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ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICALS OF EDIBLE FLOWERS

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INTRODUCTION

Plants are major sources of nutrition and vitamins for human life. Every parts of the plant such as leaves, flowers, fruits, roots and rhizomes have been used as food or health supplements. Some edible flowers are now more popularly consumed in the form of drinks, jellies, salads and main dishes. However, there are some Thai traditional plants which their flowers are edible for a long time, but there is no report concerning about the phytochemicals and biological activities of them. Therefore, this experiment was set up in order to screen the free radical scavenging activities of extracts from 11 edible flowers using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Then, quantitative analysis of total phenolic and total flavonoid contents of the extracts using spectrophotometric methods was conducted. Finally, phytochemical analysis of all flower extracts by thin layer chromatography was performed.

MATERIALS AND METHODS

Plant materials Eleven edible flowers from various plants including broccoli, (*Brassica oleracea*), cauliflower (*B. oleracea*), Chinese cabbage (*B. chinensis* var. *parachinensis*), tamarind (*Tamarindus indica*), banaba (*Musa sp.*), rose (*Rosa sp.*), ixora (*Ixora sp.*), cowslip creeper (*Telosma minor*), agasta (*Sesbania grandiflora*), Siamese neem tree (*Azadirachta indica* var. *siamensis*) and garlic chive (*Allium tuberosum*) were purchased from local market, Bangkok, Thailand in June 2013. The samples were cleaned and dried in hot air oven (50°C) for 6 hours and powdered with electronic mill (20 mesh sieve). Each sample was prepared using the extraction procedures shown below.

Decoction: Dried powder of each flower sample was separately boiled (80°C) with distilled water (plant/water ratio = 1:10 w/v) for 3 h, then filtered. The filtrate was dried upon a water bath to obtain dried decoction extract.

Maceration: Dried powder of each flower sample was separately macerated with 95% ethanol (plant/water ratio = 1:10 w/v) for 7 day with frequent shaking, then filtered. The filtrate was dried upon a water bath to obtain dried ethanol extract.

Determination of free radical scavenging activity using DPPH scavenging assay The free radical scavenging effect of flower extracts as well as standard trolox corresponding to the quenching ability of 1,1-diphenyl-2-picryl hydrazyl (DPPH) was carried out as described by Yamasaki et al.[1]. Each sample was assayed in triplicate and the average percentage of inhibition at the concentration of 100 µg/ml was calculated.

Determination of total phenolic content using Folin-Ciocalteu method Using the method modified from Naithani et al.[2], solutions of plant extracts were oxidized with Folin-Ciocalteu reagent and the reactions were neutralized by sodium carbonate solution. The absorbance of the resulting blue colored solution was measured at 765 nm after 60 min. Each sample was assayed in duplicate. Total phenolic content was expressed as g gall acid equivalent in 100 g extract (g% GAE).

Determination of total flavonoid content Total flavonoid content was investigated using the method previously described [3]. Solutions of plant extracts were reacted with an equal volume of aluminium chloride solution. Absorbances were read at 415 nm after 10 min, and flavonoid con tent was expressed as grams quercetin equivalent in 100 g of plant extracts (g% QE).

Thin layer chromatographic fingerprints Thin layer chromatography of all flower extracts was performed on TLC pre-coated silica gel 60 GF_{254} plate, using ethyl acetate-formic acid-acetic acid-water (137:11:11:26) as the solvent system. TLC plates were detected under UV at 254 and 366 nm, NP/PEG and DPPH spray reagents.

RESULTS

Free radical scavenging activity: DPPH scavenging assay Comparing among all flower extracts at the concentration of 100 µg/ml, decoction and maceration extracts from the flowers of rose, ixora and Siamese neem tree exhibited high free radical scavenging activity. Rose flower extracts prepared from

decoction and maceration showed the strongest free radical scavenging activity with percentages of inhibition of 90.25 ± 0.52 and 94.00 ± 0.98 µg/ml, respectively (Table 1).

Determination of total phenolic content: Folin-Ciocalteu method As shown in Table 1, rose flower decoction extract exhibited the highest total phenolic content of 23.03 ± 0.93 g% GAE. Other extracts which displayed high total phenolic contents were decoction extract from the flowers of Siamese neem tree and maceration extracts from the flowers of banana and garlic chive, with total phenolic contents of 18.05 ± 0.76, 17.73 ± 2.82 and 20.34 ± 3.92 g% GAE in the dried extracts, respectively.

Determination of total flavonoid: aluminium chloride method Rose flower decoction extract also contained the highest amount of total flavonoids (2.60 ± 0.09 g% QE), followed by the maceration and decoction extracts from the flowers of Siamese neem tree (Table 1). From these results, DPPH scavenging activities of decoction extracts showed higher correlations to total phenolic and total flavonoid contents, with coefficient determination values (R^2) of 0.664 and 0.455, respectively, while maceration extracts displayed low correlation ($R^2 < 0.2$) between the activities and total phenolic and flavonoid contents.

Thin layer chromatographic fingerprints As shown in Figure 1, the decoction extracts from 11 edible flowers showed specific TLC fingerprints to NP/PEG spraying reagent. The rose and ixora flower extracts (track numbers 8 and 10) displayed chromatographic bands corresponding to standard phenolics and flavonoids, including quercetin and chlorogenic acid which are antioxidant compounds, suggesting their influences on the strong *in vitro* antioxidant effects.

Table 1 Free radical scavenging activity, total phenolic and total flavonoid contents of extracts from 11 edible flowers

No.	Sample	Extraction solvent	Yield (%w/w)	DPPH scavenging activity*	Total phenolic (g% GAE)	Total flavonoid (g% QE)
1	Chinese cabbage	Water	1.46	17.65 ± 1.86	5.79 ± 1.17	0.72 ± 0.13
		Alcohol	0.89	13.85 ± 2.57	8.09 ± 1.47	1.29 ± 0.17
2	Banana	Water	1.34	8.11 ± 1.11	2.39 ± 0.39	0.72 ± 0.17
		Alcohol	0.65	Inactive	**17.73 ± 2.82**	0.53 ± 0.17
3	Cowslip creeper	Water	3.17	13.68 ± 3.61	4.34 ± 0.03	0.96 ± 0.06
		Alcohol	1.59	2.08 ± 0.66	6.83 ± 0.15	0.85 ± 0.14
4	Rose	Water	3.00	**90.25 ± 0.52**	**23.03 ± 0.93**	**2.60 ± 0.09**
		Alcohol	1.64	**94.00 ± 0.98**	13.46 ± 0.57	0.93 ± 0.47
5	Tamarind	Water	4.70	23.06 ± 0.93	3.93 ± 0.39	0.61 ± 0.10
		Alcohol	4.81	26.69 ± 5.42	7.47 ± 0.53	0.88 ± 0.01
6	Ixora	Water	3.99	**73.15 ± 8.41**	14.41 ± 0.70	0.96 ± 0.47
		Alcohol	2.41	**94.24 ± 0.49**	14.03 ± 1.22	0.73 ± 0.06
7	Broccoli	Water	2.19	21.58 ± 0.31	5.34 ± 0.80	0.47 ± 0.08
		Alcohol	1.33	1.18 ± 0.34	5.95 ± 1.80	0.28 ± 0.05
8	Cauliflower	Water	3.14	20.72 ± 1.87	5.15 ± 0.44	0.63 ± 0.08
		Alcohol	0.93	9.99 ± 0.61	11.83 ± 1.70	1.35 ± 0.12
9	Garlic chive	Water	0.48	19.62 ± 0.61	5.76 ± 0.51	0.72 ± 0.17
		Alcohol	0.53	2.27 ± 1.50	**20.34 ± 3.92**	1.11 ± 0.06
10	Agasta	Water	2.08	21.97 ± 1.30	6.95 ± 0.39	1.10 ± 0.10
		Alcohol	2.50	5.20 ± 1.52	7.68 ± 2.72	1.29 ± 0.01
11	Siamese neem tree	Water	3.34	**84.91 ± 0.10**	**18.05 ± 0.76**	**1.45 ± 0.03**
		Alcohol	1.35	29.28 ± 1.75	8.87 ± 2.20	**2.26 ± 0.03**
	Trolox	-	-	29.98 ± 0.63**	-	-

*% inhibition at the concentration of 100 µg/ml, ** IC_{50} value

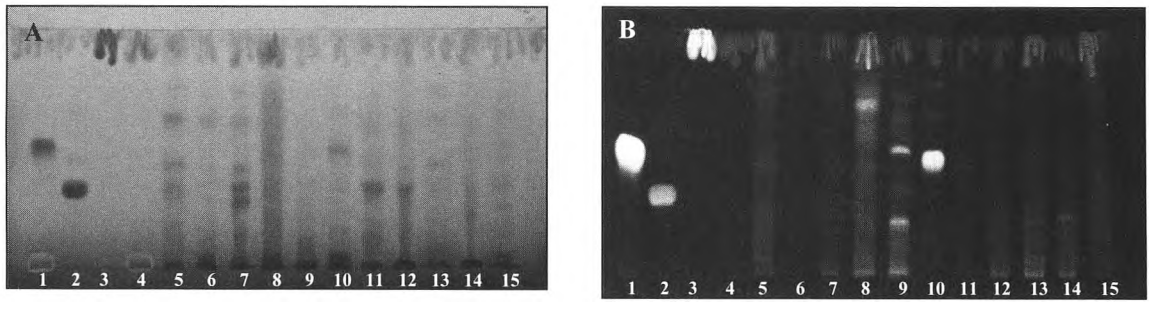

Figure 1 TLC fingerprints of decoction extracts from 11 edible flowers; 1 = standard chlorogenic acid, 2 = standard rutin, 3 = standard quercetin, 4 = standard gallic acid, 5-15 = water extracts; 5 = Chinese cabbage, 6 = banana, 7 = cowslip creeper, 8 = rose, 9 = tamarind, 10 = ixora, 11 = broccoli, 12 = cauliflower, 13 = garlic chive, 14 = agasta, 15 = Siamese neem tree
Stationary phase: silica gel GF_{245}
Solvent system: ethyl acetate-formic acid-acetic acid-water (137:11:11:26)
Detection: A= UV 254 nm, B= NP/PEG spraying reagent under UV 366 nm

DISCUSSION

All extracts from edible flowers exhibited *in vitro* free radical scavenging activities and contained high amounts of phenolic and flavonoid compounds. The antioxidant activities can be correlated to the phenolic and flavonoid contents, especially in decoction extracts. This result suggested that decoction could be the suitable extraction method to prepare health supporting products from flowers. Extracts from rose and ixora flowers showed the bands corresponding to flavonoid and phenolic acid which could be responsible for their strong free radical scavenging activities. Moreover, these two flowers have pink to red color, indicating the presence of anthocyanins which could also promote antioxidant effects.

CONCLUSION

Thai edible flowers showed *in vitro* free radical scavenging activities, suggesting health promotion through their consumption. Spectrophotometric and thin layer chromatographic results indicated the presence of phenolic and flavonoid compounds. Rose and ixora flowers exhibited the strongest antioxidant effects with high phenolic and flavonoid contents, and should therefore be studied for their phytochemicals and other related biological activities.

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