CHAPTER V
RESULTS

5.1 Antioxidant Activities

5.1.1 Optimized extraction solvents of aqueous ethanol extracted teas

In this study, different extraction solvents (0, 30, 50, 70 and 90% (v/v) ethanol in deionized (DI) water) were evaluated using five conventional teas including green tea, white tea, oolong tea, black tea and pu–erh tea as well as Thai herbal teas from different parts of plants including Indian gooseberry, chrysanthemum, stevia, lemon grass and ginger herbal teas. Antioxidant assays (ORAC, FRAP and DPPH radical scavenging assays) and total phenolic contents were employed for determination and comparison of antioxidant capacity of these tea extracts.

The effects of different extraction solvents of all conventional teas and five Thai herbal teas that represented different plant parts (Indian gooseberry for fruits, chrysanthemum for flowers, stevia for leaves, lemon grass for leaves and stems, and ginger for roots) suggested that antioxidant activities were optimized under the range of 30–70% (v/v) ethanol in DI water as being detected by ORAC and FRAP assays (Figure 5.1 and 5.2). Since extraction solvent of 50% (v/v) ethanol in DI water provided the highest antioxidant activity for most tea extracts, this solvent system was chosen for further comparison of antioxidant activity between herbal teas and conventional teas.

Under this optimized extraction solvent system, white tea and green tea exhibited the highest antioxidant capacity among conventional teas, followed by oolong tea and pu–erh tea ~ black tea, respectively, as being detected by ORAC and FRAP assays (~1803–7671 μmol TE/g by ORAC assay and ~398–1430 μmol TE/g by FRAP assay) (Table 5.1). However, DPPH radical scavenging assay could not differentiate these results (91–95% radical scavenging activity). Nevertheless, these antioxidant activities were consistent with the total phenolic contents (48–140 mg

GAE/g), in which green tea exhibited the highest quantity of phenolic compounds, followed by white tea, oolong tea, black tea and pu–erh tea, respectively.

As for Thai herbal teas, ORAC and FRAP assays suggested that Indian gooseberry, stevia and cat's whisker provided comparable antioxidant activities to those of conventional teas (~2196–3482 μmol TE/g by ORAC assay and ~336–1290 μmol TE/g by FRAP assay) (Table 5.1). On the other hand, the DPPH radical scavenging assay suggested a wider range of herbal teas including Indian gooseberry, beal fruit, rosella, chrysanthemum, Asiatic pennywort, stevia and cat's whisker that possessed similar antioxidant activities (>90% radical scavenging activity) that could prevent free radical damage as those of conventional teas. These antioxidant activities were consistent with the total phenolic contents (21–155 mg GAE/g), in which Indian gooseberry exhibited the highest quantity of total phenolic compounds among fifteen Thai herbal teas, followed by stevia, beal fruit and cat's whisker, respectively. It was also observed that these teas extracts provided comparable phenolic contents to those of conventional teas.

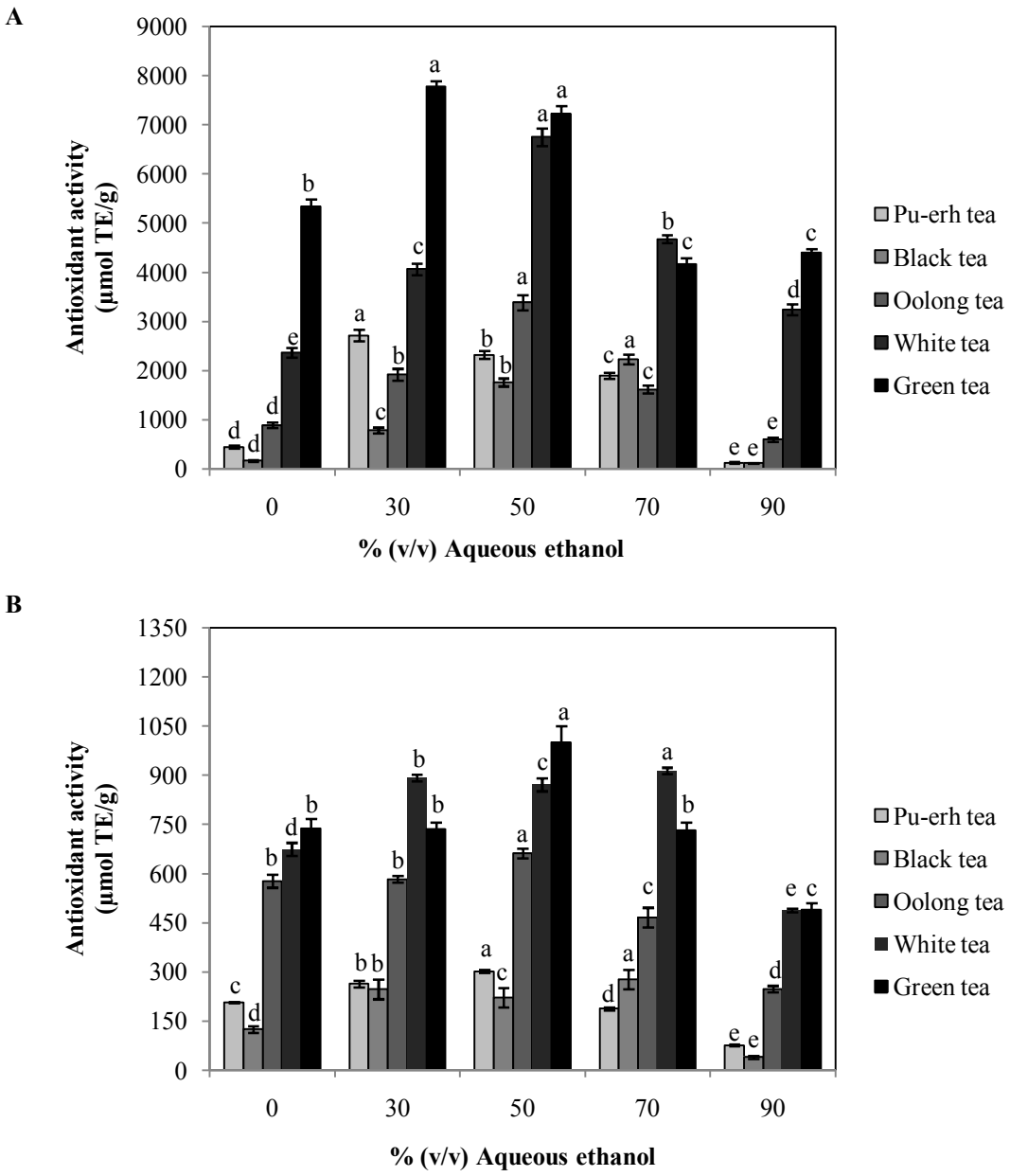

Figure 5.1 The effect of extraction solvents on conventional teas including green tea, white tea, oolong tea, black tea and pu–erh tea was monitored by (A) ORAC and (B) FRAP assays. All analytical data were represented as mean values of three independent samples (n=3) ± standard deviation. Values with different letters (a–e) of each conventional tea were significantly different at $p < 0.05$.

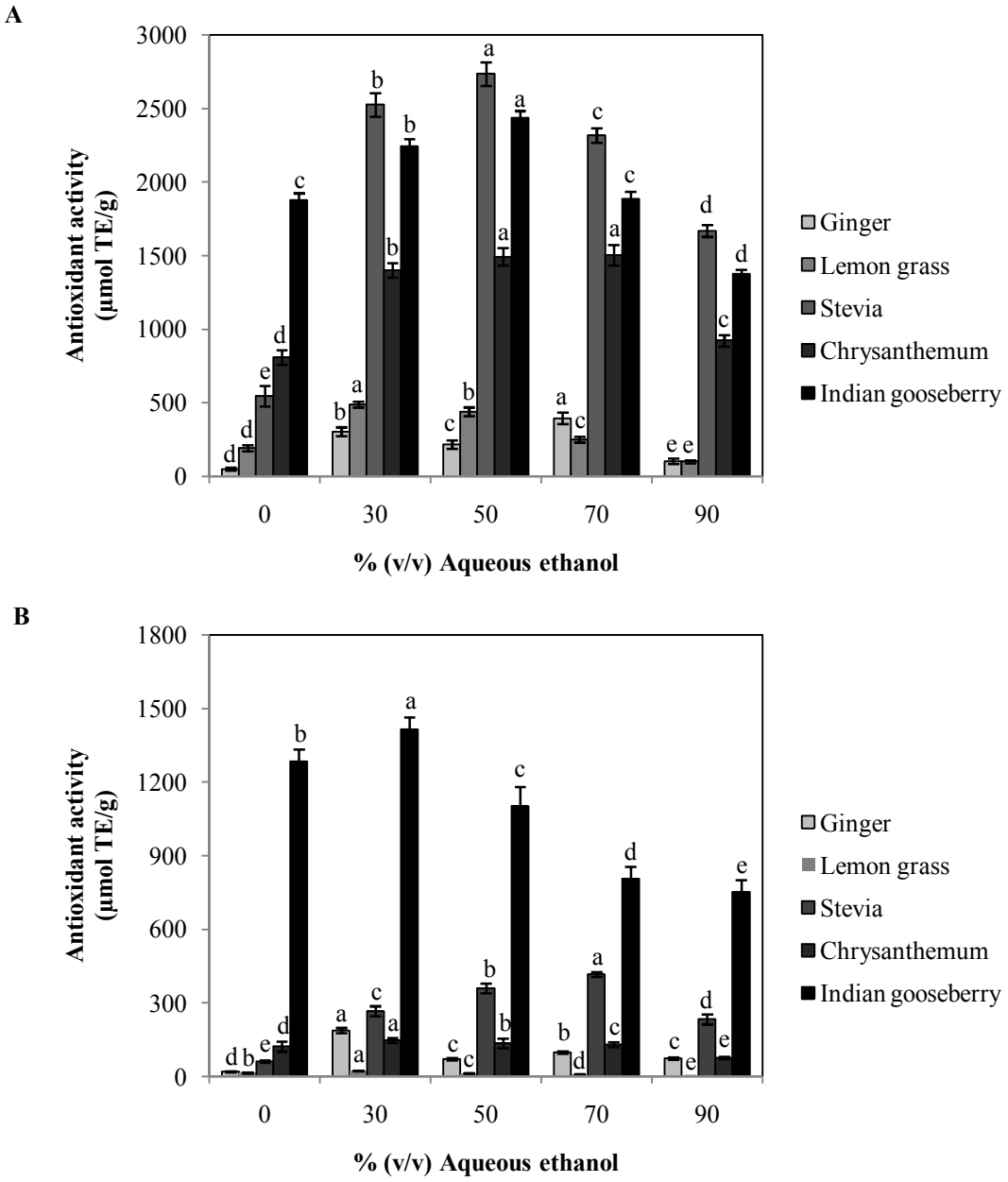

Figure 5.2 The effect of extraction solvents on Thai herbal teas was monitored by (A) ORAC and (B) FRAP assays. Thai herbal teas were selected from various plant parts including Indian gooseberry (fruits), chrysanthemum (flowers), stevia (leaves), lemon grass (leaves and stems) and ginger (roots). All analytical data were represented as mean values of three independent samples (n=3) ± standard deviation. Values with different letters (a–e) of each herbal tea were significantly different at $p < 0.05$.

Table 5.1 Antioxidant activity and total phenolic contents of different teas extracted with 50% (v/v) aqueous ethanol were evaluated by ORAC, FRAP, DPPH radical scavenging and Folin–Ciocalteu assays

Type of teas*	Abbreviation	Antioxidant activity			Folin-Ciocalteu assay (mg GAE/g)
		DPPH assay (% scavenging)	ORAC assay (µmol TE/g)	FRAP assay (µmol TE/g)	
Conventional tea					
Green tea	GT	93.21 ± 0.58[a]	7671.96 ± 129.47[a]	1430.10 ± 39.54[a]	140.25 ± 5.40[b]
White tea	WT	92.71 ± 1.07[a]	4382.30 ± 149.08[b]	1100.51 ± 67.35[c]	109.97 ± 6.20[c]
Oolong tea	OT	91.96 ± 0.87[a]	3068.21 ± 141.48[d]	723.84 ± 53.31[d]	71.36 ± 6.28[d]
Black tea	BT	95.44 ± 0.45[a]	1803.04 ± 109.73[f]	421.16 ± 23.85[e]	69.90 ± 7.13[d]
Pu-erh tea	PT	91.44 ± 0.82[a]	025.99 ± 95.80[e]	398.47 ± 42.30[e]	48.06 ± 2.53[e]
Thai herbal tea					
Bael fruit	BF	93.77 ± 0.16[a]	1591.45 ± 20.23[f]	124.65 ± 6.39[h]	42.51 ± 5.32[e]
Indian gooseberry	IG	96.47 ± 0.28[a]	2207.36 ± 123.36[e]	1290.58 ± 59.28[b]	154.77 ± 9.55[a]
Bitter cucumber	BC	45.34 ± 3.16[e]	107.78 ± 5.68[i]	16.05 ± 0.58[i]	1.73 ± 0.97[g]
Rosella	RS	97.63 ± 2.05[a]	503.38 ± 23.38[h]	118.47 ± 5.03[h]	9.66 ± .62[g]

*Extractions were performed under 50% (v/v) aqueous ethanol at 30 °C for 2 hours.
All analytical data were mean values of three independent sample (n=3) ± standard deviation.
Values with different letter (a–i) within the same column were significantly different with $p < 0.05$.
TE: Trolox equivalent, GAE: Gallic acid equivalent

Table 5.1 Antioxidant activity and total phenolic contents of different teas extracted with 50% (v/v) aqueous ethanol were evaluated by ORAC, FRAP, DPPH radical scavenging and Folin–Ciocalteu assays (cont.)

Type of teas*	Abbreviation	Antioxidant activity			Folin-Ciocalteu assay (mg GAE/g)
		DPPH assay (% scavenging)	ORAC assay (µmol TE/g)	FRAP assay (µmol TE/g)	
Thai herbal tea					
Safflower	SF	76.19 ± 2.28^{c}	1557.79 ± 7.72^{f}	88.66 ± 7.59^{h}	18.81 ± 1.31^{f}
Chrysanthemum	CS	89.69 ± 0.24^{a}	1627.07 ± 40.11^{f}	220.64 ± 1.29^{g}	27.14 ± 2.56^{f}
White mulberry	MB	84.42 ± 1.01^{b}	825.46 ± 29.30^{g}	51.84 ± 3.97^{h}	8.75 ± 0.47^{g}
Asiatic pennywort	AP	93.38 ± 0.48^{a}	853.11 ± 4.85^{g}	78.95 ± 1.52^{h}	8.16 ± 0.56^{g}
Pandanus	PD	79.36 ± 0.24^{b}	154.99 ± 0.68^{i}	25.99 ± 1.90^{i}	6.68 ± 0.45^{g}
Jiaogulan	JL	82.27 ± 4.80^{b}	619.93 ± 19.80^{g}	29.13 ± 2.39^{g}	4.44 ± 0.08^{g}
Stevia	ST	90.89 ± 0.26^{a}	3482.22 ± 110.92^{c}	452.18 ± 26.06^{e}	74.07 ± 2.54^{d}
Cat's whisker	CW	91.27 ± 0.25^{a}	2196.61 ± 58.61^{e}	336.78 ± 12.36^{f}	20.79 ± 0.45^{f}
Lemon grass	LG	65.03 ± 3.30^{d}	387.65 ± 7.19^{h}	26.20 ± 1.07^{h}	20.36 ± 1.23^{f}
Jewel vine	JV	82.36 ± 1.62^{b}	263.57 ± 21.80^{h}	37.48 ± 2.97^{h}	12.97 ± 0.61^{g}
Ginger	GG	73.18 ± 0.92^{c}	450.38 ± 23.10^{h}	147.84 ± 12.36^{g}	13.17 ± 0.14^{f}

*Extractions were performed under 50% (v/v) aqueous ethanol at 30 °C for 2 hours.
All analytical data were mean values of three independent sample (n=3) ± standard deviation.
Values with different letter (a–i) within the same column were significantly different with $p < 0.05$.
TE: Trolox equivalent, GAE: Gallic acid equivalent

5.1.2 Tea infusions

The results of tea infusions (Table 5.2) that were extracted with 95 °C DI water (a general condition for making tea) indicated green tea, white tea and black tea exhibited higher antioxidant capacity (~93% radical scavenging activity) than those of oolong tea and pu–eh tea (~89% radical scavenging activity) as being detected by DPPH radical scavenging assay. Similar results were also observed in ORAC and FRAP experiments, in which green tea exhibited the highest antioxidant capacity (~1829 μmol TE/g by ORAC assay and ~621 μmol TE/g by FRAP assay), followed by white tea, pu–eh tea, oolong tea and black tea, respectively. These antioxidants were consistent to the total phenolic contents in tea extract, in which green tea (~54 mg GAE/g) exhibited the highest quantity of total phenolic contents, while black tea was the poorest source (~16 mg GAE/g).

Likewise, DPPH radical scavenging assay was first employed to screen antioxidant activity of all fifteen herbal teas in comparison to five conventional teas (Table 5.2). The results suggested that tea infusions from Indian gooseberry, chrysanthemum, Asiatic pennywort, stevia and cat's whisker possessed similar quantity of antioxidants that could prevent free radical damage as those of conventional teas (>87% radical scavenging activity). These results were confirmed by the ORAC and FRAP assays, in which Indian gooseberry, chrysanthemum, stevia and cat's whisker herbal teas were found to exhibited comparable antioxidant activities to those of conventional teas (~1019–3282 μmol TE/g by ORAC assay and ~211–1520 μmol TE/g by FRAP assay). These antioxidants were also consistent with the total phenolic contents, in which Indian gooseberry exhibited the highest quantity of phenolic compounds (126 mg GAE/g), followed by stevia, cat's whisker, beal fruit and chrysanthemum (~22–78 mg GAE/g).

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Table 5.2 Antioxidant activity and total phenolic contents of different tea infusions were evaluated by ORAC, FRAP, DPPH radical scavenging and Folin–Ciocalteu assays

Type of teas	Abbreviation	Antioxidant activity			Folin-Ciocalteu assay (mg GAE/g)
		DPPH assay (% scavenging)	ORAC assay (µmol TE/g)	FRAP assay (µmol TE/g)	
Conventional tea					
Green tea	GT	92.35 ± 0.63^{a}	1828.87 ± 141.53^{b}	620.97 ± 18.39^{b}	54.03 ± 2.39^{c}
White tea	WT	92.23 ± 1.00^{a}	1268.91 ± 26.54^{c}	650.18 ± 37.72^{b}	47.98 ± 1.30^{d}
Oolong tea	OT	89.29 ± 1.02^{b}	723.96 ± 22.46^{d}	357.79 ± 12.33^{d}	37.67 ± 2.27^{f}
Black tea	BT	93.27 ± 0.39^{a}	498.77 ± 3.22^{d}	167.08 ± 17.94^{e}	15.66 ± 2.09^{h}
Pu-erh tea	PT	89.94 ± 0.73^{b}	1243.00 ± 84.30^{c}	352.05 ± 8.20^{d}	36.71 ± 0.19^{f}
Thai herbal tea					
Bael fruit	BF	77.28 ± 2.32^{c}	2070.51 ± 358.72^{b}	87.79 ± 5.25^{f}	41.97 ± 2.45^{e}
Indian gooseberry	IG	96.43 ± 0.13^{a}	1753.82 ± 17.05^{b}	1520.76 ± 67.41^{a}	126.45 ± 4.85^{a}
Bitter cucumber	BC	25.99 ± 1.84^{g}	68.29 ± 0.92^{f}	4.63 ± 0.58^{g}	3.51 ± 0.33^{i}
Rosella	RS	74.67 ± 4.05^{c}	362.31 ± 16.21^{e}	85.12 ± 3.16^{f}	14.13 ± 2.06^{h}

All analytical data were mean values of three independent sample (n=3) ± standard deviation.
Values with different letter (a–i) within the same column were significantly different with $p < 0.05$.
TE: Trolox equivalent, GAE: Gallic acid equivalent

Table 5.2 Antioxidant activity and total phenolic contents of different tea infusions were evaluated by ORAC, FRAP, DPPH radical scavenging and Folin–Ciocalteu assays (cont.)

Type of teas	Abbreviation	Antioxidant activity			Folin-Ciocalteu assay (mg GAE/g)
		DPPH assay (% scavenging)	ORAC assay (µmol TE/g)	FRAP assay (µmol TE/g)	
Thai herbal tea					
Safflower	SF	75.60 ± 1.42^{c}	1103.71 ± 44.23^{c}	72.18 ± 2.23^{f}	22.25 ± 1.43^{g}
Chrysanthemum	CS	86.40 ± 1.14^{b}	1019.86 ± 55.85^{c}	210.95 ± 3.78^{e}	22.65 ± 0.56^{g}
White mulberry	MB	51.02 ± 2.28^{e}	481.23 ± 15.44^{d}	76.01 ± 2.64^{f}	12.61 ± 0.17^{h}
Asiatic pennywort	AP	87.77 ± 2.77^{b}	305.53 ± 41.42^{e}	75.62 ± 3.82^{f}	8.19 ± 0.81^{i}
Pandanus	PD	56.13 ± 1.34^{d}	173.51 ± 21.22^{e}	18.42 ± 1.93^{g}	7.62 ± 0.20^{i}
Jiaogulan	JL	58.49 ± 3.62^{d}	262.63 ± 20.63^{e}	25.78 ± 3.75^{f}	3.09 ± 0.37^{i}
Stevia	ST	87.77 ± 2.77^{b}	3281.77 ± 138.23^{a}	549.74 ± 9.64^{c}	78.05 ± 1.41^{b}
Cat's whisker	CW	90.32 ± 0.96^{b}	2030.11 ± 47.95^{b}	328.81 ± 9.64^{d}	44.64 ± 2.70^{e}
Lemon grass	LG	38.91 ± 2.01^{f}	99.74 ± 12.19^{e}	22.74 ± 0.92^{f}	5.46 ± 0.20^{i}
Jewel vine	JV	75.07 ± 1.14^{c}	337.76 ± 32.02^{e}	43.48 ± 2.90^{f}	11.16 ± 0.41^{h}
Ginger	GG	51.82 ± 1.05^{e}	500.42 ± 96.77^{d}	12.70 ± 0.89^{f}	5.86 ± 0.29^{i}

All analytical data were mean values of three independent sample (n=3) ± standard deviation.
Values with different letter (a–i) within the same column were significantly different with $p < 0.05$.
TE: Trolox equivalent, GAE: Gallic acid equivalent

5.2 Anti–glycation Activity

The anti–glycation activity was evaluated according to the ability to fight against fluorescent AGEs formation in BSA/glucose and BSA/MG systems. It was found that all tea infusions were able to inhibit the formation of fluorescent AGEs in a dose–dependent manner in both systems as being represented as IC_{50} (concentration of tea extract that inhibited the glucose– or MG–mediated formation of fluorescent AGEs by 50%) (Table 5.3). Anti-glycation activity using BSA/glucose and BSA/MG systems of five conventional teas were in a range of IC_{50} 0.45–1.08 and 0.77–1.39 μg/mL, respectively. Among five conventional teas, white tea exhibited the highest anti–glycation capacity in both systems, followed by green tea, oolong tea ~ pu-erh tea and black tea, respectively.

Likewise, the anti–glycation capacities of fifteen Thai herbal teas were investigated in comparison to those of five conventional teas (Table 5.3). The results suggested that IC_{50} of all Thai herbal teas in BSA/glucose and BSA/MG systems were in a range of 0.12–1.29 and 0.82–1.72 μg/mL, respectively. Among these herbal teas, stevia exhibited the strongest anti–glycation capacity in both systems. Interestingly, the IC_{50} values of stevia were even lower than those of conventional teas in both glycation systems, suggesting that it could effectively function against glycation with a greater capacity than conventional teas. Moreover, cat's whisker and Indian gooseberry also possessed the IC_{50} values that were comparable to those of conventional teas. Indian gooseberry possessed comparable anti–glycation capacities to those of green tea in both glycation systems. Anti–glycation capacity of cat's whisker, however, was lower than those of conventional teas in BSA-glucose system but was higher in BSA-MG system. On the contrary, tea extracts from bael fruit, bitter cucumber, white mulberry, Asiatic pennywort, pandanus, jiaogulan, lemon grass, jewel vine and ginger possessed only trace anti–glycation activity and were incapable of being monitored IC_{50} values under experimental conditions (concentration of tea extract was 0–3.33 mg/mL).

Table 5.3 Inhibitory activities of different tea infusions on the formation of AGEs were represented as IC_{50} values as being investigated in BSA–glucose and BSA–methylglyoxal systems.

Type of teas	Abbreviation	IC_{50} (mg/mL)	
		BSA–glucose system	**BSA–methylglyoxal system**
Conventional tea			
Green tea	GT	0.5045 ± 0.03	0.9354 ± 0.02
White tea	WT	0.4574 ± 0.02	0.7757 ± 0.01
Oolong tea	OT	0.9033 ± 0.04	1.3486 ± 0.04
Black tea	BT	1.0837 ± 0.02	1.3924 ± 0.05
Pu–erh tea	PT	0.9254 ± 0.04	1.3234 ± 0.02
Thai herbal tea			
Bael fruit	BF	NA	NA
Indian gooseberry	IG	0.4993 ± 0.02	1.0138 ± 0.02
Bitter cucumber	BC	NA	NA
Rosella	RS	0.8759 ± 0.01	3.0364 ± 0.02
Safflower	SF	1.1193 ± 0.05	1.6183 ± 0.01
Chrysanthemum	CS	0.6947 ± 0.02	1.7237 ± 0.03
White mulberry	MB	NA	NA
Asiatic pennywort	AP	NA	NA
Pandanus	PD	0.8233 ± 0.01	NA
Jiaogulan	JL	1.2958 ± 0.03	NA
Stevia	ST	0.1200 ± 0.02	0.8264 ± 0.01
Cat's whisker	CW	0.2361 ± 0.02	1.6175 ± 0.03
Lemon grass	LG	NA	NA
Jewel vine	JV	NA	NA
Ginger	GG	NA	NA

All analytical data were mean values of three independent samples (n=3) ± standard deviation.
IC_{50} is the concentrations of tea extracts that inhibited the glucose– or methyglyoxal–mediated formation of AGEs by 50%.
NA: Not assessable

5.3 Lipase Inhibitory Activity

5.3.1 Aqueous ethanol extracted tea

The lipase inhibitory activity of different teas extracted with 50% (v/v) aqueous ethanol (Table 5.4) suggested that conventional teas exhibited a narrow range of lipase inhibitory activity (66–85% inhibition) with black tea and pu–erh tea providing the highest lipase inhibitory activities, followed by green tea, white tea and oolong tea, respectively (Figure 5.3). These inhibitory activities could be compared to the relative concentration of orlistat, a commercial lipase inhibitor, using a standard plot of orlistat concentrations and their corresponded percentages of lipase inhibition (Figure 5.4). As results, it was suggested that the lipase inhibitions of conventional teas were equivalent to 1115–4610 nM of orlistat that used to inhibit lipase enzyme under similar conditions. On the contrary, the comparison of IC_{50} (concentrations of tea extracts that inhibited lipase activities by 50%) suggested that green tea, white tea and oolong tea (IC_{50} of 0.06–0.08 mg/mL) were more effective toward lipase inhibitory activity than black tea and pu–erh tea (IC_{50} of 0.27–0.36 mg/mL). Nevertheless, these IC_{50} values were 500–3000 folds higher than that of orlistat (0.24 μM or 0.12 μg/mL) as being investigated under similar experimental conditions.

Likewise, lipase inhibitory activities of fifteen Thai herbal teas were investigated in comparison to five conventional teas. The results showed that ginger exhibited the highest lipase inhibitory activity (~72% inhibition), followed by cat's whisker and stevia (62 and 49% inhibition, respectively). These herbal teas provided comparable lipase inhibitory activities to those of conventional teas, which were equivalent to 500–1565 nM of orlistat. The IC_{50} values of these teas were in a range of 0.92–1.15 mg/mL. On the other hand, Indian gooseberry herbal tea that provided the highest antioxidant activities and total phenolic contents exerted the lowest lipase inhibitory activity among fifteen Thai herbal tea extracts (34% inhibition) with the IC_{50} of 2.45 mg/mL and an equivalent 263 nM of orlistat (Figure 5.3).

Table 5.4 Lipase inhibitory activities of different teas extracted with 50% (v/v) aqueous ethanol

Type of teas*	Abbreviation	% Inhibition of lipase activity	Relative to orlistat concentration (nM)	IC_{50} (mg/mL)
Conventional tea				
Green tea	GT	73.95 ± 1.93[b]	1739.02	0.08 ± 0.01
White tea	WT	79.84 ± 2.53[b]	2142.75	0.06 ± 0.01
Oolong tea	OT	66.33 ± 0.83[c]	1115.52	0.08 ± 0.01
Black tea	BT	84.79 ± 2.41[a]	4610.82	0.36 ± 0.05
Pu–erh tea	PT	84.11 ± 3.41[a]	4226.72	0.27 ± 0.03
Thai herbal tea				
Bael fruit	BF	53.29 ± 0.24[d]	602.35	1.53 ± 0.09
Indian gooseberry	IG	34.34 ± 1.29[f]	262.89	2.45 ± 0.17
Bitter cucumber	BC	40.60 ± 0.23[e]	347.84	2.29 ± 0.18
Rosella	RS	51.59 ± 3.07[d]	559.24	1.70 ± 0.01
Safflower	SF	53.14 ± 3.83[d]	598.28	1.53 ± 0.06
Chrysanthemum	CS	52.88 ± 0.86[d]	591.61	1.03 ± 0.10
White mulberry	MB	50.74 ± 2.67[d]	538.89	1.63 ± 0.01
Asiatic pennywort	AP	54.59 ± 0.62[d]	637.75	1.21 ± 0.01
Pandanus	PD	46.43 ± 2.02[e]	447.63	1.89 ± 0.55
Jiaogulan	JL	41.57 ± 0.55[e]	362.83	2.40 ± 0.05
Stevia	ST	49.01 ± 1.27[d]	500.13	1.51 ± 0.01
Cat's whisker	CW	61.94 ± 0.38[c]	894.56	1.18 ± 0.01
Lemon grass	LG	46.82 ± 0.50[e]	455.23	2.17 ± 0.05
Jewel vine	JV	53.32 ± 1.19[d]	602.98	1.30 ± 0.01
Ginger	GG	72.29 ± 2.37[b]	1564.63	0.92 ± 0.05

*Extractions were performed under 50% (v/v) aqueous ethanol at 30 °C for 2 hours.

All analytical data were mean values of three independent sample (n=3) ± standard deviation.

Values with different letter (a–i) within column of each sample were significantly different with $p < 0.05$.

"–" indicates a promotion of lipase activity and the final concentration of the extracts used in this screening was 1.67 mg/mL

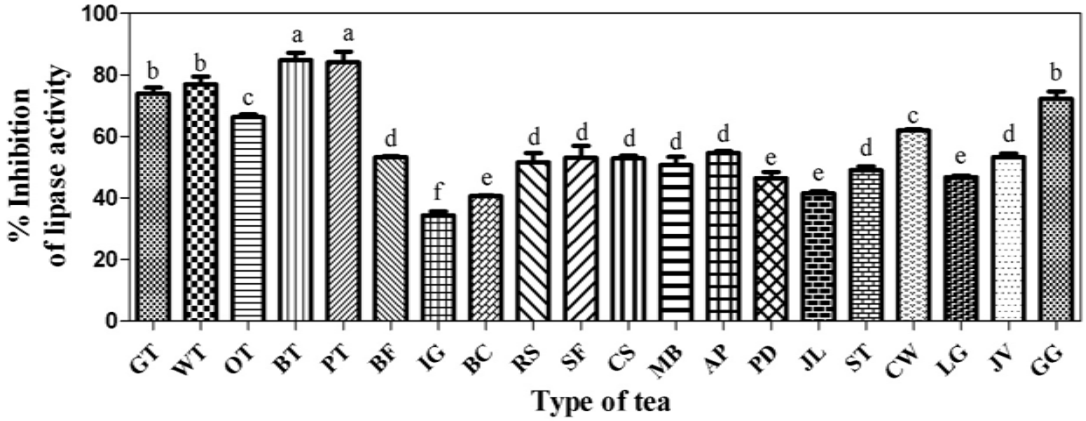

Figure 5.3 Lipase inhibitory activities of different teas extracted with 50% (v/v) aqueous ethanol were expressed as inhibitory percentage of lipase activity. All analytical data were mean values of three independent sample (n=3) ± standard deviation. The values with different letters (a–f) within the bars were significantly different ($p < 0.05$). The final concentration of the extracts used in this screening was 1.67 mg/mL. Abbreviations of all tea extracts were referred to Table 5.4.

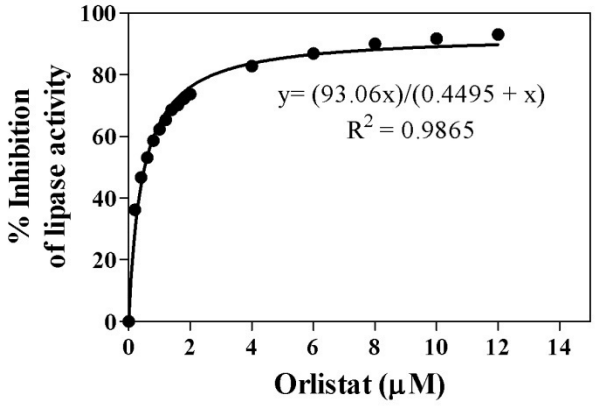

Figure 5.4 A standard plot of lipase inhibitory activities that were corresponded to different orlistat concentrations, a commercial lipase inhibitor, were expressed as inhibitory percentage of lipase activity.

5.3.2 Tea infusions

The lipase inhibitory activity of tea infusions (1 mg/mL) was followed the similar trend as those of aqueous ethanol extracted teas. It was suggested that lipase inhibitory activities of five conventional tea infusions were in the range of 47–66% inhibition with green tea, white tea and oolong tea exhibiting higher inhibitory activity than those of pu–erh tea and black tea (Table 5.5, Figure 5.5). These inhibitions were equivalent to 464–1103 nM of orlistat with the IC_{50} values of 0.12–0.93 mg/mL.

Among fifteen Thai herbal teas, tea infusions of stevia and cat's whisker showed the highest lipase inhibitory activities (22% and 18%, respectively) with the equivalent 109–145 nM of orlistat. Unlike its aqueous ethanol extracted counterpart with the highest lipase inhibitory activity among Thai herbal teas, ginger tea infusion only provided trace inhibitory activity against lipase enzyme (7% inhibition with equivalent 35 nM of orlistat). Safflower tea infusion, on the other hand, exerted the lowest activity (–9% inhibition), which seemed to promote enzymatic reaction of lipase. Nevertheless, all herbal teas were incapable of detecting IC_{50} value under experimental conditions, which might be due to low concentration of tea extracts (0–2 mg/mL).

Table 5.5 Lipase inhibitory activities of different tea infusions

Type of teas	Abbreviation	% Inhibition of lipase activity	Relative to orlistat concentration (nM)	IC_{50} (mg/mL)
Conventional tea				
Green tea	GT	66.12 ± 2.75[a]	1103.09	0.18 ± 0.01
White tea	WT	64.73 ± 1.63[a]	1026.93	0.12 ± 0.01
Oolong tea	OT	63.56 ± 0.91[a]	968.34	0.26 ± 0.03
Black tea	BT	47.26 ± 5.56[c]	463.92	0.93 ± 0.08
Pu–erh tea	PT	52.83 ± 2.63[b]	590.32	0.69 ± 0.01
Thai herbal tea				
Bael fruit	BF	6.51 ± 0.28[e]	33.79	NA
Indian gooseberry	IG	4.65 ± 0.63[f]	23.64	NA
Bitter cucumber	BC	4.81 ± 0.92[f]	24.63	NA
Rosella	RS	10.95 ± 0.71[e]	59.96	NA
Safflower	SF	– 9.24 ± 0.41[g]	–40.59	NA
Chrysanthemum	CS	9.42 ± 0.29[e]	50.63	NA
White mulberry	MB	7.14 ± 1.07[e]	37.35	NA
Asiatic pennywort	AP	8.06 ± 1.64[e]	42.62	NA
Pandanus	PD	5.59 ± 0.50[f]	28.72	NA
Jiaogulan	JL	3.26 ± 0.24[f]	16.33	NA
Stevia	ST	22.71 ± 0.89[d]	145.10	NA
Cat's whisker	CW	18.20 ± 0.47[d]	109.28	NA
Lemon grass	LG	3.56 ± 0.25[f]	17.86	NA
Jewel vine	JV	7.38 ± 1.08[f]	38.73	NA
Ginger	GG	6.67 ± 0.12[e]	34.72	NA

All analytical data were mean values of three independent sample (n=3) ± standard deviation

Values with different letter (a–i) within column of each sample were significantly different with $p < 0.05$

"–" indicates a promotion of lipase activity and the final concentration of the extracts used in this screening was 1 mg/mL.

NA: Not assessable

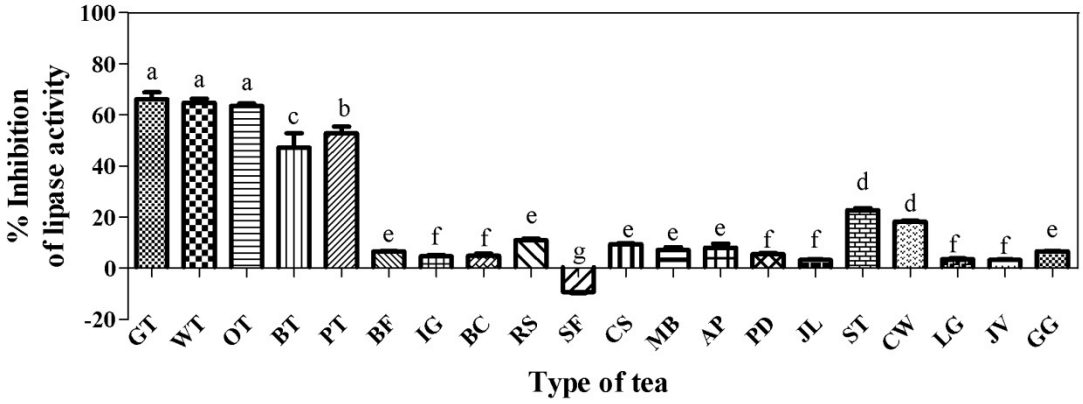

Figure 5.5 Lipase inhibitory activities of different tea infusions were expressed as inhibition percentage of lipase activity. The values with different letter (a–g) within the bars were significantly different ($p < 0.05$). All analytical data were mean values of three independent sample (n=3) ± standard deviation. The final concentration of the extracts used in this screening was 1 mg/mL. Abbreviations of all tea extracts were referred to Table 5.5.

5.4 ACE Inhibitory Activity

5.4.1 Aqueous ethanol extracted tea

The inhibition of ACE activity of different teas (0.835 mg/mL) extracted with 50% (v/v) aqueous ethanol suggested that all conventional teas exhibited high ACE inhibitory activities (>85% inhibition) (Table 5.6). However, unlike other biological properties of conventional teas that highly related to the quantity of antioxidants and phenolic compounds, these results could not be differentiated between samples that undergo different fermentation stages (Figure 5.6).

These inhibitory activities could be compared to the relative concentration of lisinopril, a commercial ACE inhibitor, using a standard plot of ACE concentrations and their corresponded percentages of ACE inhibition (Figure 5.7). As results, it was suggested that the ACE inhibitions of conventional teas were equivalent to 12–29 nM of lisinopril that used to inhibit ACE under similar conditions.

Likewise, the ACE inhibitory activities of fifteen Thai herbal teas were investigated in comparison to five conventional teas (Figure 5.6). Interestingly, among fifteen Thai herbal teas, safflower, stevia, cat's whisker and chrysanthemum showed the highest inhibitory activities (81–89% inhibition). These herbal teas provided comparable ACE inhibitory activities to those of conventional teas, which were equivalent to 10–16 nM of lisinopril. On the other hand, bitter cucumber herbal tea exerted the lowest inhibitory activity (19% inhibition) with the equivalent 1 nM of lisinopril.

Table 5.6 ACE inhibitory activities of different teas extracted with 50% (v/v) aqueous ethanol

Type of teas*	Abbreviation	% Inhibition of ACE activity	Relative to lisinopril concentration (nM)
Conventional tea			
Green tea	GT	91.29 ± 2.09[a]	18.70
White tea	WT	92.35 ± 1.19[a]	20.43
Oolong tea	OT	93.02 ± 0.80[a]	21.67
Black tea	BT	95.90 ± 1.34[a]	28.98
Pu–erh tea	PT	85.48 ± 0.11[a]	12.45
Thai herbal tea			
Bael fruit	BF	50.82 ± 1.65[c]	2.72
Indian gooseberry	IG	62.96 ± 8.27[c]	4.33
Bitter cucumber	BC	19.11 ± 2.27[e]	0.65
Rosella	RS	37.35 ± 2.52[d]	1.60
Safflower	SF	86.04 ± 3.00[ab]	12.89
Chrysanthemum	CS	81.42 ± 5.42[b]	9.87
White mulberry	MB	39.46 ± 6.27[d]	1.75
Asiatic pennywort	AP	57.89 ± 2.03[c]	3.56
Pandanus	PD	30.27 ± 3.02[d]	1.18
Jiaogulan	JL	30.03 ± 3.98[d]	1.16
Stevia	ST	89.28 ± 0.59[ab]	16.03
Cat's whisker	CW	88.33 ± 3.53[ab]	14.99
Lemon grass	LG	61.45 ± 5.04[c]	4.08
Jewel vine	JV	26.14 ± 4.47[d]	0.96
Ginger	GG	48.74 ± 1.28[c]	2.51

*Extractions were performed under 50% (v/v) aqueous ethanol at 30 °C for 2 hours.
All analytical data were mean values of three independent sample (n=3) ± standard deviation
Values with different letter (a–e) within column of each sample were significantly different at $p < 0.05$
The final concentration of the extracts used in this screening was 0.835 mg/mL.

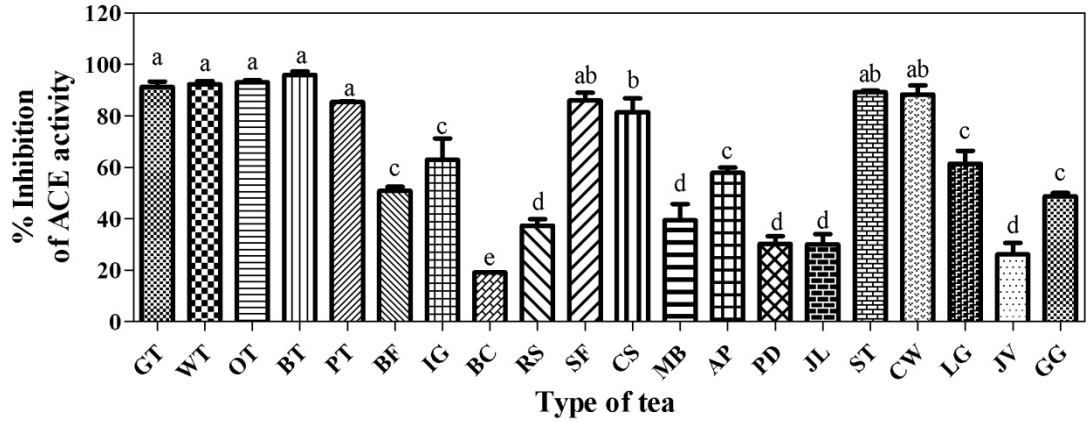

Figure 5.6 ACE inhibitory activities of different teas extracted with 50% (v/v) aqueous ethanol were expressed as inhibitory percentage of ACE activity. All analytical data were mean values of three independent sample (n=3) ± standard deviation. The values with different letters (a–e) within the bars were significantly different ($p < 0.05$). The final concentration of the extracts used in this screening was 0.835 mg/mL. Abbreviations of all tea extracts were referred to Table 5.6.

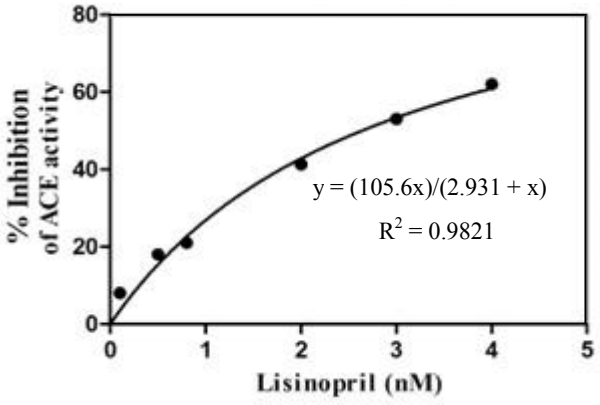

Figure 5.7 A standard plot of ACE inhibitory activities that were corresponded to different lisinopril concentrations, a commercial ACE inhibitor, were expressed as inhibitory percentage of ACE activity.

5.4.2 Tea infusions

The inhibition of ACE activity of different tea infusions (0.5 mg/mL) suggested that among five conventional teas, green tea and white tea exhibited the highest ACE inhibitory activities (>90% inhibition), followed by oolong tea, black tea ~ pu–erh tea, respectively (Figure 5.8). These inhibitions were equivalent to 6–28 nM of lisinopril (Table 5.7). These results were followed the similar trends as other biological properties of the conventional teas, where fermentation processes seemed to affect their activities against the disease-controlled enzymes.

Likewise, the results of ACE inhibitory activity of herbal teas infusions suggested that stevia, Asiatic pennywort and cat's whisker provided high anti-ACE activity with 80–88% inhibition with the equivalent 9–15 nM of lisinopril. Stevia with the highest ACE inhibitory capacity among all herbal teas might be the comparable source of anti-ACE agents as green tea and white tea. In addition, Asiatic pennywort and cat's whisker herbal teas showed superior ACE inhibitory activity to black tea and pu–erh tea. On the other hand, bitter cucumber herbal tea was found to exert the lowest activity (5% inhibition).

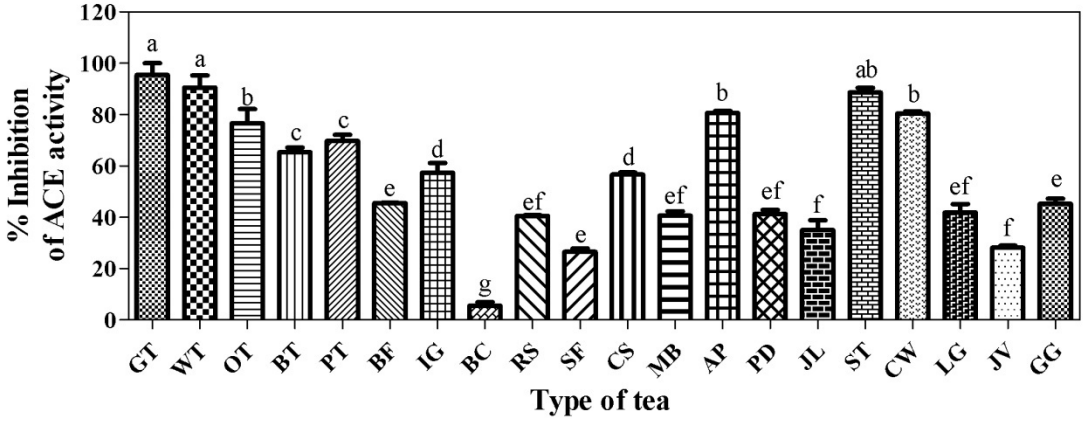

Figure 5.8 ACE inhibitory activities of different teas infusions were expressed as inhibition percentage of ACE activity. The values with different letter (a–g) within the bars were significantly different ($p < 0.05$). All analytical data were mean values of three independent sample (n=3) ± standard deviation. The final concentration of the extracts used in this screening was 0.5 mg/mL. Abbreviations of all tea extracts were referred to Table 5.7.

Table 5.7 ACE inhibitory activities of different tea infusions

Type of teas	Abbreviation	% Inhibition of ACE activity	Relative to lisinopril concentration (nM)
Conventional tea			
Green tea	GT	95.49 ± 4.54[a]	27.68
White tea	WT	90.55 ± 4.67[a]	17.63
Oolong tea	OT	76.64 ± 5.51[b]	7.76
Black tea	BT	65.13 ± 1.79[c]	4.72
Pu–erh tea	PT	69.78 ± 2.34[c]	5.71
Thai herbal tea			
Bael fruit	BF	45.59 ± 0.12[e]	2.23
Indian gooseberry	IG	57.40 ± 3.75[d]	3.49
Bitter cucumber	BC	5.53 ± 1.37[g]	0.16
Rosella	RS	40.56 ± 0.37[ef]	1.83
Safflower	SF	26.54 ± 1.31[e]	0.98
Chrysanthemum	CS	56.65 ± 0.82[d]	3.39
White mulberry	MB	40.68 ± 1.70[ef]	1.84
Asiatic pennywort	AP	80.65 ± 0.76[b]	9.47
Pandanus	PD	41.24 ± 1.63[ef]	1.88
Jiaogulan	JL	35.00 ± 3.89[f]	1.45
Stevia	ST	88.63 ± 1.84[ab]	15.31
Cat's whisker	CW	80.41 ± 0.83[b]	9.36
Lemon grass	LG	41.87 ± 3.26[ef]	1.93
Jewel vine	JV	28.16 ± 0.77[f]	1.07
Ginger	GG	45.25 ± 1.99[e]	2.20

All analytical data were mean values of three independent sample (n=3) ± standard deviation

Values with different letter (a–g) within column of each sample were significantly different at $p < 0.05$

The final concentration of the extracts used in this screening was 0.5 mg/mL.