CHAPTER III

RESULTS AND DISCUSSION

1. Determination of Total Polyphenolic Contents in Fruit Extracts
2. Determination of Free Radical Scavenging Activity Using DPPH Method
3. Determination of Superoxide Anion Scavenging Activity of Fruit Extracts
4. Determination of Hydroxyl Radical Scavenging Activity of Fruit Extracts
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1. Determination of Total Polyphenolic Contents in Fruit Extracts

By using gallic acid as a standard in Folin-Ciocalteu method, the data showed that the amount of total polyphenolic contents varied greatly from 3.243 ± 0.303 to 2120.526 ± 141.244 mg GAE/g wet weight (Table 5). The standard curve of gallic acid was illustrated in Fig. 16. Low levels (< 30 mg GAE/g wet weight) were found in watermelon, rose apple, fragrant banana, grape pulp, lemon juice, mango (Rad), papaya, rambutan, pineapple, banana (Kai), jackfruit, durian, orange juice, mango (Kiow-Sa-Wooei), pomelo, and sa-la. Moderate levels (30-80 mg GAE/g wet weight) were found in mangosteen pulp, longan, mango (Nam-Dok-Mai), ma-fai, zalacca, indian mulberry, apple, litchi, guava, longkong pulp, orange, carambola, custard apple, jujube, santol (white pulp), plum mango, and grape skin (White Malaca). High levels (> 80 mg GAE/g wet weight) were found in grape skin (White Spain), banana (Nam-Var), longkong seed, grape skin (Pok-Dum), santol (brown pulp), and mangosteen hull.
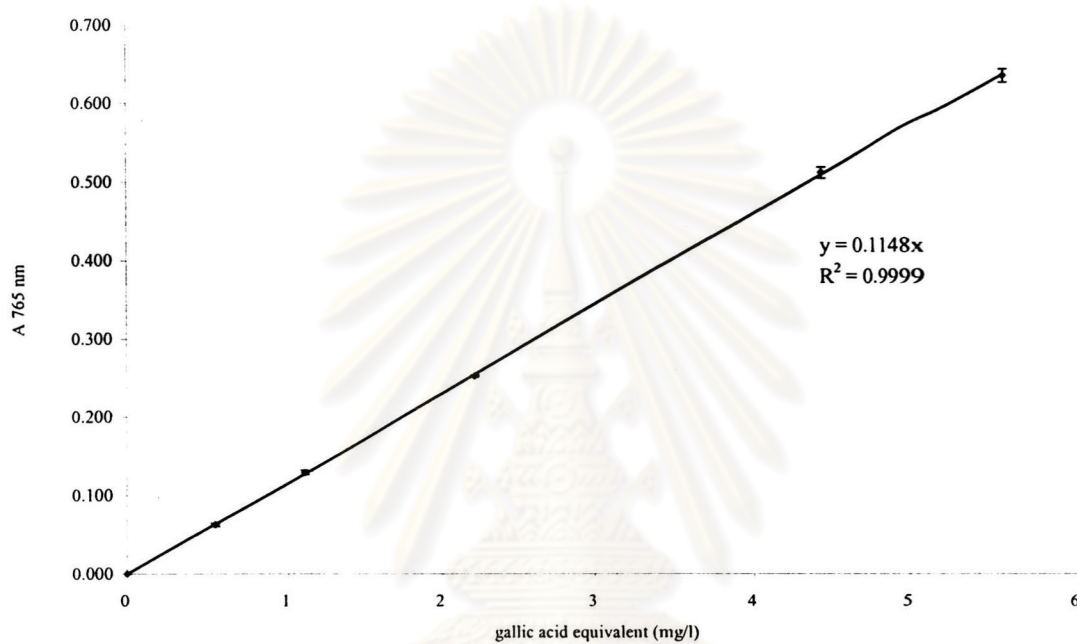
Fig. 16 Standard curve of gallic acid

Table 5. Total polyphenolic contents in fruit extracts

Sample	Total GAE (mg/g wet weight)
Watermelon	3.243 ± 0.303
Rose apple *	6.009 ± 0.208
Fragrant banana	8.773 ± 0.784
Grape pulp (Pok-Dum)	8.876 ± 0.291
Grape pulp (White Malaca)	9.700 ± 0.000
Grape pulp (White Spain)	11.984 ± 0.000
Lemon juice	12.281 ± 0.230
Mango (Rad)	14.100 ± 0.455
Papaya	14.128 ± 0.426
Rambutan *	14.310 ± 0.378
Pineapple (See-Ra-Cha)	14.778 ± 1.923
Banana (Kai)	16.276 ± 0.493
Jackfruit	16.559 ± 0.518
Durian *	18.059 ± 0.617
Pineapple (Poo-Lae)	19.864 ± 0.550
Orange juice	20.023 ± 0.828
Mango (Kiow-Sa-Wooei)	21.683 ± 0.175
Pomelo	28.384 ± 1.931
Sa-la	29.480 ± 2.484
Mangosteen pulp	35.807 ± 1.132
Longan	36.846 ± 1.154
Mango (Nam-Dok-Mai, ripe)	38.472 ± 0.550
Ma-fai	40.109 ± 0.832
Zalacca	40.193 ± 1.377
Indian mulberry	40.879 ± 0.806
Mango (Nam-Dok-Mai, unripe)	42.804 ± 0.686
Apple	43.423 ± 2.325
Litchi	44.225 ± 0.292
Guava *	45.036 ± 0.955
Longkong pulp	47.502 ± 2.763
Orange	50.016 ± 0.304
Carambola	54.274 ± 1.417
Custard apple	54.304 ± 0.828
Jujube	57.209 ± 0.241
Santol (white pulp)	58.043 ± 1.174
Plum mango *	60.878 ± 0.149
Grape skin (White Malaca)	62.780 ± 2.314
Grape skin (White Spain)	94.935 ± 1.447
Banana (Nam-Var) *	94.961 ± 0.116
Longkong seed *	109.221 ± 3.791
Grape skin (Pok-Dum)	236.364 ± 16.578
Santol (brown pulp)	255.549 ± 2.531
Mangosteen hull	2120.526 ± 141.244

   * evaporated fruit extract.

In the present work, the non-edible part of some fruits, especially grape skin and mangosteen hull contained much higher amount of polyphenolic than of grape pulp and mangosteen pulp. Small differences of total polyphenolic levels were found in different varieties of mangoes, Nam-Dok-Mai, Kiow-Sa-Wooei, and Rad. The total polyphenolic content in different varieties of banana ranged from 8.773 ± 0.784 to 94.961 ± 0.116 mg GAE/g wet weight. Total polyphenolic content in white grape was lower than of red grape.

Total phenolic contents of 11 cultivars of fresh plum varied widely from 125.0 to 372.6 mg / 100 g expressed as gallic acid equivalents (Kim *et al.*, 2003).

The total phenolic content of 28 plant products, including sunflower seeds, flaxseeds, wheat germ, buckwheat, several fruits, vegetables, and medicinal plants varied from 169-10548 mg/ 100 g of dry weight assayed by Folin-Ciocalteu reagent using frerulic acid as standard (Velioglu *et al.*, 1998).

Total polyphenols, using the Folin-Ciocalteu method, of several kinds of apple (*Malus spp.*) were tested by Sanoner and colleagues (Sanoner *et al.*, 1999).

The antioxidant activity of phenolic contents that presented in berries was investigated. The amount of total phenolics varied between 617 and 4350 mg/kg in fresh berries, as gallic acid equivalents (GAE) (Heinonen *et al.*, 1998).

Total phenols and antioxidant activity were measured in fruits, using the Folin-Ciocalteu reagent and inhibition of low-density lipoprotein oxidation promoted by cupric ion, respectively. Cranberry had the highest total phenols, and was distantly followed by red grape (Vinson *et al.*, 2001).

Several researches reported that fruits have much higher polyphenolic contents in peel than their pulps. For example, Banana (*Musa cavendish*) peel has antioxidant activity more than banana pulp and it has much gallocatechin (Someya *et al.*, 2002). The peel portion of guava (*Psidium guajava* L.) contained higher amounts of polyphenols than its pulp (Jimenez-Escrig *et al.*, 2001). Peels of apple and pear have a significantly higher positive influence on plasma lipid levels and on plasma antioxidant capacity of rats than apple and pear pulps (Leontowicz *et al.*, 2003).

2. Determination of Free Radical Scavenging Activity Using DPPH Method

DPPH is a free radical that has been widely used to measure the free radical scavenging activity of various plant extracts (Du *et al.*, 2001; Okawa *et al.*, 2001). This method is based on the reduction of DPPH, stable free radical. The odd electron of DPPH shows a strong absorption maximum at 517 nm and its solution is deep purple color. As the odd electron of the radical becomes paired off, the absorption strength is decreased. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The mechanism of the DPPH radical and various oxidizable groups were shown in Fig. 17 (Blois, 1958).

(a) $(DPPH)^{\cdot} + R - SH \longrightarrow (DPPH){:}H + R - S^{\cdot}$

$R - S^{\cdot} + R - S^{\cdot} \longrightarrow R - S - S - R$

(b) $(DPPH)^{\cdot} + R - \overset{\overset{\displaystyle OH}{|}}{C} = \overset{\overset{\displaystyle OH}{|}}{C} - R \longrightarrow (DPPH){:}H + R - \overset{\overset{\displaystyle O^{\cdot}}{|}}{C} = \overset{\overset{\displaystyle OH}{|}}{C} - R'$

$(DPPH)^{\cdot} + R - \overset{\overset{\displaystyle O^{\cdot}}{|}}{C} = \overset{\overset{\displaystyle OH}{\|}}{C} - R' \longrightarrow (DPPH){:}H + R - \overset{\overset{\displaystyle O}{\|}}{C} = \overset{\overset{\displaystyle O}{\|}}{C} - R'$

(c) $(DPPH)^{\cdot} + HO - R - OH \longrightarrow (DPPH){:}H + HO - R - O^{\cdot}$

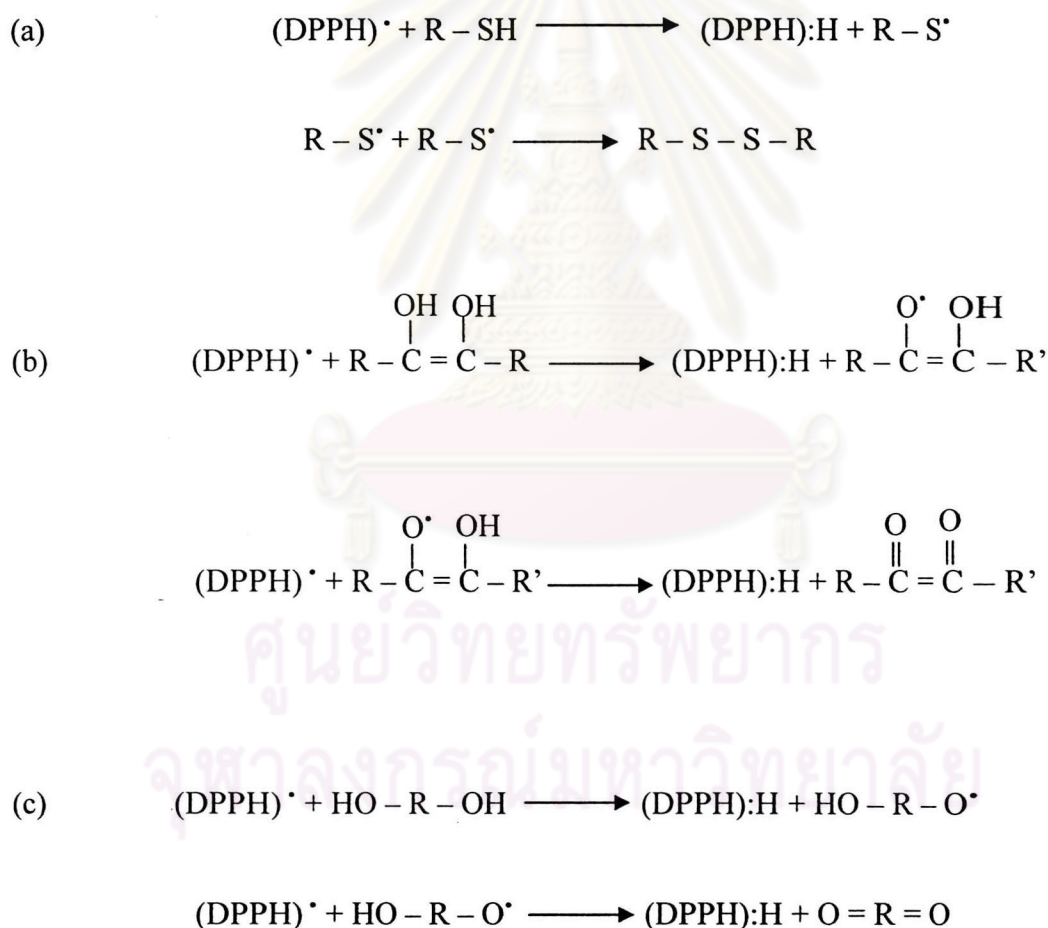$(DPPH)^{\cdot} + HO - R - O^{\cdot} \longrightarrow (DPPH){:}H + O = R = O$

Fig. 17 Schema of the reactions between the DPPH radical and various oxidizable groups. (a) Sulphydryl groups interacting in the ratio of (1:1); (b) oxidation of the conjugated group of ascorbic acid to the dehydro form (2:1); (c) oxidation of a hydroquinone (2:1)

The data demonstrated that the ability of fruit extracts to scavenge the DPPH radicals measured as IC_{50} varied significantly from 0.149 to 513.974 mg/ml. Gallic acid was used in all parallel experiments and a logarithmic curve was plotted to calculate the value of IC_{50} (Fig. 18). The results were shown in Table 6. IC_{50} value was calculated by a logarithmic regression curve. The highest DPPH radical scavenging properties ($IC_{50} < 20$ mg/ml) were found in mangosteen hull, grape skin, santol, banana (Nam-Var), plum mango, mango, longkong seed, carambola, guava, ma-fai, litchi, apple, jujube, zalacca, sa-la, mangosteen pulp, longan, indian mulberry, custard apple, orange, and pomelo. Durian, longkong pulp, grape pulp, banana (Kai), papaya, rambutan, orange juice, pineapple, fragrant banana, rose apple, and lemon juice showed moderate DPPH radical scavenging activities (IC_{50} from 20 - 100 mg/ml). Jackfruit and watermelon showed the lowest DPPH radical scavenging activities ($IC_{50} > 100$ mg/ml). The non-edible part of some fruits, especially grape skin, mangosteen hull, and longkong seed had much DPPH scavenging activity than their pulps. Little difference of DPPH scavenging activity was found in different varieties of mangoes. The ability of different varieties of banana to scavenge the DPPH radicals widely varied from 2.084 - 71.996 mg/ml.

$$y = 23.866Ln(x) + 188.98$$
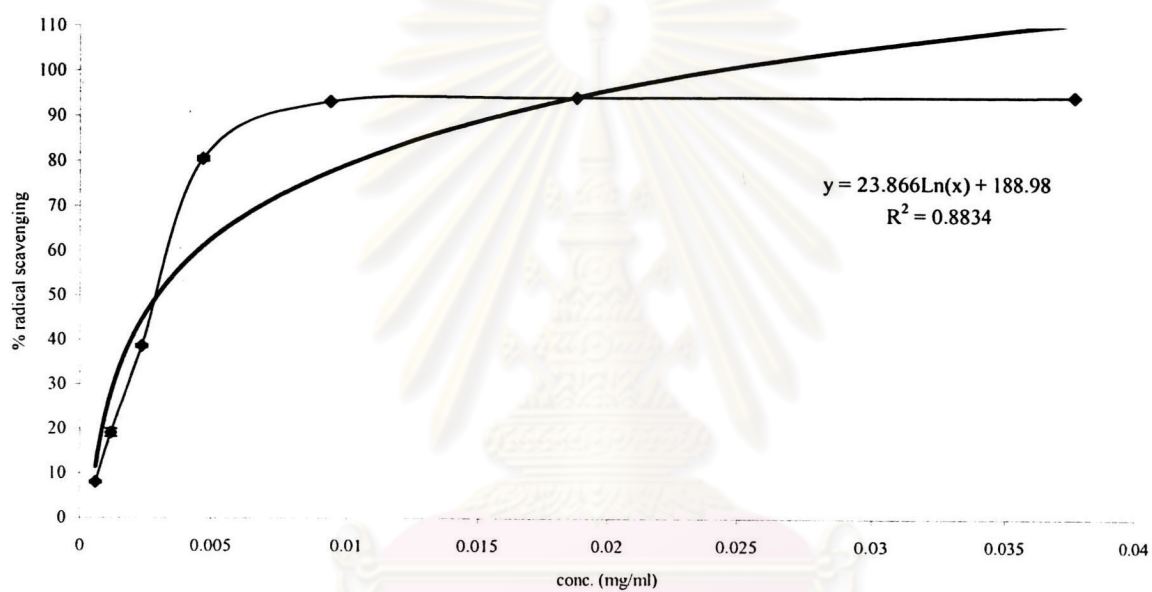$$R^2 = 0.8834$$

Fig. 18 A logarithmic regression curve of gallic acid

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Table 6. IC$_{50}$ values of DPPH radical scavenging activities in fruit extracts

sample	DPPH scavenging activity IC$_{50}$ (mg/ml)
Mangosteen hull	0.149
Grape skin (Pok-Dum)	1.104
Santol (brown pulp)	1.155
Banana (Nam-Var) *	2.084
Grape skin (White Spain)	2.838
Plum mango *	3.517
Mango (Nam-Dok-Mai, unripe)	4.184
Grape skin (White Malaca)	4.214
Longkong seed *	4.462
Mango (Nam-Dok-Mai, ripe)	5.642
Santol (white pulp)	6.229
Carambola	6.726
Guava *	7.764
Ma-fai	8.375
Litchi	8.788
Apple	9.557
Jujube	9.709
Zalacca	10.292
Sa-la	10.765
Mangosteen pulp	11.300
Longan	11.328
Mango (Kiow-Sa-Wooei)	12.853
Indian mulberry	13.190
Custard apple	14.609
Orange	17.754
Pomelo	19.409
Mango (Rad)	19.412
Durian *	25.709
Longkong pulp	27.824
Grape pulp (White Spain)	28.133
Banana (Kai)	30.047
Papaya	31.621
Rambutan *	32.730
Grape pulp (White Malaca)	32.800
Orange juice	46.322
Pineapple (See-Ra-Cha)	47.123
Grape pulp (Pok-Dum)	53.422
Pineapple (Poo-Lae)	61.769
Fragrant banana	71.996
Rose apple *	76.718
Lemon juice	95.976
Jackfruit	280.328
Watermelon	513.974

* evaporated fruit extract.

Edible portion of fruits, especially strawberry and grape juice had the highest antioxidant activity, using automated oxygen radical absorbance capacity (ORAC) assay (Wang *et al.*, 1996).

The antioxidant activities of pulp and peel of guava (*Psidium guajava* L.), using DPPH radical scavenging method, were evaluated (Jimenez-Escrig *et al.*, 2001).

Garcinol from *Garcinia indica* fruit rind showed nearly 3 times greater DPPH free radical scavenging activity than DL-α-tocopherol (Yamaguchi *et al.*, 2000). The antioxidant potentials measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) were tested in apple and pear (Leontowicz *et al.*, 2003).

Good correlation levels between total phenol content and SC_{50} value of DPPH test ($R^2 = 0.866$) were observed in orange juices (Rapisarda *et al.*, 1999).

A positive relationship of fresh plums (correlation coefficient $r^2 = 0.977$) was presented between total phenolics and antioxidant capacity, expressed as gallic acid equivalents and vitamin C equivalent antioxidant capacity, respectively (Kim *et al.*, 2003).

The data suggests an inverse correlation between the amount of polyphenolic compound and the IC_{50} value of DPPH test (correlation coefficient $R^2 = 0.8434$, Fig. 19). It implies that polyphenolic contents in fruits might contribute to their radical scavenging activity.
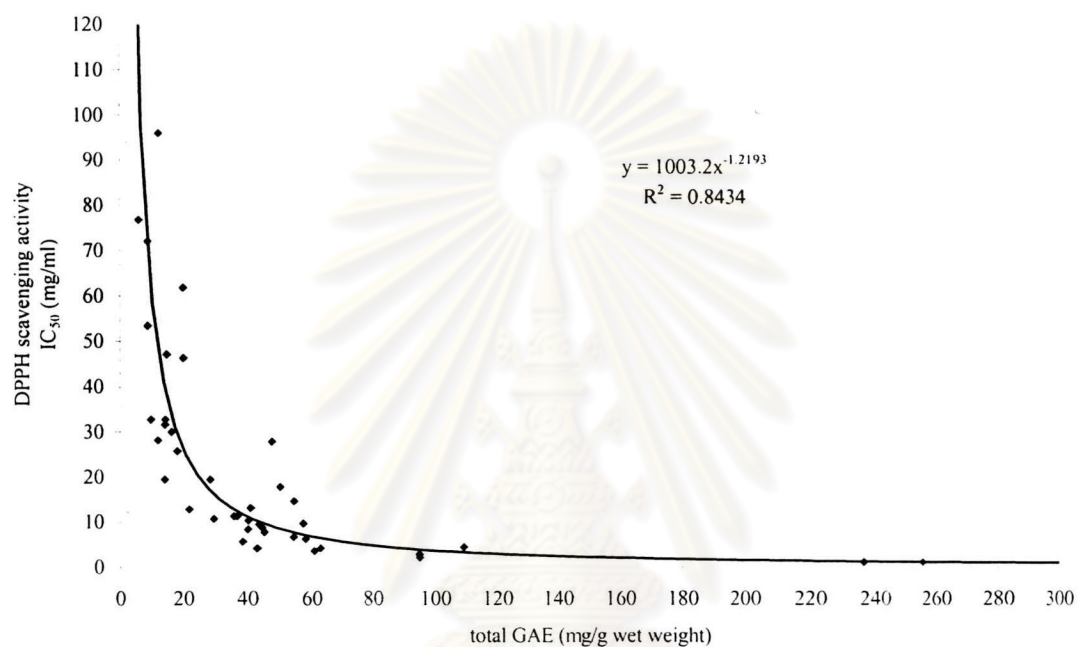
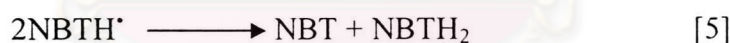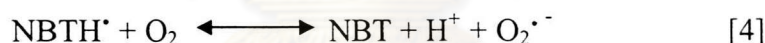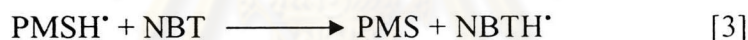Fig. 19 Correlation between total GAE (mg/g wet weight) and IC_{50} value of DPPH radical scavenging activity

3. Determination of Superoxide Anion Scavenging Activity of Fruit Extracts

Nitro blue tetrazolium (NBT) is often used as an indicating scavenger for $O_2^{\cdot-}$, in which role it offers several advantages. Thus, it is reduced by $O_2^{\cdot-}$ and produces a water-insoluble formazan (Bielski and Richter, 1977). The superoxide radicals were generated in a PMS/NADH system and assayed by the reduction of NBT according to the method of Valentao *et al.* (2001), except NADPH was used instead of NADH.

$$NADPH + H^+ + PMS \longrightarrow NADP^+ + PMSH_2 \qquad [1]$$

When NBT was present with PMS+NADPH, it was reduced (reaction [2] and [3]). The NBT radical (NBTH$^{\cdot}$) causes the univalent reduction of dioxygen (reaction [4]). We therefore write the following reactions:

$$PMSH_2 + NBT \longrightarrow PMSH^{\cdot} + NBTH^{\cdot} \qquad [2]$$

$$PMSH^{\cdot} + NBT \longrightarrow PMS + NBTH^{\cdot} \qquad [3]$$

$$NBTH^{\cdot} + O_2 \longleftrightarrow NBT + H^+ + O_2^{\cdot-} \qquad [4]$$

$$2NBTH^{\cdot} \longrightarrow NBT + NBTH_2 \qquad [5]$$

Reaction [4] is an equilibrium which can be displaced to the right by the removal of $O_2^{\cdot-}$. Antioxidant compound would lower the steady-state concentration of NBTH$^{\cdot}$ and there decrease the rate of production of the formazan by reaction [5] (Picker and Fridovich, 1984).

The superoxide anion scavenging activity was plotted against the concentration of fruit extract. A logarithmic regression curve was established. The results are shown in Fig 20, 21, 22, 23, 24, and 25.
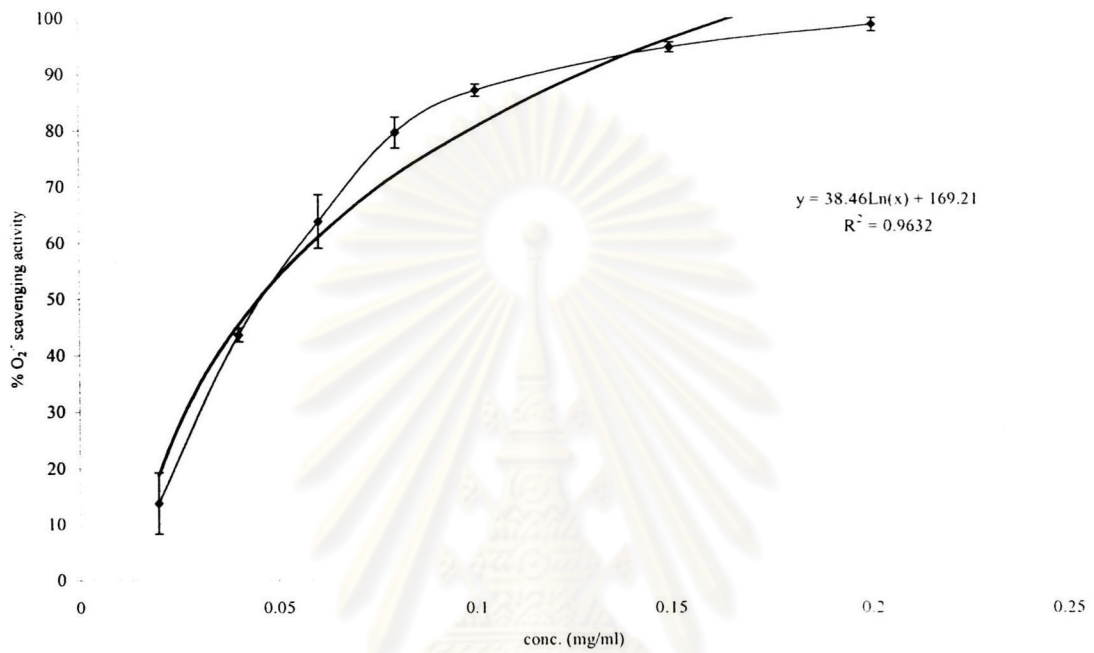
Fig. 20 A logarithmic regression curve of mango (Nam-Dok-Mai) on
superoxide scavenging activity

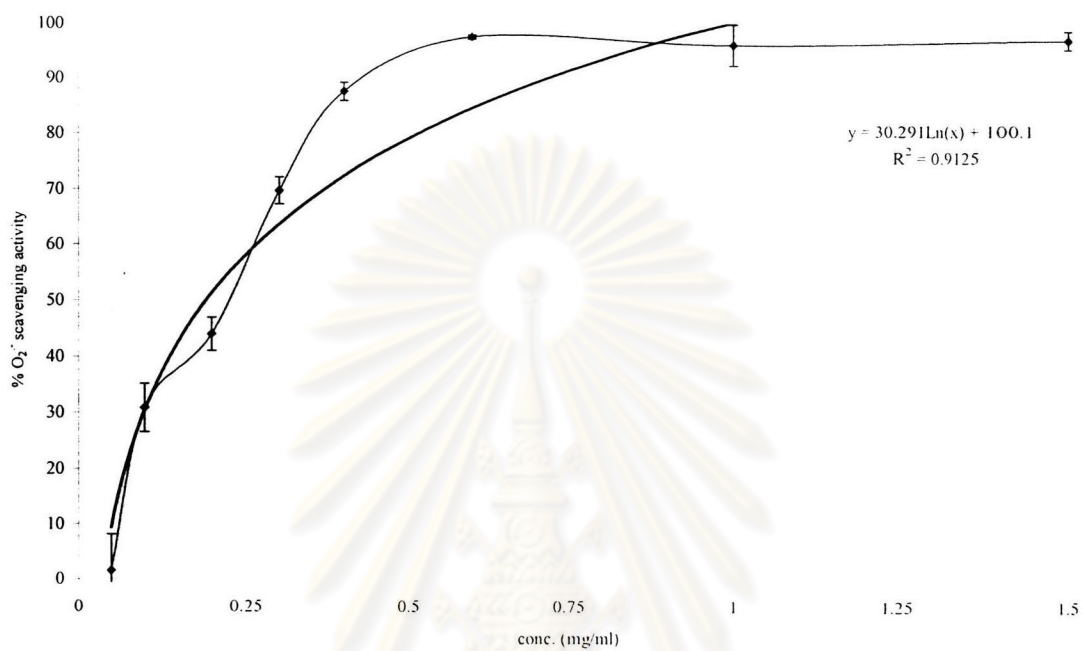Fig. 21 A logarithmic regression curve of longkong seed on superoxide scavenging activity
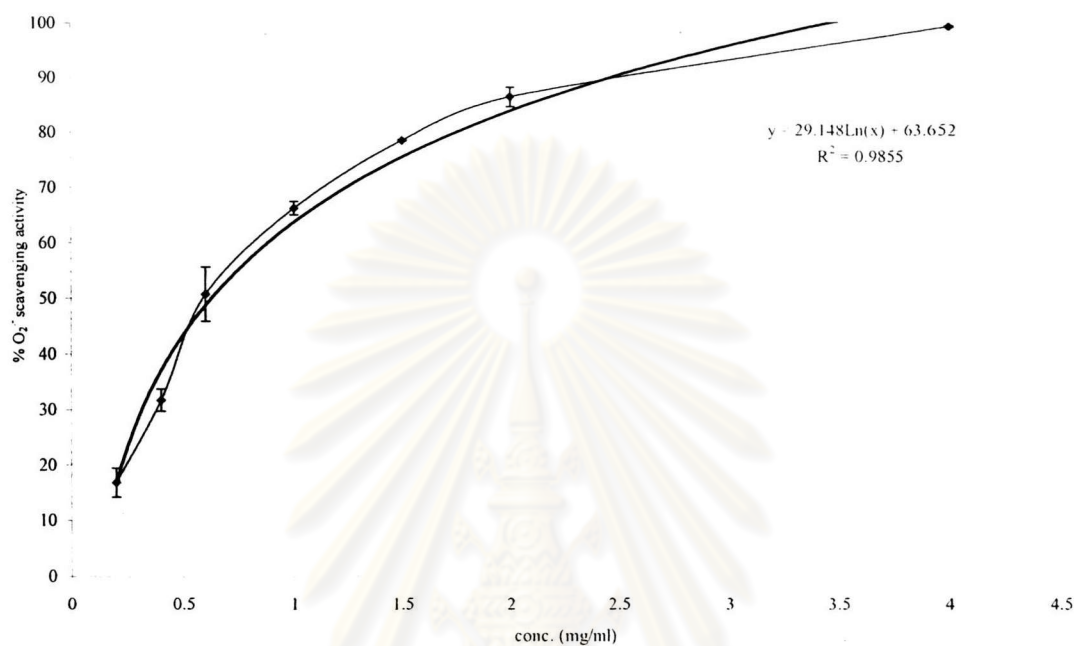
Fig. 22 A logarithmic regression curve of longkong pulp on superoxide
scavenging activity
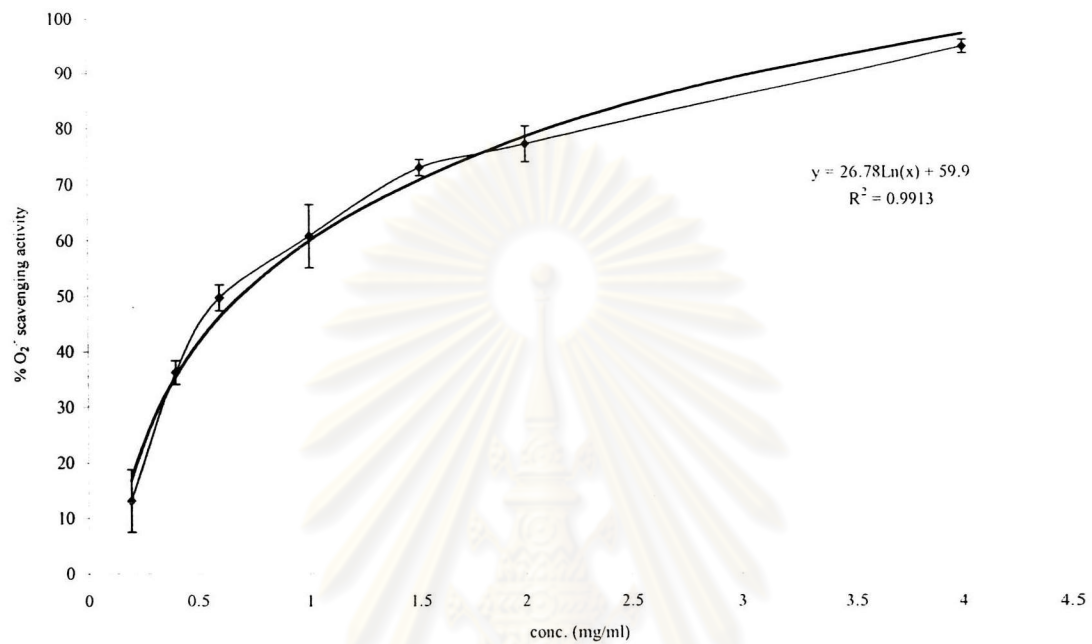
Fig. 23 A logarithmic regression curve of guava on superoxide scavenging
activity
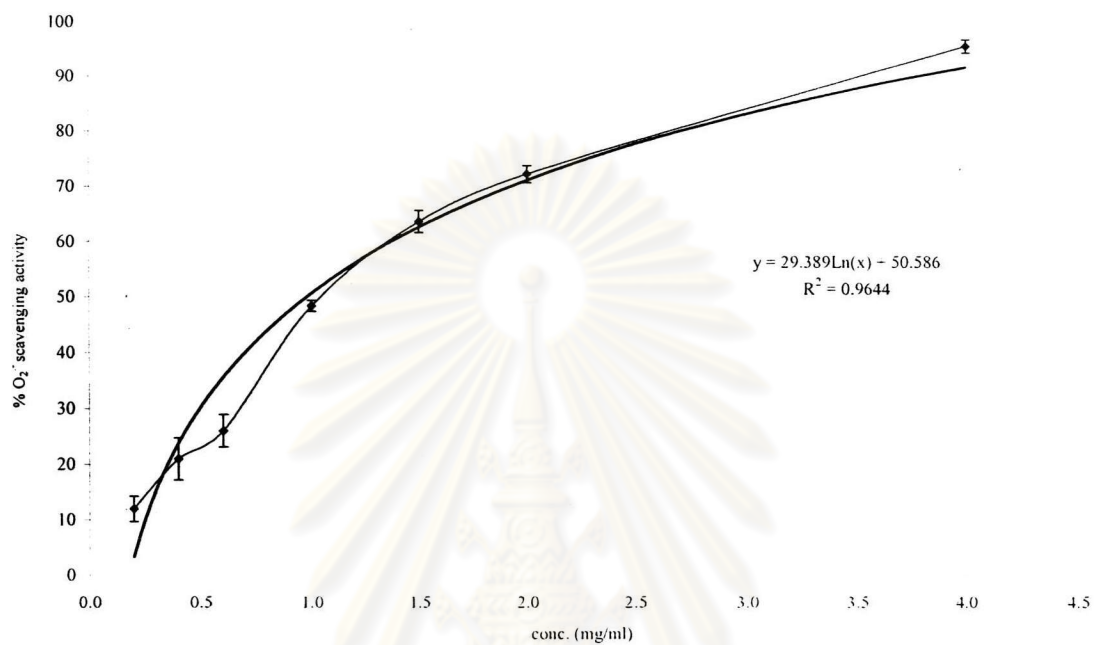
Fig. 24 A logarithmic regression curve of banana (Nam-Var) on superoxide
scavenging activity
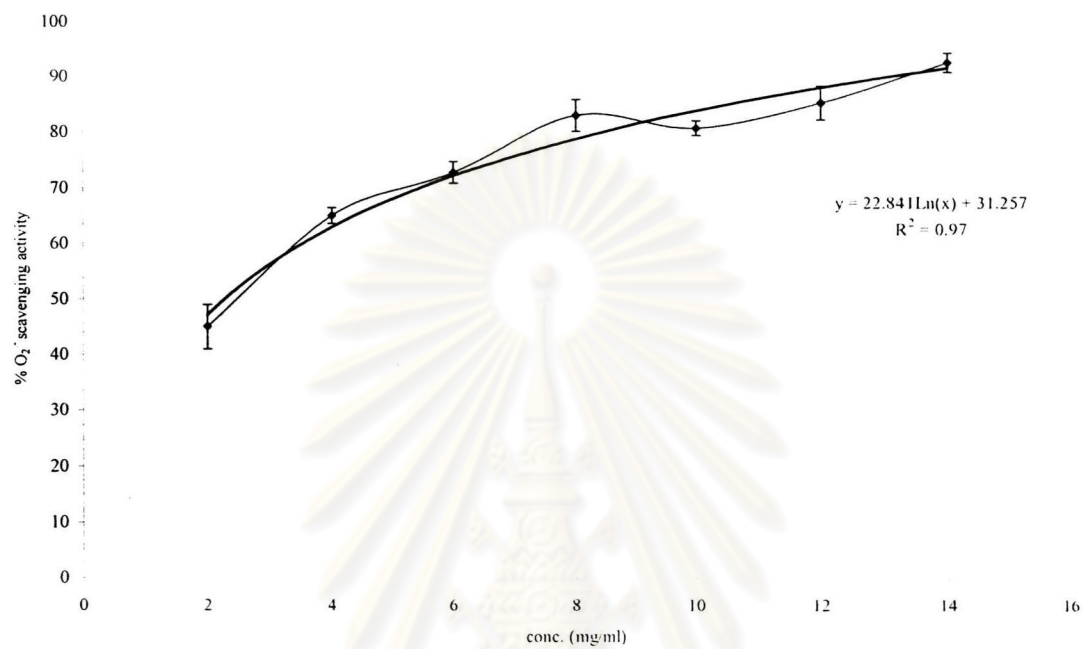
Fig. 25 A logarithmic regression curve of durian on superoxide scavenging
activity

IC$_{50}$ is the concentration of each fruit extract which necessary to decrease by 50% the absorbance value of the control. The results were shown in Table 7.

Table 7. IC$_{50}$ values of the superoxide anion scavenging activities of fruit extracts

Fruit extracts	$O_2^{\cdot-}$ scavenging activity IC$_{50}$ (mg/ml)
Mango (Nam-Dok-Mai)	0.045 ± 0.002
Longkong seed	0.191 ± 0.012
Longkong pulp	0.626 ± 0.027
Guava	0.691 ± 0.017
Banana (Nam-Var)	0.980 ± 0.015
Durian	2.273 ± 0.194

Each value is the mean $\pm$ S.D. of three replicates.

The superoxide scavenging abilities of these six fruit extracts are as follows: mango (Nam-Dok-Mai) > longkong seed > longkong pulp > guava > banana (Nam-Var) > durian. Mango (Nam-Dok-Mai) exhibited the strongest superoxide radical scavenging activity. Durian has the least ability to scavenge superoxide radical. We have not yet determined the exact compounds in each fruit that contribute to the scavenging activity of superoxide radical.

Lin and colleagues reported the six compounds in tea that showed superoxide scavenging abilities in the following order: (-)-epigallocatechin-3-gallate (EGCG) > theaflavin-3-gallate (TF$_2$) > theaflavin (TF$_1$) > gallic acid (GA) > theaflavin-3,3'-digallate (TF$_3$) > propyl gallate (PG) (Lin *et al.*, 2000).
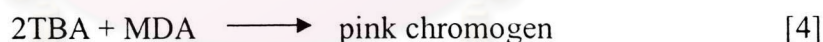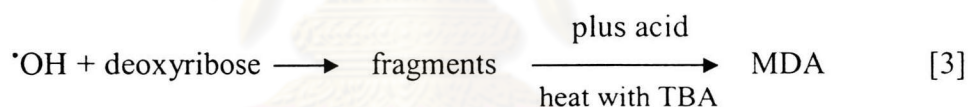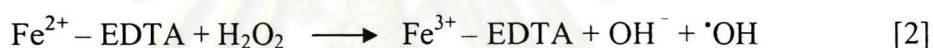
Anthocyanidins: delphinidin, cyanidin, and pelargonidin in pomegranate fruit extract scavenged $O_2^{\cdot-}$ in dose-dependent manner (Noda *et al.*, 2002).

4. Determination of Hydroxyl Radical Scavenging Activity of Fruit Extracts

Deoxyribose (2-deoxy-D-ribose) is degraded into malonaldehyde on exposure to hydroxyl radicals that generated by the Fenton system. The reaction mixture is heated under acid conditions and may be detected by its ability to react with thiobarbituric acid (TBA) to form a pink chromogen (Halliwell *et al.*, 1987; Aruoma, 1994). Including a reducing agent, such as ascorbic acid, in the reaction mixture may increase the rate of deoxyribose degradation and maintaining a supply of Fe^{2+}:

$$Fe^{3+}\text{-EDTA} + ascorbate \longrightarrow Fe^{2+}\text{-EDTA} + oxidized\ ascorbate \qquad [1]$$
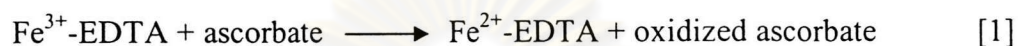
The oxidation of deoxyribose proceeds by the following mechanism:

$$Fe^{2+} - EDTA + H_2O_2 \longrightarrow Fe^{3+} - EDTA + OH^- + {}^{\bullet}OH \qquad [2]$$

$$^{\bullet}OH + deoxyribose \longrightarrow fragments \xrightarrow[\text{heat with TBA}]{\text{plus acid}} MDA \qquad [3]$$

$$2TBA + MDA \longrightarrow pink\ chromogen \qquad [4]$$

The hydroxyl radicals were generated by the Fenton system. Hydroxyl radicals detected by their ability to degrade the sugar deoxyribose into fragments, on heating with thiobarbituric acid at low pH, which generated a pink chromogen. Scavenging effects of the six fruit extracts on hydroxyl radical were shown in table 8 - 13.

Table 8. Scavenging effect of mango (Nam-Dok-Mai) on hydroxyl radical

conc. (mg/ml)	Mango (Nam-Dok-Mai)		
	ABS	ABS (-AA)	ABS (-EDTA)
0.00	0.452 ± 0.003	0.095 ± 0.005	0.414 ± 0.005
0.02	0.471 ± 0.013	0.105 ± 0.008	0.412 ± 0.005
0.04	0.481 ± 0.016	0.109 ± 0.010	0.413 ± 0.005
0.06	0.487 ± 0.013	0.115 ± 0.010	0.397 ± 0.004
0.08	0.488 ± 0.025	0.113 ± 0.013	0.391 ± 0.007
0.10	0.397 ± 0.005*	0.116 ± 0.014	0.385 ± 0.008
0.15	0.090 ± 0.007*	0.115 ± 0.009	0.378 ± 0.021
0.20	0.242 ± 0.006*	0.106 ± 0.003	0.378 ± 0.033

Each value is the mean ± S.D. of three replicates.; ABS (-AA) is the absorbance value of the reaction mixture without ascorbic acid.; ABS (-EDTA) is the absorbance value of the reaction mixture without EDTA. Significance of the mean differences from each set of the control (0 mg/ml of mango extract) values: * $P < 0.05$.

Table 9. Scavenging effect of longkong pulp on hydroxyl radical

conc. (mg/ml)	Longkong pulp		
	ABS	ABS (-AA)	ABS (-EDTA)
0.00	0.439 ± 0.017	0.110±0.013	0.367±0.009
0.20	0.436 ± 0.004	0.121±0.029	0.390±0.004
0.40	0.377 ± 0.012*	0.104±0.006	0.380±0.010
0.60	0.342 ± 0.018*	0.106±0.004	0.324±0.013*
1.00	0.252 ± 0.010*	0.095±0.003	0.297±0.017*
1.50	0.224 ± 0.007*	0.094±0.004	0.260±0.000*
2.00	0.192 ± 0.009*	0.095±0.002	0.238±0.010*
4.00	0.098 ± 0.009*	0.109±0.005	0.219±0.011*

Each value is the mean ± S.D. of three replicates.; ABS (-AA) is the absorbance value of the reaction mixture without ascorbic acid.; ABS (-EDTA) is the absorbance value of the reaction mixture without EDTA. Significance of the mean differences from each set of the control (0 mg/ml of longkong pulp extract) values: * $P < 0.05$.

Table 10. Scavenging effect of longkong seed on hydroxyl radical

conc. (mg/ml)	Longkong seed		
	ABS	ABS (-AA)	ABS (-EDTA)
0.00	0.415$\pm$0.003	0.062$\pm$0.023	0.361$\pm$0.010
0.20	0.540$\pm$0.009*	0.092$\pm$0.021	0.382$\pm$0.039
0.40	0.519$\pm$0.011*	0.088$\pm$0.002	0.303$\pm$0.028
0.60	0.464$\pm$0.013*	0.090$\pm$0.005	0.261$\pm$0.012*
1.00	0.293$\pm$0.005*	0.086$\pm$0.001	0.195$\pm$0.024*
1.50	0.110$\pm$0.002*	0.061$\pm$0.005	0.205$\pm$0.011*
2.00	ND	ND	ND
4.00	ND	ND	ND

Each value is the mean $\pm$ S.D. of three replicates.; ND, not determined; ABS (-AA) is the absorbance value of the reaction mixture without ascorbic acid.; ABS (-EDTA) is the absorbance value of the reaction mixture without EDTA. Significance of the mean differences from each set of the control (0 mg/ml of longkong seed) values: * P < 0.05.

Table 11. Scavenging effect of banana (Nam-Var) on hydroxyl radical

conc. (mg/ml)	Banana (Nam-Var)		
	ABS	ABS (-AA)	ABS (-EDTA)
0.00	0.445 ± 0.004	0.081 ± 0.003	0.374 ± 0.004
0.20	0.407 ± 0.006*	0.119 ± 0.003*	0.322 ± 0.015*
0.40	0.397 ± 0.013*	0.138 ± 0.002*	0.328 ± 0.008*
0.60	0.366 ± 0.003*	0.139 ± 0.006*	0.268 ± 0.001*
1.00	0.299 ± 0.010*	0.140 ± 0.016*	0.258 ± 0.009*
1.50	0.288 ± 0.005*	0.167 ± 0.004*	0.236 ± 0.006*
2.00	0.263 ± 0.013*	0.144 ± 0.014*	0.216 ± 0.006*
4.00	0.165 ± 0.006*	0.110 ± 0.002*	0.202 ± 0.016*

Each value is the mean ± S.D. of three replicates.; ABS (-AA) is the absorbance value of the reaction mixture without ascorbic acid.; ABS (-EDTA) is the absorbance value of the reaction mixture without EDTA. Significance of the mean differences from each set of the control (0 mg/ml of banana extract) values: * $P < 0.05$.

Table 12. Scavenging effect of guava on hydroxyl radical

conc.	Guava		
(mg/ml)	ABS	ABS (-AA)	ABS (-EDTA)
0.00	0.472 ± 0.007	0.120 ± 0.004	0.357 ± 0.009
0.20	0.528 ± 0.004*	0.249 ± 0.003*	0.364 ± 0.002
0.40	0.533 ± 0.011*	0.311 ± 0.012*	0.287 ± 0.013*
0.60	0.521 ± 0.004*	0.327 ± 0.017*	0.266 ± 0.006*
1.00	0.424 ± 0.007*	0.328 ± 0.009*	0.207 ± 0.011*
1.50	0.442 ± 0.012*	0.386 ± 0.080*	0.210 ± 0.006*
2.00	0.376 ± 0.002*	0.331 ± 0.013*	0.202 ± 0.004*
4.00	0.241 ± 0.008*	0.263 ± 0.006*	0.260 ± 0.010*

Each value is the mean ± S.D. of three replicates.; ABS (-AA) is the absorbance value of the reaction mixture without ascorbic acid.; ABS (-EDTA) is the absorbance value of the reaction mixture without EDTA. Significance of the mean differences from each set of the control (0 mg/ml of guava extract) values: * $P < 0.05$.

Table 13. Scavenging effect of durian on hydroxyl radical

conc.	Durian		
(mg/ml)	ABS	ABS (-AA)	ABS (-EDTA)
0	0.457 $\pm$ 0.012	0.108 $\pm$ 0.003	0.355 $\pm$ 0.009
2	0.390 $\pm$ 0.012*	0.354 $\pm$ 0.003*	0.282 $\pm$ 0.017*
4	0.318 $\pm$ 0.000*	0.306 $\pm$ 0.015*	0.277 $\pm$ 0.010*
6	0.290 $\pm$ 0.007*	0.272 $\pm$ 0.015*	0.265 $\pm$ 0.028*
8	0.255 $\pm$ 0.015*	0.258 $\pm$ 0.005*	0.266 $\pm$ 0.011*
10	0.243 $\pm$ 0.004*	0.270 $\pm$ 0.017*	0.288 $\pm$ 0.011*
12	0.252 $\pm$ 0.006*	0.246 $\pm$ 0.010*	0.290 $\pm$ 0.003*
14	0.251 $\pm$ 0.015*	0.251 $\pm$ 0.004*	0.306 $\pm$ 0.019

Each value is the mean $\pm$ S.D. of three replicates.; ABS (-AA) is the absorbance value of the reaction mixture without ascorbic acid.; ABS (-EDTA) is the absorbance value of the reaction mixture without EDTA. Significance of the mean differences from each set of the control (0 mg/ml of durian extract) values: * $P < 0.05$.

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The abilities of these six fruit extracts to act as hydroxyl scavenger are followed: mango (Nam-Dok-Mai) > longkong pulp > banana (Nam-Var) > longkong seed > guava > durian. Mango (Nam-Dok-Mai) exhibited the strongest hydroxyl radical scavenging activity while durian has the least ability to scavenge hydroxyl radical.

To evaluate the pro-oxidant potential of fruit extracts, this assay was conducted in the absence of ascorbic acid (Valentao *et al.*, 2002). As shown in table 8, 9, and 10, mango (Nam-Dok-Mai), longkong pulp, and longkong seed have no pro-oxidant effect. However, pro-oxidant properties were observed in banana (Nam-Var), guava and durian (Table 11, 12 and 13).

Deoxyribose was also damaged by the reaction mixture that absence of EDTA, because omission of the chelator allows iron ions to bind directly to the sugar. Compounds which can inhibit deoxyribose degradation in the absence of EDTA are those with iron ion-binding capacity and which can withdraw the iron ions and render them inactive or poorly active in Fenton reaction (Paya *et al.*, 1992). The assay performed in the absence of EDTA showed that the five fruit extracts (banana (Nam-Var), longkong pulp, longkong seed, guava, and durian) have weak metal chelation potential. However, metal chelation potential was not found in mango (Nam-Dok-Mai).

No pro-oxidation activity in copper-mediated oxidation was observed in elderberry (*Sambucus nigra*) (Abuja *et al.*, 1998) and vegetables but tea showed a pro-oxidant activity (Cao *et al.*, 1996).

Anthocyanidins: delphinidin, cyanidin, and pelargonidin in pomegranate fruit extract inhibited a Fenton reagent $^{\cdot}$OH generating system possibly by chelating with ferrous ion (Noda *et al.*, 2002).

Tea polyphenols from both black and green teas inhibited the $^{\cdot}$OH fluxes in a concentration-dependent manner (Grinberg *et al.*, 1997).
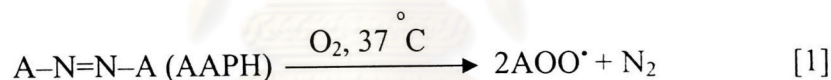
The tea extracts, especially the green, pouching, and oolong tea extracts markedly stimulated the oxidation of deoxyribose in the presence of Fe^{3+} and H_2O_2. However, the oxidation was decreased by a high dosage of tea extracts (Yen *et al.*, 1997).

5. Inhibitory Effects of Fruit Extracts on Human Erythrocyte Hemolysis

Increasing evidence suggests that oxidative damage to cell component may have an important pathophysiological role in several types of human disease (Ames *et al.*, 1993). RBCs are highly susceptible to oxidative damage as a result of high polyunsaturated fatty acid content of their membranes and the high cellular concentrations of oxygen (Clemens and Waller, 1987).

Hemolysis of human erythrocyte membrane was induced by 2,2'-azo-bis(2-methylpropionamidine) dihydrochloride (AAPH). AAPH decomposes unimolecularly to give nitrogen and initiating free radicals in the aqueous phase. An initiating radical attacks membrane component and produces many lipid hydroperoxides and eventually causes membrane damage and hemolysis (Miki *et al.*, 1987). The oxidation of lipid (LH) in RBC membranes proceeds by the following mechanism:

Chain initiation:

$$A\text{–}N\text{=}N\text{–}A \text{ (AAPH)} \xrightarrow{O_2,\ 37\ ^\circ C} 2AOO^\bullet + N_2 \qquad [1]$$

$$AOO^\bullet + LH \longrightarrow AOOH + LOO^\bullet \qquad [2]$$

Chain propagation:

$$LOO^\bullet + LH \longrightarrow LOOH + L^\bullet \qquad [3]$$

$$L^\bullet + O_2 \longrightarrow LOO^\bullet \qquad [4]$$

Chain termination:

$$LOO^\bullet + LOO^\bullet \longrightarrow \text{molecular products} \qquad [5]$$

A is HCl– NH = $C(NH_2)$ – $C(CH_3)_2$–. AAPH decompose to give nitrogen and initiating radical $(AOO^{\bullet})$ (reaction [1]). An initiating free radical attacks the polyunsaturated lipid (LH) in RBC membrane and generates lipid peroxyl radical (reaction [2]). The lipid peroxyl radical attacks another lipid molecule to yield lipid hydroperoxide and new lipid radical. Reaction [3] and [4] take place repeatedly to generate a free radical chain (Miki *et al*., 1987; Lanping *et al*., 2000).
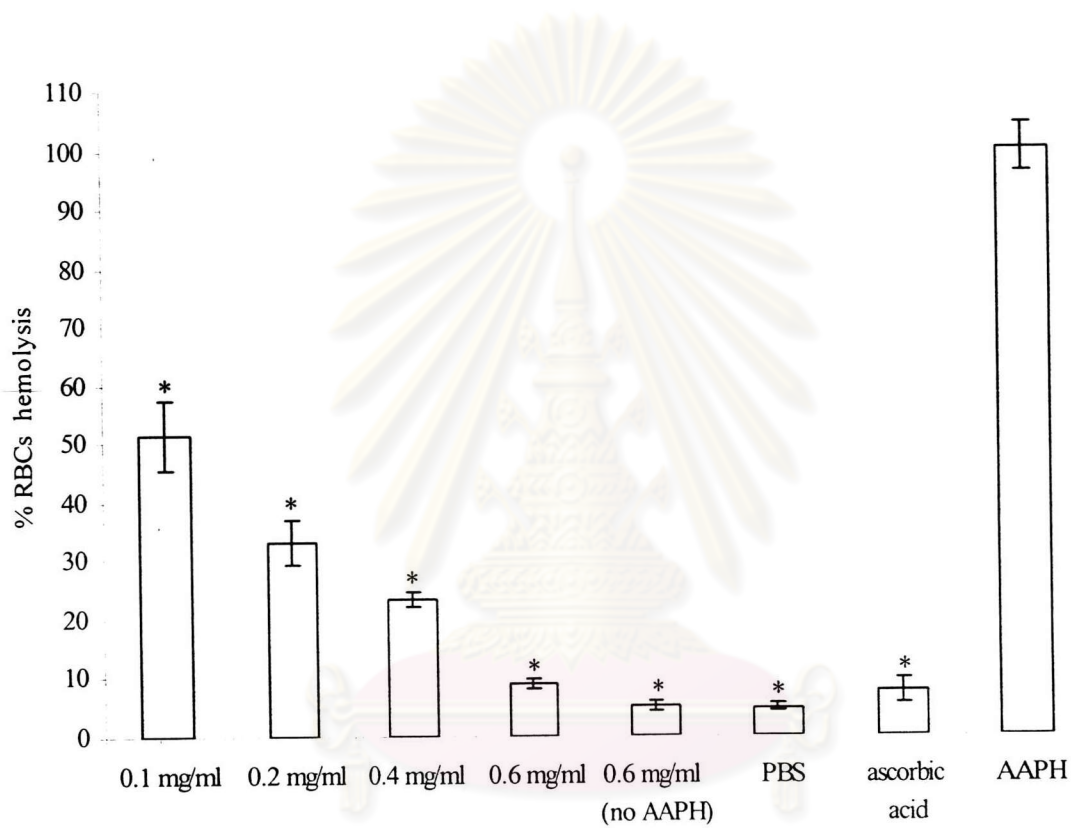
Fig. 2 6 E ffects o f m ango (Nam-Dok-Mai) e xtract o n h uman e rythrocyte h emolysis initiated by AAPH at 37 $^\circ$C for 1 hour. Each value is the mean $\pm$ S.D. of three replicates. Significance of the mean differences from the control (AAPH) values: * P < 0.05.
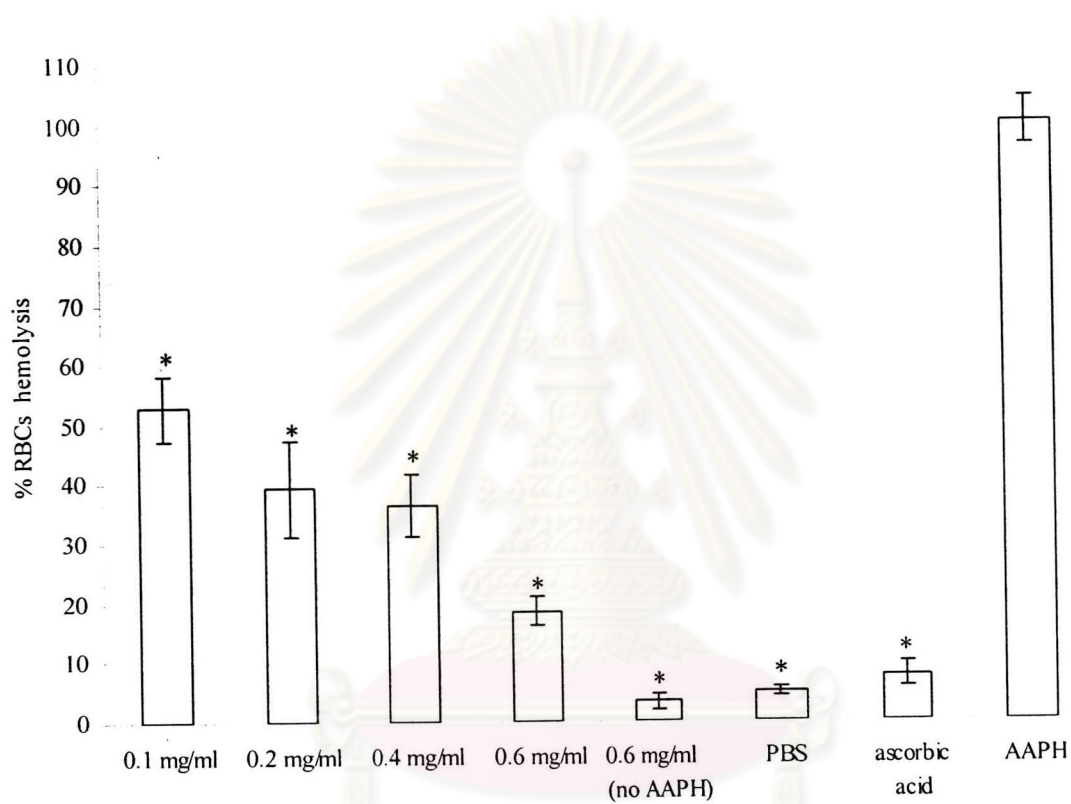
Fig. 27 Effects of banana (Nam-Var) extract on human erythrocyte hemolysis initiated by AAPH at 37 $^\circ$C for 1 hour. Each value is the mean $\pm$ S.D. of three replicates. Significance of the mean differences from the control (AAPH) values: * P < 0.05.
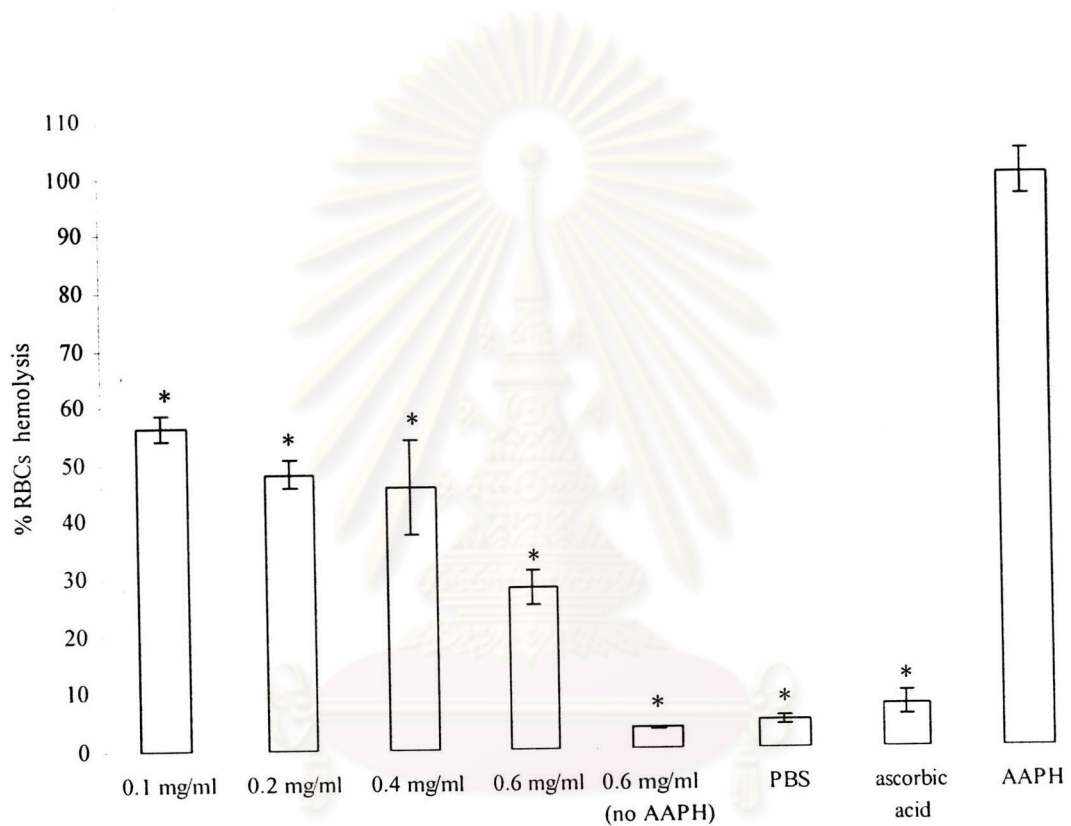
Fig. 28 Effects of guava extract on human erythrocyte hemolysis initiated by AAPH at 37 °C for 1 hour compared with control (AAPH). Each value is the mean ± S.D. of three replicates. Significance of the mean differences from the control (AAPH) values: * $P < 0.05$.
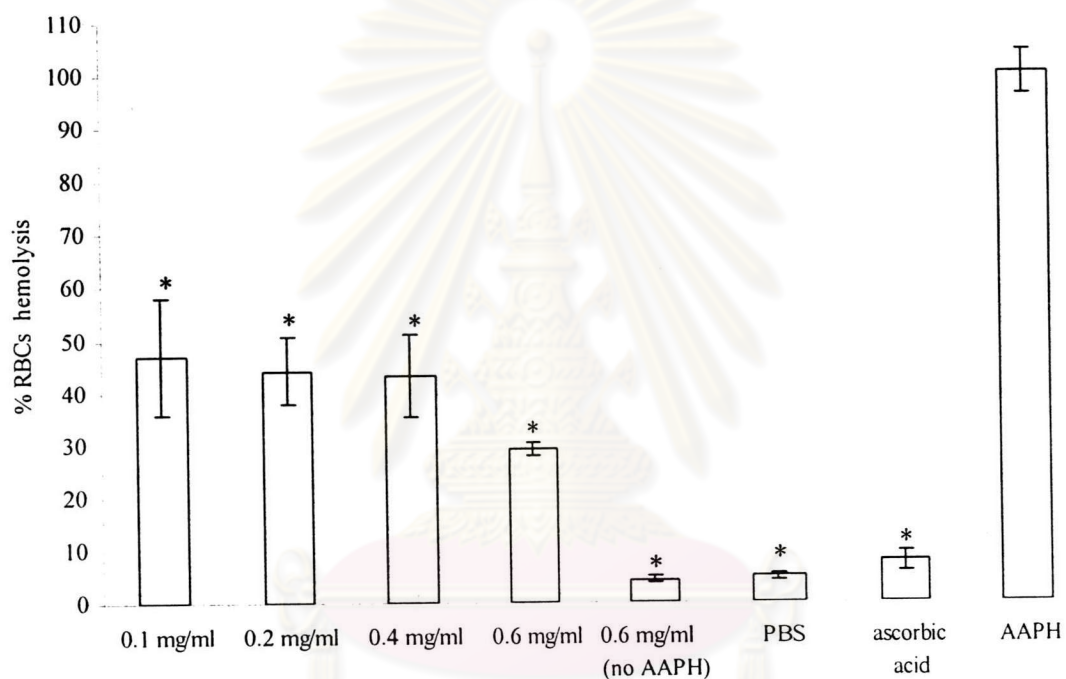
Fig. 29 Effects of longkong pulp extract on human erythrocyte hemolysis initiated by AAPH at 37 °C for 1 hour. Each value is the mean ± S.D. of three replicates. Significance of the mean differences from the control (AAPH) values: * P < 0.05.

Fig. 30 Effects of longkong seed extract on human erythrocyte hemolysis initiated by
AAPH at 37 $^{\circ}$C for 1 hour. Each value is the mean $\pm$ S.D. of three replicates.
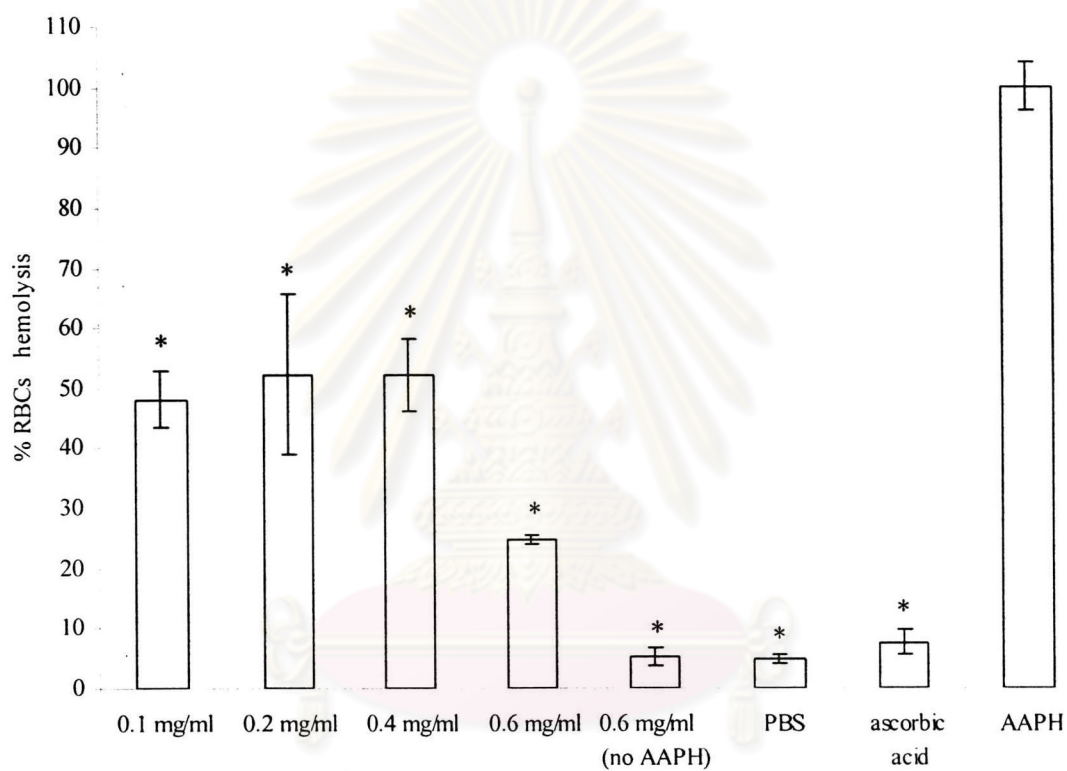Significance of the mean differences from the control (AAPH) values: * P <
0.05.

Fig. 31 Effects of durian extract on human erythrocyte hemolysis initiated by AAPH at 37 $^{\circ}$C for 1 hour.   Each value is the mean $\pm$ S.D. of three replicates. Significance of the mean differences from the control (AAPH) values: * P < 0.05.
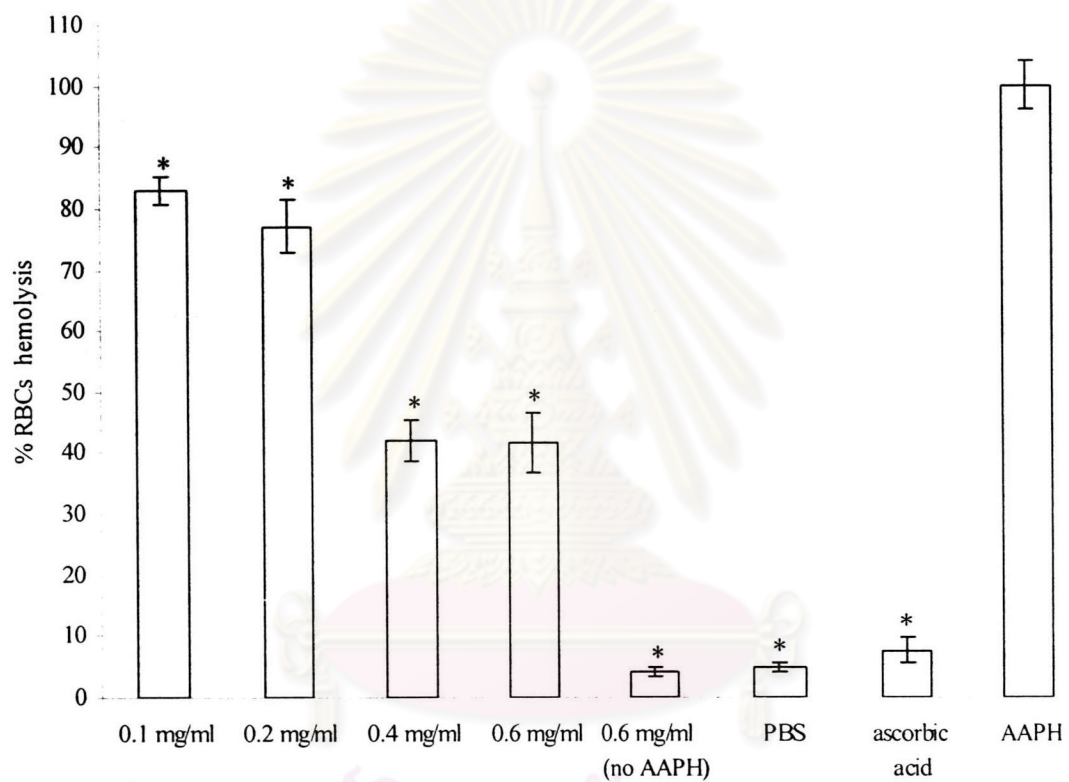
The present study demonstrated the dose-dependent protective effects of all the six fruit extracts (mango (Nam-Dok-Mai), banana (Nam-Var), guava, longkong pulp, longkong seed, and durian) on AAPH-induced RBCs hemolysis (Fig 26 - 31). All concentration of fruit extracts (from 0.1 to 0.6 mg/ml) significantly inhibited hemolysis of human RBCs compared with control (AAPH).

To elucidate whether there is any harmful effect of the fruit extract itself on human RBCs membrane damage. At the highest concentration of each fruit extract (0.6 mg/ml) to the RBCs suspension, we found that no significant RBCs hemolysis was observed.

Comparison of the inhibitory effects of the six varieties of fruit extracts on human erythrocyte hemolysis initiated by AAPH was shown in Table 14. Among the six fruit extracts, mango (Nam-Dok-Mai) possesses the strongest effect to protect human erythrocyte membrane from hemolysis while durian has the weakest scavenger to quench the oxyradical caused by AAPH. Mango (Nam-Dok-Mai) extract (at 0.6 mg/ml) can inhibit human erythrocyte hemolysis up to 91%. Banana, guava, longkong pulp, and longkong seed have moderate antioxidant capability to prevent human erythrocyte membrane hemolysis.

The water, methanol, and dichloromethane extracts of brown seaweed (*Sargassum siliquastrum*) exhibited 11.1 $\pm$ 0.8, 54.2 $\pm$ 2.6, and 78.6 $\pm$ 0.1 % hemolysis inhibition on rat RBCs hemolysis induced by AAPH, respectively at 10 µg/ml (Lim *et al.*, 2002).

Protection effects of polyphenols from green tea, black tea, and jasmine green tea against oxidative damage to RBCs were studied (Grinberg *et al.*, 1997; Zhang *et al.*, 1997).

The present work have not yet determined the exact compounds in each fruit extract that contribute to the protective action on human RBCs hemolysis induced by AAPH. However, the strongest ability to inhibit RBCs hemolysis in mango (Nam-Dok-Mai) extract correlate with its best antioxidant potency. In addition, the weakest antioxidant activities in durian exhibited the lowest effect to protect human RBCs hemolysis. These works suggest that antioxidant compounds in fruits have the capability to prevent RBCs membrane damage, caused by ROS. In other words, the inhibitory effects of fruit extracts on human RBCs hemolysis might due to the antioxidant properties present in the fruit extracts.

Table 14. Inhibitory effect of fruit extracts on human erythrocyte hemolysis initiated by AAPH.

conc. (mg/ml)	% Inhibition of RBCs hemolysis					
	Mango (Nam-Dok-Mai)	Banana (Nam-Var)	Guava	Longkong pulp	Longkong seed	Durian
0.1	48.69 ± 5.89	47.14 ± 5.43	43.70 ± 2.42	53.19 ± 11.11	51.77 ± 4.77	16.99 ± 2.21
0.2	66.83 ± 3.93	60.72 ± 8.04	51.88 ± 2.45	55.85 ± 6.54	47.70 ± 13.35	22.88 ± 4.28
0.4	76.47 ± 1.30	63.67 ± 5.20	54.17 ± 8.10	56.74 ± 7.97	47.70 ± 5.98	57.84 ± 3.40
0.6	91.18 ± 0.85	81.51 ± 2.47	71.85 ± 3.16	71.10 ± 1.23	75.18 ± 0.81	58.17 ± 4.91
0.6 (no AAPH)	94.93 ± 0.75	96.73 ± 1.42	96.40 ± 0.28	95.74 ± 0.53	94.68 ± 1.41	95.92 ± 0.75

Each value is the mean ± S.D. of three replicates.