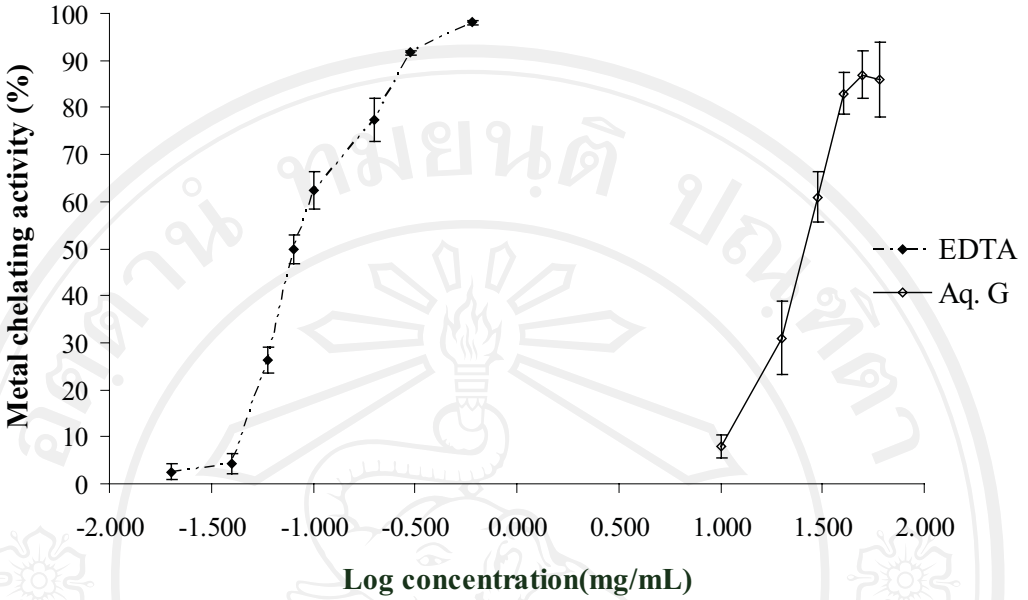

Figure 18 Concentration-response (metal chelating) curves of the Aq. *G* and EDTA

3.1.6 Reducing power

The Aq. *G* and gallic acid possessed a reducing capacity. Figure 19 illustrates a relationship of absorbance value (Y-axis) and concentrations (X-axis) of the Aq. *G* and gallic acid. Higher absorbance value means stronger reducing power of samples. The potency of the Aq. *G* was lower than that of gallic acid. The GAE value of the Aq. *G* was found to be 1.48 ± 0.03.

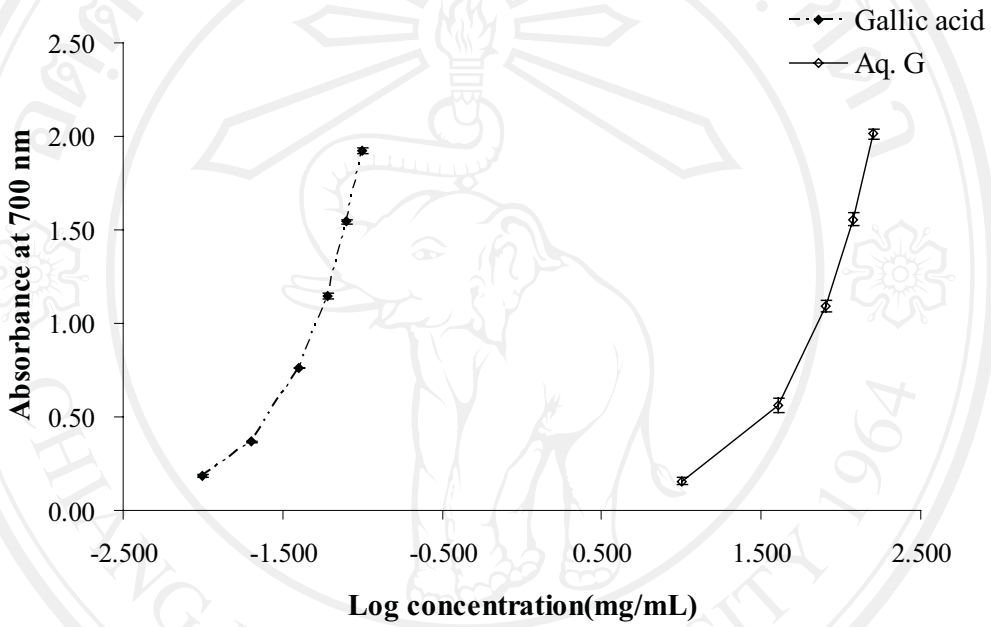

Figure 19 Concentration-response (absorbance, reducing power) curves of the Aq. *G* and gallic acid

3.2 Determination of phenolic contents in *G. fisheri*

Phenolic contents expressed as absorbance values determined at wave length of 765 nm. Figure 20 depicts the relationship of absorbance values (phenolic contents) and concentrations of gallic acid and the Aq. *G*. The amount of phenolics increased with higher concentrations. It was found that the GAE value of the Aq. *G* was 0.28 ± 0.01.

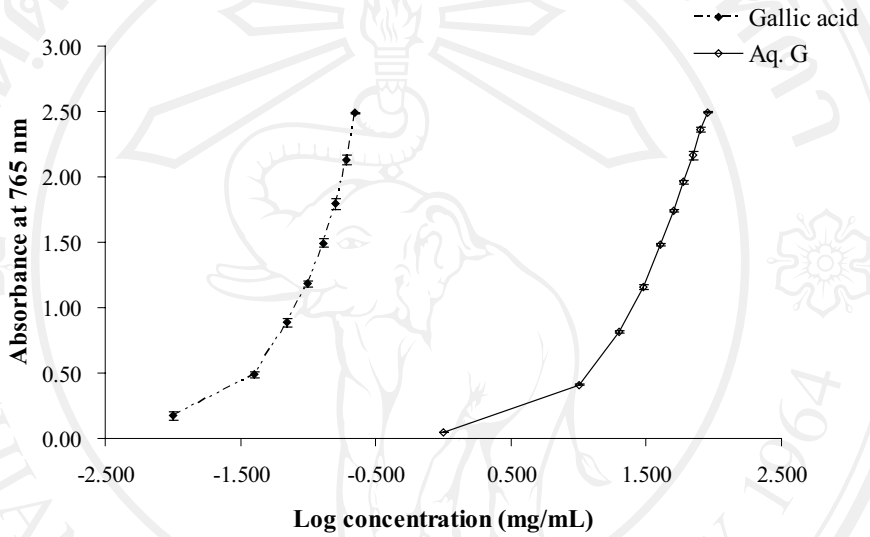

Figure 20 Concentration-response (absorbance at 765 nm, phenolic contents) curves of the Aq. *G* and gallic acid

EC_{50} of the Aq. *G* and standard antioxidants in various assays are summarized in Table 2. Efficacy of the Aq. *G* was highest (lowest EC50) in the lipid peroxidation assay. The order of efficacy is: lipid peroxidation < superoxide anion radical < hydroxyl radical < metal chelating < ABTS cation radical.

Table 2 EC_{50} values of the Aq. *G* and standard antioxidants in various assays

	EC_{50} (mg/mL)				
Sample	$ABTS^{+\bullet}$	$O_2^{-\bullet}$	$^{\bullet}OH$	LPO	MC
Aq. *G*	282.08±9.74	3.03±0.23	3.58±0.24	0.97±0.01	26.69±1.90
Trolox	0.003±0.0001	–	1.02±0.24	0.11±0.002	–
Gallic acid	–	0.23±0.01	–	–	–
EDTA	–	–	–	–	0.08±0.002

Data expressed as mean ± S.D. of triplicate measurements. LPO = lipid peroxidation, MC = metal chelating activity

Antioxidant capacity of the Aq. *G* expressed as GAE, TEAC and EDTAE values of the Aq. *G* obtained from various assays for antioxidant activity is shown in Table 3.

Table 3 GAE, TEAC and EDTAE values of the Aq. *G* obtained from various assays for antioxidant activity

Assays for antioxidant activity	GAE[a] or TEAC[b] or EDTAE[c] values of the Aq. *G*
$ABTS^{+\bullet}$ scavenging activity	0.04 ± 0.003[b]
$O_2^{-\bullet}$ scavenging activity	0.01 ± 0.001[a]
$^{\bullet}OH$ scavenging activity	1452.23 ± 393.70[b]
Anti-lipid peroxidation	464.10 ± 6.66[b]
Metal chelating activity	3047.29 ± 156.61[c]
Reducing power	1.48 ± 0.03[a]

Data expressed as mean ± S.D. of triplicate measurements

[a]GAE expressed as g of Aq. *G* per mg of gallic acid

[b]TEAC expressed as mM Trolox per g of Aq. *G*

[c]EDTAE expressed as μg EDTA per g of Aq. *G*

3.3 Cardiovascular effects of the Aq. *G*

3.3.1 Effect of the Aq. *G* on blood pressure and heart rate of thiopental anesthetized hypertensive rats

An intravenous administration of L-NAME at the dose 3 mg/kg caused hypertensive response. The hypertension reached the peak approximately 5 min after L-NAME administration and maintained at least 60 min. Typical blood pressure response to the L-NAME administration is depicts in Figure 21. Both systolic and diastolic blood pressure were elevated accompanying by reflex bradycardia. The Aq. *G* at the dose of 5-40 mg/kg were given 10 min after L-NAME administration. The results obtained are summarized in Table 4. Mean arterial blood pressure (MABP) before and after L-NAME induced hypertensive rats were 130-150 and 178-185 mmHg, respectively. The Aq. *G* showed a decrease blood pressure and heart rate in a dose-dependent manner. The Aq. *G* at the dose of 5-40 and 10-40 mg/kg caused a significant decrease blood pressure and heart rate in L-NAME induced hypertension experiment, respectively.

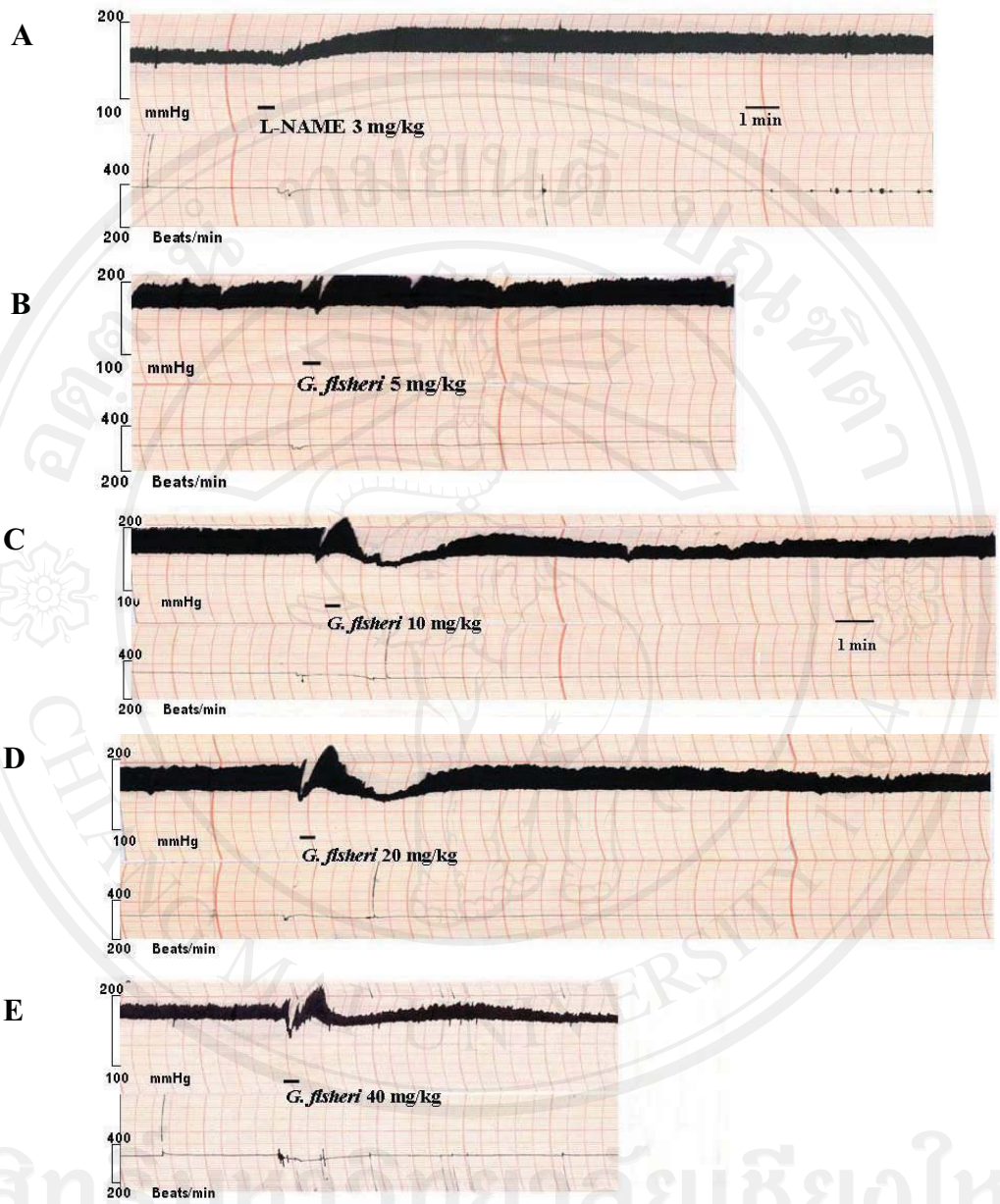

Figure 21 Effect of the Aq. *G* on mean arterial blood pressure (MABP) and heart rate of L-NAME-induced hypertension in rats under thiopental anesthesia. The Aq. *G* at the dose of 5-40 mg/kg were given 10 min after L-NAME administration. A: L-NAME administration (3 mg/kg, i.v injection); B, C, D, and E: The Aq. *G* at the doses of 5 (B), 10 (C), 20 (D) and 40 (E) mg/kg

Table 4 Effect of the Aq. *G* on MABP and heart rate of L-NAME-induced hypertensive in rats under thiopental anesthesia

Dose	MABP after L-NAME induced hypertension (mmHg)			Heart rate (beats/min)		
(mg/kg)	Control	Experiment	% Decrease	Control	Experiment	% Decrease
5	180.3 ± 7.0	159.0 ± 11.1*	12.18 ± 3.32	336.0 ± 15.7	318.0 ± 9.2	5.08 ± 1.69
10	184.7 ± 9.2	146.0 ± 12.6*	21.47 ± 2.72	334.0 ± 6.8	311.7 ± 4.9*	10.33 ± 2.18
20	178.1 ± 5.3	134.4 ± 7.1*	24.69 ± 2.35	311.7 ± 19.9	270.0 ± 18.3*	13.46 ± 1.45
40	181.3 ± 7.0	82.3 ± 23.1*	55.09 ± 12.03	320.0 ± 9.5	242.0 ± 28.7*	23.22 ± 10.60

Data expressed as mean ± S.E.M. n = 6

Significantly different from control group: *$p < 0.05$

MABP before L-NAME administration was 130-150 mmHg

3.3.2 Effects of the Aq. *G* on force and rate of contraction of the isolated rat atria

The Aq. *G* at the concentrations of 1, 2, and 4 mg/mL caused a significant decrease in both force and heart rate of atrial contraction (Figure 22). The results obtained are summarized in Table 5. The effects of the Aq. *G* on the force and rate of atrial contractions are concentration-dependent. The high concentration of 4 mg/mL caused percent decrease of force and rate of contraction of 91.71% and 54.26%, respectively.

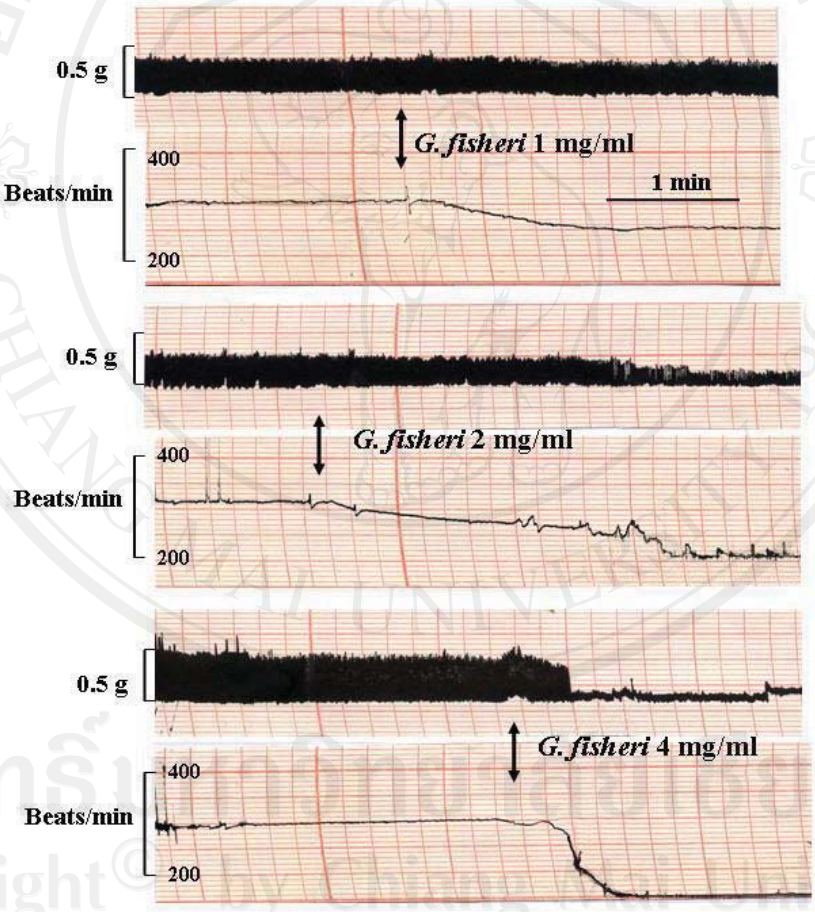

Figure 22 Effect of the Aq. *G* on force and rate in isolated rat atria

Table 5 Effect of the Aq. *G* on force and rate of contraction of the isolated rat atria

Doses (mg/mL)	Force of contraction (g)			Rate of contraction (beats/min)		
	Control	Experiment	% Decrease	Control	Experiment	% Decrease
1	0.33 ± 0.26	0.29 ± 0.03*	12.69 ± 3.41	311.0 ± 5.1	267.0 ± 7.7*	14.04 ± 2.99
2	0.32 ± 0.03	0.18 ± 0.03*	45.54 ± 4.99	317.5 ± 5.7	200.0 ± 7.2*	36.89 ± 2.69
4	0.38 ± 0.05	0.03 ± 0.01*	91.71 ± 1.91	324.2 ± 11.7	147.5 ± 9.8*	54.26 ± 3.42

Data expressed as mean ± S.E.M. n = 6

Significantly different from control: *$p < 0.05$

3.3.3 Effect of the Aq. *G* on the isolated rat aorta

The Aq. *G* exhibited an inhibitory effect on the contractions of the aorta induced by phenylephrine (10 μM) as well as by high K^+ (80 mM).

The relaxant effect (causing a decrease of contraction) of the Aq. *G* on phenylephrine (PE 10 μM) induced contractions of the aorta with endothelium intact and denuded is shown in Figure 23. The relaxant effect of the Aq. *G* increased with the increasing concentrations (Table 6). The effect was marked in the intact aorta. At the high concentration (20 mg/mL), % relaxation of the aortae with intact and denude endothelium were 79.23 and 11.40, respectively.

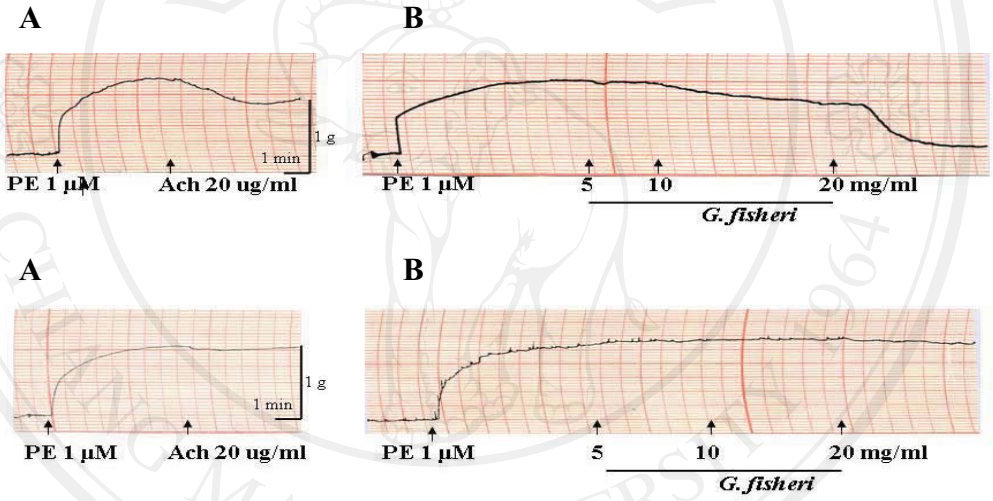

Figure 23 Effect of acetylcholine (ACh, 20 μg/mL) and the Aq. *G* (cumulative administration) on phenylephrine (PE, 10 μM) induced contraction isolated rat aorta with endothelium intact and endothelium denuded

Upper Panel : endothelium intact aorta

A: Effect of ACh

B: Effect of the Aq. *G* at the concentrations of 5, 10 and 20 mg/mL

Lower Panel: endothelium denuded aorta

A: Effect of ACh

B: Effect of the Aq. *G* at the concentrations of 5, 10 and 20 mg/mL

Table 6 Effect of the Aq. *G* on phenylephrine (10 μM) induced contraction isolated rat aorta with endothelium intact and endothelium denuded

Doses (mg/mL)	% Relaxation	
	Endothelium intact	Endothelium denude
5	1.00 ± 1.00	0.00 ± 0.00
10	32.97 ± 1.35*	2.45 ± 1.58
20	79.23 ± 12.13*	11.40 ± 3.58

Data expressed as mean ± S.E.M. n = 6

Significantly different from endothelium denude group: *$p < 0.05$

Figure 24 and Table 7 illustrate the relaxant effect of the Aq. *G* (at the concentrations of 5, 10 and 20 mg/mL) on the high K^+ (80 mM) induced contractions of the aorta with endothelium intact. The relaxant effect of the Aq. *G*. increased with the increasing concentrations. However, significant effect was found only with the concentration of 20 mg/mL.

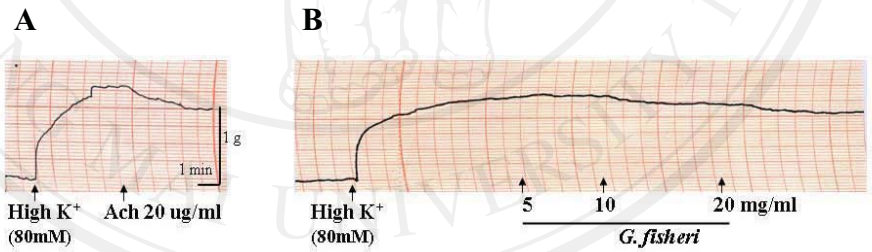

Figure 24 Effect of acetylcholine (ACh, 20 μg/mL) (A) and the Aq. *G* (cumulative administration) at the concentrations of 5, 10 and 20 mg/mL on high K^+ (80 mM) induced contraction isolated rat aorta with endothelium intact (B)

Table 7 Effect of the Aq. *G* on high K^+ induced contraction isolated rat aorta with endothelium intact

Doses (mg/mL)	% Relaxation
5	4.29 ± 3.22
10	16.90 ± 6.78
20	32.52 ± 8.26*

Data expressed as mean ± S.E.M. n = 6. *$p < 0.05$

The relaxant activity of the Aq. *G* was greater when tested against phenylephrine than against high K^+ induced contraction of the endothelium intact aorta. As illustrated in Table 8, at the concentration of 20 mg/mL of the Aq. *G*, % relaxation of the phenylephrine induced contraction of the aorta was 79.23 ± 12.13 whereas that of high K^+ induced contraction was 32.52 ± 8.26.

Table 8 Effect of the Aq. *G* on phenylephrine (10 μM) and high K^+ (80 mM) induced contraction isolated rat aorta with endothelium intact

Doses (mg/mL)	% Relaxation	
	Phenylephrine (10 μM)	High K^+ (80 mM)
5	1.00 ± 1.00	4.29 ± 3.22
10	32.97 ± 1.35*	16.90 ± 6.78
20	79.23 ± 12.13*	32.52 ± 8.26*

Data expressed as mean ± S.E.M. n = 6. * p <0.05