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Phenolic compounds and antioxidant activities of edible flowers from Thailand

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ABSTRACT

We investigated the phenolic compounds and antioxidant capacities of free and bound phenolics from 12 available Thai edible flowers which have long been consumed as vegetable and used as ingredients in cooking. *Cassia siamea* showed the highest value of total phenolic content (TPC) (88 mg gallic acid equivalents (GAE)/g dry weight). *Tagetes erecta* had the highest total flavonoid content (TFC) (68.9 mg RE/g dry weight). *Antigonon leptopus* and *T. erecta* had the highest ferric reducing antioxidant power (FRAP) value (62.0 and 60 mmol$FeSO_4$/g 100 dry weight). Major phenolic acids identified in these analyses were gallic acid, ferulic acid and sinapic acid, while predominant flavonoids were quercetin and rutin. The results of this study showed that soluble as well as bound fractions of edible flowers are rich sources of phenolic compounds with antioxidant, *DPPH radical-scavenging activity* and reducing power. This study has provided useful information for screening edible flowers as potential sources of bioactive components with high antioxidant properties that may be of interest to consumers and public health workers.

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1. Introduction

Consumption of various types of fruits and vegetables provides excellent health benefits because they are a rich source of phytochemicals that are good for disease risk reduction. High intake of fruits and vegetables has been reported to be associated with a lower incidence of chronic diseases such as cardiovascular disease (Hu, 2003; Ikram et al., 2009) and cancer (Ikram et al., 2009; Riboli & Norat, 2003). These health benefits are attributed to the antioxidant capacity derived from the phenolic compounds present in edible plants (Salta et al., 2010). Phenolic compounds are a large and diverse group of phytochemicals, which includes many different families of aromatic secondary metabolites in plants (Harborne & Williams, 2000). They exist in three forms, namely, free, soluble conjugated and insoluble bound; this last form is found in dietary fiber (Sosulski, Krygier, & Hogge, 1982). They are known to exert various physiological effects in humans, such as inhibiting platelet aggregation (Daniel, Meier, Schlatter, & Frischknecht, 1999), reducing the risk of coronary heart disease and cancer and preventing oxidative damage of lipid and low-density lipoprotein (Morton, Abu-Amsha, Puddey, & Croft, 2000; Shahidi, 2000; Shui & Leong, 2006). Phenolic compounds have strong *in vitro* and *in vivo* antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions and chelate metals (Shahidi & Naczk, 2004). Increased consumption of phenolic compounds has been correlated with a reduced risk of cardiovascular disease

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and certain cancers (Barreira, Ferreira, Oliveira, & Pereira, 2008). Flavonoids and other classes of phenolic compounds are important phytochemicals (Johnson, 2001; Meyers, Watkins, Pritts, & Liu, 2003). Flavonoids are very effective antioxidants (Yanishlieva-Maslarova, 2001) that constitute a large group of naturally occurring plant phenolic compounds including flavones, flavonols, isoflavones, flavonones and chalcones. Flavonoids contain a characteristic C6–C3–C6 structure, with free hydroxyl groups attached to aromatic rings, and they inhibit lipid oxidation by scavenging free radicals or by other mechanisms such as singlet oxygen quenching, metal chelation, and lipoxygenase inhibition (Yanishlieva-Maslarova, 2001). Many plant phenolics exhibiting antioxidant properties have been studied and proposed for protection against oxidation (Oktay, Guloin, & Kufrevioglu, 2003; Van der Sluis, Dekker, Skrede, & Jongen, 2002). Natural antioxidants occur in all parts of the plant (wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen, and seeds) (Pratt, 1992). Flower is an important part of plant which contains a great variety of natural antioxidants, such as phenolic acids, flavonoids, anthocyanin and many other phenolic compounds (Kaur, Alamb, Jabbar, Javed, & Athar, 2006; Youwei, Jinlian, & Yonghong, 2008).

Edible flowers are becoming more popular as evidenced by an increase in the number of edible flower cookbooks, culinary magazine articles, and television shows. Consumers purchase packaged flowers for use in meals as a garnish or ingredients in salads, soups, entrees, desserts, and drinks (Barash, 1997; Kelley, Cameron, Biernbaumb, & Poff, 2003). Flowers have traditionally been used in many types of cooking such as European, Asian, East Indian, Victorian English, and Middle Eastern. Early American settlers also used flowers as food. Edible flowers can be used fresh as a garnish or as an integral part of a dish, such as a salad. Some flowers can be stuffed or used in stir-fry dishes (Belsinger, 1991). In Thailand, many flowers have been eaten since ancient times, and some have medicinal properties as well as nutritional value (Institute of Nutrition, 1999; Wongwattanasathien, Kangsadalampai, & Tongyonk, 2010). It is believed that consumption of these flower vegetables can cure illness and diseases. They also help individuals who suffer from diarrhea, thus indicating the anti-microbial activity of these flowers (Boonyaprapatsara, 2000; Vachirasup, 1995). Therefore, the objective of the present study was to generate information about the phenolic compounds, antioxidant properties and nutritional value of 12 edible flowers from northeastern Thailand; we expect to shed light on their potential health benefits that could be useful for consumers and public health workers.

2. Materials and methods

2.1. Edible flowers

Twelve types of Thai cultivate edible flowers (Table 1) were studied, namely, Puangchompoo (*Antigonon leptopus*), Fueangfa (*Bougainvillea hybrida*), Khee lek (*Cassia siamea*), Aunchan (*Clitorea ternatea*), Dawkajay (*Cosmos sulphureus*), Chaba (*Malvaviscus arboreus*), Kem (*Ixora chinensis*), Katin (*Leucaena leucocephala*), Bua Luang (*Nelumbo nucifera*), Leelawadee (*Plumeria obtusa* L.), Daao rueang (*Tagetes erecta*) and Kajorn (*Telosma minor*). The fresh edible flowers were collected from northeastern region of Thailand. The plant samples were carried to the laboratory within a maximum of 6 h for experimentation. Their names, characteristics and biological activities are presented in Table 1.

2.2. Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), Folin–Ciocalteu reagent, phenolic compounds (gallic acid (GA), protocatechuic acid (PCCA), *p*-hydroxybenzoic acid (*p*-HO), chorogenic acid (ChA), vanilic acid (VA), caffeic acid (CFA), syringic acid (SyA), *p*-coumaric acid (*p*-CA), ferulic acid (FA), sinapic acid (SNA), rutin, myricetin, quercetin, apigenin and kaempferol were obtained from Fluka (Neu-Ulm, Germany). HPLC-grade methanol, acetonitrile and other solvents and reagents were purchased from Merck (Darmstadt, Germany). All chemicals and reagents used in the study were of analytical grade.

2.3. Determination of phenolic compounds

2.3.1. Phenolic compounds extraction

Phenolic compounds in the samples were extracted using a modification of the procedure described by Bengoechea et al. (1997) as adapted from Uzelac, Pospišil, Levaj, and Delonga (2005). Each sample (5 g) was mixed with 50 mL of methanol/HCl (100:1, v/v) which contained 2% tert-butylhydroquinone in an inert atmosphere (N_2) during 12 h at 35 °C in the dark. The extract was then centrifuged at 4000*g*, and the supernatant was evaporated to dryness under reduced pressure (35–40 °C). The residue was redissolved in 25 mL of water/ethanol (80:20, v/v) and extracted four times with 25 mL of ethyl acetate. The organic fractions were combined, dried for 30–40 min using anhydrous sodium sulfate, filtered through a Whatman-40 filter, and evaporated to dryness under vacuum (35–40 °C). The extracts were used to determine total anti-oxidant activity, total phenolics, total flavonoids and phenolic compounds by using HPLC method.

2.3.2. Extraction of bound phenolic compounds

The bound phenolic contents were extracted according to the method of Butsat, Weerapreeyakul, and Siriamornpun (2009) with minor modifications. Briefly, the residue from soluble fractions described above were drained off and hydrolyzed directly with 2 M sodium hydroxide at room temperature for 1 h with shaking under nitrogen gas, and the solution was neutralized with an appropriate amount of hydrochloric acid and extracted with hexane to remove lipids. The final solution was extracted five times with ethyl acetate. The ethyl acetate fraction was evaporated to dryness. Phenolic compounds were dissolved with 5 mL of methanol and analyzed by HPLC. All analyses were performed in triplicate.

2.3.3. Determination of total phenolic contents

Total phenolic content was determined using Folin–Ciocalteu reagent as followed by Abu Bakar, Mohamed, Rahmat, and Fry (2009) as adapted from Velioglu, Mazza, Gao, and Oomah (1998). Briefly, 300 μL of extract was mixed with 2.25 ml of

Table 1 – The characteristics and biological activities literature of the selected edible flowers.

Scientific name	Thai name	Common name	Edible parts	Cooking style	Activities	References
Antigonon leptopus	Puangchompoo	Coral vine	Flower	Salad, frying	Prevention and treatment of cough and flu-related pain, anti-thrombin, analgesic, anti-inflammatory, anti-diabetic and lipid peroxidation inhibitory	Mitchell & Ahmad, 2006; Chistokhodova et al., 2002; Lans, 2006; Mamidipalli et al., 2008; Vanisree et al., 2008
Bougainvillea hybrida	Fueangfa	Paper flower	Flower	Salad, frying	Analgesic, antidiabetic, antiinflamatory, antimicrobial, astringent and diuretic effects	Iribarren & Pomilio, 1983; Braca et al., 2001
Cassia siamea	Khee lek	Siamese senna	Flower	Light curry, curry	Treatment of fever, skin disease, constipation, diabetes, hypertension and insomnia	Kinghorn & Balandrin, 1992; Deachapunya et al., 2005
Clitorea ternatea	Aunchan	Asian pigeonwing	Flower	Salad, dessert, vegetable	Applied for sore throat and fever (root), hypoglycemic and hypolipidemic effects (flower), hectic fever, severe bronchitis, asthma, remedy for snakebite, scorpion sting, antihyperglycaemic and antihyperlipidaemic	Chopra et al., 1982; Daisy et al., 2007; Rajathi & Daisy, 2000; Daisy1 et al., 2009
Cosmos sulphureus	Dawkajay	Cosmos	Flower	Salad	Jaundice, intermittent fever, splenomegaly, anti-inflammatory activity, antioxidant and antigenotoxic activity, antimicrobial	Jang et al., 2008; Alexandros, 2007; Akihisa et al.1996; Rasdi et al., 2010
Malvaviscus arboreus	Chaba	Queen of tropic flower	Flower	Salad, light curry	Antifungal activity	Kobaisy et al., 2001; Boughalleb et al., 2005
Ixora chinensis	Kem	West Indian jasmine	Flower	Salad, frying	Hepatoprotective, Chemoprotective, antimicrobial, anti-oxidant, antinociceptive, anti-mitotic, anti-inflammatory activities	Vadivu, Jayshree, Kasthuri, Rubhini, & Rukmankathan, 2009
Leucaena leucocephala	Katin	White popinacs	Flower	Salad, vegetable	Anti-diabetes	Darmono & Simanjuntak, 2006
Nelumbo nucifera	Bua luang	Sacred lotus	Flower	Salad, frying	Anti-obesity, anti-oxidant, anti-diabetic activity, anti-inflammatory, antipyretic activity and antifungal activity	Ono et al., 2006; Sohn et al., 2003; Mukherjee et al., 1997a; ; Mukherjee et al., 1997b; Agnihotri et al., 2008
Plumeria obtusa L.	Leelawadee	pagoda tree	Flower	Salad, frying	Antifouling, anticancer and algicidal	Coppen, 1983; Coppen et al., 1983; Fujimoto & Made, 1988
Tagetes erecta	Daao rueang	Marigold	Flower	Salad, frying, light curry	For skin complaints, wounds and burns, conjunctivitis and poor eyesight, menstrual regularities, inflammation, antiviral and antitomer	Cetkovic et al., 2004; Wichtl, 1994; Ostad et al., 2004; Khalil et al., 2007
Telosma minor	Kajorn	Cowslip creeper	Flower	Light curry, steaming, frying	Antimicrobial activity	Krasaekoopt & Kongkarnchanatip, 2005

Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min; 2.25 mL of sodium carbonate (60 g/L) solution were added to the mixture. After 90 min at room temperature, absorbance was read at 725 nm using spectrophotometer. Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g dry weight).

2.3.4. *Determination of total flavonoid content*

Total flavonoid content was determined using the colorimetric method described by Abu Bakar et al. (2009) as adapted from Dewanto, Wu, Adom, and Liu (2002). Briefly, 0.5 mL of the extract was mixed with 2.25 mL of distilled water in a test tube followed by addition of 0.15 mL of 5% $NaNO_2$ solution. After 6 min, 0.3 mL of a 10% $AlCl_3.6H_20$ solution was added and allowed to stand for another 5 min before 1.0 mL of 1 M NaOH was added. The mixture was mixed well by vortex. The absorbance was measured immediately at 510 nm using a spectrophotometer. Results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g dry weight).

2.4. *Identification and quantification of phenolic compounds*

RP-HPLC analysis of phenolic compounds was performed using Shimadzu LC-20AC pumps (Shimadzu Co., Kyoto, Japan), SPD-M20A with a diode array detector, and chromatographic separations were performed on a LUNA C-18 column (4.6 × 250 mm, i.d. 5 μm). The composition of solvents and the gradient elution conditions used were as described by Uzelac et al. (2005) and Butsat et al. (2009) with some modifications. The mobile phase consisted of purified water with acetic acid (pH 2.74) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 mL/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5 to 9% solvent B; from 5 to 15 min, 9% solvent B; from15 to 22 min, linear gradient from 9% to 11% solvent B; from 22 to 38 min, linear gradient from 11% to 18% solvent B; from 38 to 43 min, from 18% to 23% solvent B; from 43 to 44 min, from 23% to 90% solvent B; from 44 to 45 min, linear gradient from 90% to 80% solvent B; from 45 to 55 min, isocratic at 80% solvent B; from 55 to 60 min, linear gradient from 80% to 5% solvent B and a re-equilibration period of 5 min with 5% solvent B used between individual runs. Operating conditions were as follows: column temperature, 38 °C, injection volume, 20 μL, and UV-diode array detection at 280 nm (hydroxybenzoic acids), 320 nm (hydroxycinnamic acids) and 370 nm (flavonols) at a flow-rate of 0.8 mL/min. Spectra were recorded from 200 to 600 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of standard compounds and were detected using an external standard method.

2.5. *Determination of antioxidant activity*

2.5.1. *DPPH radical-scavenging activity*

The hydrogen atom or electron-donation ability of the corresponding extracts was measured from the bleaching of a purple-colored methanol solution of DPPH (Gulluce, Sahin, Sokmen, et al., 2007). The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, was determined according to the method described by Braca et al. (2001). The extract (0.1 mL) was added to 2.9 mL of a 0.004% DPPH solution in methanol. Absorbance at 517 nm was read after 30 min, and the percent inhibition (I%) of activity was calculated as $[(A_o - A_e)/A_o] \times 100$ (A_o = absorbance without extract; A_e = absorbance with extract).

2.5.2. *Ferric reducing/antioxidant power (FRAP)*

FRAP assay was based on the reduction of Fe^{3+}–TPTZ to a blue colored Fe^{2+}–TPTZ (Butsat & Siriamornpun, 2010). The FRAP assay was adapted from Benzie and Strain (1996). The antioxidant potential of the extract was determined against a standard curve of ferrous sulfate (FeII, 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mM) in Milli-Q water or methanol with 0.1% (v/v) HCl.

The FRAP reagent was freshly prepared by mixing 100 mL of acetate buffer (300 mM, pH 3.6), 10 mL TPTZ solution

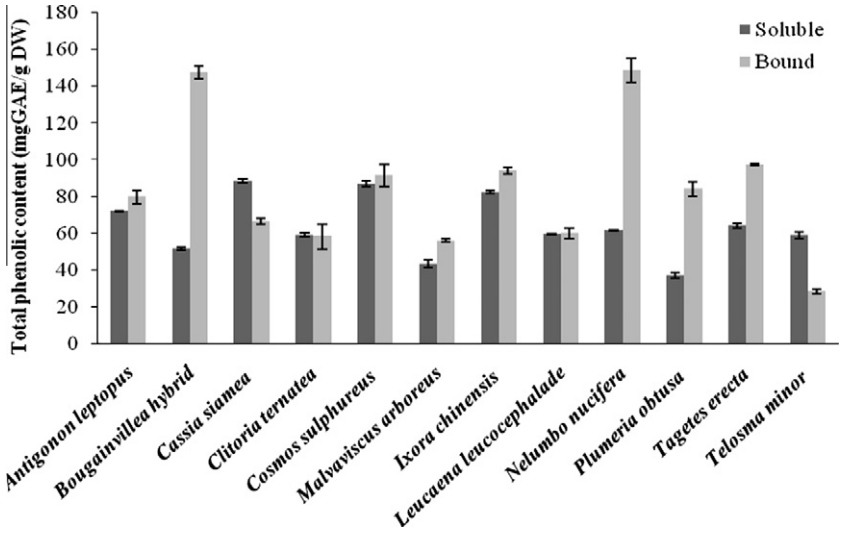

Fig. 1 – Total phenolic content of the extracts from 12 flowers.

(10 mM TPTZ in 40 mM HCl), 10 mL $FeCl_3.6H_2O$ (20 nM) at a ratio of 10:1:1 (v/v/v) and 12 mL distilled water, at 37 °C. To perform the assay, 1.8 mL of FRAP reagent, 180 μL Milli-Q water and 60 μL sample, standard or blank were then added to the same test tubes, and incubated at 37 °C for 4 min; absorbance was read at 593 nm, using the FRAP working solution as a blank. The reading of relative absorbance should be within the range 0–2.0; otherwise, the sample should be diluted. In the FRAP assay, the antioxidant potential of sample was determined from a standard curve plotted using the $FeSO_4.7H_2O$ linear regression equation to calculate the FRAP values of the sample.

2.6. Statistical analyses

Statistical analyses were conducted using SPSS software. Analysis of variance (ANOVA) in a completely randomized design and Duncan's multiple range test were used to compare any significant differences between samples. Values were expressed as means ± standard deviations. All determinations were done at least in triplicate and all were averaged. The confidence limits used in this study were based on 95% ($P < 0.05$).

3. Results and discussion

3.1. Total phenolic content (TPC)

Phenolic compounds are widely distributed in fruits, vegetables and cereals (Bonolia, Marconib, & Caboni, 2004; Li, Smith, & Hossain, 2006). These components have received considerable attention, due to their antioxidant activities and free-radical scavenging abilities, which potentially have beneficial implications in human health (Imeh & Khokhar, 2002; Lopez-Velez, Martinez-Martinez, & Del Valle-Ribes, 2003). The total phenolic content differed among the different types of plant and each plant extract contained a lower total flavonoid content than the total phenolic content, due to the presence of non-flavonoid phenolic substances in plants (Maisuthisakul, Suttajit, & Pongsawatmanit, 2007; Pietta, 2000). The TPC values expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight are shown in Fig. 1. There were significant differences amongst different varieties tested. The soluble TPC of edible flowers ranged from 37 to 89 mg GAE/g dry weight. *C. siamea* and *C. sulphureus* had the highest soluble TPC with concentration 88.5 and 86.8 mg GAE/g dry weight, followed by *I. chinensis* (82.4 mg GAE/g dry weight) and *A. leptopus* (72.1 mg GAE/g dry weight), while *P. obtusa* had the lowest soluble TPC (37.0 mg GAE/g dry weight). According to Kaur et al. (2006) reported that *C. siamea* (Indian variety) had high levels on phenolic compounds. The ethanolic extract was found to contain 257 mg/g of GAE. Total phenol content has been reported to be associated with antioxidant activity and in various plants. It was observed that three flowers contained remarkably high phenolic contents (>80 mg GAE/g dry weight): *C. siamea*, *C. sulphureus* and *I. chinensis*. Elzaawely, Xuan, Koyama, and Tawata (2007) studied the total phenolics from *Alpinia zerumbet* growing in Japan. The results confirmed that flower extracts contained higher amounts of total phenolic than seed of *A. zerumbet*. In addition, Falleh et al. (2008) found that the total phenolic was lower in flowers of *Cynara cardunculus* than in leaves and seeds. Bano et al. (2003) reported that the distribution of secondary metabolites may change during plant development. However, flowers may be considered suitable for further investigation of their potential antioxidant activity in the human body because usually they can be consumed fresh without any concern for their toxicity. Bound TPC of edible flowers ranged from 56.04 to 148.73 mg of GAE/g dry weight. *B. hybrida* and *N. nucifera* had had the highest bound TPC with concentration 147.72 and 148.73 mg of GAE/g dry weight. The lowest bound TPC was found in *T. minor* (28.37 mg of GAE/g dry weight). *B. hybrida* and *N. nucifera* exhibited 2.5 times higher phenolic content in bound phenolic extract compared to its soluble phenolic counterpart. This

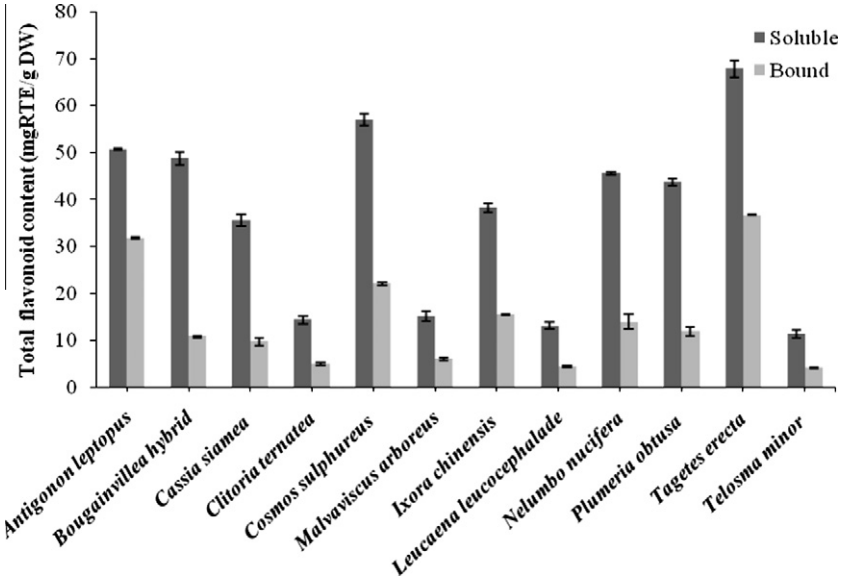

Fig. 2 – Total flavonoid content of the extracts from 12 flowers.

Table 2 – Soluble phenolic acid contents (micrograms per gram dry weight) of the extracts from 12 flowers.

Scientific name	Soluble phenolic acids (μg/g DW)									
	Hydrobenzoic acids				Hydrocinnamic acids					
	GA	PCCA	*p*-OH	VA	ChA	CFA	SyA	*p*-CA	FA	SNA	Total
Antigonon leptopus	25.3 ± 1.0	7.2 ± 1.7	7.5 ± 0.8	3.3 ± 0.1	14.8 ± 1.6	8.20 ± 0.34	5.7 ± 0.3	4.6 ± 0.3	13.7 ± 1.5	26.3 ± 0.9	116.6 ± 8.5
Bougainvillea hybrida	25.8 ± 1.5	4.2 ± 2.3	6.5 ± 1.0	7.5 ± 0.8	16.1 ± 1.0	11.73 ± 1.58	6.3 ± 0.4	5.2 ± 0.2	12.0 ± 0.4	25.4 ± 0.9	120.7 ± 10.1
Cassia siamea	30.3 ± 2.6	638.4 ± 11.4	29.4 ± 1.9	4.2 ± 0.8	20.6 ± 1.3	14.93 ± 1.85	16.0 ± 0.5	17.5 ± 1.1	11.6 ± 1.1	10.4 ± 0.9	793.3 ± 23.5
Clitoria ternatea	33.2 ± 1.7	1.8 ± 0.1	Nd	Nd	Nd	10.03 ± 0.28	Nd	11.6 ± 0.4	34.8 ± 1.6	152.1 ± 11.1	243.5 ± 15.2
Cosmos sulphureus	30.0 ± 0.4	2.2 ± 0.3	10.2 ± 0.7	9.3 ± 1.0	72.8 ± 3.9	13.47 ± 0.11	81.0 ± 1.9	11.6 ± 0.5	76.3 ± 2.6	308.2 ± 7.0	615.1 ± 18.4
Malvaviscus arboreus	23.2 ± 1.5	2.2 ± 0.1	52.0 ± 1.26	Nd	14.8 ± 1.7	Nd	Nd	5.6 ± 1.0	11.0 ± 1.5	7.9 ± 0.8	116.7 ± 7.9
Ixora chinensis	66.3 ± 2.3	13.1 ± 0.4	26.2 ± 1.13	15.6 ± 0.4	54.0 ± 2.8	11.41 ± 0.16	6.0 ± 0.2	7.5 ± 0.1	19.56 ± 1.2	31.4 ± 0.6	251.1 ± 9.3
Leucaena leucocephalade	27.9 ± 1.4	64.6 ± 4.0	10.1 ± 0.6	32.9 ± 2.0	26.5 ± 1.3	18.21 ± 1.87	6.9 ± 0.5	5.2 ± 0.2	10.4 ± 1.5	51.8 ± 0.3	254.5 ± 13.7
Nelumbo nucifera	26.7 ± 1.6	9.9 ± 0.4	49.2 ± 1.7	2.8 ± 0.1	15.8 ± 0.6	9.32 ± 0.30	8.7 ± 1.2	5.4 ± 0.2	8.2 ± 0.4	11.9 ± 1.4	147.9 ± 7.9
Plumeria obtusa	25.5 ± 0.2	3.5 ± 0.1	21.7 ± 0.2	20.4 ± 0.7	22.8 ± 0.1	12.80 ± 1.53	7.4 ± 0.2	8.3 ± 0.6	9.8 ± 0.1	149.3 ± 6.7	281.5 ± 10.4
Tagetes erecta	416.4 ± 4.0	6.7 ± 0.9	4.1 ± 0.2	Nd	17.3 ± 0.3	12.31 ± 0.58	8.2 ± 0.4	6.2 ± 0.2	38.7 ± 1.7	39.3 ± 2.2	549.2 ± 10.5
Telosma minor	25.2 ± 2.2	1.3 ± 0.0	3.6 ± 0.1	6.4 ± 0.2	23.9 ± 0.6	10.13 ± 1.01	7.2 ± 0.5	5.1 ± 0.4	10.4 ± 1.2	23.5 ± 2.2	116.7 ± 8.4

Values are expressed as mean ± SD of triplicate measurement.
Nd: not detected; GA: gallic acid; PCCA: protocatechuic; *p*-OH: *p*-hydroxy benzoic acid; ChA: chorogenic acid; VA: vanilic acid; CFA: caffeic acid; SyA: syringic acid; *p*-CA: *p*-coumaric acid; FA: ferulic acid; SNA: sinapic acid.

finding was in agreement with the results of Madhujith and Shahidi (2009) that the content of bound phenolic was significantly higher than free phenolic of barley. The comparison between soluble and insoluble bound phenolic contents showed an inconsistency in their trend among different millet types.

3.2. *Total flavonoid content (TFC)*

Flavonoids, as one of the most diverse and widespread groups of natural compounds, are probably the most important natural phenolics (Prasad et al., 2009). A multitude of biological effects *in vitro* and *in vivo* results from the consumption of flavonoid-containing foods. Epidemiologic studies show that increased consumption of flavonoids reduces the risk of cardiovascular disease and certain types of cancer (Arts & Hollman, 2005; Koga & Meydani, 2001). Significant differences were detected amongst varieties of edible flowers. The soluble TFC of edible flowers ranged from 11.4 mg RE/g dry weight in *T. minor* to 68 mg RE/g dry weight in *T. erecta* (Fig. 2). *T. erecta* had the highest soluble TFC, with a concentration of 67.9 mg RE/g dry weight, followed by *C. sulphureus* (57.0 mg RE/g dry weight), *A. leptopus* (50.8 mg RE/g dry weight) and *B. hybrida* (48.8 mg RE/g dry weight). In addition, *T. minor* had the lowest soluble TFC, with concentration 11.4 mg RE/g dry weight. However, the extracts with higher flavonoid content did not always have a higher phenolic content, as was evident for *T. erecta* which had a higher total flavonoid content (67.9 mg RE/g dry weight) compared with that of *C. siamea* (35.6 mg RE/g dry weight), although the total phenolic content was lower (64.3 and 88.5 mg RE/g dry weight, respectively). The TFC varied significantly ($P < 0.05$) between soluble and bound fractions for all edible flowers in this study. In general, all soluble extracts had higher TFC than their corresponding bound extracts. The bound TFC of *T. erecta* had the highest (36.72 mg RE/g dry weight) TFC, whereas *T. minor* showed the lowest (4.20 mg RE/g dry weight). According to Chandrasekara and Shahidi (2010) reported that the soluble extracts in millets had higher TFC than bound extracts. In contrast, Adom and Liu (2002) reported that soluble extracts of corn, wheat, oat, and rice contained a lesser TFC than their bound counterparts.

3.3. *Identification and quantification of phenolic compounds*

RP-HPLC analysis was used to identify the phenolic compounds of edible-flowers extracts, by comparison with standard compounds. Phenolic acids are hydroxylated derivatives of hydrobenzoic and hydrocinnamic, which often occur in plants as esters, glycosides and bound complexes (Germano et al., 2006). In the 12 edible flower varieties analyzed, it was possible to identify 10 phenolic acid: gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, chorogenic acid, vanilic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid as well as five flavonoids, namely rutin, myricetin, quercetin, apigenin and kaempferol. The distribution of soluble and bound phenolic acids in all flowers is presented in Tables 2 and 3. The highest concentrations of total soluble phenolic acids were found in flowers of *C. siamea*

Table 3 – Bound phenolic acids contents (micrograms per gram dry weight) of the extracts from 12 flowers.

Scientific name	Bound phenolic acids (μg/g DW)										
	Hydrobenzoic acids				Hydrocinnamic acids						
	GA	PCCA	*p*-OH	VA	ChA	CFA	SyA	*p*-CA	FA	SNA	Total
Antigonon leptopus	34.8 ± 0.9	66.2 ± 2.7	25.5 ± 3.6	Nd	Nd	Nd	Nd	15.8 ± 0.6	16.3 ± 0.2	25.3 ± 1.1	183.9 ± 9.1
Bougainvillea hybrida	Nd	32.0 ± 0.2	Nd	Nd	Nd	Nd	Nd	Nd	196.6 ± 7.2	119.5 ± 10.5	348.1 ± 17.8
Cassia siamea	Nd	Nd	8.8 ± 0.4	3.4 ± 0.1	Nd	Nd	4.7 ± 0.1	93.2 ± 4.5	128.6 ± 6.6	216.8 ± 8.6	455.5 ± 20.3
Clitoria ternatea	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	108.0 ± 4.5	153.5 ± 12.5	261.5 ± 17.0
Cosmos sulphureus	Nd	21.8 ± 0.1	Nd	Nd	Nd	Nd	5.5 ± 0.1	54.7 ± 1.8	132.1 ± 10.4	418.5 ± 13.3	418.5 ± 25.7
Malvaviscus arboreus	77.5 ± 4.6	4.4 ± 0.5	Nd	Nd	Nd	Nd	Nd	7.3 ± 0.4	20.9 ± 1.4	214.3 ± 8.2	324.4 ± 15.1
Ixora chinensis	29.8 ± 0.3	39.9 ± 5.6	58.7 ± 1.5	Nd	Nd	Nd	57.2 ± 0.3	104.1 ± 9.1	26.9 ± 2.1	64.8 ± 6.7	381.4 ± 25.6
Leucaena leucocephalade	Nd	Nd	15.0 ± 0.9	Nd	Nd	Nd	Nd	37.1 ± 2.1	57.1 ± 1.2	Nd	134.9 ± 4.2
Nelumbo nucifera	Nd	22.7 ± 1.1	18.8 ± 1.7	Nd	Nd	Nd	Nd	Nd	149.0 ± 7.5	312.4 ± 12.3	502.9 ± 22.6
Plumeria obtusa	2.7 ± 0.1	Nd	Nd	Nd	Nd	Nd	Nd	17.8 ± 1.3	144.9 ± 15.0	164.5 ± 9.5	329.9 ± 25.9
Tagetes erecta	28.9 ± 0.5	25.0 ± 0.8	Nd	Nd	145.8 ± 7.8	Nd	66.8 ± 1.4	Nd	314.9 ± 25.3	491.1 ± 38.2	1072.5 ± 74.0
Telosma minor	Nd	13.6 ± 0.2	Nd	Nd	26.5 ± 2.9	Nd	Nd	Nd	13.5 ± 0.2	16.8 ± 0.8	70.4 ± 4.1

Values are expressed as mean ± SD of triplicate measurement.
Nd: not detected; GA: gallic acid; PCCA: protocatechuic; *p*-OH: *p*-hydroxy benzoic acid; ChA: chorogenic acid; VA: vanilic acid; CFA: caffeic acid; SyA: syringic acid; p-CA: *p*-coumaric acid; FA: ferulic acid; SNA: sinapic acid.

(739 μg/g dry weight), followed by *C. sulphureus* (615 μg/g dry weight), *T. erecta* (549 μg/g dry weight) and *P. obtusa* (281 μg/g dry weight). The main soluble phenolic acids in these flowers were gallic acid, ferulic acid and sinapic acid. Gallic acid and ferulic acid have attracted considerable attention due to their reported health benefits (Lu, Nie, Belton, Tang, & Zhao, 2006; Roche, Dufour, Mora, & Dangles, 2005). The *p*-hydroxybenzoic acid, chorogenic acid, vanilic acid, syringic acid and *p*-coumaric acid occurred in small quantities, but not in all flowers investigated. In flowers of *Clitoria ternatea*, no *p*-hydroxybenzoic acid, chorogenic acid, vanilic acid and syringic acid were detected (Table 2). The smallest amounts of phenolic acids were found in *A. leptopus*, *M. arboreus* and *T. minor* (Table 2). The contents of bound phenolic acids are listed in Table 3. With respect to variation of bound phenolic acid content in all extracts, *T. erecta* exhibited the highest total bound phenolic acids (1072.5 μg/g dry weight). The most abundant bound phenolic acid in all extracts was sinapic acid, followed by ferulic acid along with traces of vanilic acid, chorogenic acid, *p*-hydroxy benzoic acid and syringic acid. Compared to soluble phenolic acids, the contents of bound phenolics were 12-fold greater for sinapic acid and 8-fold greater for ferulic acid. These results agree with previous studies of other grains, such as wild rice (Bunzel, Allerdings, Sinwell, Ralph, & Steinhart, 2002). Phenolic acids can be classified as free, soluble conjugated and bound phenolic acids (Regnier & Macheix, 1996). Bound phenolic acids are typically involved in cell wall structure (Bunzel et al., 2002) where the cross-linking esters of lignin components via phenolic acids appear to have a profound effect on the growth of the cell wall and its mechanical properties and biodegradability. For flavonoids, the major of flavonoid in flowers were quercetin, ranging from 33.6 μg/g dry weight in *M. arboreus* to 1024 μg/g dry weight in *T. erecta* (Table 4). While, apigenin was found in smaller amount ranged from 0.17 μg/g dry weight (*T. minor*) to 0.72 μg/g dry weight (*C. sulphureus*). However, apigenin not

Table 4 – Soluble flavoniod contents (micrograms per gram dry weight) of the extracts from 12 flowers.

Sample	Soluble flavonoid contents (μg/g DW)					Total
	Rutin	Myricetin	Quercetin	Apigenin	Kaempferol	
Antigonon leptopus	5.7 ± 0.3	4.50 ± 0.0	294.1 ± 45.0	Nd	3.06 ± 0.0	307.4 ± 45.3
Bougainvillea hybrida	51.5 ± 2.4	5.60 ± 0.3	79.7 ± 4.9	Nd	3.54 ± 0.2	140.3 ± 7.8
Cassia siamea	64.0 ± 0.1	4.56 ± 0.2	61.9 ± 3.2	Nd	3.21 ± 0.1	133.7 ± 3.6
Clitoria ternatea	38.1 ± 2.9	4.85 ± 0.0	68.9 ± 2.2	Nd	3.65 ± 0.0	115.5 ± 5.2
Cosmos sulphureus	7.0 ± 0.2	4.90 ± 0.1	485.9 ± 49.5	0.72 ± 0.0	3.54 ± 0.1	502.1 ± 50.0
Malvaviscus arboreus	27.7 ± 2.6	5.05 ± 0.1	33.6 ± 2.0	Nd	3.18 ± 0.0	69.5 ± 4.7
Ixora chinensis	139.0 ± 2.6	5.18 ± 0.1	102.4 ± 3.2	0.64 ± 0.0	3.77 ± 0.0	251.0 ± 5.9
Leucaena leucocephalade	16.2 ± 1.5	5.72 ± 0.0	67.1 ± 5.5	Nd	4.23 ± 0.1	93.3 ± 7.1
Nelumbo nucifera	23.1 ± 3.8	5.00 ± 0.1	237.8 ± 16.8	0.62 ± 0.	3.79 ± 0.4	270.3 ± 21.1
Plumeria obtuse	500.3 ± 32.0	5.06 ± 0.1	193.6 ± 8.4	Nd	3.58 ± 0.0	702.5 ± 40.6
Tagetes erecta	156.6 ± 3.8	5.39 ± 0.2	1024.7 ± 52.0	Nd	3.43 ± 0.0	990.1 ± 56.0
Telosma minor	Nd	4.76 ± 0.1	117.6 ± 8.0	0.17 ± 0.0	3.57 ± 0.1	126.1 ± 8.2

Values are expressed as mean ± SD of triplicate measurement.
Nd: not detected.

Table 5 – Bound flavoniod contents (micrograms per gram dry weight) of the extracts from 12 flowers.

Sample	Bound flavonoid contents (μg/g DW)					Total
	Rutin	Myricetin	Quercetin	Apigenin	Kaempferol	
Antigonon leptopus	56.1 ± 1.1	Nd	56.1 ± 1.6	18.2 ± 2.0	Nd	130.4 ± 4.7
Bougainvillea hybrida	4.1 ± 1.0	Nd	14.1 ± 3.0	8.9 ± 0.5	Nd	27.1 ± 4.4
Cassia siamea	32.0 ± 1.6	Nd	10.9 ± 0.4	10.0 ± 8.8	Nd	52.9 ± 10.7
Clitoria ternatea	113.2 ± 11.8	Nd	11.1 ± 1.9	17.2 ± 1.9	Nd	141.4 ± 15.6
Cosmos sulphureus	31.7 ± 2.9	2.8 ± 0.6	109.2 ± 10.3	14.5 ± 1.5	Nd	158.1 ± 15.1
Malvaviscus arboreus	Nd	Nd	9.1 ± 0.3	25.7 ± 5.3	Nd	34.8 ± 5.5
Ixora chinensis	28.5 ± 1.5	3.0 ± 0.4	13.6 ± 0.8	14.5 ± 2.5	Nd	59.6 ± 5.2
Leucaena leucocephalade	7.7 ± 0.2	2.83 ± 0.4	12.6 ± 0.2	15.5 ± 0.9	Nd	38.6 ± 1.6
Nelumbo nucifera	Nd	Nd	2.1 ± 2.0	9.6 ± 0.3	Nd	11.7 ± 2.2
Plumeria obtuse	Nd	Nd	41.7 ± 1.9	5.1 ± 4.5	Nd	46.8 ± 6.5
Tagetes erecta	139.8 ± 7.6	4.0 ± 0.6	145.6 ± 2.4	23.7 ± 2.3	Nd	313.1 ± 12.9
Telosma minor	Nd	Nd	Nd	3.2 ± 0.3	Nd	3.2 ± 0.3

Values are expressed as mean ± SD of triplicate measurement.
Nd: not detected.

detected in *A. leptopus, B. hybrida, C. siamea, C. ternatea, M. arboreus, Plumeria obtuse, T. erecta* and *T. minor*. Quercetin is common flavonoid found in edible plants and is usually present in glycosylated form (Chen, Zhang, & Ye, 2000). According to our present data, the content of flavonoids and antioxidant activities were associated with the amount of quercetin present in the flowers studied. This may indicate that quercitin could be a potential active compound in the flowers. Natural antioxidants are important ingredients that facilitate the control of the oxidative deterioration of foods. Foods containing these antioxidants are expected to have preventive activity against oxidation-related diseases (Gills, 1964; Vanisree, Alexander-Lindo, DeWitt, & Nair 2008). Flower extracts, which exhibited strong antioxidant activities, were found to contain high amounts of total and individual phenolics that may contribute to this activity. Phenolic compounds are commonly found in plants and they have been reported to have a strong antioxidant activity (Elzaawely, Xuan, & Tawata, 2005; Mansouri, Embarek, Kokkalou, & Kefalas, 2005). The antioxidant potential of phenolic compounds is dependent on the number and arrangement of the hydroxyl groups as well as the presence of electron donating substituent in the ring structure (Elzaawely et al., 2007; Lapornik, Prosek, & Wondra, 2005) The contents of bound flavonoids are listed in Table 5. With respect to variation of bound flavonoids content in all extracts, *T. erecta* exhibited the highest total bound flavonoids (313.1 μg/g dry weight). The most abundant bound flavonoids in all extracts were quercetin and apigenin. Compared to soluble flavonoids, the contents of bound flavonoids were lower.

3.4. *Antioxidant activity*

3.4.1. *DPPH radical scavenging activity*

The DPPH assay is a preliminary test to investigate the antioxidant potential of extracts. This assay has been widely used to test the free radical scavenging ability of various samples (Sakanaka, Tachibana, & Okada, 2005; Shimoji et al., 2002). DPPH, a free radical compound, is a stable organic radical with a characteristic absorption at 517 nm; it was used to study the radical scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases. Antioxidants, on interaction with DPPH, either transfer an electron or hydrogen atom to DPPH, thus neutralizing its free radical character (Naik et al., 2003). The color changes from purple to yellow and its absorbance at wavelength 517 nm decreases. The DPPH radical-scavenging activity (percentage inhibition) of soluble and bound phenolic fractions from twelve edible flowers is given in Table 6. The percent inhibition (DPPH radical scavenging activity) of soluble phenolic fractions was in a wide range of 31 in *M. arboreus* to 97% in *C. siamea*. As observed from result, *C. siamea* had obviously stronger antioxidant activity than others which supports previous findings (Thongsaard, Chainakul, Bennett, & Marsden, 2001; Wongwattanasathien et al., 2010). Kaur et al. (2006) evaluated the antioxidant activity of *C. siamea* flower (Indian variety) and found that the extract neutralized 96% of DPPH radicals at a concentration of 0.25 mg/mL. *C. siamea* flowers bear a potent antioxidant activity. Their constituents scavenge free radicals, chelate the catalytic metal ions and exert a protective effect against oxidative damage induced to cellular macromolecules (Kaur et al., 2006). Thongsaard et al. (2001) reported an active compound present in leaves and flowers of *C. siamea* is namely known as barakol (2,5-dimethyl-3aH-pyrano[2,3,4-de]-1-benzopyran-3a,8-diol).
The varied radical scavenging activity of the extracts depended on the amount of total phenolic content and total flavonoid content in each variety (Butsat & Siriamornpun, 2010; Cetkovic, Djilas, Canadanovic Brunet, & Tumbas, 2004). DPPH radical scavenging capacity of bound phenolic fraction ranged from 17.6% in *C. ternatea* to 85.7% in *T. erecta*. Bound phenolics of all edible flowers exhibited a lower DPPH radical scavenging capacity than their soluble counterparts.

3.4.2. *Ferric reducing/antioxidant power (FRAP) assay*

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe^{2+}–TPTZ) (Benzie & Strain 1996). Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through

Table 6 – Antioxidant activity of the extracts from 12 flowers.

Color of petal /scientific name	Common name	DPPH (% inhibition)		FRAP (mmol FeSO4/100 g dry weight)	
		Soluble	Bound	Soluble	Bound
Antigonon leptopus	Coral vine	89.36 ± 3.51^{de}	34.14 ± 1.87^{de}	61.97 ± 0.10^{a}	91.5 ± 10.4^{c}
Bougainvillea hybrida	Paper flower	91.44 ± 0.44^{d}	79.62 ± 3.54^{a}	58.80 ± 0.14^{b}	126.6 ± 11.2^{b}
Cassia siamea	Siamese senna	97.64 ± 0.28^{a}	38.30 ± 2.39^{d}	7.30 ± 0.22^{h}	26.6 ± 2.5^{e}
Clitoria ternatea	Asian pigeonwing	32.7 ± 2.75^{h}	17.59 ± 2.91^{h}	16.37 ± 0.78^{f}	7.7 ± 0.5^{i}
Cosmos sulphureus	Cosmos	87.04 ± 0.57^{e}	33.41 ± 2.29^{e}	53.86 ± 2.05^{c}	21.2 ± 2.0^{f}
Malvaviscus arboreus	Queen of tropic flower	31.39 ± 2.17^{h}	21.03 ± 0.10^{g}	27.13 ± 1.00^{e}	Nd
Ixora chinensis	West Indian jasmine	62.52 ± 3.61^{g}	33.67 ± 2.13^{e}	14.45 ± 0.57^{g}	15.8 ± 1.3^{g}
Leucaena leucocephalade	White popinacs	92.73 ± 3.80^{cd}	29.41 ± 1.51^{f}	38.48 ± 1.47^{d}	10.5 ± 0.9^{h}
Nelumbo nucifera	Sacred lotus	96.9 ± 0.14^{b}	68.64 ± 0.88^{b}	58.54 ± 0.53^{b}	90.1 ± 2.1^{c}
Plumeria obtusa	pagoda tree	69.65 ± 1.76^{f}	60.52 ± 0.78^{c}	26.03 ± 0.80^{e}	61.0 ± 2.2^{d}
Tagetes erecta	Marigold	94.32 ± 0.75^{c}	85.70 ± 2.65^{a}	60.92 ± 1.06^{a}	203.8 ± 8.8^{a}
Telosma minor	Cowslip creeper	34.08 ± 4.89^{h}	18.12 ± 1.04^{h}	16.26 ± 0.71^{f}	Nd

Values are expressed as mean ± SD (n = 3) of triplicate measurement, TPC = total phenolic content, TFC = total flavonoid content, FRAP = ferric reducing antioxidant activity. Different letters in the column indicate significant differences at $P < 0.05$.

donating a hydrogen atom (Duh, Du, & Yen, 1999; Gordon, 1990). According to Benzie and Strain (1996), the reduction of Fe^{3+}–TPTZ complex to blue colored of Fe^{2+}–TPTZ occurs at low pH. The ferric reducing ability powers of soluble and bound phenolic fractions from edible flowers extracts expressed as FRAP values (mmol $FeSO_4$/100 g dry weight) are shown in Table 6. The soluble phenolic fractions of *A. leptopus* and *T. erecta* had the highest FRAP value of 62.0 and 61.0 mmol $FeSO_4$/100 g dry weight, followed by *B. hybrida* (58.8 mmol $FeSO_4$/100 g dry weight) and *N. nucifera* (58.5 mmol $FeSO_4$/100 g dry weight). Thus, *A. leptopus* stood out as having significantly stronger ($P < 0.05$) reducing power than all other varieties. Vanisree et al. (2008) reported that the compounds isolated from the aerial parts of *A. leptopus* plant (Jamaica variety) have the ability to scavenge reactive oxygen species and reduce oxidative stress in *vitro* and *vivo*. As the edible flowers studied in our research have been shown to be contain high amount of phenolic compounds and exhibited strong antioxidant activities. Therefore, products prepared from traditional plants such as *A. leptopus* and their use as ingredients in herbal supplements are probably appealing to consumers. The reducing power of bound phenolic fraction ranged from 7.7 to 203.8 mmol $FeSO_4$/100 g dry weight with the highest efficacy rendered by *T. erecta* extract. Bound phenolics of the five edible flowers exhibited a lower FRAP value than their soluble counterparts. Reducing power of bound phenolic extracts showed a significant ($P < 0.05$) difference from that of soluble phenolic extracts except for *C. ternatea*, *C. sulphureus*, *M. arboreus*, *Leucaena leucocephalade* and *T. minor*. In the present study, bound extracts of *T. erecta* showed generally ~3-fold higher FRAP value than their soluble counterparts.

4. Conclusion

The data of twelve edible flower samples in the present study indicate that edible flowers were a rich source of phytochemicals, with high levels of phenolic compounds and antioxidant activities. Furthermore, our observations of 12 varieties, which were investigated in this study, indicated that yellow flowers exhibiting higher flavonoid content were likely to have higher antioxidant potential than other colors. This study generated useful information for consumers and many encourage researchers to utilize edible flowers as sources of phytochemicals. However, the toxicity of the plant extracts with high antioxidant activity should be tested, to confirm their safety for use as food additives. In addition, the antioxidant mechanisms and the anti-proliferative of the extracts should be further studied to gain more application for use as natural antioxidants.

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