Several antioxidant vitamins have shown protective role against doxorubicin cardiotoxicity. Vitamin E decreased cardiac lipid peroxidation and delayed the lethality of a single dose of doxorubicin at 24 hr (Mimnaugh et al., 1979). Daily vitamin E administration 4 days prior to a single i.v. dose of doxorubicin injection prevented cardiac damage and improved survival of rabbits, which was related to cardiac glutathione oxidation (Wang et al., 1980). However, other studies suggest that co-administration of vitamin E during chronic DOX treatment improved survival of animals, but failed to protect animals from cardiotoxicity (Breed et al., 1980; Shinozawa et al., 1988). Although vitamin C also prevented cardiac lipid peroxidation and structural damage in mice and guinea pigs treated with one or multiple doses of DOX, its effect on the prolongation of survival time of the animals is controversial. (Fujita et al., 1982; Shimpo K et al., 1991). Another standard antioxidant trolox, a water soluble analogue of α-tocopherol, has also shown cytoprotective role in various settings such as cisplatin-induced ototoxicity (Teranishi and Nakashima, 2003) and oxidative damage from ischemia/reperfusion (Molyneux et al., 2002; Sagach et al., 2002). In this study, the greatest benefits form vitamin C and NAC were observed at 100 μM while trolox provided insignificant cytoprotective effect. The conflicting results may occur because of the differences in models of study, oxidative insults, and time course of measurements. Additionally, target specificity of the oxidative damage may different among individual radicals generated. Therefore, there are opportunities for therapeutic implication of new antioxidants for the prevention of DOX cardiotoxicity.

All plant extracts increased IC50 except CL-EtOH since the extract produced highest toxicity compared to others. PE showed the most protective effect on DOX cardiotoxicity at the concentration of 100 μM (~12-fold IC50 increase). The potential antioxidant constituents of PE include ascorbic acid, polyphenols, flovonoids, and tannoids (Bhattacharya et al., 2000a). Recent studies demonstrate antioxidantive effects of PE in several model of oxidative stress including lipid peroxidation (Kumar et al., 1999) cyclophophamide toxicity (Haque et al., 2001), diabetes (Sabu and Kuttan, 2002), hepatotoxicity (Bhattacharya et al., 2000b). Despite the high vitamin C content in PE, it does not completely account for the cytoprotective effects since standard vitamin C at non-toxic concentrations showed far less effectiveness.

Although antioxidant capacity may predict the cytoprotective effectiveness against DOX cardiotoxicity, the correlation analysis of antioxidant capacity and effectiveness in cardioprotection (fold increases in DOX IC50) is insignificant. This is probably due to the small

number of tested compounds. In addition to the cytoprotective effect investigated in this study, further tests for selectivity (tumor cells remain vulnerable while normal cells are protected), broad-spectrum activity (protect various tissues from toxicity), and a favorable side effect profile should be addressed according to the concept of ideal cytoprotective agent (Griggs, 1998).

In summary, a large body of evidence suggests that ROS play a major role in DOX-induced cardiotoxicity. Despite standard antioxidant vitamins show some benefits in animal model of DOX cardiotoxicity their cardioprotective effect still controversial. Our study provides a basic pharmacological screening for alternative antioxidants from Thai medicinal plants. Further study should suggest the molecular mechanisms by which the extracts exert their cardioprotective role since recent studies have identified several oxidative pathways involved in the cellular oxidative damage at the molecular levels. In addition, other mechanisms of DOX cardiotoxicity have been proposed, including intracellular Ca^{2+} overload, the toxicity of DOX metabolites, and the direct interaction with the actin-myosin contractile system (De Beer et al. 2001). Therefore, a better understanding of causes of DOX-induced cardiotoxicity and protective-adaptive responses could lead to new target interventions to protect cardiomyocyte toxicity.

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TABLES

Table 1. Total antioxidant capacity of the plant extracts evaluated by ferric reducing/antioxidant power (FRAP) assay

Plant Extracts (1 mg/mL)	Total Antioxidant Capacity (μM vitamin C equivalence)*
Curcuma longa L.(CL-EtOH)	712.54 ± 45.00
Curcuma longa L.(CL- H_2O)	1541.32 ± 41.26
Morus alba L. (MA)	2273.99 ± 120.22
Phyllanthus emblica L.(PE)	1923.70 ± 112.03
Piper rostratum Roxb. (PR).	281.93 ± 2.92

*Data is presented as Mean±SEM

Table 2. Cardioprotective effect of standard antioxidants (vitamin C, Trolox, and N-acetylcysteine)

ANTIOXIDANT	IC50 (μM) of DOX (In the present of antioxidant at different concentrations)		
	Antioxidant 10 μM	Antioxidant 100 μM	Antioxidant 1000 μM
CONTROL IC50 = 0.177 ± 0.014 μM			
VITAMIN C	0.127 ± 0.022	0.569 ± 0.034**	0.214 ± 0.027
TROLOX	0.204 ± 0.006	0.187 ± 0.010	0.134 ± 0.005
N-acetylcysteine	0.309 ± 0.029	0.711 ± 0.027**	0.281 ± 0.008**

*Data is presented as Mean±SEM. ** Statistically significant difference from CONTROL ($p < 0.05$)

Table 3. Cardioprotective effect of plant extracts.

PLANT EXTRACT	IC50 (μM) of DOX (In the presence of plant extract at different concentrations)*		
	Extract 1 μg/mL	Extract 10 μg/mL	Extract 100 μg/mL
CONTROL IC50 = 0.177 ± 0.014 μM			
Curcuma longa L. (CL-EtOH)	0.160 ± 0.013	0.259 ± 0.010	-
Curcuma longa L. (CL- H_2O)	-	0.352 ± 0.003**	1.360 ± 0.455**
Morus alba L. (MA)	-	0.368 ± 0.044**	1.08 ± 0.354**
Phyllanthus emblica L. (PE)	0.331 ± 0.002**	0.635 ± 0.112**	2.190 ± 0.323**
Piper rostratum Roxb. (PR).	0.281 ± 0.007**	0.420 ± 0.054**	0.494 ± 0.037**

*Data is presented as Mean±SEM. ** Statistically significant difference from CONTROL ($p < 0.05$)

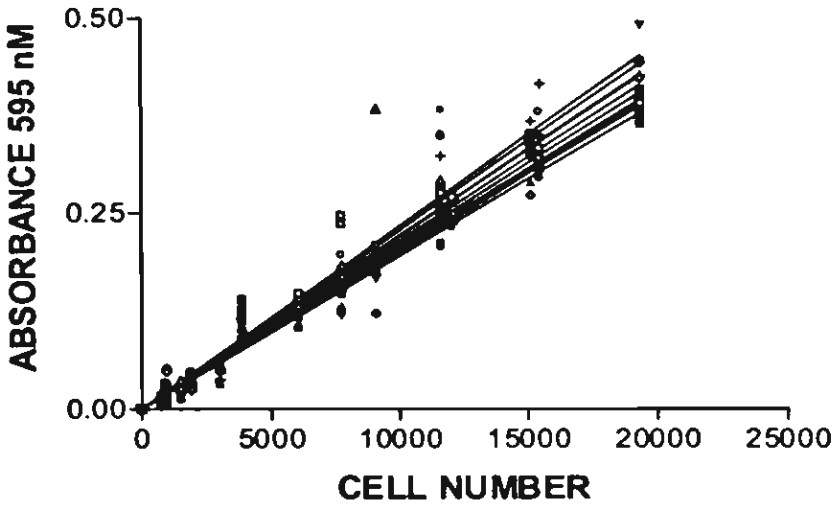


Figure 2. H9c2 cell standard curve using crystal violet method. The graph represents 12 set of data points.

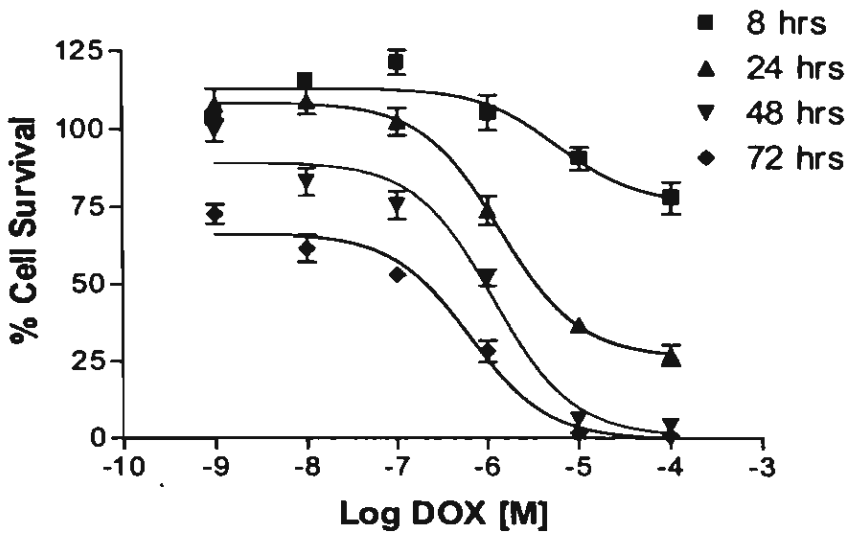


Figure 3. Time-dependent DOX-induced H9c2 cytotoxicity.

FIGURES

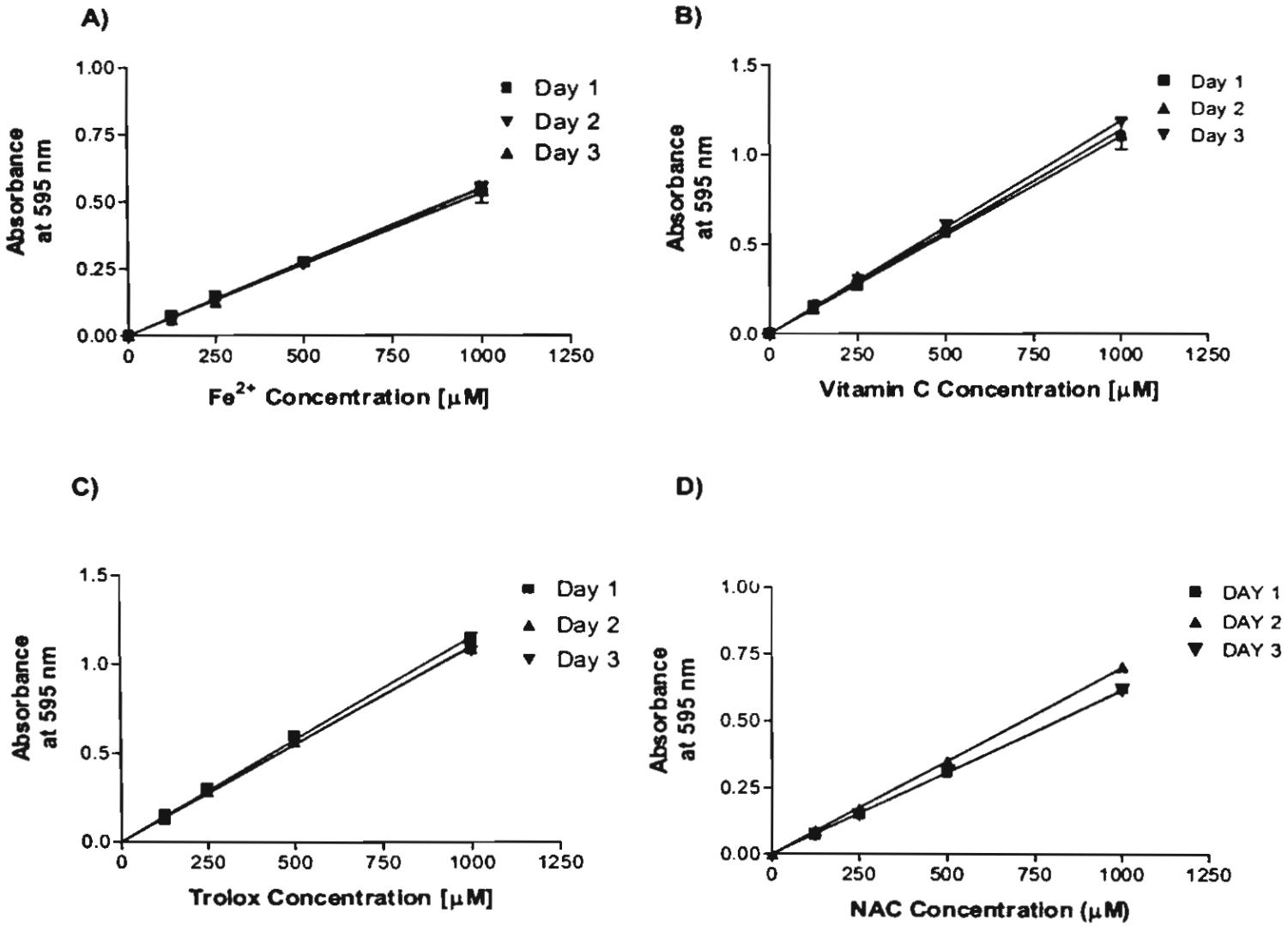
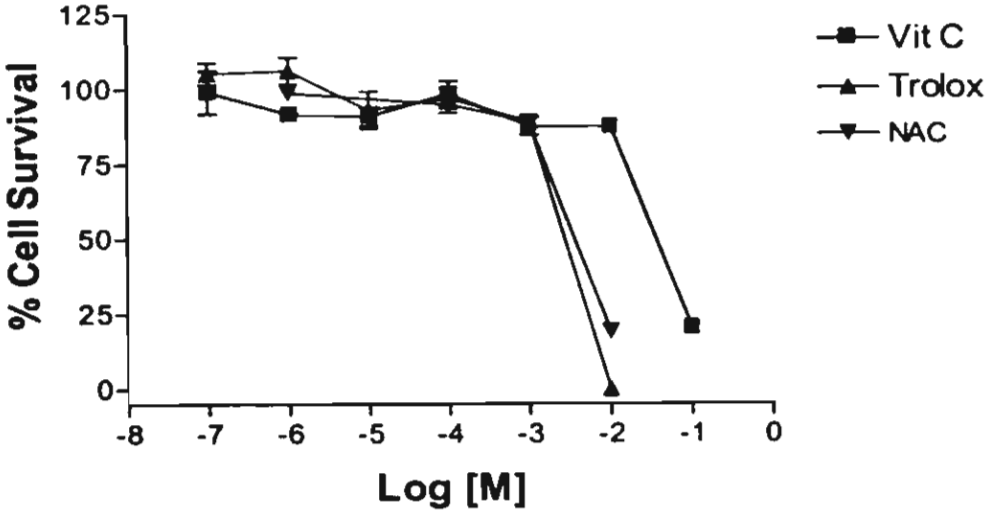


Figure 1. Inter-day variability of FRAP assay. A) Fe^{2+} standard curves; B) Vitamin C standard curves; C) Trolox standard curves; and D) NAC standard curves.

A)



B)

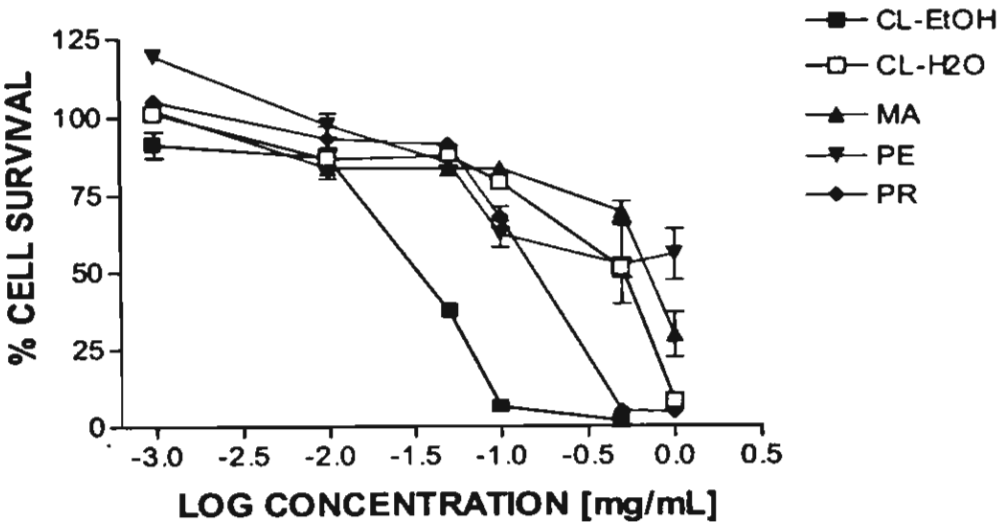


Figure 4. Effects of standard antioxidants and plants extracts on the viability of H9c2. The toxicity of each compound was tested with **A)** standard antioxidants vitamin C (VIT C), Trolox, and N-acytlycysteine (NAC) or **B)** the ethanolic plant extracts *Curcuma longa* L.(CL-EtOH), *Phyllanthus emblica* L.(PE), *Piper rostratum* Roxb. (PR), and the water extracts of *Curcuma longa* L. (CL-H_2O) and *Morus alba* L. (MA).

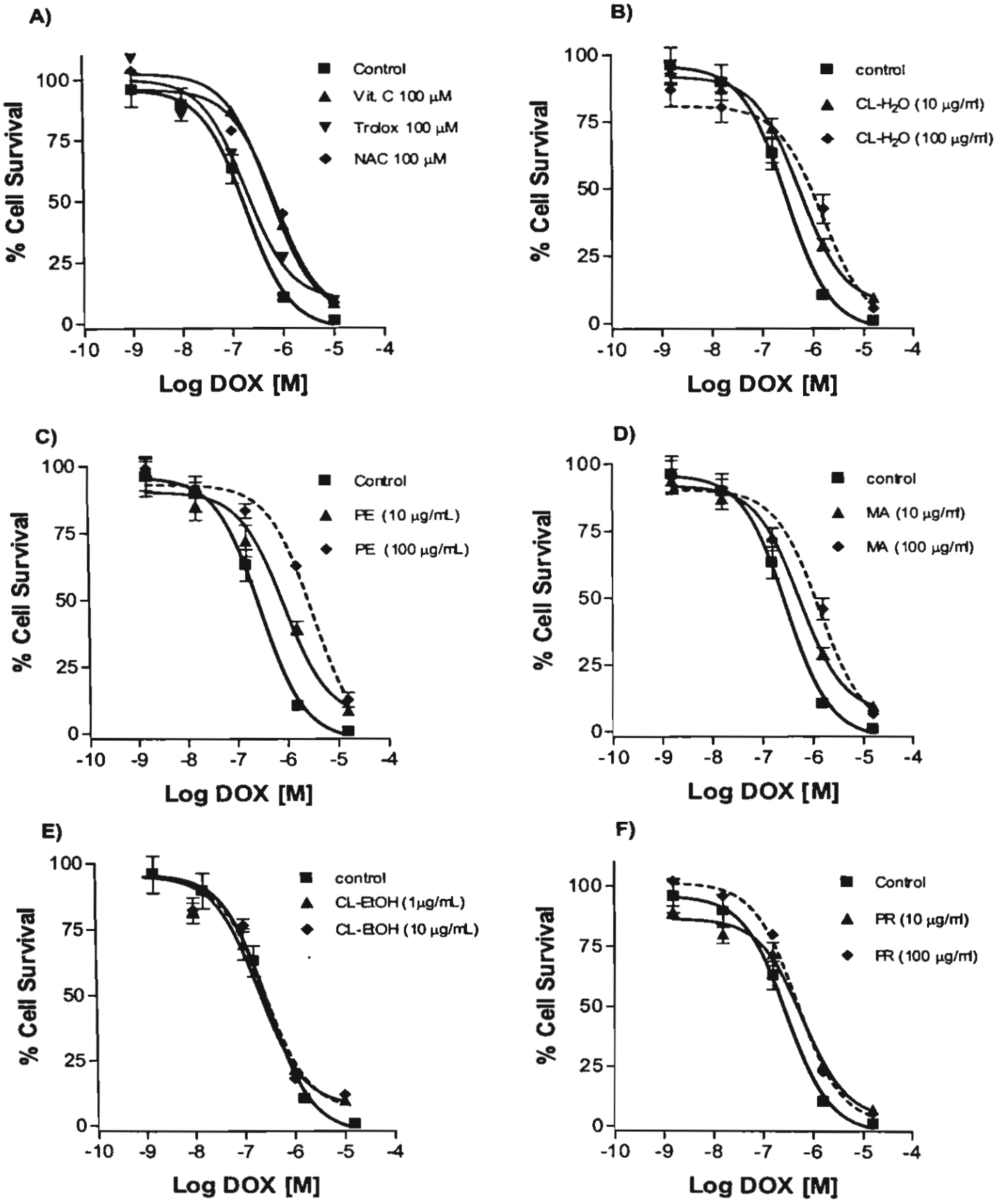
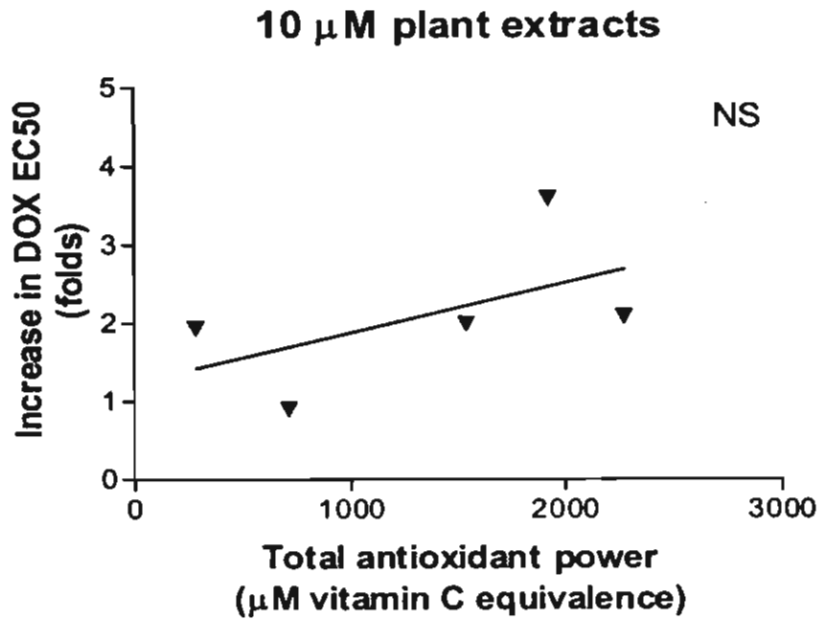

A)
% Cell Survival
Log DOX [M]
Control
Vit. C 100 µM
Trolox 100 µM
NAC 100 µM
B)
% Cell Survival
Log DOX [M]
control
CL-H2O (10 µg/ml)
CL-H2O (100 µg/ml)
C)
% Cell Survival
Log DOX [M]
Control
PE (10 µg/mL)
PE (100 µg/mL)
D)
% Cell Survival
Log DOX [M]
control
MA (10 µg/ml)
MA (100 µg/ml)
E)
% Cell Survival
Log DOX [M]
control
CL-EtOH (1µg/mL)
CL-EtOH (10 µg/mL)
F)
% Cell Survival
Log DOX [M]
Control
PR (10 µg/ml)
PR (100 µg/ml)

Figure 5. Cytoprotective effects of standard antioxidants and plants extracts on DOX-induced cardiotoxicity (H9c2). H9c2 cells were co-incubated with; standard antioxidants **(A)** including vitamin C (VIT C), Trolox, and N-acytlycysteine (NAC); or the ethanolic plant extracts from *Curcuma longa* L.(CL-EtOH) **(B)**, *Phyllanthus emblica* L.(PE) **(C)**, *Piper rostratum* Roxb. (PR) **(D)**, and the water extracts of *Curcuma longa* L. (CL-H_2O) **(E)** and *Morus alba* L. (MA) **(F)**.

A)



B)

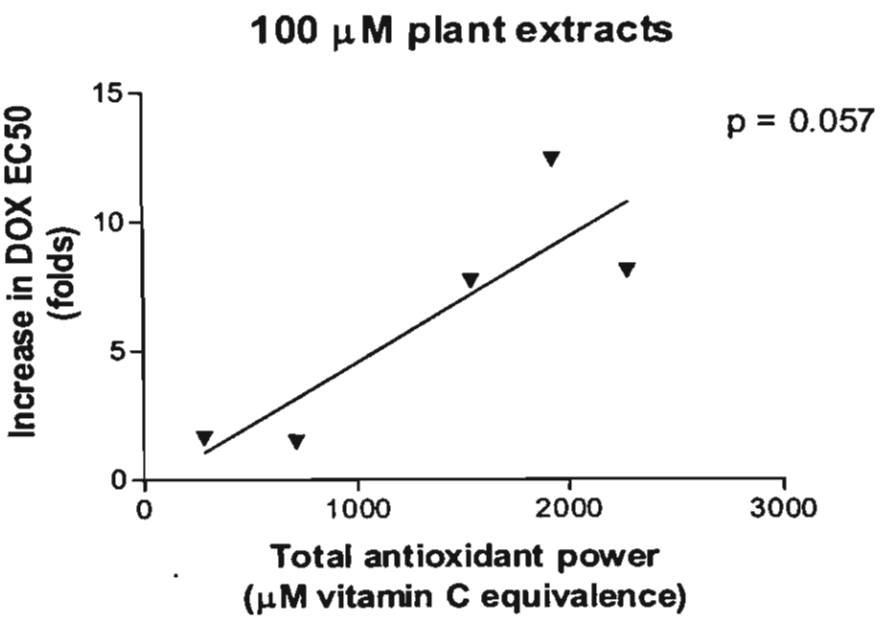


Figure 6. Correlation analysis of total antioxidant power and fold increases in DOX IC50. Tow concentrations of plants extracts were compared; **A)** 10 μg/mL, and **B)** 100 μg/mL. The solid line shown is fitted to the presented data using linear regression. NS means the slope of the regression line is not significantly different from zero.

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

ขณะนี้กำลังทำการทดลองเพื่อเก็บข้อมูลเพิ่มเติม เพื่อให้มีศักยภาพในการได้รับการตอบรับการตีพิมพ์ในวารสารวิชาการนานาชาติที่มี impact factor สูง คาดว่าจะสามารถส่ง manuscript ได้ภายในเดือนธันวาคม 2546 ถ้าได้รับการตอบรับแล้วจะส่งสำเนาให้กับทาง สกว. ต่อไป

2. การนำผลงานวิจัยไปใช้ประโยชน์

- เชิงพาณิชย์ : สมุนไพรตัวที่น่าสนใจที่สุดจากการศึกษานี้คือ มะขามป้อม ซึ่งต้องมีการศึกษาทางคลินิกต่อไปเพื่อเป็นหลักฐานสนับสนุนการใช้เป็นยาต่อไปในอนาคต
- เชิงวิชาการ : มีความพร้อมของห้องปฏิบัติการสำหรับการตรวจฤทธิ์แอนติออกซิแดนซ์ของสารสกัดสมุนไพรที่ได้ผลสัมฤทธิ์สูง และใช้สำหรับการเรียนการสอนในระดับบัณฑิตศึกษา สามารถขยายขอบเขตการศึกษาไปสู่การทำปริญญานิพนธ์ได้